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Original Article **Open Access**

Prevalence of high-risk HPV types 16 and 18 in relation to immune status and cervical cytological profile of HIV-infected women on antiretroviral therapy in northcentral Nigeria

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Abstract:

Background: Human papillomavirus (HPV) is a well-established causal agent of cervical cancer, and the first group of viruses to have been acknowledged to prompt carcinogenesis. They are linked with cancers of the uterine cervix, anogenital tumours, and head and neck malignancies. Cervical cancer is by far the most common HPV-related disease, with about 99% of cervical cancer cases caused by persistent genital high-risk (HR) HPVs, especially types 16 and 18.
Methodology: A hospital-based descriptive analytical study of 300 consenting HIV-infected women on anti-retroviral therapy (ART), selected from the three senatorial districts of Plateau State, Nigeria, was conducted over a period of 24 months (November 2018 to November 2020). Blood and cervical specimens were collected from each participant. HIV status was confirmed by standard rapid test on serum sample, $CD4^{+}$ cell count was determined by flow cytometry and HIV viral load estimation was done by GeneXpert nucleic acid amplification technique. Cervical cytology was performed by Papanicolaou (Pap smear) on the cervical specimen and reported according to the 2004 Bethesda system classification. HPV antigen was first detected on the cervical specimen using ELISA, and samples positive for HPV antigen were then subjected to multiplex PCR amplification of E6 and E7 genes to detect HR-HPV (16 and 18) and other HPV types. Standard questionnaires were administered to obtain information on biodata, risk factors and clinical presentations. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.0, and significance level was determined at $p<0.05$.
Results: Of the 300 participants, 84 were positive for HPV of any type, giving an overall prevalence of HPV infection of 28.0%. The prevalence of HPV-16 and HPV-18 types were 5.0% (15/300) and 5.3% (16/300) respectively. Cytological analysis showed that 36.3% (109/300) of the participants had cervical abnormalities ranging from low-grade to high grade squamous intraepithelial lesions and cervical intraepithelial neoplasia. HPV prevalence of 46.8% (51/109) in women with cervical abnormalities was significantly higher than 17.3% (33/191) in women with normal cervical cytology (OR 4.2, $p<0.0001$). HPV prevalence was higher in women with AG-US (100.0%), ASC-US (78.8%), AC-US (66.7%), ASC-H (33.3%), HSIL (33.3%), HSIL (23.8%), and LSIL (41.2%) compared with women with normal cervical cytology ($p<0.001$). Aside educational level ($p=0.03$), none of the analyzed sociodemographic characteristics or risk factors for cervical cancer was significantly associated with HPV infection in the study ($p>0.05$).
Conclusion: This study showed high prevalence of HPV infections among HIV-infected patients on ART in Plateau State, north-central Nigeria including detection of high-risk HPV types 16 and 18, which are major risk factors for progression of cervical intraepithelial neoplasia to cervical cancer.

Keywords: High-risk HPV; Cervical cytology; Pap smear, HIV; ART; Multiplex PCR

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Prévalence des types de VPH 16 et 18 à haut risque en relation avec le statut immunitaire et le profil cytologique cervical des femmes infectées par le VIH sous traitement antirétroviral dans le centre-nord du Nigeria

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Résumé:

Contexte: Le virus du papillome humain (VPH) est un agent causal bien établi du cancer du col de l'utérus et le premier groupe de virus à avoir été reconnu comme pouvant provoquer la cancérogenèse. Ils sont liés aux cancers du col de l'utérus, aux tumeurs anogénitales et aux tumeurs malignes de la tête et du cou. Le cancer du col de l'utérus est de loin la maladie liée au VPH la plus courante, avec environ 99 % des cas de cancer du col de l'utérus provoqués par des VPH génitaux persistants à haut risque (HR), en particulier les types 16 et 18.
Méthodologie: Une étude analytique descriptive en milieu hospitalier portant sur 300 femmes consentantes infectées par le VIH sous traitement antirétroviral (TAR), sélectionnées dans les trois districts sénatoriaux de l'État du Plateau, au Nigeria, a été menée sur une période de 24 mois (novembre 2018 à novembre 2020). Des échantillons de sang et de col de l'utérus ont été prélevés sur chaque participant. Le statut VIH a été confirmé par un test rapide standard sur un échantillon de sérum, le nombre de cellules $CD4^+$ a été déterminé par cytométrie en flux et l'estimation de la charge virale du VIH a été effectuée par la technique d'amplification de l'acide nucléique GeneXpert. La cytologie cervicale a été réalisée par Papanicolaou (test Pap) sur l'échantillon cervical et rapportée selon la classification du système Bethesda 2004. L'antigène HPV a été détecté pour la première fois sur l'échantillon cervical à l'aide d'ELISA, et les échantillons positifs pour l'antigène HPV ont ensuite été soumis à une amplification PCR multiplex des gènes E6 et E7 pour détecter le HR-HPV (16 et 18) et d'autres types de HPV. Des questionnaires standard ont été administrés pour obtenir des informations sur les données biologiques, les facteurs de risque et les présentations cliniques. Les données ont été analysées à l'aide du progiciel statistique pour les sciences sociales (SPSS) version 26.0 et le niveau de signification a été déterminé à $p < 0,05$.
Résultats: Sur les 300 participants, 84 étaient positifs pour le VPH de tout type, ce qui donne une prévalence globale de l'infection au VPH de 28,0%. La prévalence des types HPV-16 et HPV-18 était respectivement de 5,0% (15/300) et 5,3% (16/300). L'analyse cytologique a montré que 36,3% (109/300) des participants présentaient des anomalies cervicales allant de lésions intraépithéliales squameuses de bas grade à haut grade et d'une néoplasie intraépithéliale cervicale. La prévalence du VPH de 46,8% (51/109) chez les femmes présentant des anomalies cervicales était significativement supérieure à 17,3% (33/191) chez les femmes présentant une cytologie cervicale normale (OR 4,2, $p<0,0001$). La prévalence du VPH était plus élevée chez les femmes atteintes d'AG-US (100,0%), d'ASC-US (78,8%), d'AC-US (66,7%), d'ASC-H (33,3%), de HSIL (33,3%), de HSIL (23,8%) et LSIL (41,2%) par rapport aux femmes présentant une cytologie cervicale normale ($p<0,001$). Hormis le niveau d'éducation ($p=0,03$), aucune des caractéristiques sociodémographiques analysées ou des facteurs de risque de cancer du col de l'utérus n'était significativement associée à l'infection par le VPH dans l'étude ($p>0,05$).
Conclusion: Cette étude a montré une prévalence élevée d'infections par le VPH chez les patients infectés par le VIH sous TAR dans l'État du Plateau, au centre-nord du Nigeria, y compris la détection des types 16 et 18 du VPH à haut risque, qui sont des facteurs de risque majeurs de progression de la néoplasie intraépithéliale cervicale vers le col utérin cancer.

Mots clés: VPH à haut risque; Cytologie cervicale; Test Pap; VIH; ART; PCR multiplexe

Introduction:

Human papillomavirus (HPV) is the most common sexually transmitted pathogen, and it cause cervical lesions and cancers in both males and females. Accumulating epidemiological evidence supports a strong association between HPV and genital warts, as well as cancer of the cervix, vulva, anus, and penis (1). Cervical cancer is the most common genital tract malignancy among women in developing countries, including Nigeria accounting for more than 266,000 deaths annually (2).

Globally, there are over 528,000 new cases of cervical cancer with 85% of them occurring in developing countries (3). Although most infections with HPV show no symptoms, persistent genital HPV infection results in cervical cancer and virtually all cases (approximately 99%) of cervical cancer are associated with genital infection with high-risk (HR) HPV infection as reported in a previous study by Ajang et al., (4).

In sub-Saharan Africa, cervical cancer is the leading cause of cancer death among women as previously reported by Garcia-Espinosa et al., (5). Current data indicate that cervical cancer ranks as the second most common cancers among Nigerian women, with 7,968 deaths annually (6). There is a significant difference in terms of cervical cancer mortality and morbidity between developing and developed countries, with significant reduction in cervical cancer cases and associated deaths in develo-ped countries due to implementation of effective screening programs and HPV vaccination (7). In the United States, cervical cancer has decreased in incidence and mortality since the mid-19th century primarily due to screening programs (8). However, even with the intro- duction and widespread use of the pap smear test, cervical cancer still ranks among the top ten cancers diagnosed in the US within minority populations, which include Blacks, Indian Americans and Hispanics (8).

Cervical cancer is both preventable and curable. This is because, cervical cancer has a relatively long lead time and in general

precancerous lesions slowly progress through identifiable and recognizable stages before transforming into invasive disease. If the disease could therefore be identified before progressing to advanced stages, it can effectively be regarded as curable (7). Accumulating evidence suggests a strong correlation between HPV and genital warts as well as cancers of the cervix, vulva, vagina, anus and penis (1).

Human papillomaviruses are broadly categorized into high-risk (HR) and low-risk (LR) HPV types, depending on their association with malignancy. While the HR types e. g. types 16 and 18 are associated with different forms of malignancy, the LR types such as types 6 and 11, are associated with genital warts. HPV is considered as one of the viral infections associated with cancers and other diseases worldwide as reported by Tulay and Serakinci (9). Persistent HR HPV infections, specifically types 16 and 18, has been strongly linked to the development of cervical cancer, anogenital cancers, and oropharyngeal cancers (8).

Genital HPV is highly prevalent among women of reproductive age because they are mostly sexually active. Due to biological and physiological differences in their cervical epithelium with columnar or plastic epithelium as against squamous epithelium in older adults, young women are more vulnerable to HPV infections (10). However, most HPV infections regress spontaneously, and only in a small proportion of cases, the infection persists as low-grade intraepithelial lesion (LSIL), progressing to high-grade intraepithelial lesions (HSIL), and ultimately to invasive cervical carcinoma (10). It is now established that persistent infection with HR HPV is a necessary but not-sufficient cause of cervical cancer (10). The central factor in progression to cervical cancer is persistent HPV infection. Persistence in this case refers to detecting a similar HPV genotype in the same person twice or more within 6 months to one year.

HPV type 16 is the most common HPV infection in invasive cervical cancers but HPV type 18 has been shown to play a more significant role in the development of cervical adenocarcinoma, with a prevalence of nearly 40% in these tumours (2). Although HPV 16 is still the most prevalent HPV infection in adenocarcinoma, infection with HPV 18 confers a higher risk of development of adenocarcinoma. In younger women, HPV 18 has been found in up to 34% of cervical adenocarcinoma and 35% of cervical adenosquamous carcinomas (2).

HPV 6 and 11 are HPV types that cause 90% of all anogenital warts and most cases of oropharyngeal papillomatoses. Most early HPV infections, especially LR types, are self-limiting and often do not result in clinical disease. The HPV types contained in the quadrivalent vaccine (HPV 16, 18, 6, and 11), are implicated in 30% of all cervical intraepithelial neoplasia (CIN) 1 disease. While uncommon in early CIN, HPV 16 and 18 are found in 50%–60% of CIN 2 and CIN 3 disease (2).

Women living with HIV are at increased risk for HPV infection and HPV-related tumours, including CIN2/3 and invasive cervical carcinoma (11). The prevalence of HPV and CIN has been reported to increase with increase in immunosuppression (11). Combination antiretroviral therapies (cART) against HIV have greatly reduced the incidence of opportunistic infections, Kaposi's sarcoma, and non-Hodgkin's lymphoma, but not the incidence of HPV-associated cervical and anal carcinomas (12). This is perhaps not surprising as HPV-associated carcinomas have a long latent phase. A favorable effect of cART on HPV infection and cervical precancerous lesions has been shown in some but not all studies (13-16). In 2006, the United States Food and Drug Administration (FDA) approved HPV vaccine, *Gardasil*, against HR-HPV types 16 and 18 as well as LR types 6 and 11, for all females aged 9-26 years. In 2009, the FDA also approved the use of this vaccine in males aged 9-26 years. Another HPV vaccine, *Cervarix*, which aims to protect against HR-HPV types 16 and 18 only, was approved by the FDA in the Fall of 2009 for females aged 10-25years.

The oncogenic potential of a particular HPV type highlights the importance of detecting and genotyping HR HPV types, especially types 16 and 18. The results of HPV testing may have significant therapeutic and prognostic implications, providing clinicians with valuable information for deciding the most appropriate course of action for each patient. The information could also provide data necessary for implementation of vaccination programs against cervical cancer (1). The objectives of this study therefore are to determine the prevalence of HR-HPV types 16 and 18 in relation to immune status and cervical cytological profiles of selected HIV-infected women on antiretroviral therapy in Plateau State, northcentral Nigeria.

Materials and method:

Study setting and design:

This study was conducted in six major hospitals comprising teaching, specialist and general hospitals across the three senatorial zones of Plateau State, northcentral Nigeria. It is a hospital-based descriptive analytical design among HIV-infected women aged 15-74 years on ART irrespective of background and socio-economic status attending the selected hospitals during the study period (Nov 2018 to November 2020).

Study population and participants:

The study population comprised of HIV

positive women on ART who are undergoing blood sampling for $CD4^+$ cell count and viral load estimations, and those coming for routine cervical screening test. A total of 300 HIV-positive females within reproductive age were recruited as the participants into the study.

Ethical consideration:

The approval for the study was obtained from the Institutional Review Board (IRB) of Jos University Teaching Hospital and the Ethical Committee of Plateau State Specialist Hospital, while informed consent was obtained from all participants and/or their legal guardians.

Sample size determination:

The sample size was determined by using the formula described by Naing et al., (17); $n=Z^2 p(1-p)/d^2$, where 'n' is the sample size, 'p' is the prevalence from a previous study of 15.0% (0.150) by Musa et al., (18), 'Z' is the standard normal distribution at 95% confidence interval (1.96) and 'd' is the absolute desired precision at 5% (0.05). Therefore, the calculated sample size was 195.9, however, in order to account for attrition, the sample size was adjusted for 10% attrition, to give a total of 300 samples.

Sampling method and data collection:

Systematic random sampling method was used to select the 300 HIV-positive participants at the various sampling points across the three senatorial zones of Plateau State, Nigeria. A structured questionnaire containing both closed and open-ended questions was interviewer-administered on each participant to obtain relevant information on socio-demographic data, socioeconomic status, behavioral and sexual habits, clinical presentations and risk factors of HPV infections and cervical cancer.

Sample collection, transportation and storage:

Blood and cervical swab samples were collected from each participant. Swab samples were collected from the endocervix using liquid-based cytology technique after exposing the cervix with Cusco speculum by an experienced cytopathologist. First, excess mucus was removed from the cervix and surrounding mucosa using cleaning swabs, and thereafter, a collection swab was first used to obtain samples for cytology before using a cytobrush to collect swabs for HPV detection. This was done by turning the cytobrush clockwise for approximately 15 seconds to ensure adequate sampling. The head of the cytobrush containing the swab sample was then dropped off inside the sample tube containing the liquid preservative and transported to the histopathology unit of Jos University Teaching Hospital for analysis.

Blood samples collected into sterile specimen bottles were centrifuged and serum separated. Together with the cervical samples, they were stored at -4^0C until analysis for HIV detection, $CD4^+$ profiling and viral load estimations, HPV detection, and cervical cytological analysis

HIV detection by rapid test:

The serum sample of each participant was tested for HIV antibodies using the rapid diagnostic test in accordance with the national (serial) algorithm for HIV testing in Nigeria. This required the use of a first line test kit (Determine®) and confirming with a second line test kit (Unigold®) before finally tie breaking with the third test kit (Stat pak®) in cases where there are discrepancies in the results between the first and the second test kits.

The test procedure involved applying 10 ml of serum sample on the test pad of the kit and allowing it to flow through the chromatographic pad and the results read after 10 minutes and interpreted as positive, negative or invalid.

$CD4^+$ count profiling and viral load estimation:

The flow cytometry (Sysmex cyflow counter) method was used for $CD4^+$ count and the results expressed as cells/mm³. The viral load of each HIV positive participant was estimated using GeneXpert technology and the results expressed as virus copies/ml.

Cytological analysis:

The standard Pap smear procedure for cervical cells profiling was performed on all the participating women to assess any cytological changes likely associated with HPV infection. The slides were prepared, fixed and stained using the Papanicolaou (Pap smear) technique. The stained smears were examined, and the results reported accordingly to the 2004 Bethesda system classification as reported by Musa et al., (17). Except for participants with normal cytology results, all others were referred to the gynaecology clinic for further evaluation.

Viral antigen detection using ELISA:

All the cervical specimens were tested for the presence of HPV antigens and further characterized using type-specific ELISA for HR HPV (HPV 16 and 18) using commercially available enzyme immune assay (Diagnostic Automation/Cortez Diagnostics Inc., USA) ELISA kits. The assay was performed according to the manufacturer's instructions to determine HPV positive samples.

DNA extraction and PCR assay:

The DNA extraction, pre-amplification procedures and HPV genotyping were perfor-

med using standard methods. The DNA preparation kit (Inqaba biotech, South Africa) was used to extract DNA, according to the manufacturer's instructions at the AIDS Prevention Initiative in Nigeria (APIN) laboratory in Jos, Nigeria. PCR was conducted on the extracted DNA using multiplex PCR detection and genotyping kit (MaxyGene Gradient Thermal Cycler Canada).

Polymerase chain reaction assay:

Multiplex PCR amplification of E6 and E7 genes was done for all 84 identified HPV positive samples, as described by Shahi et al., (19), and following the manufacturer's instructions, using primers (forward and reverse) and conditions outlined in Table 1. During the amplification phase all the PCR reagents were spun out and a mixture of PCR master mix and DNA Taq polymerase were prepared per PCR reaction tube. One microlitre of DNA template was then added to each PCR tube. The solution was centrifuged for a few seconds and placed in the thermal cycler for DNA amplification.

Each PCR was carried out in the thermal cycler (MaxyGene Gradient Thermal cycler, USA) with the following conditions: initial step at 95^{0}C for 15 min, 10 cycles of 30s at 94^{0}C, 90s at 65^{0}C, and 90s at 72^{0}C, followed by 30 cycles of 30s at 94^{0}C, 90s at 63^{0}C, and 90s at 72^{0}C, with a final extension at 72^{0}C for 10 min.

Gel electrophoresis of PCR amplicons:

The PCR products were resolved by gel electrophoresis on a 2% agarose gel stained with ethidium bromide, and the band sizes were estimated by comparison with 100bp molecular weight marker (GeneRuler 100bp DNA ladder, Fermentas International, Canada). The gels were photographed in a UV transilluminator (UVP, USA) with a Canon PowerShot A60 digital camera.

HPV types were adequately assigned based on the amplification pattern. However, in cases where band amplification was not clear, an additional PCR amplification with specific primers was performed to confirm the HPV type.

Statistical analysis:

Data were analysed using Statistical Package for the Social Sciences (SPSS) version 26.0. Significant differences between variables were tested using Chi-square test (for categorical variables) and student's *t*-test (for comparing means). Statistical significance was determined at $p<0.05$ with 95% confidence interval. Results were presented in frequency tables, ratios and percentages.

Table 1: Primer sequences used for the study

Name	Forward primer sequence (5'-3')	Reverse primer sequence (3'-5')	Lane	Size bp
16-1/F	TTAGGCAGCACTTGGCCAACCA	TAATCCGTCCTTTGTGTGAGCT	2	207
16-2/R	ACTGCAATGTTTCAGGACCCAC	CGAAGCGTAGAGTCACACTTGC	1	661
18-1/F	TCGCGTCCTTTATCACAGGGCGA	TGCCCAGGTACAGGAGACTGTG	2	536
18-2/R	TCCGTGGTGTGCATCCCAGCAG	CACTTGTGCATCATTGTGGACC	7	274
β-globin (internal control)	GAAGAGCCAAGGACAGGTAC	CAACTTCATCCACGTTCACC	7	286

Results:

Sociodemographic and clinical characteristics of the study participants:

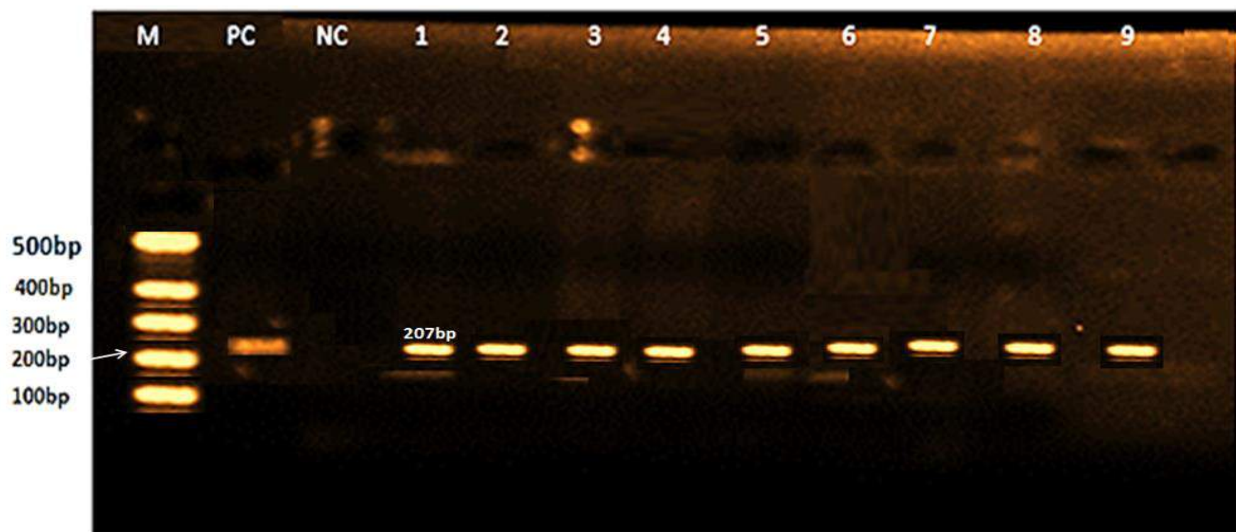
Table 2 shows socio-demographic and clinical characteristics including risk factors of the study participants. Most of the participants are married (42.0%), with 30.7% being widowed, 17.3% single and 10.0% divorced/separated. Majority (189, 63%) are in monogamous marriage. Most (109, 36.3%) had secondary and tertiary education each while only 10 (3.3%) had no formal education.

Most occupational groups among the participants are civil servants (27.7%) followed by traders (26.3%), full housewives (14.0%), farmers (8.7%), artisans (8.3%), students (5.0%), applicants (2.3%), retires (2.0%) and teachers (2.0%).

Table 2: Sociodemographic characteristics of HIV-infected women with respect to HPV infection in Plateau State, Nigeria

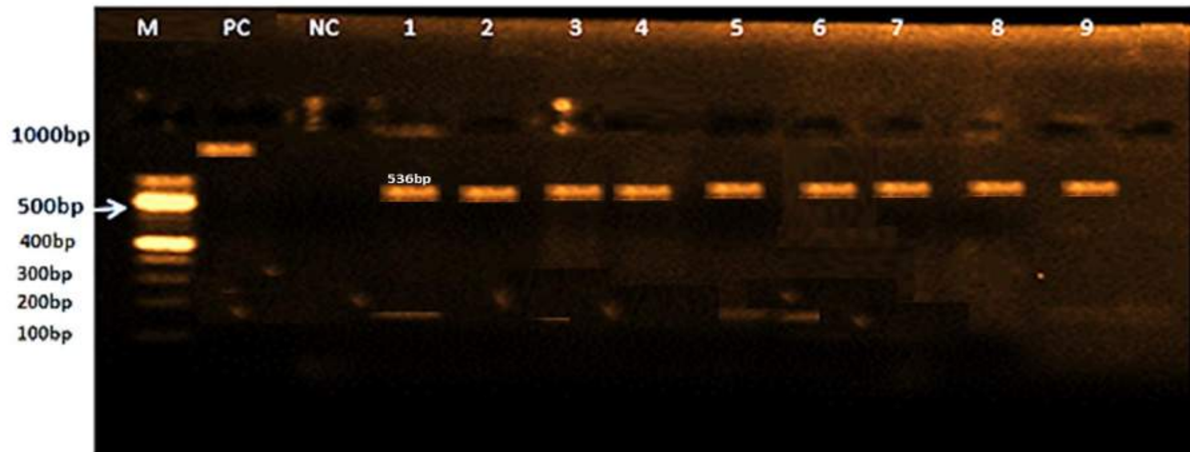
Socio-demographics	No. screened (n=300)	No positive for HPV (n=84, 28.0%)	*p*-value
Marital status			
Single	52	15 (28.8)	0.914
Married	126	33 (26.2)	
Divorced/separated	30	8 (26.7)	
Widow	92	28 (30.4)	
Type of family			
Monogamy	189	58 (30.7)	0.525
Polygamy	55	11 (20.0)	
Others	56	15 (26.8)	
Level of education			
No formal education	10	0	0.03*
Primary	72	25 (34.7)	
Secondary	109	24 (22.0)	
Tertiary	109	35 (32.1)	
Occupation			
Applicants	7	0	0.313
Civil servants	83	26 (31.3)	
Farmers	26	05 (19.2)	
Housewives	42	11 (26.2)	
Artisans	25	10 (40.0)	
Retirees	06	2 (33.3)	
Students	15	1 (6.7)	
Tailors	11	4 (36.4)	
Teachers	06	1 (16.7)	
Traders	79	24 (30.4)	

* = significant at p≤0.05, % = percentage, No = number, HPV= human papillomavirus



Lane M: 100-1000bp DNA ladder. Lane PC: Positive control; Lane NC: Negative control; Lanes 1-9: Samples' labels.

Plate 1: Agarose gel electrophoresis of PCR products after amplification of HPV 16 gene at 207bp



Lane M: 100-1000bp DNA ladder. Lane PC: Positive control; Lane NC: Negative control; Lanes 1-9: Samples' labels.

Plate 2: Agarose gel electrophoresis of PCR products after amplification of HPV 18 gene at 536bp

Prevalence of HPV and high-risk HPV 16 and 18 infections:

Of the 300 participants, 84 were HPV-positive by PCR, giving an overall prevalence of HPV infection of 28.0%. The prevalence of high-risk HPV types 16 and 18 was 10.3% (31/300), with HPV-16 (5.0%) and HPV-18 (5.3%). Plate 1 is the agarose gel electrophoresis of HPV-16 PCR amplicon size of 207bp while plate 2 is that of HPV 18 with amplicon size of 536bp.

HPV infection also occurred more frequently among civil servants, traders and full-time housewives than among artisans, tailors and teachers, students and retirees as shown in Fig 1. HPV-16 infection occurred more frequently among HIV-infected women of age group 45-54 years and least or no infection observed among early sexual debutants (15-24 years old) and among the elderly (65-74 years) as presented in Table 3.

For HPV-18 infection, the highest prevalence (14.3%) was recorded among the age group 55-64 years and 0% among the very elderly (65-74 years) although the association between age group and HPV-16/18 infections was not statistically significant ($p>0.05$).

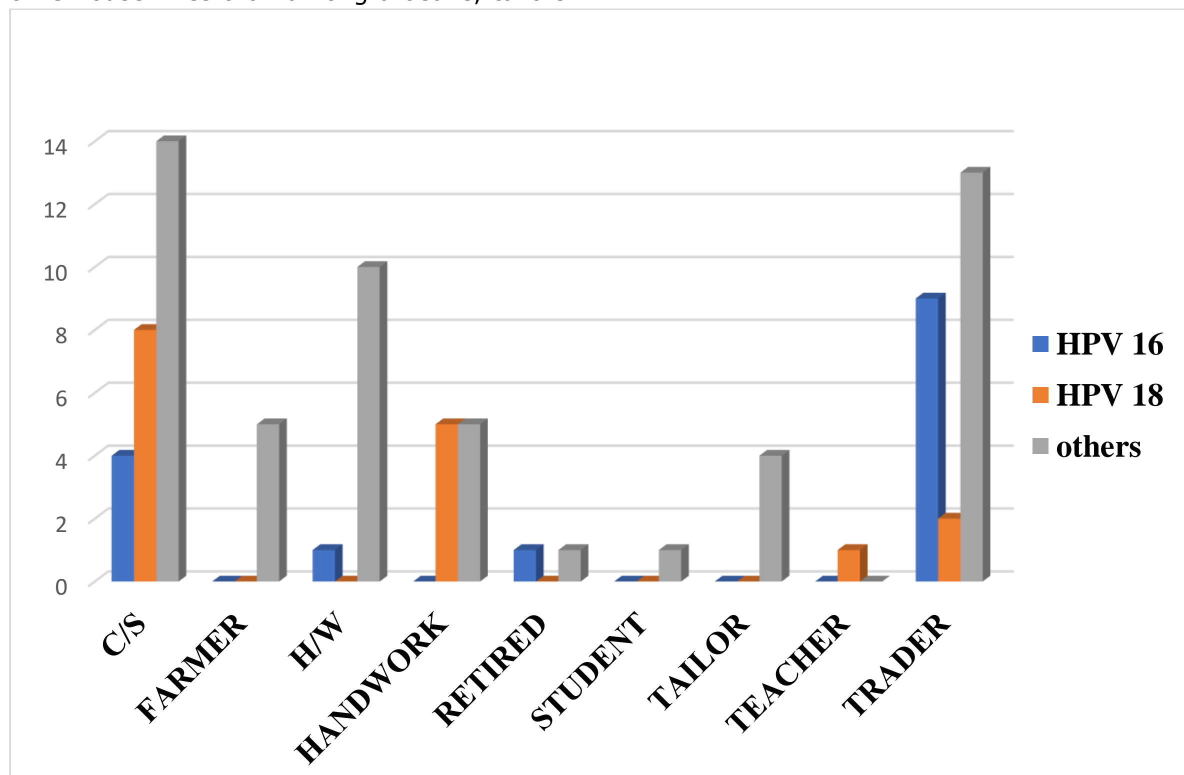


Fig. 1: Frequency distribution of HPV infections with respect to occupations of HIV-infected women in Plateau State, Nigeria

Sociodemographic characteristics and risk factors of HPV infections:

There only sociodemographic characteristic significantly associated with HPV infection in the study was the participant's level of education, with lower HPV prevalence (0%) among those with no formal education compared to those with primary (34.7%), secondary (22.0%) and tertiary education (32.1%) levels (*p*=0.03). There was no statistically significant associations (*p*>0.05) between marital status, type of family and occupation, with HPV infections (Table 2), as well as with the age group of participants (Table 3).

Of all potential risk factors for cervical cancer analyzed among the study participants, no significant statistical association (*p*>0.05) was established between HPV infections and the analyzed risk factors both in the bivariate analysis (Table 4) and in the multiple logistic regression analysis (Table 5).

HPV infection and immune status ($CD4^+$ cell count):

Analysis of immune status of the parti cipants in relation to HPV infection showed that robust immune system (as determined by $CD4^+$ cells) was associated with lower HPV prevalence. In other words, the HPV prevalence of 31.9% (53/166) was higher in HIV-infected women with lowest $CD4^+$ count of 0-200 cells/mm^3 compared to HPV prevalence of 25.0% (6/24) in those with $CD4^+$ cell count of >600 cells/mm^3 (Table 6). However, the difference is not statistically significant (*p*=0.383).

HPV infection and cervical cytology:

The cytological analysis showed that a total of 109 (36.3%) participants had cervical abnormalities, 51 (46.8%) of whom had HPV infections while 33 of 191 (17.3%) women with normal cervical cytology had HPV infections. The HPV prevalence was highest in women with advanced forms of cervical abnormalities such as AGUS endocx (100.0%, 1/1) and AGUS endomet favor neoplastic (100.0%, 1/1) (*p*<0.001). On the other hand, women with milder forms of cytological abnormality such as LSIL, HSIL and HSIL susp for invasion, have lower HPV prevalence of 41.2%, 33.3% and 23.8% respectively, compared to those with advanced cervical abnormality, while women with harsher forms of cervical abnormalities such as ASC-H, AC-US, and ASC-US have low to high HPV prevalence of 33.3%, 66.7% and 78.8% respectively. Conversely, women with normal cervical cytology have lower HPV prevalence of 17.3% (33/191), which shows significantly lower HPV prevalence compared to women with abnormal cervical cytology (OR 4.2, *p*<0.0001) (Table 7).

The prevalence of HR HPV (HPV16 and 18) infections in women with abnormal cervical cytology was 8.2% (8/109) for HPV-16 and 10.1% (11/109) for HPV-18 while for other HPV types, the prevalence was 28.4% (31/109) (Table 8). These prevalence rates are comparatively lower for women with normal cervical cytology, with rates of 3.1% (6/191), 2.6% (5/191) and 11.5% (22/191) respectively, although the differences are not statistically significant (x^2=0.546, *p*=0.760).

HPV co-infections with other sexually transmitted infections:

There were more co-infections of HPV with bacterial vaginosis and other yeast infections than with *Candida* and herpes simplex virus (HSV) infections as shown Fig 2.

Table 3: Prevalence of HPV infections in relation to age of HIV-infected women on ART in Plateau State, Nigeria

Age group (years)	No of women tested	No positive (%) HPV-16	No positive (%) for HPV-18	No positive (%) for other HPV types	*p*-value
15-24	10	0	1 (10.0)	0	0.450
25-34	46	0	1 (2.2)	10 (21.7)	
35-44	131	8 (6.1)	6 (4.6)	19 (14.5)	
45-54	82	6 (7.3)	4 (4.9)	16 (19.5)	
55-64	28	1 (3.6)	4 (14.3)	7 (25.0)	
65-74	3	0	0	1 (33.3)	
Total	**300**	**15 (5.0)**	**16 (5.3)**	**53 (18.3)**	

% = percentage, No = number, HIV=human immunodeficiency virus, ART=antiretroviral therapy, HPV=human papillomavirus

Table 4: HPV infection in relation to risk factors of cervical cancer among HIV-infected women in Plateau State, Nigeria

Variable	No of sample (n=300)	No positive for HPV (%) (n=84, 28.0%)	χ^2	*p*-value
History of immunisation				
Yes	17	6 (35.3)	0.476	0.490
No	283	78 (27.6)		
If yes, number of doses				
One	14	4 (25.2)	1.570	0.210
Two	3	26 (31.7)		
Heard of cervical cancer?				
Yes	291	82 (28.2)	0.032	0.844
No	8	2 (25.0)		
Heard of pap smear test?				
Yes	276	77 (27.9)	0.018	0.894
No	24	7 (29.2)		
Have you been screened?				
Yes	115	33 (28.7)	0.045	0.832
No	185	51 (27.6)		
Parity				
0 – 5	241	67 (27.8)	0.024	0.877
6 – 10	59	17 (28.8)		
Vaginal herbs/douching?				
Yes	67	21 (31.3)	0.478	0.489
No	233	63 (27.0)		
If yes, how long? (years)				
1 – 5	28	8 (28.6)	0.667	0.881
6 – 10	27	8 (29.6)		
11 – 15	5	2 (40.0)		
>15	7	3 (42.9)		
Do you use contraceptives?				
Yes	166	40 (24.1)	2.809	0.094
No	134	44 (32.8)		
Had STI?				
Yes	220	66 (30.0)	1.637	0.201
No	80	18 (22.5)		
Name of STI				
Others	80	18 (22.5)	10.480	0.313
Bacterial vaginosis	81	30 (37.0)	9.177	0.164
Candidiasis	20	7 (37.0)		
Gonorrhoea	1	0		
HSV	3	1 (33.3)		
Yeast	115	28 (24.3)		

x^2 = Chi square, % = percentage, No = number, STI=sexually transmitted infections, HSV=Herpes simplex virus

Table 5: Multiple regression analysis of independent risk factors for HPV infection in the study participants

Risk factors	Odd Ratio	95% CI	*p*-value
No of sex partners	0.19	(0.01-1.32)	0.342
Condom usage	0.95	(0.52-1.72)	0.855
Cigarette smoking	1.33	(0.52-3.42)	0.555
Alcohol intake	0.95	(0.52-1.72)	0.855
Immune status	1.56	(0.87-2.81)	0.136
Immunization status	1.43	(0.51-4.01)	0.492
Vaccine Dose	1.43	(0.51-4.01)	0.492
Parity	1.01	(0.23-4.23)	0.111
Other STIs	1.48	(0.81-2.69)	0.202
Age at sexual debut	1.21	(0.628-2.13)	0.139

CI = Confidence interval; % = percentage, No = number, STI = Sexually transmitted infections, HPV=Human papillomavirus

Table 6: Relationship between HPV infection and immune ($CD4^+$) status of HIV-infected women

$CD4^+$ count (cells/mm³)	Number tested	No of HPV positive (%)	*p*-value
0-200	166	53 (31.9)	0.383
201-400	70	15 (21.4)	
401-600	40	10 25.0)	
≥ 601	24	6 (25.0)	
Total	**300**	**84 (28.0)**	

% = percentage, No = number, HIV=human immunodeficiency virus, ART=antiretroviral therapy, HPV=human papillomavirus

Table 7: Prevalence of HPV in relation to cervical cytology of HIV-infected women in Plateau State, Nigeria

Cytological changes	No examined	No of HPV positive (%)	*p*-value
AGUS, endocx	1	1 (100)	<0.001*
AGUS, endomet favor neoplastic	1	1 (100)	
ASC-US	33	25 (78.8)	
AC-US	6	4 (66.7)	
ASC-H	3	1 (33.3)	
HSIL	6	2 (33.3)	
HSIL, susp for invasion	42	10 (23.8)	
LSIL	17	7 (41.2)	
Negative for intraepithelial lesion or malignancy	191	33 (17.3)	
Total	**300**	**84 (28.0)**	

* = significant at $p \leq 0.05$, % = percentage, No = number, AGUS=Atypical glandular cells of undetermined significance, ASC-US=Atypical squamous cells of undetermined significance, HSIL=High-grade squamous intraepithelial lesion, LSIL=Low-grade squamous intraepithelial lesion, ASC-H= Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion, AC-US=Atypical cell of undetermined significance.

Table 8: Statistical analysis of HPV infections with cervical cytology of HIV-infected women on ART in Plateau State, Nigeria

Cervical status	No. examined	Total HPV (%)	x^2	OR (95% CI)	p value	HPV types			x^2	p-value
						HPV 16 (%)	HPV 18 (%)	Other HPV types (%)		
Normal cervix	191	33 (17.3)	28.534	4.210 (2.475-7.163)	<0.0001*	6 (3.1)	5 (2.6)	22 (11.5)	0.546	0.760
Abnormal cervix	109	51 (46.8)				9 (8.2)	11 (10.1)	31 (28.4)		
Total	**300**	**84 (28.0)**				**15 (5.0)**	**16 (5.3)**	**43 (14.3)**		

χ^2 = Chi square, OR = Odds ratio, CI = Confidence interval, * = Significant at $p \leq 0.05$, % = percentage, No = number, HIV=human immunodeficiency virus, ART=antiretroviral therapy, HPV=human papillomavirus

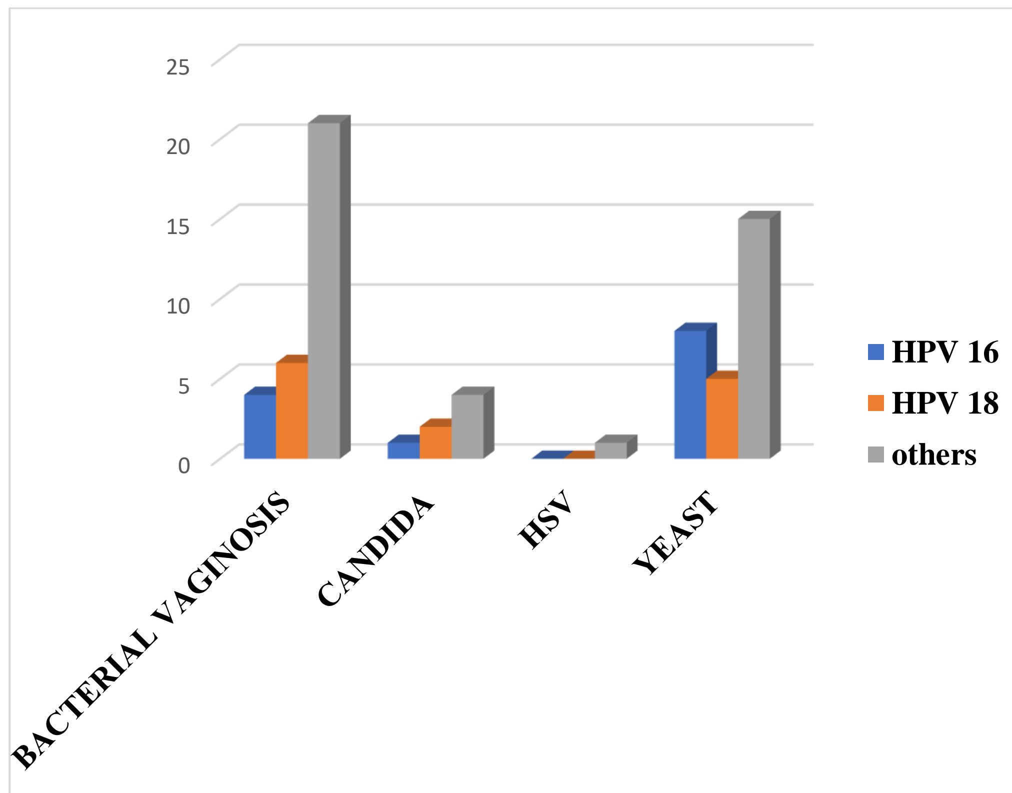


Fig. 2: Co-infection of HPV and other sexually transmitted infections among HIV-infected women in Plateau State, Nigeria

Discussion:

This study was designed to determine the prevalence of high-risk HPV infection, with particular interest in highly oncogenic types 16 and 18 infection and its association with immune status and cervical cell abnormalities and risk of cervical cancer among HIV-infected women in northcentral Nigeria. The population studied are considered at high risk of cervical cancer due to their HIV status. The demographic analysis showed that majority of the participants were married (126/300), and in monogamous family (63.0%). Most of the participants (27.6%) were civil servants and majority (36.3%) had secondary and tertiary education. However, only education was significantly associated with HPV infections (*p*=0.03) with 0% HPV prevalence in participants with no formal education compared to 34.7%, 22% and 32.1% in those with primary, secondary and tertiary education respectively. This finding implies that higher educational status may increase the odd of acquiring HPV infection.

The low HPV prevalence in HIV-infected women without formal education in our study is in contrast to the findings of a multivariate analysis of previous studies conducted by Stephen et al., (20) that showed strong association of HPV awareness with higher level of education. Part of the reasons for this may be due to the fact the risk of HPV infection is a complex phenomenon where aside educational status, there are biological and psychosocial variables that are equally important determinants of the risk of HPV infection.

Globally, HPV testing for HR HPV especially types 16 and 18 is now acceptable as a viable and validated option in the management of women with abnormal cervical cytology results. Recently, interest is increasingly mounting for the use of HPV testing from cervical samples of asymptomatic women with normal cytology results. This is to enable early identification of different levels of risk for cervical neoplasia especially from high-risk population such as HIV-positive women, due to the close relationship between HPV infection and cervical cancer development. In spite of this need, there are few reliable data on the prevalence of HR HPV among high-risk population in Nigeria.

Our results showed an overall prevalence of 28.0% for HPV infection among HIV-infected women 15 years and older attending various ART and gynecological units in Plateau State, northcentral Nigeria. This is however lower than the 37.0% HPV prevalence in the general population in Nigeria. Our finding is similar to 28.5% HPV prevalence reported among HIV-positive women in Eastern Cape, South Africa, and also agrees with the findings of Sarkar et al., (21) in India who reported the HPV prevalence among HIV-infected females of 32.2%, and Cubie et al., (22) who reported

HPV prevalence of 25.0%. However, the HPV prevalence in our study is much lower than the prevalence of 63.3% reported by Monteiro et al., (23) in Brazil.

The prevalence of 5.0% for HPV-16 and 5.3% for HPV-18 in our study are comparable to the prevalence of 2.7% and 3.1% reported by Ahmed et al., (24) for HPV 16 and 18 in Saudi Arabia, and the prevalence of 7.1% and 10.1% for HPV-16 and 18 in northern Ethiopia by Temesgan et al., (25). The probable reasons for the similarity in HPV prevalence in our study with theirs may be due in part to the common risk factors for HPV infections in the population of these two countries. More so that all the studies included women without apparent cervical cancer risks i.e. no known cervical abnormality. On the other hand, the prevalence in our study are lower than those reported by Xiang et al., (26) in China and Kulkarni et al., (27) in India who reported as high as 18.0% and 22.0% for HPV 16 and 18 respectively. This difference may partly be due to the differences in the population characteristics, as both of these researchers conducted their studies on women known to have abnormal cervical cytology in contrast to our study that did not concentrate on such subpopulation. It may also be due to differences in the diagnostic sensitivities of the detection methods used in the studies.

The analysis of association of risk factors of cervical cancer with prevalence of HPV infection in our study did not show significant association with any factor ($p>0.05$). However, there were higher odds for HPV infection among women who debuted sexual relationships at an early age, women co-infected with other STIs, cigarettes smokers and women with low immune status while on the other hand, women with multiple sex partners, who consumed alcohol and who regularly used condoms during sex, had lower odds of HPV infection, although these odds did not attain statistical significance ($p>0.05$). It is evident that many studies evaluating influence of risk factors on both HPV infections and cervical abnormalities have produced conflicting results as reported by Bahmanyar et al., (28).

Some of the reasons for conflicting findings in studies that investigate influence of risk factors on HPV and cervical abnormality may be due to differences in study designs including the diagnostic sensitivity and specificity of methods used in detecting HPV infection. While some researchers used ELISA technique, others including ours used the more sensitive and specific technique such as PCR assay. Another possible reason maybe the study population involved. For instance, while some studies were based on the general population with perceived low risk, our study was conducted among the most-at-risk-population (HIV-infected women) thereby contributing to the observed differences. However, in agreement with our findings, several other studies have documented significant associations of cigarette smoking, level of education, low immune status and lower age at sexual debut with both HPV infection, cervical abnormality and cervical cancer. Bacterial vaginosis and yeast infections were the commonest STIs co-infecting with HPV among the HIV-infected women in this study while co-infections with others STIs such as *Candida* and HSV occurred less frequently. It is well established that other STIs may increase the risk of persistent HPV infection and invasive cervical cancer.

The implementation of highly active antiretroviral therapy (HAART) among HIV-infected persons results in immune reconstitution, slower progression of HIV disease and decrease in occurrence of opportunistic infections. However, the impact of HAART on cervical HPV infection, clearance, and persistence in high-risk adolescents remains controversial (16). Our study showed that majority (55.3%) of the HIV-infected women had very low $CD4^+$ cells (0-200 cells/µl of blood) while only 8.0% had robust $CD4^+$ cells of >600/µl of blood. Generally, the immune status of HIV-infected persons is measured by the level of their $CD4^+$ cells. Therefore, the level of the immunity as measured by $CD4^+$ cell count shows how effective they will be in resisting any form of infection or minimizing its impact after infection. In the case of HPV infection and by extension cervical cancer, the immune status may not prevent HPV infection, but can go a long way in clearing the virus thereby preventing its persistence and by implication progression to cervical cancer. Adequate immunosurveillance is crucial for viral elimination and preventing disease establishment or persistence. When immunosurveillance is compromised, HPV disrupts critical signaling pathways and apoptosis. resulting in immune evasion (29).

The relationship between HPV infection and immune status showed that the weaker the immune status, the higher the chances of HPV acquisition and persistence. Hence, in our study, women with the lowest immunity ($CD4^+$ count of 0-200 cells/µl) have the highest prevalence of HPV infection (31.9%) and those with the most robust immune system ($CD4^+$ count >600 cells/µl) have correspondingly lower HPV infection (25.0%) although the prevalence difference was not statistically significant ($p=0.383$). This aligns with literature that the immune status determines vulnerability to HPV infection and other infections as well. Our study also agrees with the findings of others that lower immunity is associated with high incidence of HPV infection, that can lead to CIN and invasive cancer as reported in Aminu et al., (30). Women with low $CD4^+$ cell count and high viral load have elevated risk of

HPV acquisition. Low $CD4^+$ count is also associated with decreased HPV clearance, and a compromised immune response is a pre-requisite for disease progression. One unique feature of HPV infection is that it can affect the immune system in such a way that it presents a much more tolerant state, which facilitates persistent HR HPV infection and cervical lesion progression to cervical cancer (31).

The prevalence of abnormal cervical cytology in our study, in accordance with the Bethesda system of classification, was 36.3%. Cervical smear test is routinely done to screen for cervical cancer since the clinical disease is preventable through early detection and appropriate management of the major precursors (CIN and persistent HR HPV infection). There is abundant evidence to suggest that regular screening of sexually active women confers an overall public health benefit in reducing morbidity and mortality from cervical cancer as reported by Yousif et al., (32). The analysis of the relationship between HPV infection and cervical cytology in our study showed strong association between cervical cytological status and HPV infections ($p<0.0001$). This finding is similar to the study of Jaya et al., (33) who reported strong association between HPV infection and cervical cytological changes. Gui et al., (34) also reported that HIV-positive women have higher risk of acquiring HPV infection, pre-cancerous lesions and cervical cancer. They further showed that HIV infection was associated with higher incidence of and reduced clearance of HPV infection. A similar study conducted by Burd (35) showed that HIV-infected women are 5 times more likely than HIV-negative women to have lower genital tract neoplasia, a precursor of cervical cancer, and Yakub et al., (36) in Nigeria showed that HPV infection correlates with cervical changes in HIV-infected women. The similarities in findings between our study and those of many others in Nigeria and other countries proves that indeed HPV infection significantly affects the integrity of the cervix and the ability to clear the infection.

A relatively large proportion of HIV-infected women in our study had low $CD4^+$ cell counts, suggesting poor adherence to ART guidelines. Expectedly, the same sub-population (low $CD4^+$ counts) also have the highest HPV infection. Also, many of the participants used different forms of contraceptives and other practices such as douching and vaginal herbs as preventive measures against unplanned pregnancies and STIs. These may have contributed to the overall risk of cervical cancer. Majority of the women had also initiated sex at early age and had multiple sex partners, and were involved in unprotected sex. Since HPV infection often occur through sexual intercourse and other sexual behaviors, it clearly shows that HPV infection is influenced by the sexual practices of the participants.

Conclusion:

Our study showed high prevalence of HPV infections among HIV-infected patients on ART in Plateau State, north-central Nigeria, with detection of HR HPV types 16 and 18, which are major risk factors for progression of CIN to cervical cancer. We therefore recommend routine cervical cancer screening among HIV-infected women. Furthermore, we suggest better monitoring of ART regimen to improve the immune status of HIV-infected women. Further prospective study to establish the role of ART on HPV infection and cervical cancer progression in HIV-infected women is recommended.

Contributions of authors:

AAY was involved in research conceptualization, design formulation, methodological activities, and writing of the original draft; EEE was involved in research design, supervision and review of the manuscript; OAO was part of the design, supervision, validation of cytological/histological analysis and review of the manuscript; IE was actively involved in the cytological/histological and molecular analysis; AM was involved in the design, supervision, validation of research data and review of the manuscript. All authors read and approved the manuscript for submission.

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Conflict of interest:

Authors declare no conflict of interest.

References:

1. Krashias, G., Kpotides, D., and Christodoulou, C. HPV Prevalence and Type Distribution in Cypriot Women with Cervical Cytological Abnormalities. BMC Infect Dis. 2017; 17(346): 2-10. doi: 10.1186/s12879-017-2439-0.
2. Chinaka, C. C., and Nwazue, U. C. Awareness of Cervical Cancer and its Screening in Abakalili, Nigeria. Afr J Cellular Pathol. 2013; 1 (1): 47-51.
3. Sule A. A., and Ochichia, O. A Histologic Review of Cervical Cancer in Kano, Nigeria. Sahel Med J. 2017; 20:16-20. doi: 10.4103/1118-8561.204331
4. Ajang, A. Y., Elijah, E. E., Jatau, E. D., Aminu, M., and Longwap, A. S. Serologic Evidence of High-Risk Human Papillomavirus 16 and 18 Infections and Risk of Prostate Cancer in Northwestern Nigeria. J Med Sci Clin Res. 2021; 9(4):130-139.

https://dx.doi.org/10.18535/jmscr/v9i4.21

5. Garcia-Espinosa, B., Nieto-Bona, M., Reuda. S., et al. Genotype distribution of cervical Human papilloma-virus DNA in women with Cervical lesions in Bioko, Equatorial Guinea. Diagnost Pathol. 2009: 1186: 4-31. doi: 10.1186/1746-1596-4-31
6. Castellsage, X., Bosch, F.X., Munoz, N. Environmental Co-factors in HPV Carcinogenesis. Virus Res. 2002; 89: 191-199. doi: 10.1016/s0168-1702(02)00188-0
7. Beyazit, F., Silan, F., Gencer, M., et al. The Prevalence of HPV Genotypes Detected by PCR in Women with Normal and Abnormal Cervico-vaginal cytology. Gynaecol. 2018; 89(2): 62-67. doi: 10.5603/GP.a2018.0011
8. Blackman, E., Thurman, N., Halliday, D., et al. Multi-center Study of HPV and the HPV Vaccine: Knowledge and Attitudes Among People of African Descent. Infect Dis Obstetr Gynaecol. 2013; 2013: 1-8. doi: 10.1155/2013/428582
9. Tulay, P., and Serakinci, N. The Role of Human Papillo-maviruses in Cancer Progression. J Cancer Metastasis Treatment. 2016; 2: 201-213. http://dx.doi.org/10.20517/2394-4722.2015.67
10. Silva, L., Miranda, A., Batalha, R., Ferreira, L., Santos, M., and Talhari, S. High-Risk Human Papillomavirus and Cervical Lesions among Women living with HIV/AIDS in Brazilian Amazon, Brazil. Braz J Infect Dis. 2015; 19(6): 557-562. doi: 10.1016/j.bjid.2015.07.001
11. Vuyst, H. D., Mugo, N. R., Chung, M. H., et al. Pre-valence and Determinants of Human Papilloma- virus Infection and Cervical Lesions in HIV-Positive Women in Kenya. Br J Cancer. 2012; 107(9):16-30. doi: 10.1038/bjc.2012.441
12. Ferenczy, A., Coutlee, F., Franco, E., and Hankins, C. Human Papillomavirus and HIV Coinfection and the Risk of Neoplasias of the Lower Genital Tract: A review of Recent Developments. Canad Med Assoc J. 2003; 169 (5): 431-434.
13. Paramsothy, P., Denise, J. P, Charles, H. M., et al. The Effect of Highly Active Antiretroviral Therapy on Hum-an Papillomavirus Clearance and Cervical Cytology. Obstetr Gynaecol. 2010; 113(1): 26-31. doi:10.1097/AOG.0b013e31819225cb
14. Mikoff, H., Zhong, Y., Burk, R. D., et al. Influence of Adherent and Effective Antiretroviral Therapy Use on Human Papillomavirus Infection and Squamous Intraepithelial Lesions In HIV-Positive Women. J Infect Dis. 2010; 201: 681-690. doi: 10.1086/650467
15. Bratcher, L., and Sahasrabuddhe, V. The Impact of Antiretroviral Therapy on HPV and Cervical Intra-epithelial Neoplasia: Current Evidence and Directions for Future Research. Infect Agents Cancer. 2010; 5(1): 8. doi: 10.1186/1750-9378-5-8.
16. Shrestha, S., Sudenga, S. L., Smith, J. S., Bachman, L. H, Wilson, C. M., and Mirjam, C. K. The Impact of Highly Active Antiretroviral Therapy on Prevalence and Incidence of Cervical Human Papillomavirus Infection In HIV-Positive Adolescents. BMC Infect Dis. 2010; 10:295. doi: 10.1186/1471-2334-10-295.
17. Niang, L., Winn, T., and Rushi, B. N. Practical issues in Calculating the Sample Size for Prevalence Studies. Arch orofacial Sci. 2006; 1 (3): 9-14.
18. Musa, J., Achenbach, C., Taiwo, B., et al. High-risk human papillomavirus and cervical abnormalities in HIV-infected women with normal cervical cytology. Infect Agents Cancer. 2014; 9:36. doi: 10.1186/1750-9378-9-36.
19. Shahi, Z., Edaletmanash, M. A., and Kheikhah, B. Molecular Detection of Human Papillomavirus (Type 16, 18) using PCR and its Frequency in Patients with Cervical Cancer in Iranian Women. J Obstet Gynecol Cancer Res. 2020; 5(3): 110-114. https://doi.org/10.30699/jogcr.5.3.110
20. Stephen, E. S., Dema, E., McGee-Avila, J. K., Shiels, M. S. et al. Human Papillomavirus Awareness by Educational Level and by Race and Ethnicity. JAMA Netw Open. 2023; 1(6): 10-11. doi: 10.1001/jamanetworkopen.2023.43325
21. Sarkar, K., Pal, R., Bal, B., et al. Oncogenic Human papillomavirus among HIV infected female population in west Bengal, India. BMC Infect Dis. 2011; 11(72): 86. doi: 10.1186/1471-2334-11-72
22. Cubie, H. A., Seagar, A. L., Beattie, G. J., Monaghan, S., and Williams, A. R. A longitudinal Study of HPV Detection and Cervical Pathology in HIV Infected Women. Sex Transm Inf. 2000; 76: 257-261. doi: 10.1136/sti.76.4.257
23. Monteiro, J. C., Fonseca, R. B., Ferreira, T. C., et al. Prevalence of High-risk HPV in HIV-infected Women from Belem, Para, Amazon Region of Brazil: A Cross-Sectional Study. Front Publ Health.2021; 9 (6): 1-8. doi: 10.3389/fpubh.2021.649152.
24. Ahmed, H. G., Bensumaida, S. H., Alshammari, F. D., et al. Prevalence of Human Papillomavirus subtypes 16 and 18 among Yemeni patients with Cervical cancer. Asian Pac J Cancer Prev. 2017; 18 (6): 1543-1548. doi: 10.22034/APJCP.2017.18.6.1543.
25. Temesgan, M. M., Alemu, T., Shiferaw, B., et al. Prevalence of oncogenic Human papillomavirus (HPV 16 and 18) infection, cervical lesions and its associated factors among women aged 21-49 years in Amhara region, northern Ethiopia. PLoS One. 2021; 10 (13): 1371. https://doi.org/10.1371/journal.pone.0248949
26. Tao, X., Zhang, H., Wang, S., Cheng, et al. Prevalence and Carcinogenic Risk of High-risk Human Papillo-mavirus Subtypes in Different Cervical Cytology: A study of 124,251 Cases from the Largest Academic Center in China. J Am Soc Cytopathol. 2021; 10(4): 391-398. doi: 10.1016/j.jasc.2021.03.006.
27. Kulkarni, S. S., Kulkarni, S. S., Vastrad, P. P., et al. Prevalence and distribution of high-risk human papillomavirus (HPV) Types 16 and 18 in Carcinoma of cervix, saliva of patients with oral squamous cell carcinoma and in the general population in Karnataka, India. Asian Pac J Cancer Prev. 2011; 12 (3): 645-648.
28. Bahmanyar, E., Paavonen, J., Naud, P., et al. HPV PATRICIA Study Group. Prevalence and risk factors for cervical HPV infection and abnormalities in young adult women at enrolment in the multinational PATRICIA trial2012). Gynaecol. Oncol.2013; 127 (3): 440-450. doi: 10.1016/j.ygyno.2012.08.033
29. Hewavisenti, R. V., Arena, J., Ahlenstiel, C. L., and Sasson, S. C. Human papillomavirus in the setting of Immunodeficiency: pathogenesis and the emergence of next-generation therapies to reduce high asso-ciated risk. Front Immunol. 2023; 14: 1-24. doi: 10.3389/fimmu.2023.1112513.
30. Aminu, M., Gwafan, J. Z., Inabo, H. I., Oguntayo, A. O., Ella, E. E. and Koledade, A. K. Seroprevalence of Human Papillomavirus Immunoglobulin G Antibodies among Women Presenting at the Reproductive Health Clinic of a University Teaching Hospital in Nigeria. Int J Women Health. 2014; 6: 479-487. doi: 10.2147/IJWH.S56388
31. Song, D., Li, H., and Dai, J. Effect of Human Papillo-mavirus Infection on the Immune System and Its Role in the Course of Cervical Cancer (review). Oncol Lett. 2015; 10: 600-606. doi: 10.3892/ol.2015.3295
32. Yousif, M. G., Al-Amran, F. G., and Yousif, N. G. Prevalence and Associated Factors of Human Papillomavirus Infection Among Iragi Women. Med Adv Innov J. 2023; 1 (1): 1-10.
33. Jaya, S. and Latha, M. Channel based Threshold Segmen-tation of Multi-Class Cervical Cancer using Mean and Standard Deviation on Pap Smear Images. International Conference on Electronics and Sustainable Communication Systems (ICESC), India. 2020; 721-726. doi:10.1109/ICESC48915.2020.9156020.
34. Lui, G., Sharma, M., Tan, N., and Barnabas, R. V. HIV-positive women have higher risk of human papillomavirus infection, precancerous lesions, and cervical cancer. AIDS. 2018; 32 (6): 795 -808. doi: 10.1097/QAD.0000000000001765
35. Burd, M. E. Human papillomavirus and cervical cancer. Clin Microbiol Rev. 2003; 16(1): 1-17. https://doi.org/10.1128/cmr.16.1.1-17.2003
36. Yakub, M., Fowotade, A., Anaedobe, C., Manga, M., Bakare, R., and Abimiku, B. HPV Correlates of High-Grade Cervical Dysplasia among HIV-infected Women at a Major Treatment Centre in Nigeria. A Cross-Sectional Study. Pan Afr Med J. 2019; 33: 125. doi:10.11604/pamj.2019.33.125.17589

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Original Article **Open Access**

Phenotypic detection of extended-spectrum β-lactamase Enterobacterales isolated from people living with HIV/AIDS in Kinshasa, Democratic Republic of the Congo

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Abstract:

Background: People living with HIV/AIDS (PLWHA) are prone to opportunistic bacterial infections caused by multidrug-resistant organisms. The aim of this study was to determine the susceptibility of Enterobacterales isolated from urine and stool samples of PLWA attending the BOYAMBI Hospital Center, Kinshasa, Democratic Republic of the Congo to commonly used antibiotics and to detect extended-spectrum β-lactamases (ESBLs) producers among the isolates.
Methodology: A total of 163 HIV-infected patients attending the BOYAMBI Hospital, Kinshasa, DRC, were randomly selected for this study. Urine samples were collected from 108 patients, while stool samples were collected from 55 of them. Samples were cultured in MacConkey agar and identified using conventional microbiological methods. Antibiotic susceptibility on each isolate to selected antibiotics was performed by the disc diffusion method. Phenotypic detection of ESBL was done by the double-disc synergy test.
Results: A total of 120 Enterobacterales were isolated from the samples of the 163 HIV-infected patients with 65 of 108 (60.2%) urine and 55 of 55 (100.0%) stool samples. *Escherichia coli* was the most frequent bacterial species from both urine and stool with 84 (70.0%), followed by *Klebsiella*specieswith23 (19.2%). Other bacterial pathogens were *Citrobacter* (n=6, 5.0%), *Enterobacter* (n=4, 3.3%), *Proteus* (n=2, 1.7%) and *Morganella* (n=1, 0.8%) species. *Escherichia coli* isolates were resistant to amoxicillin (90.5%), sulfamethoxazole-trimethoprim (81.0%), ciprofloxacin (77.4%), ceftriaxone (77.4%), ceftazidime (73.8%), amoxicillin–clavulanic acid (61.9%), imipenem (60.7%), and cefotaxime (50.0%). *Klebsiella pneumoniae* isolates were resistant to ceftazidime (95.7%), ceftriaxone (91.3%), imipenem (91.3%), ciprofloxacin (87%), sulfamethoxazole-trimethoprim (78.3%), cefotaxime (56.5%), and amoxicillin–clavulanic acid (52.2%). *Citrobacter, Enterobacter, Morganella* and *Proteus* species were resistant to the majority of antibiotics. The rate of ESBL production was 23.0% (28/120) with *Citrobacter* spp being the most frequent ESBL-producer, followed *Klebsiella* spp, *Enterobacter* spp and *E. coli*.
Conclusion: The results obtained showed a high rate of ESBL-producing Enterobacterales isolates which were multi-drug resistant. Nitrofurantoin, gentamicin, chloramphenicol, cefixime and nalidixic acid were the most active antibiotics against the isolates.

Keywords: Extended-spectrum β-lactamase, Enterobacterales, HIV/AIDS, Democratic Republic of the Congo

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Détection phénotypique des β-lactamases à spectre etendu chezles Entérobactérales isolées des personnes vivant avec le VIH/SIDA à Kinshasa, République Démocratique du Congo

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Résumé:

Contexte: Les personnes vivant avec le VIH/SIDA (PLWHA) développent les infections bactériennes opportunistes causées par des organismes multi-résistants. L'objectif de cette étude était de déterminer la sensibilité aux antibiotiques des Entérobacterales isolées à des échantillons d'urine et de selles de personnes vivant avec le VIH/SIDA fréquentant le Centre Hospitalier BOYAMBI, à Kinshasa, en République démocratique du Congo, et de détecter les souches productrices de bêta-lactamases à spectre étendu (BLSE).
Méthodologie: Un total de 163 patients infectés par le VIH fréquentant l'hôpital BOYAMBI été sélectionnés au hasard pour cette étude. Des échantillons d'urine ont été prélevés sur 108 patients, tandis que des échantillons de selles ont été prélevés sur 55 d'entre eux. Les échantillons ont été mis en culture sur une gélose MacConkey et identifiés à l'aide de méthodes microbiologiques conventionnelles. La sensibilité de chaque isolat aux antibiotiques sélectionnés a été déterminée par la méthode de diffusion en milieu gélosé de Mueller Hinton. La détection phénotypique des BLSE a été effectuée par le test de double synergie de disque.
Résultats: Au total, 120 Entérobactérales ont été isolées à partir des échantillons des 163 patients infectés par le VIH, 65 des 108 (60,2%) échantillons d'urine et 55 des 55 (100,0%) échantillons de selles. *Escherichia coli* était l'espèce bactérienne la plus fréquente à la fois dans l'urine et dans les selles avec 84 souches (70,0%), suivie par les espèces de *Klebsiella* avec 23 souches (19,2%). Les autres bactéries pathogènes étaient *Citrobacter* (n=6, 5,0%), *Enterobacter* (n=4, 3,3%), *Proteus* (n=2, 1,7%) et *Morganella* (n=1, 0,8%). Les isolats d'*Escherichia coli* étaient résistants à l'amoxicilline (90,5%), au sulfaméthoxazole-triméthoprime (81,0%), à la ciprofloxacine (77,4%), à la ceftriaxone (77,4%), à la ceftazidime (73,8%), à l'amoxicilline-acide clavulanique (61,9%), à l'imipénème (60,7%) et à la céfotaxime (50,0%). Les isolats de *Klebsiella pneumoniae* étaient résistants à la ceftazidime (95,7%), à la ceftriaxone (91,3%), à l'imipénème (91,3%), à la ciprofloxacine (87.0%), au sulfaméthoxazole-triméthoprime (78,3%), à la céfotaxime (56,5%) et à l'amoxicilline-acide clavulanique (52,2%). Les espèces *Citrobacter*, *Enterobacter*, Morganella et *Proteus* étaient résistantes à la majorité des antibiotiques. Le taux de production de BLSE était de 23,0% (28/120), *Citrobacter* spp étant le producteur de BLSE le plus fréquent, suivi de *Klebsiella* spp, *Enterobacter* spp et *E. coli*.
Conclusion : Les résultats obtenus ont montré un taux élevé d'isolats d'Entérobactérales producteurs de BLSE qui étaient multi-résistants. La nitrofurantoïne, la gentamicine, le chloramphénicol, le céfixime et l'acide nalidixique étaient les antibiotiques les plus actifs contre les isolats.

Mots-clés: β-lactamase à spectre Etendu, Enterobacterales, VIH/SIDA, République Démocratique du Congo.

Introduction:

People living with HIV/AIDS (PLWHA) develop opportunistic infections which remain a cause of mortality and morbidity in the majority of patients. These infections are the result of severe immunosuppression very often due to the absence, failure or non-compliance with anti-HIV treatment. Majority of the opportunistic infections are caused by mycobacteria, parasites, fungi and bacteria. Among the bacteria involved in opportunistic infections, Enterobacterales play important role (1,2,3). Members of the order Enterobacterales have developed resistance to commonly prescribed antibiotics. One of the resistance mechanisms is the production of the extended spectrum β-lactamases (ESBLs), which are enzymes that confer resistance to penicillins, cephalosporins and monobactams.

The global prevalence of ESBL-producing Enterobacterales is increasing (4-8). Worldwide, the prevalence of ESBL differs between regions and varies between 0 and 100% (9), and it is estimated that 5% of urinary tract infections (UTIs) in pregnant women in Europe and 45% in Africa is caused by ESBL-producing pathogens (10). Among PLWHA, some studies have reported prevalence of ESBL among Enterobacterales in Nigeria (21.0%), in Ethiopia (99.0%), Zimbabwe (3.0%), and Germany (4.0%) (11-14). The production of these enzymes by bacteria leads to the hydrolysis of the beta-lactam nucleus and renders the treatment ineffective, which constitutes a real public health problem (6,11,15).

Data on the prevalence of ESBL-producing Enterobacterales in the general population are very limited in Democratic Republic of the Congo. People living with HIV/AIDS have a high risk of developing multiple infections, some of which are due to multi-drug resistant ESBL-producing bacteria. The prevalence of ESBL in this category of patients is also not very well studied in the DRC. Thus,

this study aimed to assess the antibiotic susceptibility of Enterobacterales isolates and to determine the rate of ESBL-production among these isolates in HIV-infected patients at the BOYAMBI Hospital center in Kinshasa, DRC.

Materials and method:

Study setting, participants and samples:

This study was conducted between January and March 2022 at BOYAMBI Hospital Center in Kinshasa, Democratic Republic of the Congo. A total of 163 HIV/AIDS patients were randomly selected for the study and urine samples were collected from 108 and stool samples from 55 of the patients. The samples were analyzed at the University Reference Center of Antimicrobial Resistance Surveillance (URC-AMRS) of Faculty of Pharmaceutical Sciences, University of Kinshasa.

Isolation and identification of bacteria

The isolation of bacteria was done by culturing the stool samples on Hektoen and MacConkey agar plates (Liofilchem, Roseto Degli Abbruzzi, Italy). For urine samples, the enumeration of bacterial colonies was performed on Cysteine Lactose Electrolyte Deficient (CLED) agar plates (Liofilchem, Roseto Degli Abbruzzi, Italy) which were incubated for 24 hours at 37°C. Cultures were considered positive when 10^5 colony forming units (CFU)/ml of urine were counted.

Isolated Enterobacterales were identified by conventional microbiological identification methods including Gram staining, oxidase tests, indole and urease production, citrate utilization, hydrogen sulphide gas production and fermentation of sugars, as well as lysine decarboxylase (LDC), ornithine decarboxylase (ODC) and arginine dihydrolase (ADH) tests (17-19).

Antibiotic susceptibility tests:

Antimicrobial susceptibility test (AST) of each Enterobacterales isolate was done by the disc diffusion method on Mueller-Hinton (MH) agar using the following antibiotic disks (Liofilchem, Roseto Degli Abruzzi, Italy); cefotaxime (30µg), ceftriaxone (30µg), amoxicillin (30µg), amoxicillin-clavulanic acid (20/10µg), ceftazidime (20µg), cefixime (10µg), cefuroxime (15µg), co-trimoxazole (23.75/1.25µg), imipenem (10µg), nalidixic acid (10 µg), ciprofloxacin (5µg), nitrofurantoin (300 µg), aztreonam (30µg), gentamicin (20µg), and chloramphenicol (10µg). The interpretation of the results as sensitive, intermediate or resistant was done according to the criteria of the Clinical and Laboratory Standards Institute (16,20,21). *E. coli* ATCC 25922 was used for quality control.

Phenotypic screening and confirmation of ESBL production:

The screening for ESBLs production from each bacterial isolate was determined from the AST result of ceftazidime and cefotaxime. Thus, all isolates that were resistant to ceftazidime and/or cefotaxime were suspected to be ESBL producers (16,21). Phenotypic confirmation of ESBLs production was done by the 'double-disc' synergy test. Four antibiotic discs were used, with amoxicillin-clavulanic acid in the centre of the plate, surrounded by two discs of 3rd generation cephalosporins (cefotaxime and ceftazidime) and a disc of aztreonam. The discs were placed at a distance of 25 mm on MH agar plate that has been inoculated with bacterial suspension equivalent 0.5McFarland turbidity standard, and incubated for 18-24 hours at 37°C (16, 21). The characteristic image of 'champagne button' appearance confirms the presence ESBL. *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative control strains respectively.

Statistical analysis:

Statistical analysis of the data (descriptive analysis) was carried out using the Microsoft Excel 2010 and R software. Statistical comparisons of antibiotic resistance and prevalence of ESBL producing isolates in stool and urine samples was done using Chi-square test, with a significance threshold of 5% (95% CI, $p \leq 0.05$).

Results:

Enterobacterales isolates:

Of the total of 163 HIV/AIDS patients from whom urine (n=108) and stool (n=55) samples were collected and analyzed, 120 Enterobacterales were isolated, with 65 from 108 (60.2%) urine and 55 from 55 (100.0%) stool samples. *Escherichia coli* was the most frequent bacterial species isolated from both urine and stool (Table 1). The isolated bacterial species belong to six bacterial genera; *Escherichia* (n=84, 70.0%), *Klebsiella* (n=23, 19.2%), *Citrobacter* (n=6, 5.0%), *Enterobacter* (n=4, 3.3%), *Proteus* (n=2, 1.7%) and *Morganella* (n=1, 0.8%).

Table 1: Frequency distribution of the Enterobacterales isolates

Enterobacterales isolates	Biological specimen		Total	%
	Urine	Stool		
Escherichia coli	40	44	84	70.0
Klebsiella spp	16	7	23	19.2
Citrobacter spp	3	3	6	5.0
Enterobacter spp	3	1	4	3.3
Morganella morganii	1	0	1	0.8
Proteus spp	2	0	2	1.7
Total (%)	**65 (54.2)**	**55 (45.8)**	**120**	**100.0**

Results of antibiotic susceptibility test:

The results in Tables 2 and 3 showed that the Enterobacterales isolates were resistant to most of the antibiotics tested. High resistance was observed to amoxicillin (92%), ceftriaxone (82.0%), sulfamethoxazole-trimethoprim (81.0%), ceftazidime (80.0%), ciprofloxacin (78.0%), imipenem (71.0%), cefuroxime (65.0%), amoxicillin-clavulanic acid (58.0%) and cefotaxime (53.0%). High sensitivity was obtained with nitrofurantoin (88%), gentamicin (77.0%), chloramphenicol (72%), cefixime (61.0%) and nalidixic acid (51.0%).

Escherichia coli isolates (n=84) exhibited high resistance to amoxicillin (90.5%), sulfamethoxazole-trimethoprim (81.0%), cipr rofloxacin (77.4%), ceftriaxone (77.4%), ceftazidime (73.8%), amoxicillin–clavulanic acid (61.9%), imipenem (60.7%) and cefotaxime (50.0%). The highest sensitivity of *E. coli* isolates was observed with nitrofurantoin (99.0%) followed by cefixime (61.0%).

Klebsiella isolates (n=23) were resistant to ceftazidime (95.7%), ceftriaxone (91.3%), imipenem (91.3%), ciprofloxacin (87%), sulfamethoxazole-trimethoprim (78.3%), cefotaxime (56.5%), and amoxicillin–clavulanic acid (52.2%) and were sensitive to cefixime (65.0%), nalidixic acid (60.0%) nitrofurantoin (60.0%), gentamicin (61.0%) and chloramphenicol (61.0%). The combination of amoxicillin and clavulanic acid showed average activity of 50.0% against *Klebsiella* isolates. *Citrobacter, Enterobacter, Morganella* and *Proteus* species were highly resistant and exhibited multiple resistance to the antibiotics tested.

Citrobacter isolates (n=6) were fully (100.0%) resistant to cefuroxime, ceftriaxone, ceftazidime, cefotaxime and imipenem, and 83.0% were resistant to nalidixic acid, ciprofloxacin, gentamicin and sulfamethoxazole-trimethoprim combination. High sensitivity was only observed with nitrofurantoin (83.0%). *Enterobacter* strains (n=4) exhibited high resistance to imipenem (75.0%), ceftazidime (75.0%) and sulfamethoxazole-trimethoprim (75.0%), but were 100.0% susceptible to nalidixic acid and 75.0% to cefixime, ciprofloxacin, gentamicin and cefotaxime.

The resistance rate of the urinary Enterobacterales isolates of 67.7% to amoxicillin-clavulanic acid was significantly higher than 47.3% for the stool isolates (p=0.0038), and the resistance rate of 40.0% for the urinary isolates to gentamicin was significantly higher than 25.5% for the stool isolates (p= 0.0283). Conversely, the resistance rate of 30.8% for urinary Enterobacterales isolates to chloramphenicol was significantly lower than 47.3% for the stool isolates (p=0.0167), and 75.4% resistance rate of the urinary isolates to sulfamethoxazole-trimethoprim was also significantly lower than 87.3% for the stool isolates (p=0.0309).

Results of ESBL detection:

Out of the 120 Enterobacterales isolates, 98 showed resistance to at least one 3rd generation cephalosporin (ceftriaxone, ceftazidime and cefotaxime) and 112 isolates to penicillins (amoxicillin). Of the 120 isolates, 28 showed an ESBL phenotype after 'double disc synergy test' by the appearance of a 'champagne button' (Fig 1), giving an overall ESBL rate of 23.0%. The most frequent ESBL producing bacteria was *Citrobacter* spp (50%, 3/6) followed *Klebsiella* spp (26.1%, 6/23), *Enterobacter* spp (25.0%, 1/4) and *E. coli* (21.4%, 18/84) (Table 4). ESBL were detected in 15 of 65 (23.1%) Enterobacterales isolates from urine samples, while they were detected in 13 of 55 (23.6%) isolates from stool samples (x^2=0.0052, p=0.9424).

Table 2: Susceptibility of Enterobacterales isolates to selected antibiotics

Antibiotics	**Enterobacterales isolates**											
	***Citrobacter* spp (n=6)**		***Enterobacter* spp (n=4)**		***Escherichia coli* (n=84)**		***Klebsiella* spp (n=23)**		***Morganella* spp (n=1)**		***Proteus* spp (n=2)**	
	R (%)	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
AMC	3 (50.0)	3 (50.0)	2 (50.0)	2 (50.0)	52 (61.9)	32 (38.1)	12 (52.2)	11 (47.8)	1 (100.0)	0	0	2 (100.0)
NA	5 (83.3)	1 (16.7)	0	4 (100.0)	44 (52.4)	40 (47.6)	9 (39.1)	14 (60.9)	0	1(100.0)	0	2 (100.0)
AML	ND	ND	ND	ND	76 (90.5)	8 (9.5)	ND	ND	ND	ND	ND	ND
CXM	6 (100.0)	0	2 (50.0)	2 (50.0)	49 (58.3)	35 (41.7)	19 (82.6)	4 (17.4)	1 (100.0)	0	1 (50.0)	1 (50.0)
CFM	4 (66.7)	2 (33.3)	1 (25.0)	3 (75.0)	33 (39.3)	51 (60.7)	8 (34.8)	15 (65.2)	0	1(100.0)	1 (50.0)	1 (50.0)
CRO	6 (100.0)	0	3 (75.0)	1 (25.0)	65 (77.4)	19 (22.6)	21 (91.3)	2 (8.7)	1 (100.0)	0	2 (100.0)	0
IMI	6 (100.0)	0	3 (75.0)	1 (25.0)	51 (60.7)	33 (39.3)	21 (91.3)	2 (8.7)	1 (100.0)	0	2 (100.0)	0
CIP	5 (83.3)	1 (16.7)	1 (25.0)	3 (75.0)	65 (77.4)	19 (22.6)	20 (87.0)	3 (13.0)	1 (100.0)	0	1 (50.0)	1 (50.0)
CN	5 (83.3)	1 (16.7)	1 (25.0)	3 (75.0)	25 (29.8)	59 (70.2)	9 (39.1)	14 (60.9)	0	1(100.0)	0	2 (100.0)
C	3 (50.0)	3 (50.0)	2 (50.0)	2 (50.0)	29 (34.5)	55 (65.5)	9 (39.1)	14 (60.9)	1 (100.0)	0	2 (100.0)	0
CAZ	6 (100.0)	0	3 (75.0)	1 (25.0)	62 (73.8)	22 (26.2)	22 (95.7)	1 (4.3)	1 (100.0)	0	2 (100.0)	0
CTX	6 (100.0)	0	1 (25.0)	3 (75.0)	42 (50.0)	50 (50.0)	13 (56.5)	10 (43.5)	0	1(100.0)	2 (100.0)	0
SXT	5 (83.3)	1 (16.7)	3 (75.0)	1 (25.0)	68 (81.0)	16 (19.0)	18 (78.3)	5 (21.7)	1 (100.0)	0	2 (100.0)	0
F	1 (16.7)	5 (83.3)	1 (25.0)	3 (75.0)	1 (1.2)	83 (98.8)	9 (39.1)	14 (60.9)	0	1(100.0)	2 (100.0)	0

R: Resistant; S: Susceptible; ND: Not determined; AMC: Amoxicillin-clavulanic acid; NA: Nalidixic acid; AML: Amoxicillin; CXM: Cefuroxime; CFM: Cefixime; CRO: Ceftriaxone; IMI: Imipenem; CIP: Ciprofloxacin; CN: Gentamicin; C: Chloramphenicol; CAZ: Ceftazidime; CTX: Cefotaxime; SXT: Sulfamethoxazole trimethoprim; F: Nitrofurantoin.

Table 3: Comparative resistance of Enterobacterales isolates from stool and urine samples

Antibiotics tested	**Resistance of Enterobacterales isolates to the tested antibiotics**				***p* value**
	Urine		**Stool**		
	No of isolates	**Number (%) of resistant isolates**	**No of isolates**	**Number (%) of resistant isolates**	
AMC	65	44 (67.7)	55	26 (47.3)	0.003814*
NA	65	30 (46.2	55	28 (50.9)	0.5007
AML	40	37 (92.5)	44	39 (88.6)	0.5469
CXM	65	42 (64.6)	55	36 (65.5)	0.902
CFM	65	25 (38.5)	55	22 (40.0)	0.8235
CRO	65	56 (86.2)	55	42 (76.4)	0.0761
IMI	65	46 (70.8)	55	39 (70.9)	0.9826
CIP	65	49 (75.4)	55	44 (80.0)	0.4326
CN	65	26 (40.0)	55	14 (25.5)	0.02833*
C	65	20 (30.8)	55	26 (47.3)	0.01676*
CAZ	65	54 (83.1)	55	42 (76.4)	0.2373
CTX	65	36 (55.4)	55	28 (50.9)	0.5265
SXT	65	49 (75.4)	55	48 (87.3)	0.03098*
F	65	10 (15.4)	55	4 (7.3)	0.07036

* = statistically significant at $p<0.05$; R: Resistant; S: Susceptible; ND: Not determined; AMC: Amoxicillin-clavulanic acid; NA: Nalidixic acid; AML: Amoxicillin; CXM: Cefuroxime; CFM: Cefixime; CRO: Ceftriaxone; IMI: Imipenem; CIP: Ciprofloxacin; CN: Gentamicin; C: Chloramphenicol; CAZ: Ceftazidime; CTX: Cefotaxime; SXT: Sulfamethoxazole trimethoprim; F: Nitrofurantoin.

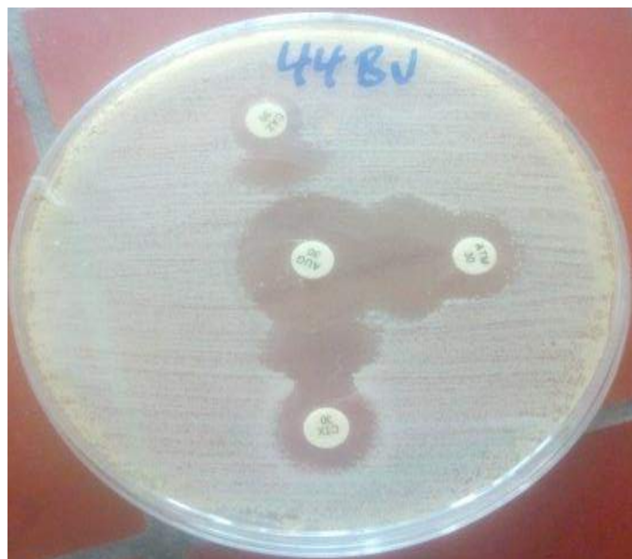

Fig 1: Champagne button appearance in a positive ESBL isolate by double-disc synergy test

Table 4: Frequency of ESBL phenotypes in Enterobacterales isolates

Enterobacterales isolates	Total number of isolates	Number (%) with ESBL phenotype
Escherichia coli	84	18 (21.4)
Klebsiella spp	23	6 (26.1)
Citrobacter spp	6	3 (50.0)
Enterobacter spp	4	1 (25.0)
Proteus spp	2	0
Morganella spp	1	0
Total	**120**	**28 (23.3)**

Discussion:

In this study, microbiological analysis of urine (n=108) and stool (n=55) samples obtained from 163 PLWHA in BOYAMBI Hospital center in Kinshasa resulted in isolation of 120 Enterobacterales isolates, with 65 from 108 (60.2%) urine and 55 from 55 (100.0%) stool samples. These isolates belong to six major genera of Enterobacterales; *Escherichia*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Proteus* and *Morganella*. *Escherichia coli* was the most frequently isolated bacterial species in both stool (80%, n=44) and urine (61%, n=40) samples, followed by *Klebsiella* spp with 13.0% and 25.0% respectively. These results agree with that of Iyamba et al., (1) in 2013 in Democratic Republic of the Congo, which reported the predominance of *E. coli* in both stool (87.0%) and urine (62.5%) samples. Our results also agree with that of Bayleyegn et al., (12) in Ethiopia in 2021 that reported 60.0% *E. coli* and 16.1% *Klebsiella*, Olaru (13) in 2021 in Zimbabwe with 75.2% *E. coli*, and Salah et al., (23) in Lome Togo. Our results are also consistent with studies from Nigeria in 2021 by Oluwaseun et al., (22) with 23.0% *E. coli* and 11.8% *Klebsiella* spp, and Mofolorunsho et al., (15) with 69.0% *E. coli* and 31.0% *Klebsiella pneumoniae*.

The susceptibility test of the Enterobacterales isolates to 14 antibiotics showed high resistance to many antibiotics tested such as the 3rd cephalosporins, sulfamethoxazole-trimethoprim, ciprofloxacin, imipenem, cefuroxime, and amoxicillin-clavulanic acid. But they showed high sensitivity to nitrofurantoin, gentamicin, chloramphenicol, cefixime, and nalidixic acid. These results disagree with those of Liesse et al., (1) in 2014 which reported that cefotaxime, ceftriaxone and gentamicin were still the most effective antibiotics with *in vitro* sensitivity of over 74.1% by the bacterial isolates. These antibiotic susceptibility results showed that Enterobacte-

rales in general show high rate of resistance to the antibiotics commonly used in human health. In Nigeria, Ifeoma et al., (24) reported resistance rates of 99.0%, 97.1%, 88.2%, 82.4%, 81.4%, 65.7%, 54.9%, 46.1%, 46.1%, and 23.5% for ampicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, ceftriaxone, ceftazidime, amoxicillin-clavulanate, gentamicin, cefoxitin, nitrofurantoin and imipenem respectively among Enterobacterales isolates (24). Our results also disagree with those obtained in Ethiopia by Bayleyegn et al., (12) in 2021 where bacterial isolates were highly susceptible to chloramphenicol, cefotaxime and ceftazidime. Our results confirm the high level of resistance among Enterobacterales reported in many African countries (9,11), although the study by Ali et al., (11) in Nigeria in 2020 reported that the addition of clavulanic acid has allowed the restoration of amoxicillin activity on some bacterial strains such as *Klebsiella* species.

Our study also showed several multidrug-resistant isolates, which can be explained by the fact that the genes carrying these resistance traits are found on the same plasmids, coexistence of several resistance mechanisms, and by multiplicity of enzyme types (42). The rate of ESBL producers in our study was 23.3% (28/120). *Citrobacter* spp was the most predominant ESBL producer (50.0%, 3/6) followed *Klebsiella* spp (26.1%, 6/23), *Enterobacter* spp (25.0%, 1/4) and *E. coli* (21.4%, 18/84). Sami et al., (40) reported a prevalence of 24.7% (61/246) of ESBL-producing *Citrobacter* spp, which is lower than the rate in our study. The distribution of ESBL-producing Enterobacterales in our study is also at odd with the results of Choi et al., (41), which reported ESBL rates of 12.8%, 12.4%, 4.9%, and 0% in *Enterobacter* spp., *S. marcescens*, *C. freundii*, and *M. morganii* respectively. The ESBL rates in our study are higher than that reported by Tshilumbu (25) in Democratic Republic of the Congo in 2012, with 19.2% and predominance among hospitalized patients (10.1%) than those living in the community (9.1%). Dikoumba et al., (26) in Gabon reported ESBL prevalence among *Enterobacteriaceae* of 11.8% with predominance of *E. coli* and *K. pneumoniae*. Olaru et al., (13) in 2021 reported a prevalence of 19.3% in people living with and without HIV/AIDS in primary clinics in Zimbabwe. Utulo et al., (27) in 2021 reported 17% ESBL prevalence in some health facilities in Nigeria.

In a systematic review by Mansouri (10) in 2019 on the prevalence of ESBL in *Enterobacteriaceae* from urine of pregnant and postpartum women, the pooled prevalence of 25.0% was reported in all the studies (6 in Africa, 2 in North America, 12 in Asia, 1 in South America and 2 in Europe). The estimated prevalence rates were 45.0% in Africa, 33.0% in India, 5.0% in Europe, 4.0% in South America and 3.0% in North America. The rate of ESBL obtained in our study is lower than that reported in some countries around the world. This is particularly true in certain countries such as Nigeria (with 31.2 - 51.1% rate), Central African Republic (50.8%), Nepal (37.8% in PLWHA compared to 68.9% in non-PLWHA); Tanzania (50.4% in hospitalized children compared to 11.6% among children in the community, 89.7% in PLWHA children versus 16.9% in non-PLWHA), Ethiopia (73.9%) and 44.0% in Chad (72.0%) (4,5,14,22,28-35). It is also slightly lower than that reported by Bajpai et al., (36) in 2017 and Braide et al., (37) in 2018, with ESBL prevalence of 45.0% among urinary isolates.

The ESBL rate in Enterobacterales isolates from urine samples in our study was 23.1% (15/65) and 23.6% (13/55) for the stool samples. These prevalence rates are in agreement with those of Iliyasu et al., (6) who reported 28.2% for urine samples in UTIs and 27.1% in stool samples, but contradicted those of Utulo et al., (27) who reported higher ESBL prevalence in health facilities in Nigeria of 22.0% from stool samples than from blood (21.4%) and urine (13.8%) samples. In our study however, there was no significant difference in the ESBL rate between Enterobacterales isolates from urine and stool samples (x^2=0.0052, p=0.9424).

Conclusion:

The results of our study showed a relatively high rate of ESBL-producing Enterobacterales isolates with high *in vitro* and multi-drug resistance to commonly used antimicrobials. Certain β-lactam agents (penicillins, cefuroxime, ceftriaxone, cefotaxime, ceftazidime) should no longer be used as first-line empirical antibiotic therapy of infections of the urinary and gastrointestinal systems, where Enterobacterales are involved in the Democratic Republic of the Congo. Nitrofurantoin, gentamicin and chloramphenicol are the most active antibiotics *in vitro* against the isolates, and should be considered as firstline drugs in the empirical treatment of infections caused by these organisms in DRC.

Our study was limited by the relatively small sample which may not allow us to give accurate estimate of the true prevalence of ESBL-producing Enterobacterales in our environment.

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Contributions of authors:

MVG was involved in the study conceptualization, methodology, laboratory analysis, interpretation of the results, and original drafting of the manuscript. ILJ and MML were involved in sample collection. MLC and NNJ were involved in the processing of the samples. NPO and BMT were involved in the statistical analysis of the data. LIJM and MWJ corrected the study protocol and revised and finalized the manuscript. TK and OD participated in the critical review of the manuscript. All authors have read and agreed to the final version of this manuscript.

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Conflict of interest:

Authors declare no conflict of interest

References:

1. Iyamba J. M., Wambale, J. M., and Takaisi-Kikuni, N. Z. Antimicrobial susceptibility patterns of enterobacteriaceae isolated from HIV-infected patients in Kinshasa. Pan Afr Med J. 2014; 17: 179.doi:10.11604/pamj.2014.17.179.3788
2. Wembulua, S., Lakhe, A., VMP, C.D., Ka, D., Fortes, L., and Seydi, M. Antibacterial Susceptibility Patterns of Bloodstream Isolates in 74 HIV-Infected Patients Hospitalized at the Clinic of Infectious and Tropical Diseases of Fann University Hospital, Dakar from 2013 to 2016. Medecine Tropicale et Sante Internationale. 2021; 1 (2): doi:10.48327/mtsibulletin.n1.2021.88
3. Vandenhende, M. A., Blanc, P., Bessede, E., et al. Infections bactériennes chez les patients infectés par le VIH: Profil de résistance aux antibiotiques et évolution au cours du temps. La Revue de Médecine Interne, 2021; 42: A34. https://doi.org/10.1016/j.revmed.2021.03.23.
4. Sanke-Waïgana, H., Mbecko, J. R., Ngaya, G., Manirakiza, A., and Alain, B. A. 2003-2019: explosive spread of enterobacteria producing extended-spectrum beta- lactamases in Bangui Central African Republic. Pan Afr Med J. 2021; 39: 22 https://doi.org/10.11604/pamj.2021.39.22.28812
5. Tadesse, S., Mulu, W., Genet, C., Kibret, M., and Belete, M. A. Emergence of high prevalence of extended-spectrum beta-lactamase and carbapenemase-producing Enterobacteriaceae species among patients in Northwestern Ethiopia Region BioMed Res Int. 2022; 5727638, doi: 10.1155/2022/5727638
6. Iliyasu, M. Y., Uba, A., and Agbo, E. B. Phenotypic detection of multidrug resistant extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* from clinical samples. Afr J Cellular Pathol. 2018; 10 (2): 25-32. doi:10.5897/AJCPath2018.0004
7. Nossair, M. A., AbdElBaqy, F. A., Rizk, M. S., et al. Prevalence and Molecular Characterization of Extended-Spectrum β-Lactamases and AmpC β-lactamase-Producing Enterobacteriaceae among Human, Cattle, and Poultry. Pathogens. 2022; 11 (8): 852. doi: 10.3390/pathogens11080852
8. Paulitsch-Fuchs, A. H., Melchior, N., Haitzmann, T., et al. Analysis of Extended Spectrum Beta Lactamase (ESBL) Genes of Non-Invasive ESBL Enterobacterales in Southeast Austria in 2017. Antibiotics. 2022; 12 (1): 1. doi: 10.3390/antibiotics12010001.
9. Ouedraogo, A. S., Pierre, H. J., Banuls, A. L., Ouédraogo, R., and Godreuil, S. Émergence et diffusion de la résistance aux antibiotiques en Afrique de l'Ouest: Facteurs favorisants et évaluation de la menace. Médecine et Santé Tropicales. 2017; 27 (2): 147-154. http://dx.doi.org/10.1684/mst.2017.0678.
10. Mansouri, F., Sheibani, H., Javedani Masroor, M., and Afsharian, M. Extended - spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae and urinary tract infections in pregnant/ postpartum women: A systematic review and meta-analysis. Int J Clin Pract. 2019; 73 (12): e13422. doi:10.1111/ijcp.13422
11. Murna, A. A., Okojokwu, O. J., Ujeh, A. A., et al. Prevalence and drug-resistance profile of plasmid borne extended spectrum beta-lactamase (ESBLs) resistance genes in multidrug resistant *Escherichia coli* from HIV-1 positive individuals in Jos, Nigeria. Afr J Microbiol Res. 2020; 14 (10): 564-571. https://doi.org/10.5897/AJMR2020.9396
12. Bayleyegn, B., Fisaha, R., and Kasew, D. Fecal carriage of extended spectrum beta-lactamase producing Enterobacteriaceae among HIV-infected children at the University of Gondar Comprehensive Specialized Hospital Gondar, Ethiopia. AIDS Res Ther. 2021; 18 (1): 1-9 . doi:10.1186/s12981-021-00347-x
13. Olaru, I. D., Ferrand, R. A., Chisenga, M., et al. Prevalence of ESBL-producing Escherichia coli in adults with and without HIV presenting with urinary tract infections to primary care clinics in Zimbabwe. J Antimicrob Chemother. 2021; 3(2): dlab082.doi:10.1093/jacamr/dlab082
14. Reinheimer, C., Keppler, O. T., Stephan, C., Wichelhaus, T. A., Friedrichs, I., and Kempf, V. A. Elevated prevalence of multidrug-resistant Gram-negative organisms in HIV positive men. BMC Infect Dis. 2017; 17: 1-6. doi: 10.1186/s12879-017-2286-z
15. Mofolorunsho, K. C., Ocheni, H. O., Aminu, R. F., Omatola, C. A., and Olowonibi, O. O. Prevalence and antimicrobial susceptibility of extended spectrum beta lactamases producing *Escherichia coli* and *Klebsiella pneumoniae* isolated in selected hospitals of Anyigba, Nigeria. Afri Health Sci. 2021; 21(2). 505-512. doi:10.4314/ahs.v21i2.4
16. Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing, Twenty-Second Informational Supplement M100-S21, 2018, Wayne, PA, USA.
17. Cheesbrough, M. Medical Laboratory Manual for Tropical Countries (Vol. 1). Cambridgeshire, UK.
18. Greenwood, D., Slack, R. C., Barer, M. R., and Irving, W. L. Medical Microbiology e-book: A guide to microbial infections: Pathogenesis, immunity, laboratory diagnosis and control. Elsevier Health Sciences.
19. Denis, F., Ploy, M. C., Martin, C., Bingen, E., and Quentin, R. Bactériologie Médicale: Techniques usuelles 2eme édition Revue et Augmentée, Elsevier Masson 2011; 587- 591.
20. Hindler, J. F., and Munro, S. (Eds.). Antimicrobial susceptibility testing. Clinical Microbiology Procedures Handbook, 5-0. 2010.
21. Courvalin, P., Leclerq, R., and Bingen, E. Antibiogramme, 2nd edn ESKA, Paris, 2006.
22. Odetoyin, B., and Adewole, O. Prevalence of Extended-Spectrum β-Lactamase Production among Clinical Gram-Negative Bacilli Isolates in A Tertiary Hospital, Southwest Nigeria. Euro J Med Health. 2021; 3 (5): 9-13.

https://doi.org/10.24018/ejmed.2021.3.5.1031.

23. Salah, F. D., Sadji, A. Y., Akolly, K., et al. Augmentation de la résistance aux antibiotiques des Entérobactéries isolées à l'Institut National d'Hygiène de Lomé de 2010 à 2017. J Interval Epidemiol Publ Health. 2021; 4 (3): 3. https://doi.org/10.37432/jieph.supp.2021.4.3.03.3.
24. Nwafuluaku, I. G., Agbakoba, N. R., Ushie, S. N., and Dilibe, E. A. Prevalence and molecular characterization of extended spectrum beta-lactamase producing uropathogenic *Escherichia coli* in a tertiary health care facility in Anambra State, Nigeria. World J Adv Res Rev. 2021; 11 (3): 300-311. doi: 10.30574/wjarr.2021.11.3.0467
25. Tshilumbu, K. P. Epidémiologie et caractérisation moléculaire des beta lactamases à spectre élargi chez les entérobactéries de la flore intestinale de la population de Kinshasa, Thèse, Université de Kinshasa. 2021
26. Dikoumba, A. C., Onanga, R., Boundenga, L., Bignoumba, M., Ngoungou, E. B., and Godreuil, S. Prevalence and characterization of extended-spectrum beta- lactamase-producing Entero-bacteriaceae in major Hospitals in Gabon. Micro Drug Resist. 2021; 27(11): 1525-1534. doi: 10.1089/mdr.2020.0497
27. Utulo, P. S., Umeh, E. U., Gberikon, G. M., and Abba, P. O. Phenotypic Detection of Extended-Spectrum ß-lactamase Produced by *Escherichia coli* Isolates from Health facilities in Otukpo, Benue State Nigeria. J Res Basic Clin Sci. 2021; 2 (2): 7-13. doi.10.46912/jrbcs.111
28. Oluwakemi, T. A., Oluduro, A. O., and Oludare, O. T. Prevalence of extended spectrum β-lactamases in multi-drug resistant strains of Gram-negative bacteria, Afr J Microbiol Res. 2018; 12(7): 147-151. https://doi.org/10.5897/AJMR2017.8755
29. Padmavathy, K., Padma, K., and Rajasekaran, S. Extended-spectrum β-lactamase/AmpC-producing uropathogenic *Escherichia coli* from HIV patients: do they have a low virulence score? J Med Microbiol. 2013; 62 (3): 345-351. https://doi.org/10.1099/jmm.0.050013-0.
30. Hosuru Subramanya, S., Bairy, I., Nayak, N., Padukone, S., Sathian, B., and Gokhale, S. Low rate of gut colonization by extended-spectrum β-lactamase producing Enterobacteriaceae in HIV infected persons as compared to healthy individuals in Nepal. PLoS One. 2019; 14 (2): e0212042. https://doi.org/10.1371/journal.pone.0212042
31. Tellevik, M. G., Blomberg, B., Kommedal, Ø., Maselle, S. Y., Langeland, N., and Moyo, S. J. High prevalence of faecal carriage of ESBL-producing Enterobacteriaceae among children in Dar es Salaam, Tanzania. PLoS One. 2016; 11 (12):e0168024. https://doi.org/10.1371/journal.pone.0168024
32. VandenBunt, G., Liakopoulos, A., Mevius, D. J., et al. ESBL/AmpC-producing Enterobacteriaceae in households with children of preschool age: prevalence, risk factors and co-carriage. J Antimicrob Chemother. 2016; 72 (2): 589-595. doi:10.1093/jac/dkw443
33. Tilahun, M. Multi-Drug Resistance Profile, Prevalence of Extended-Spectrum Beta-Lactamase and Carbapenemase-Producing Gram-Negative Bacilli Among Admitted Patients After Surgery with Suspected of Surgical Site Nosocomial Infection North East Ethiopia. Infect Drug Resist. 2022; 3949-3965. doi:10.2147/IDR.S376622
34. Tornberg-Belanger, S. N., Rwigi, D., Mugo, M., et al. Antimicrobial resistance including Extended-Spectrum Beta Lactamases (ESBL) among *E. coli* isolated from Kenyan children at hospital discharge. PLoS Negl Trop Dis. 2022; 16 (3):e0010283. doi:10.1371/journal.pntd.0010283
35. Ouchar Mahamat, O., Lounnas, M., Hide, M., et al. High prevalence and characterization of extended-spectrum ß-lactamase producing Enterobacteriaceae in Chadian hospitals. BMC Infect Dis. 2019; 19:205. https://doi.org/10.1186/s12879-019-3838-1
36. Bajpai, T., Pandey, M., Varma, M., and Bhatambare, G. S. Prevalence of TEM, SHV, and CTX-M Beta-Lactamase genes in the urinary isolates of a tertiary care hospital. Avicenna J Med. 2017; 7(01): 12-16. doi: 10.4103/2231-0770.197508
37. Braide, W., Madu, L. C., Adeleye, S. A., Korie, M. C., and Akobondu, C. I. Prevalence of extended spectrum beta lactamase producing *Escherichia coli* and *Pseudomonas aeruginosa* isolated from clinical samples. Int J Sci. 2018; 4 (2): 89-93.doi:10.18483/ijSci.1556
38. Egbule, O. S., and Odih, E. E. Prevalence of Extended-Spectrum Beta-Lactamases (ESBLs) and Metallo-Beta Lactamases (MBLs) Among Healthy and Hospitalized Children in Abraka and Eku Communities, Delta State, Nigeria. Nig J Basic Appl Sci. 2016; 28(1): 7-14. doi:10.4314/njbas.v28i1.2.
39. Sahle, Z., Engidaye, G., Shenkute, D., Metaferia Y., and Shibabaw, A. High Prevalence of Multi-Drug Resistance and Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae Among Hospitalized Patients Presumptive for Bacterial Infection at Debre Berhan Comprehensive Specialized Hospital, Ethiopia. Infect Drug Resist. 2022; 2639-2656. doi:10.2147/IDR.S363988
40. Sami H, Sultan A, Rizvi M, Khan F, Ahmad S, Shukla I, et al. Citrobacter as a uropathogen, its prevalence and antibiotics susceptibility pattern. Chrismed J Health Res 2017; 4: 23-26. doi:10.4103/2348-3334.196037
41. Choi, SH., Lee, J.E., Park, S.J. *et al.* Prevalence, microbiology, and clinical characteristics of extended-spectrum β-lactamase-producing *Enterobacter* spp., *Serratia marcescens*, *Citrobacter freundii*, and *Morganella morganii* in Korea. Eur J Clin Microbiol Infect Dis 2007; 26, 557–561. https://doi.org/10.1007/s10096-007-0308-2
42. Adeline, M., and Mainil. J. Résistance bactériennes aux antibiotiques, les mécanismes et leur contagiosité, Ann Méd Vét. 2012; 156: 109-123

Ky/Ba et al. *Afr. J. Clin. Exper. Microbiol.* 2024; 25 (3): 271 - 276 https://www.afrjcem.org

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Vaginal carriage of *Streptococcus agalactiae* among pregnant women in Ouagadougou, Burkina Faso

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Abstract:

Background: Group B *Streptococcus* (GBS) is one of the main bacteria responsible of serious neonatal infections. Neonatal transmission is very high at the end of pregnancy between the 34th and 38th week of gestation, and the systematic screening of GBS is strongly recommended in Burkina Faso. The objective of this study was to assess the prevalence of GBS carriage among pregnant women in Ouagadougou, Burkina Faso.
Methodology: This was a cross-sectional study of 300 pregnant women in their third trimester of pregnancy, conducted between July and November 2020. Vaginal sample was collected from each of the pregnant women during prenatal consultations. The samples were cultured on blood agar and the isolated pathogens were identified using the BD Phoenix M50 automated system. Antibiotic sensitivity test was performed on each isolate according to the recommendations of the CA-SFM-EUCAST 2020.
Results: Of the 300 women, 12 samples were positive for GBS, giving a carriage rate of 4.0%. All the GBS isolates were susceptible to ampicillin, cefotaxime, vancomycin and nitrofurantoin. On the other hand, resistance was encountered against penicillin G, erythromycin and trimethoprim-sulfamethoxazole. None of the risk factors assessed was statistically predictive of vaginal carriage of GBS in the pregnant women.
Conclusion: Vaginal carriage of GBS remains relevant. Pending the introduction of an effective vaccine in Burkina Faso, a systematic screening policy for GBS in pregnant women would help reduce perinatal infections from GBS, and the attendant neonatal and infant mortality.

Keywords: *Streptococcus agalactiae*, Group B streptococcus, vaginal carriage, risk factors, pregnancy

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Portage vaginal de *Streptococcus agalactiae* chez les femmes enceintes à Ouagadougou, Burkina Faso

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Résumé:

Contexte: Le streptocoque du groupe B (SGB) est l'une des principales bactéries responsables d'infections néonatales graves. La transmission néonatale est très élevée en fin de grossesse entre la 34ème et la 38ème semaine de gestation, et le dépistage systématique du SGB est fortement recommandé au Burkina Faso. L'objectif de cette étude était d'évaluer la prévalence du portage du SGB chez les femmes enceintes à Ouagadougou, au Burkina Faso.
Méthodologie: Il s'agit d'une étude transversale portant sur 300 femmes enceintes dans leur troisième trimestre de grossesse, réalisée entre juillet et novembre 2020. Un échantillon vaginal a été prélevé sur chacune des femmes enceintes lors de consultations prénatales. Les échantillons ont été cultivés sur gélose au sang et les pathogènes isolés ont été identifiés à l'aide du système automatisé BD Phoenix M50. Un test de sensibilité aux antibiotiques a été réalisé sur chaque isolat selon les recommandations du CA-SFM-EUCAST 2020.
Résultats: Sur les 300 femmes, 12 échantillons étaient positifs au SGB, soit un taux de portage de 4,0%. Tous

les isolats de GBS étaient sensibles à l'ampicilline, au céfotaxime, à la vancomycine et à la nitrofurantoïne. En revanche, une résistance a été rencontrée contre la pénicilline G, l'érythromycine et le triméthoprime-sulfaméthoxazole. Aucun des facteurs de risque évalués n'était statistiquement prédictif du portage vaginal du SGB chez les femmes enceintes.

Conclusion: Le portage vaginal du SGB reste d'actualité. En attendant l'introduction d'un vaccin efficace au Burkina Faso, une politique de dépistage systématique du SGB chez les femmes enceintes contribuerait à réduire les infections périnatales dues au SGB et la mortalité néonatale et infantile qui en découle.

Mots-clés: *Streptococcus agalactiae,* streptococcus du groupe B, portage vaginal, facteurs de risque, grossesse

Introduction:

Pregnancy-related illnesses and deaths remain very high. In 2015, it was estimated worldwide that 303,000 women died from pregnancy-related causes; 2.7 million children died during their first 28 days of life and 2.6 million children were stillborn (1,2). Among the causes of maternal and child mortality and morbidity, infections held an important place. Indeed, bacterial, viral or parasitic infections are common during pregnancy (3). *Streptococcus agalactiae* or Lancefield group B streptococcus (GBS), is an encapsulated Gram-positive commensal bacteria of the digestive and vaginal tract found in 20 to 30% of healthy adults (30). Group B streptococcus is considered the main agent involved in maternal-fetal infections, in utero fetal death, septicemia and meningitis in full-term newborns in industrialized countries (1,4,5).

In general, the prevalence of GBS colonization varies between countries and with figures ranging from 1.8% to 81.09% (1,6-7). Carriage rates are 10% in France (8), 26.5% in the United States of America (9), 18.12% in Thailand (10) and 23.7% in Belgium (11). In West Africa, the carriage rate was 12.7% in the city of Abidjan (Ivory Coast) (12), and 18% in Nigeria (13). It is very likely that GBS colonizes virtually all women at some point, intermittently or transiently (1), with carriage increasing with the age of pregnancy (7). Invasive infection occurs after vertical transmission from the rectovaginal sphere of the mother and 1 to 2% of newborns of colonized mothers develop neonatal infections in the first seven days after birth (14,15). In a study in Tunisia, a history of spontaneous miscarriages was associated with carriage of group B streptococcus, with newborns whose mothers who had positive samples having a significantly lower birth weight than those who did not (5).

Prevention of neonatal infection is based on GBS screening in pregnant women and the administration of antibiotics intrapartum (5). Since the Millennium Development Goals (MDGs) for maternal and child health were not achieved in 2015, those for sustainable development (SDGs) were set to, among other things, offer new opportunities for improving maternal and child health by 2030 (16, 17). Given the absence of a real GBS infection prevention in children guidelines in the health system of Burkina Faso, the present study on the vaginal carriage of GBS in women in the third trimester of pregnancy in the health district of Bogodogo, which objective was to assess the prevalence of GBS carriage among these women in Ouagadougou, with the aim of contributing to the improvement of maternal and child health indicators.

Materials and method:

Study setting:

The study was conducted in the health facilities (Medical Center of Saaba, CSPS of Wemtenga, Dassagho, and sector 30) of the health district of Bogodogo, in the city of Ouagadougou (where vaginal samples were collected) and bacteriological analysis of samples at the biomedical analysis laboratory of the University Teaching Hospital of Bogodogo (CHUB).

Study design period of study:

This was a descriptive cross-sectional study that determined the prevalence of vaginal carriage of group B streptococcus in pregnant women. The study took place from July 2020 to November 2020.

Ethical consideration:

Written authorization from the Regional Director of Health of the Centre Region as well as the agreements of principle from the managers of the health centers were obtained beforehand. In addition, informed consent of the women was a prerequisite for their inclusion in the study. The anonymity of the women and confidentiality were preserved.

Study population and participants:

The study population were pregnant women receiving prenatal consultation in different health facilities in Ouagadougou. The inclusion criteria were pregnant women in the 3rd trimester of pregnancy seen in prenatal consultation and women who voluntarily agreed to participate in the study. Exclusion criteria were women under antibiotic therapy within 72 hours preceding collection, and women with a contraindication to vaginal sampling or who have not given consent to participate in the study.

Study sample size calculation:

The sample size was calculated using the formula; $n=t^2P(1-P)/e^2$ for *S. agalactiae*

carriage prevalence of 22.0% (p=0.22), the highest carriage rate in Africa (18). For a confidence level of 95%, t=1.96 and e=5% precision, giving the calculated minimum sample size of 264, but 300 women were subsequently enrolled into the study.

Data collection:

Data collection from each pregnant women was done using a designed data collection form to obtain information on sociodemographic characteristics, obstetric history, and current pregnancy parameters.

Biological samples collection and laboratory analyses:

Each woman had a vaginal sample taken using a sterile cotton swab from the distal third of the vagina. The swabs were immediately introduced into tubes containing Todd-Hewitt with Colistin and Nalidixic Acid (CNA) broth, and transported to the laboratory for incubation at 37°C for 18-24 hours.

Subculture of the incubated broth was done on agar supplemented with 5% defibrinated sheep blood containing CNA, and incubated at 37°C for 24 hours in the presence of 10% CO_2. GBS colonies on fresh sheep blood agar appeared as small (1 to 2 mm in diameter), rounded, and present a zone of β haemolysis resulting in a perfectly transparent clear halo around the colony which corresponds to total lysis of red blood cells. Preliminary identification of GBS was by Gram stain microscopy of the colonies which showed Gram-positive cocci in chains of varying length and negative catalase test. Confirmatory phenotypic identification of GBS species was carried out using the automated BD Phoenix M50 instrument.

Antibiotic susceptibility of the GBS isolates was done by the disc diffusion technique on Muller-Hinton agar medium supplemented with 5% fresh defibrinated sheep blood, and results interpreted according to the recommendations of the 2020 Antibiogram Committee of the French Society of Microbiology - European Committee on Antimicrobial Susceptibility Testing (CA-SFM-EUCAST 2020) (18).

Data analysis:

Data were entered into Excel and Microsoft Word, and statistical analysis was done using EPI-INFO software version 7.2.3.1. Pearson's Chi-square test was used to compare data with $p<0.05$ considered as statistical significance.

Results:

A total of 300 pregnant women were enrolled into the study. The mean age of the women was 25.2±5.5 years with range of 15-42 years. A total of 36 (12.0%) cultures were positive for microbial pathogens but 12 were positive for GBS isolates, giving vaginal carriage rate in the study to be 4.0%. The mean age of women with vaginal GBS is 25.1±5.5 years with range of 19-38 years (Table 1).

The antibiotic susceptibility of the GBS isolates showed 100% sensitivity to ampicillin, cefotaxime, amoxicillin-clavulanic acid, vancomycin and nitrofurantoin but 23.0% of the isolates were resistant to penicillin G. Resistance rate to macrolides was 23.0% to erythromycin and 25.0% to lincomycin), 75.0% to quinolone (ciprofloxacin), 92.0% to tetracycline and 100.0% to trimethoprim-sulfamethoxazole (Fig 1).

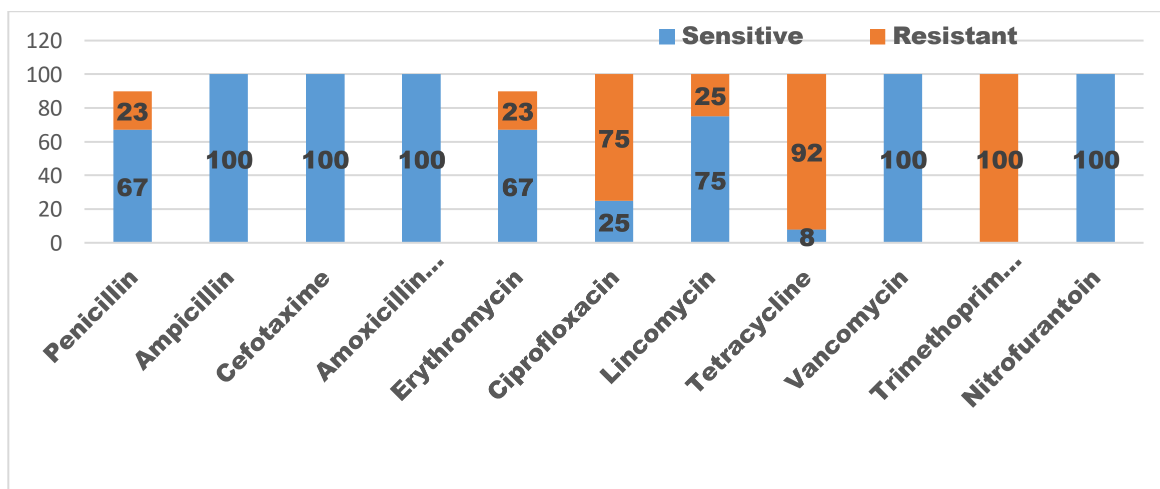

Fig 1: Susceptibility of Group B streptococcus (GBS) isolates to selected antibiotics

Table 1: Prevalence of GBS with respect to sociodemographic and obstetric characteristics of the women

Variables	Number of women (n=300)	Number of GBS isolated	Percentage (%)	*p* value
Age group (years)				
15-19	48	1	2.1	
20-34	238	8	3.4	0.054
≥ 35	14	3	21.4	
Educational level				
Not educated	156	05	3.2	
Primary	85	05	5.9	0.77
Secondary	44	01	2.3	
Tertiary	15	01	6.7	
Marital status				
Married	279	11	3.9	0.77
Single	21	1	4.8	
Occupation				
Housewife	232	8	3.4	
Employed (informal sector)	68	4	5.9	0.454
Number of gestation				
1-2	195	6	3.1	
3-4	81	3	3.7	0.09
≥ 5	24	3	12.5	
Parity				
<4	284	2	0.7	
≥ 4	16	10	62.5	0.16

Discussion:

In the present study, 300 pregnant women were enrolled, the minimum age was 15 years with an average of 25.19 years. The age group of 20 to 34 was the most represented, with 238 women or 79.33%. Among these enrolled women, 279 (93%) lived as a couple. These findings are observed in other studies in Africa, notably that carried out in Ethiopia in 2016 by Mengist et al., who found an average age of 26 years (19), as well as that carried out by Salou et al., (20) in Togo. In the African context, the early age of marriage of the girl could explain this significant frequency of young people and young adults among the participants in the present study. It appears from this study that most of these parturient women (77.33%) had no income and more than 94% of them were out of school or limited to a primary or secondary level.

Group B streptococcus was isolated from vaginal samples of the 300 participants, representing a vaginal colonization rate of 4.0%. These results are close (4.7%) to those of Nyampa, (21) in 2018 who also conducted his study on pregnant women in the city of Ouagadougou. It is the same as that led by Zaïdi, in Fez (Morocco), who also found in 2018 a GBS carriage rate (4.69%) comparable to ours (22). The Ouédraogo team, in their work in 2017 on pregnant women at all ages of pregnancy in Bobo Dioulasso, reported 6.05% (23). In addition, Mounerou et al., reported vaginal carriage rate that is a little lower than ours (2.5%) in 2015 in Togo (20).

Generally speaking, the vaginal carriage rate of GBS has been reported to vary from continent to continent, country to country, and sometimes within the same country. Thus, on the African continent, several recent studies conducted by different authors have given percentages higher than ours; Gbonon et al. reported carriage rate of 12.7% in the city of Abidjan (Ivory Coast) (12). In 2016, Bassir et al., (24) in Marrakech Morocco, reported a rate of 20.5%, Mahmoud (25) in Fez, Morocco in 2010 reported a rate of 23.0%, Mengist et al., (19) in Ethiopia reported a rate of 19% and Ezeonu in 2014 in Nigeria reported a rate of 18.0% (13). Carriage frequencies range from 10.0% in France (8), 18.12% in Thailand (10), 23.7% in Belgium (11) to 26.5% in the United States of America (9).

The variation in rates could be attributed to a number of factors including maternal hygiene and the choice of sampling site. Some researchers collected anal and/or rectal samples with vaginal samples such as in the studies carried out in Morocco and Cameroon (25,26) where high rates of GBS carriage of 23.3% and 14.0% respectively were reported. The laboratory techniques used to detect the pathogens are also factors in the variability of results between studies. Thus, researchers who used PCR reported very high prevalence of 43.8% (27).

Regarding the parameters studied in the present study which included age, level of education, marital status, profession, weight, number of gestures, parity, gestational age, high blood pressure, diabetes and anaemia, none was significantly associated with maternal carriage of GBS. Gbonon et al., (12) in Abi-

djan in 2006 and other researchers (28,29) likewise did not find statistically significant association between socio-demographic and obstetric factors, and the carriage of GBS by pregnant women. On the other hand, Joachim et al., (30) in Tanzania reported an increase in carriage when the age of pregnancy was advanced, finding 46.7% in women with gestational age of 41 to 42 weeks.

Concerning the sensitivity profile to antibiotics such as ampicillin, cefotaxime, amoxicillin-clavulanic acid, vancomycin and nitrofurantoin had very good activity on the GBS strains isolated in this study. Similar results were reported by Ferjani et al., (7). Numerous studies have reported a lack of resistance of GBS to penicillin G (7,31,20,32,33). In our series, 23.0% of the isolated strains were resistant to penicillin G. The research teams of Agricola (30) and those of Ouedraogo (23) also reported resistance of GBS to penicillin G of 9.4% and 10.8% respectively. High rates of GBS resistance were observed against macrolides (erythromycin 23%, lincomycin 25%) as well as quinolones (ciprofloxacin 75%), cyclins (92.0%), and trimethoprim-sulfamethoxazole (100.0%). This high level of GBS resistance could be a consequence of the inappropriate use of these antibiotics which are relatively easy to access for the population in Burkina Faso. Added to this is an inadequacy of regulations regarding the prescription, distribution and consumption of antibiotics in the context of Burkina Faso.

Conclusion:

Group B streptococcus can be transmitted from mother to child during or before delivery and is responsible for fetal or neonatal infection. Vaginal GBS carriage was high among women in the 3rd trimester of pregnancy in the Bogodogo health district, with no predictive factors. The most commonly used antibiotics are increasingly ineffective against these bacteria. Given the non-negligible prevalence of maternal GBS carriage in our conditions and the absence of real predictive factors for vaginal carriage, it seems desirable in the context of the prevention of maternal-fetal GBS infections to establish a policy of systematic screening near the end of pregnancy for all pregnant women in order to reduce neonatal mortality.

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Contributions of authors:

KBA, OA conceived the study idea, led the conduct of the study and editing of the manuscript. KBA, TI and KC were responsible for carrying out the bacteriological diagnostic activities. KAB and KAY were responsible for English translation activities. KAB, OA, TI, KC, KAY, SM, and SI were responsible for the final editing of the manuscript. All authors approved the final manuscript submitted.

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No conflict of interest is declared.

References:

1. Gibbs, R. S., Schrag, S., and Schuchat, A. Perinatal Infections Due to Group B Streptococci: Obstet Gynecol. 2004; 104 (5, Part 1): 1062-1076. doi:10.1097/01.AOG.0000144128.03913.c2
2. World Health Organization. Quality, equity, dignity: the network to improve quality of care for maternal, newborn and child health – strategic objectives, 2018
3. Judlin, P., and Thiébaugeorges, O. La surveillance microbiologique de la femme enceinte : quels examens réaliser durant la grossesse? Gynécologie Obstétrique Fertilité. 2005; 33 (11): 907-913. https://doi.org/10.1016/j.gyobfe.2005.08.019
4. Six, A., Joubrel, C., Tazi, A., and Poyart, C. Infections materno-fœtales à *Streptococcus agalactiae.* La Presse Médicale, 2014; 43 (6, Part 1): 706-714. https://doi.org/10.1016/j.lpm.2014.04.008
5. Jerbi, M., Hidar, S., Hannachi, N., et al. Facteurs de risque du portage du streptocoque du groupe B chez la femme enceinte à terme : étude prospective à propos de 294 cas, Gynécologie Obstétrique Fertilité. 2007; 35 (4): 312-316. https://doi.org/10.1016/j.gyobfe.2007.01.027
6. Tarana, M. N., and Shamsuzzaman, S. M. Detection of Group B Streptococcus in vaginal swab of pregnant women by culture and PCR and their Antibiotic susceptibility pattern in a Tertiary care hospital of Bangladesh. Mymensingh Med J. 2018; 27(3): 567-572.
7. Ferjani, A., Abdallah, H. B., Saida, N. B., Gozzi, C., and Boukadida. J. Portage vaginal de *Streptococcus agalactiae* chez la femme enceinte en Tunisie : facteurs de risque et sensibilité aux antibiotiques des isolats. Bull Soc Pathol Exot. 2006; 99 (2): 99-102.
8. Agence nationale d'accréditation et d'évaluation en santé. Prévention anténatale du risque infectieux bactérien néonatal précoce. J Gynecol Obstet Biol Reprod (Paris). 2003; 32: 68-74
9. Yancey, M. K., Schuchat, A., Brown, L. K., Ventura, V. L., and Markenson, G. R. The accuracy of late antenatal screening cultures in predicting genital group B streptococcal colonization at delivery. Obstet Gynecol. 1996; 88 (5): 811-815 doi: 10.1016/0029-7844(96)00320-1.
10. Kovavisarach, E., Ying, W. S., and Kanjanahareutai, S. Risk factors related to group B streptococcal colonization in pregnant women in labor. J Med Assoc Thail. 2007; 90 (7): 1287-1292.

11. Lorquet, S., Melin, P., Minon, J-M., et al. Le streptocoque du groupe B en clinique anténatale et en salle de travail : un problème d'attitude systématique. J Gynécologie Obstétrique Biol Reprod. 2005 ; 34 (2) : 115-127. doi:10.1016/S1637-4088(05)86229-7
12. Gbonon, V., Ouattara, N., Guessennd, N., Faye-Ketté, H., and Dosso, M. Facteurs de risque de portage du Streptocoque du groupe B chez la parturiente à terme : Enquête réalisée en 2006 dans les maternités d'Abidjan. Rev Bio Afr N. 2012; 40-46.
13. Ezeonu, I. M., and Agbo, M. C. Incidence and anti-microbial resistance profile of Group B Streptococcus (GBS) infection in pregnant women in Nsukka, Enugu State, Nigeria. Afr J Microbiol Res. 2014; 8 (1): 91-95. https://doi.org/10.5897/AJMR12.2307
14. De Steenwinkel, F. D. O., Tak, H. V., Muller, A. E., Nouwen, J. L., Oostvogel, P. M., and Mocumbi, S. M. Low carriage rate of group B streptococcus in pregnant women in Maputo, Mozambique, Trop Med Int Health. 2008; 13 (3): 427-429. doi:10.1111/j.1365-3156.2008.02018.x.
15. Musleh, J., and Al Qahtani, N. Group B Streptococcus colonization among Saudi women during labor. Saudi J Med Sci. 2018; 6 (1): 18-22. doi:10.4103/sjmms.sjmms_175_16.
16. Kuruvilla, S., Bustreo, F., Kuo, T., et al. The global strategy for women's, children's and adolescents' health (2016–2030): a roadmap based on evidence and country experience. Bull World Health Organ. 2016; 94(5): 398-400. doi: 10.2471/BLT.16.170431
17. United Nations, General Assembly: Transforming our world: The 2030 agenda for sustainable development, 2015
18. Gizachew M., Tiruneh, M., Moges, F., and Tessema, B. *Streptococcus agalactiae* maternal colonization, antibiotic resistance and serotype profiles in Africa: a meta-analysis. Ann Clin Microbiol Antimicrob. 2019; 18 (1): 14 doi:10.1186/s12941-019-0313-1
18. CA-SFM. Comité de l'Antibiogramme de la société Française de Microbiologie. Recommandations CA-SFM-EUCAST 2020, Version avril 2020 V.1.1.
19. Mengist, A., Kannan, H., and Abdissa, A. Prevalence and antimicrobial susceptibility pattern of anorectal and vaginal group B Streptococci isolates among pregnant women in Jimma, Ethiopia. BMC Res Notes. 2016; 9 (1): 351. doi:10.1186/s13104-016-2158-4
20. Salou, M., Dagnra, A. Y., Hondégla, A., et al. Group B Streptococcal Carriage Rate in Vaginal of Pregnant Women in Third Trimester in Lomé, Togo. World J Prev Med. 2015; 3 (1): 7-10. doi:10.12691/jpm-3-1-2
21. Nyampa, H. portage de *Streptococcus agalactiae* (groupe B) chez les femmes enceintes à Ouagadougou, Burkina Faso. [Mémoire : Master II bactériologie-virologie]. Bobo Dioulasso: Université catholique de l'Afrique de l'ouest (UCAO/UUB). 2019: 77
22. Zaidi, I, Prévalence des Streptocoques B bêta hémolytiques chez les femmes enceintes, Mémoire, Licence Science & Techniques Sciences Biologiques Appliquées et Santé. Université Sidi Mohamed Ben Abdellah, 2018
23. Ouédraogo, A. S., Sawadogo, Y., Somé, D. A., et al. Prevalence of Group B Streptococcus among Pregnant Women in Bobo-Dioulasso (Burkina Faso). Open J Med Microbiol. 2019; 9 (3): 63-76. doi:4236/ojmm.2019.93008
24. Bassir, A., Dhibou, H., Farah, M., et al. Portage vaginal du streptocoque du groupe B chez la femme enceinte au niveau de la région de Marrakech. Pan Afr Med J. 2016; 23: 107. doi:10.11604/pamj.2016.23.107.9047.
25. Mahmoud, M., Yahyaoui, G., Benseddik, N., Saadi, M., Chaara, H., and Melhouf, M. A. Group B *Streptococcus* screening during the third quarter of pregnancy in the CHU Hassan II of fez. Revue Tunisienne d'Infectiologie. 2011; 5 (1): 12-15.doi: https://doi.org/10.80481/revuetest30246
26. Nkembe, N. M., Kamga, H. G., Baiye, W. A., Chafa, A. B., and Njotang, P. N. *Streptococcus agalactiae* prevalence and antimicrobial susceptibility pattern in vaginal and anorectal swabs of pregnant women at a tertiary hospital in Cameroon. BMC Res Notes, 2018; 11: 480. https://doi.org/10.1186/s13104-018-3589-x
27. Bidgani, S., Navidifar, T., Najafian, M., and Amin, M. Comparison of group B streptococci colonization in vaginal and rectal specimens by culture method and polymerase chain reaction technique. J Chin Med Assoc. 2016; 79 (3): 141-145. doi:10.1016/j.jcma.2015.06.021
28. Jisuvei, S. C., Osoti, A., and Njeri, M. A. Prevalence, antimicrobial susceptibility patterns, serotypes and risk factors for group B streptococcus rectovaginal isolates among pregnant women at Kenyatta National Hospital, Kenya; a cross-sectional study. BMC Infect Dis. 2020; 20 (1): 302. doi:10.1186/s12879-020-05035-1.
29. Dashtizade, M., Zolfaghari, M. R., Yousefi, M., and Nazari-Alam, A. Antibiotic susceptibility patterns and prevalence of *Streptococcus agalactiae* rectovaginal colonization among pregnant women in Iran. Rev Bras Ginecol Obstetrícia. 2020; 42 (8): 454-459. doi: 10.1055/s-0040-1710299.
30. Agricola, J., Matee, M. I., Massawe, F. A., and Lyamuya, E. F. Maternal and neonatal colonisation of group B Streptococcus at Muhimbili National Hospital in Dar es Salaam, Tanzania: Prevalence, risk factors and antimicrobial resistance. BMC Publ Health. 2009; 9: 437. doi:10.1186/1471-2458-9-437
31. Onipede, A., Adefusi, O., Adeyemi, A., Adejuyigbe, E., Oyelese, A., and Ogunniyi, T. Group B Streptococcus carriage during late pregnancy in Ile-Ife, Nigeria. Afr J Clin Exper Microbiol. 2012; 13 (3): 135-143. doi:10.4314/ajcem.v13i3.2
32. Loulergue, J., Couhé, C., Grasmick, C., Laudat, P., and Quentin, R. Sensibilité aux antibiotiques des souches de streptocoque du groupe B de portage vaginal isolées en France, 2003. BEH. 2004; 18: 69-70.
33. Quentin, R., Loulergue, J., Mala, L., et al. Streptocoque du groupe B en pathologie humaine: origine des isolats et sensibilité aux antibiotiques. BEH. 2006; 45:2005-2007.

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Original Article **Open Access**

Bacteraemia at the tertiary care University Hospital Yalgado Ouedraogo: Bacterial species and their antibiotic resistance profiles

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Abstract:

Background: Bloodstream infections are serious health problem because of the significant morbidity and mortality they cause. The number of deaths increases in the presence of multidrug resistant bacteria. The aim of this study was to determine the bacteriological and resistance profiles of bacteria isolated from blood cultures.
Methodology: This is a retrospective descriptive study over 3 years (1st January 2019 to 31st December 2021) of all blood cultures received in the Bacteriology-Virology Laboratory at CHU-YO. Bacteria were isolated from blood cultures after routine processing in automated BD BACTEC FX40 machine. Bacteria identification and antibiotic susceptibility test (AST) was by conventional biochemical tests and API 20E, and Kirby-Bauer disc diffusion method respectively from Jan to Sept 2019, and by BD Phoenix M50 from Sept 2019 to Dec 2021. All proven cases of bacteraemia documented by culture, identification and AST were included in the study. Data analysis was done using EPI-INFO 7.2.4.0 software.
Results: A total of 335 pathogenic bacteria were isolated from non-duplicate blood cultures collected from a total of 2345 patients, with 1209 males and 1136 females, giving a male to female ratio of 1.06. Gram-negative bacilli accounted for 63.6% (n=213) with a predominance of Enterobacteriaceae (40.6%, n=136). The leading species were *Klebsiella* spp (18.5%, n=62) followed by *E. coli* (14.0%, n=47). Gram-positive cocci accounted for 36.4% (n=122), mostly *Staphylococcus aureus* (22.1%, n=74). *Klebsiella* spp and *E. coli* strains showed high levels of resistance to beta-lactams (60 to 71.0% for piperacillin-tazobactam, 87 to 89.0% for amoxicillin-clavulanic acid and 79 to 90.0% for ceftriaxone), fluoroquinolones (75 to 78.0% for ciprofloxacin) and sulphonamides (82 to 96.0% for cotrimoxazole). The lowest levels of resistance were observed with cefoxitin (5-20.0%) and imipenem (5-7.0%). Strain sensitivity to aminoglycosides was highly variable, ranging from 0-5.0% for amikacin to 54-67.0% for gentamicin. The main mechanism of resistance of *Klebsiella* spp and *E. coli* strains to beta-lactam antibiotics was ESBL production; 64.5% (40/62) and 60.0% (28/47), respectively. *Staphylococcus aureus* strains were resistant to methicillin (MRSA) in 13.0% of cases. All were sensitive to fusidic acid and vancomycin.
Conclusion: *Klebsiella pneumoniae, E. coli* and *S. aureus* are the main bacteria responsible for bacteremia in Burkina Faso. Their resistance to antibiotics is very high and remains of concern. The introduction of rapid tests to detect resistant bacteria directly from blood culture broths is essential for the early adaptation of empirical antibiotic therapy.

Keywords: Blood culture; Bacteraemia; Antibiotic resistance

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Bactériémies au Centre Hospitalier Universitaire Yalgado

Ouedraogo: Espèces bactériennes et leur profil de résistance aux antibiotiques

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Résumé:

Contexte: Les bactériémies sont des infections graves du fait des taux de morbidité et de mortalité significatives qu'elles engendrent. Les décès sont multipliés par deux ou trois si les patients sont atteints d'infections bactériennes résistantes aux antibiotiques. Le but de ce travail est de déterminer le profil bactériologique et de résistance actuelle des bactéries isolées dans les hémocultures.
Méthodologie: Il s'est agi d'une étude descriptive rétrospective sur 3 ans (1er Janvier 2019 au 31 Décembre 2021) de toutes les hémocultures reçues au laboratoire de bactériologie-virologie du CHU-YO. Les bactéries ont été isolées à partir des hémocultures après traitement de routine dans l'automate BD BACTEC FX40. L'identification des bactéries et l'antibiogramme ont été réalisés respectivement par des tests biochimiques classiques et API 20E, et par la méthode de diffusion du disque de Kirby-Bauer de janvier à septembre 2019, et par BD Phoenix M50 de Septembre 2019 à Décembre 2021. Tous les cas avérés de bactériémie documentés par culture, identification et antibiogramme ont été inclus dans l'étude. L'analyse des données a été réalisée à l'aide du logiciel EPI-INFO 7.2.4.0
Résultats: Au total, 335 bactéries pathogènes ont été isolées à partir d'hémocultures non redondantes sur un total de 2345 patients, dont 1209 hommes et 1136 femmes, soit un ratio homme/femme de 1,06. Les bacilles à Gram négatif représentaient 63,6% (n=213) avec une prédominance d'entérobactéries (40,6%, n=36). Les principales espèces étaient *Klebsiella* spp (18,5%, n=62) suivies par *E. coli* (14.0%, n=47). Les cocci à Gram positif représentaient 36,4% (n=122), principalement *S. aureus* (22,1%, n=74). Les souches de *Klebsiella* spp et *d'E. coli* ont montré des niveaux élevés de résistance aux bêtalactamines (60 à 71.0% pour la pipéracilline-tazobactam, 87 à 89.0% pour l'amoxicilline-acide clavulanique et 79 à 90.0% pour la ceftriaxone), aux fluoroquinolones (75 à 78.0% pour la ciprofloxacine) et aux sulfamides (82 à 96.0% pour le cotrimoxazole). Les niveaux de résistance les plus faibles ont été observés avec la céfoxitine (5 à 20.0%) et l'imipénème (5 à 7.0%). La sensibilité des souches aux aminosides était très variable, allant de 0-5% pour l'amikacine à 54-67.0% pour la gentamicine. Le principal mécanisme de résistance des souches de *Klebsiella* spp et *d'E. coli* aux bêtalactamines était la production de BLSE; 64,5% (40/62) et 60.0% (28/47), respectivement. Les souches de *S. aureus* étaient résistantes à la méthicilline (SARM) dans 13.0% des cas. Toutes étaient sensibles à l'acide fusidique et à la vancomycine.
Conclusion: *Klebsiella pneumoniae*, *E. coli* et *S. aureus* sont les principales bactéries responsables des bactériémies dans Burkina Faso. Leur résistance aux antibiotiques est très élevée et reste préoccupante. L'introduction de tests rapides de détection des bactéries résistantes directement à partir des bouillons d'hémoculture est essentielle pour l'adaptation précoce de l'antibiothérapie probabiliste.

Mots clés: Hémoculture; Bactériémie; Résistance aux antibiotiques

Introduction:

Bacteremia is defined as the presence of bacteria in the blood of a patient with systemic signs of infection. It may be secondary to a localized and documented infection or primary i.e. without an identified origin (1). In developed countries, 40% of community-acquired and nosocomial bacteremia progress to sepsis or septic shock, compared to 20% in intensive care units (2). Whatever the cause, bacteremia is most often associated with a high mortality rate of up to 36% (3). Factors associated with an increased risk of mortality include the absence of appropriate early antibiotic therapy and poor control of the infection site (3-5).

Increasing bacterial resistance to antibiotics is a major problem in the management of invasive infections. In practice, convention-

al bacteriological diagnostic methods take 48-72 hours to obtain an antibiogram, and 20-30% of patients receive inadequate initial antibiotic therapy, with a 7.6% increase in mortality for each hour of delayed antibiotic therapy (6). For clinicians, knowledge of the bacterial species most frequently encountered in infectious diseases and their susceptibility to the main antibiotics is essential for early and appropriate antibiotic therapy (7). This is more than necessary in the case of invasive infections such as sepsis which cause 30% of hospital deaths in developed countries (8) and where empirical antibiotic therapy determines the vital prognosis.

The management of sepsis is based on urgent and appropriate empirical antibiotic therapy. The choice of molecules used requires knowledge of the local bacterial ecology and their sensitivity to antibiotics, which needs to be regularly updated. The aim of this study is to describe the bacteriological and resistance profiles of the main bacteria involved in bacteraemia at the University Hospital Yalgado Ouedraogo (CHUYO) from 2019 to 2021.

Materials and method:

Study setting, design and period:

This is a retrospective descriptive study over a 3-year period (1st January 2019 to 31st December 2021) of blood cultures received at the CHUYO. All proven cases of bacteraemia documented by culture, identification and antibiotic susceptibility testing carried out and validated in the bacteriology-virology laboratory of the CHUYO were included in this study. Duplicates that had been clearly identified (the same species identified in the same patient with same sensitivity profile) were excluded.

Ethics statement:

The study was approved by the National Ethics Committee for Health Research (Ref erence no: 2023-04-096). The personal data collected on the patients were not disclosed.

Isolation and identification of bacteria:

Blood samples were routinely inoculated in BD BACTEC bottles (aerobic, anaerobic and paediatric) and incubated in the BD BACTEC FX40 (Becton Dickinson, New Jersey) following a 5-day protocol. All positive broths were cultured on Chocolate + PolyViteX agar. A selective medium was added according to the results of Gram staining (e. g. presence of Gram-negative bacilli, EMB medium was used, while in the presence of Gram-positive cocci in clusters, CHAPMAN medium was used).

From January to August 2019, identification was carried out using conventional microbiology methods, including morphological and biochemical characteristics (API20E, catalase, oxidase), and the antibiotic susceptibility testing (AST) was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton (MH) agar. From September 2019 to December 2021, identification and antibiotic susceptibility testing were performed on BD Phoenix M50 (Becton Dickinson, New Jersey) using panels for Gram-negative (NMIC) and Gram-positive (PMIC) bacteria.

The following antibiotics were routinely used for the sensitivity testing; penicillin G (PG), ampicillin (AMP), amoxicillin-clavulanic acid (AMC), ticarcillin-clavulanic acid (TCC), piperacillin-tazobactam (PTZ), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), imipenem (IMI), meropenem (MEM), ertapenem (ERT), gentamicin (GEN), tobramycin (TOB), amikacin (AN), moxifloxacin (MOX), levofloxacin (LEV), ciprofloxacin (CIP), trimethoprim-sulfamethoxazole (SXT), erythromycin (E), clindamycin (DA), fusidic acid (FA), and vancomycin (VAN). The results of AST were interpreted in accordance with the recommendations of the 2021 European Committee on Antimicrobial Susceptibility Testing (EUCAST 2021).

ESBL production was detected by the double disc synergy test involving a central amoxicillin-clavulanic acid disc placed 30 mm away from a 3rd or 4th generation cephalosporin disc (ceftazidime, ceftriaxone, cefepime) on MH agar, with characteristic 'champagne cork' appearance. For AST performed in liquid media, ESBL production was detected by the Expert System of the BD Phoenix M50 automated system (Becton Dickinson, New Jersey). Cefoxitin was used to detect methicillin resistance in *S. aureus* isolates.

Statistical analysis:

The data from the study were entered into Excel 2013 and analyzed using EPI-INFO 7.2.4 software. The Chi-square test was used to make comparisons between the different proportions, with a value of $p<0.05$ as the threshold of statistical significance.

Results:

Patient characteristics:

A total of 2345 non-redundant blood cultures from 2345 patients were included during the study period. There were a total of 1209 males, giving a sex ratio of 1.06. Nearly 64.0% (n=1498) of patients were aged ≤ 17 years. Children under one year of age were the most represented (34.6%, n=812). The majority of blood samples came from the departments of paediatrics (61.2%, n=1436), nephrology-haemodialysis (8.1%, n=189), gynaecology-obstetrics (5.1%, n=120), internal medicine (4.8%, n=112), medical emergencies (4.2%, n=99), intensive care (3.9%, n=91) and infectious diseases (2.4% n=55) (Table 1).

Microbiological profiles of the microorganisms isolated in blood cultures:

A total of 715 non-redundant bacterial and fungal species were isolated, giving a microbiological yield of 30.5% (715/2345). These isolates belonged to almost fifty different species, of which clinically significant bacteria accounted for 46.8% (n=335). Gram-negative bacilli (GNB) were the most frequent (63.6%, n=213), with predominance of Enterobacteriaceae (40.6%, n=136). More than 80.0% of the Enterobacteriaceae were *Klebsiella* spp (45.6%, n=62), followed by *Escherichia coli* (34.5%, n=47). Gram-positive cocci (GPC) accounted for 36.4% (n=122) of all clinically significant isolates, with predominance of *Staphylococcus aureus* (22.1%, n=74), followed by *Enterococcus* species (8.6%, n=29) and *Streptococcus* spp (5.7%, n=19) (Table 2). *Staphylococcus aureus* accounted for 60.7% (74/122) of clinically significant Gram-positive cocci.

The rate of potential contamination of blood cultures was about 16.0% (373/2345), which represented 52.1% of all isolates (373/715) and were essentially coagulase-negative staphylococci (38.7%, n=277) and Gram-positive bacilli of the genus *Bacillus* (10.7%, n=77) (Table 2). The trends in clinical categories show that the rate of isolation of potential contaminants decreased significantly with patients' age ($p<0.001$), from 20.0% in children aged ≤ 1 year, to 12.0% in patients aged 60 years and above (Fig 1).

Table 1: demographic characteristics of patients with clinical suspicion of bacteraemia at CHUYO, Ouagadougou, Burkina Faso

Characteristics	Number	Percentage
Age group (years)		
≤ 1	812	34.6
1-17	686	29.3
18-60	630	26.8
≥ 60	217	9.3
Gender		
Female	1136	48.4
Male	1209	51.6
Departments		
Paediatric	1436	61.2
Nephrology-haemodialysis	189	8.1
Gynecology-obstetrics	120	5.1
Internal medicine	112	4.8
Medical emergencies	99	4.2
Intensive care units	91	3.9
Other departments	69	2.9
Infectious diseases	55	2.3
Cardiology	37	1.6
Dermatology	37	1.6
Neurosurgery	32	1.4
Neurology	30	1.3
Surgery (orthopedics and visceral)	24	1.0
Hepato-Gastro-Enterology	14	0.6

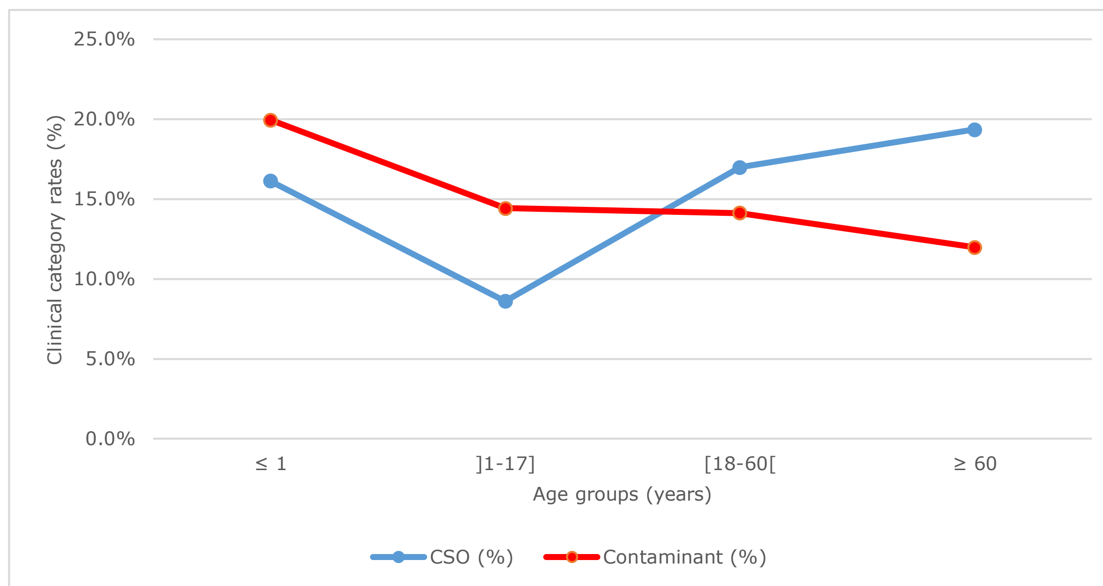
Antibiotic resistance of the main bacteria isolates:

The antibiotic resistance of the main bacteria isolated from blood cultures is shown in Table 3. *Klebsiella* spp and *E. coli* showed high levels of resistance to beta-lactam antibiotics; penicillins + beta-lactamase inhibitors (60 to 71.0% for piperacillin-tazobactam versus 87 to 89.0% for amoxicillin-clavulanic acid), 3rd generation cephalosporins (79 to 90.0% for ceftriaxone), fluoroquinolones (75 to 78.0% for ciprofloxacin) and sulphonamides (82 to 96.0% for trimethoprim-sulfamethoxazole). The lowest levels of resistance were observed with cefoxitin (5 to 20.0%) and imipenem (5 to 7.0%).

The sensitivity of isolates to aminoglycosides varied widely, ranging from 0 to 5.0% for amikacin, to 54 to 67.0% for gentamicin (Table 3). The main mechanism of resistance of *Klebsiella* spp and *E. coli* isolates to beta-lactam antibiotics was ESBL production (64.5%, 40/62) and (60%, 28/47) respectively.

Table 2: Distribution of micro-organisms isolated from blood cultures at CHUYO, Ouagadougou, Burkina Faso

Bacterial family and species		Number of isolates	Percentage	Clinical categorization
Enterobacterales (n=136)	*Klebsiella* spp	62	8.67	Clinically significant organisms (n=335)
	Escherichia coli	47	6.57	
	Enterobacter cloacae complex	15	2.10	
	Morganella morganii	3	0.42	
	Raoultella ornithinolytica	2	0.28	
	Citrobacter spp	2	0.28	
	Yersinia pseudotuberculosis	1	0.14	
	Serratia odorifera	1	0.14	
	Proteus mirabilis	1	0.14	
	Providencia stuartii	1	0.14	
	Salmonella spp	1	0.14	
Other Gram-negative bacilli (n=77)	*Acinetobacter baumannii*	38	5.31	
	Pseudomonas spp	21	2.94	
	Burkholderia cepacia	9	1.26	
	Pantoea agglomerans	4	0.56	
	Comamonas testosteroni	2	0.28	
	Aeromonas hydrophila	1	0.14	
	Chromobacterium violaceum	1	0.14	
	Tatumella ptyseos	1	0.14	
Gram-positive cocci (n=410)	*Staphylococcus aureus*	74	10.35	
	Enterococcus spp	29	4.06	
	Streptococcus spp	19	2.66	
	Coagulase negative staphylococci	277	38.74	Potential contaminants (n=373)
	Aerococcus spp	5	0.70	
	Micrococcus spp	5	0.70	
	Rhodococcus equi	1	0.14	
Gram-positive bacilli (n=85)	*Bacillus* spp	77	10.77	
	Corynebacterium spp	8	1.12	
Fungi (n=7)	*Candida* spp	7	0.98	Fungemia (n=7)
Total		**715**	**100.00**	



CSO= clinically significant organisms

Fig 1: Distribution of clinical categories of bacteria isolated according to patients' age

Table 3: Antibiotic resistance of the four main bacterial pathogens isolated from blood cultures of patients with bloodstream infection at CHUYO, Ouagadougou, Burkina Faso

Number of isolates tested to the antibiotics	**Percentage of antibiotic-resistant isolates**																
	AMP	**AMC**	**TCC**	**PTZ**	**CRO**	**CAZ**	**IMI**	**SXT**	**AN**	**GEN**	**CIP**	**FOX**	**PG**	**E**	**DA**	**FA**	**VAN**
Klebsiella spp (n=62)	-	87	87	71	90	89	7	82	0	67	75	10	-	-	-	-	-
Escherichia coli (n= 47)	96	89	89	60	79	79	5	96	5	54	78	20	-	-	-	-	-
Acinetobacter baumannii (n=38)	-	-	45	32	-	42	34	76	16	52	61	-	-	-	-	-	-
Staphylococcus aureus (n=74)	-	-	-	-	-	-	-	29	-	12	23	13	88	28	12	0	0

AMP: ampicillin; AMC: amoxicillin-clavulanic acid; TCC: ticarcillin -clavulanic acid; PTZ: piperacillin-tazobactam; CRO: ceftriaxone; CAZ: ceftazidime; IMI: imipenem; SXT: cotrimoxazole; AN: amikacin; GEN: gentamicin; CIP: ciprofloxacin; FOX: cefoxitin; PG: penicillin G; E: erythromycin; DA: clindamycin; FA: fusidic acid; VAN: vancomycin

As for Gram-negative non-Enterobacteriaceae isolates, *Acinetobacter baumannii* was particularly resistant to beta-lactam antibiotics, with 34.0% resistant to imipenem. Amikacin was the most active antibiotic, with only 16.0% of bacterial isolates resistant to it. *Staphylococcus aureus* isolates were highly resistant to penicillin G (88.0%) and cotrimoxazole (29.0%). Methicillin-resistant strains (MRSA) accounted for 13.0% of all *S. aureus* isolates. Macrolides and related drugs were relatively active, with resistance rates ranging from 12% for clindamycin to 28.0% for erythromycin. No resistance to vancomycin or fusidic acid was reported (Table 3).

Discussion:

In this study, clinically significant bacteria responsible for bacteremia were Gram-negative bacilli (63.6%) dominated by Enterobacteriaceae (40.6%), mainly *Klebsiella* spp and *E. coli*, while *S. aureus* accounted for 22.1%. The rate of potential contamination of blood cultures was very high at 16.0%. High proportion of Gram-negative bacilli in bacteraemia, particularly Enterobacteriaceae, has been reported in several studies, with rates of between 40 to 70.0% (9-12). Over 80.0% of Enterobacteriaceae isolated were *Klebsiella* species (45.6%) and *E. coli* (34.5%). This trend in the bacteriological profile of bacteraemias caused by Enterobacteriaceae had already been described by Ebongue et al., (10) in Cameroon, who reported that *Klebsiella* were in first place at 27.8%, followed by *E. coli* at 12.0% of all isolates from blood cultures. Boughattas et al., (9) reported the same observation regarding the high involvement of Enterobacteriaceae in both nosocomial and community-acquired bacteraemias, with frequencies of 33.3% and 52.5% respectively.

Among the clinically significant Gram-positive cocci, *S. aureus* was the most frequently seen in our study (60.7%). Data from the French national observatory on the epidemiology of bacterial resistance to antibiotics also show that it is the main species isolated (45.4%) among Gram-positive cocci involved in nosocomial bacteraemia (13). *Staphylococcus aureus* is generally considered to be the second pathogen involved in bacteremia after Enterobacteriaceae (14). In Morocco, Badaoui et al., (12) reported the same finding, with *S. aureus* bacteremia occurring in 26.5% of cases. In Tunisia, similar trends were also reported by Boughattas et al., (9) with 30.6% and 22.5% respectively in nosocomial and community-acquired bacteraemias. *Staphylococcus aureus* bacteraemias are common in healthcare-associated infections and is most often linked to a variety of clinical situations, the most frequent being catheter-related in poly-pathological patients (14). They constitute diagnostic and therapeutic emergencies due to their high mortality rates. Mortality is estimated at 20-25.0% at one month, and is very often associated with the initial presence of sepsis, the location of the primary infection (neurological, pulmonary, and cardiac) (15).

In this study, over 52.0% of isolates were potential contaminants, consisting mainly of coagulase-negative staphylococci (38.7%) and Gram-positive bacilli of the genus *Bacillus* (10.8%). This high frequency highlights the quality challenges in the microbiological diagnosis of bacteraemia in our context, and is attributable to two main factors; (i) the working environment where contamination of blood cultures in countries with limited resources often originates in the environment, as *Bacillus* spp are known to be present in the air (as aerobic spore bearers) and have been described in epidemics of pseudo-bacteremia originating in the environment (16), and (ii) failure to follow good blood culture practice, making it difficult to distinguish between contaminants and pathogens, because certain contaminants typical of blood cultures, such as coagulase-negative staphylococci, which accounted for over 38.0% of isolates in this study, can cause catheter-related infections.

Clinical categorization of organisms is generally established by the number of blood cultures positive for the particular organisms (16). Often, a coagulase-negative staphylococcus is only considered clinically significant if it is isolated from at least two separate blood cultures, as the probability of contaminating both cultures with the same pathogen is very low (17-18). However, this approach is often very difficult to implement in low- and middle-income countries, where in majority of cases, only a single pair of blood cultures is taken because of the low purchasing power of the population and the lack of health insurance to facilitate access to microbiological diagnosis. The time taken for detection can also help with interpretation, as it has been shown that contaminants develop more slowly than true pathogens. In this concept, it is established that a bacteraemic patient will have a much higher inoculum of bacteria than a contaminated culture. Theoretically, it follows that a larger inoculum will develop more rapidly than a smaller one (19).

The rate of isolation of potential contaminants in our study was around 16.0%, with a significant variation according to patients' age, ranging from 20.0% in children aged ≤ 1 year of age to 12.0% in patients aged 60 years and above. The high level of contamination of paediatric blood cultures was reported by Brunet et al., (20) who described contamination rates of over 10.0%, with coagulase-negative staphylococci topping the list. It is gener-

ally accepted that contamination of blood cultures by skin flora, particularly coagulase-negative staphylococci, is essentially due to poor antisepsis of the skin during venipuncture (16). This could account for the high rate of potential contamination in paediatric patients, who are less cooperative during venipuncture and are subject to a variety of manipulations that can lead to contamination of blood cultures.

In terms of antibiotic resistance, the most frequently isolated Enterobacteriaceae (*Klebsiella* spp and *E. coli*) exhibited very high levels of resistance to amoxicillin-clavulanic acid and to the 3rd generation cephalosporins (3GCs). The main mechanism of resistance was the production of extended-spectrum beta lactamase (ESBL) in 60.0% and 64.5% of cases respectively in *E. coli* and *Klebsiella* spp. Similar results were reported in a Madagascan hospital in 2020, with ESBL-producing strains occurring in 62.2% of cases (21). In Casablanca Morocco and Tunisia respectively, prevalence rates of 62.0% and 60.3% ESBL-producing Enterobacteriaceae were reported in bacteraemias (22-23). The strong involvement of ESBL-producing Enterobacteriaceae in bacteraemia no longer needs to be demonstrated, leading to the empirical prescription of carbapenems, which remain the antibiotics of 'last resort'. However, the widespread use of carbapenems has led to the emergence of carbapenemase-producing strains. In this study, the resistance of strains to imipenem was 5.0% and 7.0% for *E. coli* and *Klebsiella* spp respectively. Dridi et al., (23) reported that 6.4% of *Klebsiella* strains were resistant to carbapenems. Boughattas et al., (9) and Cattoen et al., (24) described near-total sensitivities of *E. coli* to imipenem. In developed countries, the current trend is to use therapeutic alternatives that avoid the use of carbapenems, such as piperacillin-tazobactam (25) or new antibiotics such as ceftazidime-avibactam and ceftolozane-tazobactam (26-27) which are not yet available in the West African countries.

Methicillin-resistant *S. aureus* (MRSA) accounted for 13.0% of all isolates, and 87.0% were methicillin-susceptible (MSSA). Antibiotic susceptibility data for *S. aureus* isolates from infections in France show the same trend with 86.0% of MSSA in hospital settings (14). Whereas in the case of MSSA bacteraemia, there is ample room for maneuver in terms of choice of antibiotics, the presence of MRSA bacteraemia considerably reduces the therapeutic options, with the exception of vancomycin or daptomycin-based monotherapy and the use of the new 5th generation cephalosporins such as ceftaroline and ceftobiprole (28-29). But like ceftazidime-avibactam and ceftolozane-tazobactam, 5th generation cephalosporins are not yet available in our country.

Conclusion:

Escherichia coli, K. pneumoniae and *S. aureus* are the main bacteria responsible for bacteraemia in our hospital. The antibiotic resistance of these bacteria is very high. The introduction of rapid tests to detect resistant bacteria directly from blood culture broths is proving essential for the early adaptation of empirical antibiotic therapy. However, the high level of potential contaminants in blood cultures is not conducive to the optimal use of antibiotics. There is an urgent need to improve local blood culture practice and to assess the impact of contaminants on therapeutic decisions.

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Contributions of authors:

HK, PL, SGO and IS were involved in study conceptualization; SGO, HK and IS were involved in the methodology; HK and IS were involved in study validation; HK and SGO were involved in data collection, cleaning and analysis; HK, SG, SGO and ID were involved in original manuscript draft preparation; HK, SG, ID, PL, IS and ASO reviewed the article. All authors read and agreed to the submitted version of the manuscript.

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The authors declare that they have no conflicts of interest in connection with this study.

References:

1. National HealthCare Safety Network. Bloodstream Infection Event (Central Line-Associated Bloodstream Infection and Non-central Line Associated Blood stream Infection). https://www.cdc.gov/nhsn/pdfs/pscmanual/4psc_clabscurrent.pdf; 2024. (Accessed January 22, 2024).
2. Timsit, J. F., Ruppé, E., Barbier, F., Tabah, A., and Bassetti, M. Bloodstream infections in critically ill patients: an expert statement. Intensive Care Med. 2020; 46 (2): 266-284. https://doi.org/10.1007/s00134-020-05950-6
3. Tabah, A., Koulenti, D., Laupland, K., et al. Characteristics and determinants of outcome of hospital-acquired bloodstream infections in intensive care units: the EUROBACT International Cohort Study.

Intensive Care Med. 2012; 38 (12): 1930-1945. https://doi.org/10.1007/s00134-012-2695-9
4. Adrie, C., Garrouste-Orgeas, M., Ibn Essaied, W., et al. Attributable mortality of ICU-acquired bloodstream infections: Impact of the source, causative micro-organism, resistance profile and antimicrobial therapy. J Infect. 2017; 74 (2):131-141. https://doi.org/10.1016/j.jinf.2016.11.001
5. Zahar, J. R., Timsit, J. F., Garrouste-Orgeas, M., et al. Outcomes in severe sepsis and patients with septic shock: Pathogen species and infection sites are not associated with mortality. Crit Care Med. 2011; 39 (8):1886.doi:10.1097/CCM.0b013e31821b827c
6. Kumar, A., Roberts, D., Wood, K. E., et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med. 2006; 34 (6):1589. doi:10.1097/01.CCM.0000217961.75225.E9
7. Da, G., Ra, W., Rp, W., et al. Strategies to Prevent and Control the Emergence and Spread of Antimicrobial-Resistant Microorganisms in Hospitals. A challenge to hospital leadership. JAMA. 1996; 275 (3). https://pubmed.ncbi.nlm.nih.gov/8604178/
8. Abbara, S., Guillemot, D., El Oualydy, S., et al. Caractéristiques clinico-microbiologiques et mortalité des épisodes avec bactériémie nosocomiale dans 14 hôpitaux universitaires en France, 2016-2019. MMI Formation. 2022; 1 (2): S31-32. doi: 10.1016/j.mmifmc.2022.03.069
9. Boughattas, S., El Haddad, N., Azouzi, F., et al. Bacteremia in paediatrics. Infect Dis Now. 2021; 51 (5): S140. https://doi.org/10.1016/j.idnow.2021.06.222
10. Ebongue, C. O., Mefo'o, J. N., Dongho, E. N., Moukoko, E. E., Adiogo, D., and Beyihabd, G. Bacteriological profile and antibiotic susceptibility of blood culture isolates (2006-2011) in Douala, Cameroon. Rev Mali Infect Microbiol. 2014; 27-39. https://doi.org/10.53597/remim.vi2.365
11. Savadogo, M., Diallo, I., Diendéré, A., Sondo, K., and Sawadogo, A. Sepsis observed in the infectious diseases department of the CHU Yalgado Ouédraogo in Ouagadougou: epidemiological, clinical and evolutionary aspects. Rev Mali Infect Microbiol. 2021; 16 (2):32-35. http://dx.doi.org/10.53597/remim.v16i2.1867
12. Badaoui, L., Lahsen, A. O., Daoudi, N., et al. Current profile of bacteremia in the Department of Infectious Diseases: About 216 cases. Rev Mali Infect Microbiol. 2016:7-11. http://dx.doi.org/10.53597/remim.v6i0.825
13. Bertrand, X., Costa, Y., and Pina, P. Surveillance of antimicrobial resistance of bacteria isolated from bloodstream infections: data of the French National Observatory for Epidemiology of Bacterial Resistance to Antibiotics (ONERBA), 1998-2003. Med Mal Infect. 2005; 35 (6):329-334. doi: 10.1016/j.medmal.2005.05.002.
14. Moing, V. L. *Staphylococcus aureus* bacteremia and infective endocarditis. MMI Formation. 2022; 1 (4): 172-179.doi:10.1016/j.mmifmc.2022.09.006
15. Braquet, P., Alla, F., Cornu, C., et al. Factors associaed with 12-week case-fatality in *Staphylococcus aureus* bacteraemia: a prospective cohort study. Clin Microbiol Infect. 2016; 22 (11):948.e1-948.e7. doi:10.1016/j.cmi.2016.07.034
16. Ombelet, S., Barbé, B., Affolabi, D., et al. Best Practices of Blood Cultures in Low-and-Middle-Income Countries. Front Med. 2019; 6: 131. doi: 10.3389/fmed.2019.00131
17. Weinstein, M. P. Blood Culture Contamination: Persisting Problems and Partial Progress. J Clin Microbiol. 2003; 41 (6): 2275-2278. doi: 10.1128/jcm.41.6.2275-2278.2003
18. Isabelle, A., Murielle, C., and Brigitte, L. Bacteremia and fungemia - Blood culture. In: REMIC. French Microbiology Society. 5th Edition, 2015: 125-128.
19. Hall, K. K., and Lyman, J. A. Updated Review of Blood Culture Contamination. Clin Microbiol Rev. 2006; 19 (4): 788-802. doi: 10.1128/cmr.00062-05
20. Brunet, C. Impact of contaminated blood cultures in paediatric emergencies, retrospective study at Bordeaux University Hospital. Thesis Doctorate in Medicine. 2019.
21. Rakotovao-Ravahatra, Z. D., Randriatsarafara, F. M., Rakotovao, A. L., and Rasamindrakotroka, A. Prevalence and factors associated with extended-spectrum β-lactamase producing Enterobacteriaceae bacteraemia in University Hospital of Befelatanana, Madagascar. Afr J Clin Exper Microbiol. 2021; 22 (1): 52-59.doi:10.4314/ajcem.v22i1.7
22. Jarmoumi, A., Bencharef, H., Hasnane, S., Lamchaheb, M., Zerouali, K., and Quessar, A. Bacteraemia in neutropenic patients during acute myeloblastic leukaemia at the Casablanca haematology center. Poster, Biology Innovation Day. 2019
23. Dridi, K., Gdoura, S., and Houimli, S. Bacteriological profile and sensitivity of blood culture isolates at Hôpital F.S.I la Marsa. Poster 0555, 30th National Congress of the Tunisian Society of Infectious Pathology. 2021
24. Cattoen, C., Descamps, D., Vachée, A., and Wallet, F: ESBL-producing *Escherichia coli* bacteremia in Nord - Pas-de-Calais: patient characteristics, antibiotic susceptibility, and therapeutic management. Med Mal Infect. 2014; 44(6):41. doi:10.1016/S0399-077X (14)70160-X
25. Henderson, A., Paterson, D. L., Chatfield, M. D., et al. Association Between Minimum Inhibitory Concentration, Beta-lactamase Genes and Mortality for Patients Treated With Piperacillin/Tazobactam or Meropenem From the MERINO Study. Clin Infect Dis. 2021; 73 (11):e3842-3850.doi:10.1093/cid/ciaa1479
26. Isler, B., Ezure, Y., Romero, J. L. G. F., Harris, P., Stewart, A. G., and Paterson, D. L. Is Ceftazidime/ Avibactam an Option for Serious Infections Due to Extended-Spectrum-β-Lactamase-and Amp C-Producing Enterobacterales? a Systematic Review and Meta-analysis. Antimicrob Agents Chemother. 2020; 65 (1): doi:10.1128/aac.01052-20.
27. Hirsch, E. B., Brigman, H. V., Zucchi, P. C., et al. Ceftolozane-tazobactam and ceftazidime-avibactam activity against β-lactam-resistant *Pseudomonas aeruginosa* and extended-spectrum β-lactamase-producing Enterobacterales clinical isolates from US medical centres. J Glob Antimicrob Resist. 2020; 22: 689-694. doi: 10.1016/j.jgar.2020.04.017
28. Tong, S. Y. C., Lye, D. C., Yahav, D., et al. Effect of Vancomycin or Daptomycin With vs Without an Antistaphylococcal β-Lactam on Mortality, Bacteremia, Relapse, or Treatment Failure in Patients with MRSA Bacteremia: A Randomized Clinical Trial. JAMA. 2020; 323(6):527-537.doi:10.1001/jama.2020.0103
29. Jacqueline, C., and Tattevin, P. Ceftaroline, a new cephalosporin active against methicillin-resistant *Staphylococcus aureus*: experimental and clinical data, therapeutic prospects. J Anti-Infect. 2012;14(1):3-10 doi: 10.1016/j.antinf.2012.01.001

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Original Article **Open Access**

Antibiotic resistance in uropathogenic *Escherichia coli* strains at Brazzaville University Hospital, Congo and the therapeutic consequences

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Abstract:

Background: Urinary tract infections (UTIs) are a very frequent reason for consultations and antibiotic prescriptions in everyday practice. Excessive and inappropriate use of antibiotics is responsible for the emergence and spread of multidrug-resistant (MDR) uropathogenic bacteria. The aim of this study was to determine the frequency of isolation and antibiotic resistance of uropathogenic strains of *Escherichia coli* (UPEC) isolated in the bacteriology-virology laboratory of the University Hospital Centre (CHU) in Brazzaville, Congo.
Methodology: This was a descriptive retrospective study over a 6-month period (from 1 April to 31 September 2022) that included all non-redundant uropathogenic UPEC strains isolated from urine samples of patients with UTIs referred to the bacteriology-virology laboratory of the University Hospital of Brazzaville, Congo. The strains were isolated from urine samples after inoculation onto Cystine Lactose Electrolyte Deficient agar (CLED), and incubating aerobically at 37°C for 24 hours. Identification was carried out using BioMérieux API 20 E galleries and antibiotic susceptibility testing was performed on Mueller Hinton agar medium using selected antibiotic discs. Extended spectrum β-lactamase (ESBL) production by the isolates was confirmed by double disc synergy test. Data were analysed using Microsoft Office Excel 2013.
Results: Of the 187 non-repetitive uropathogenic *Enterobacteriaceae* isolated from urine samples of 187 patients with clinical UTIs, 81 were strains of UPEC, giving an overall frequency of UPEC isolation of 43.0%. The modal age of patients from whom UPEC strains were isolated was 57 years (age range 2 to 86 years), with 49 from females and 32 from males (F: M ratio of 1.5). The UPEC strains showed high rates of resistance to amoxicillin (94.0%), amoxicillin-clavulanic acid (84.0%), piperacillin-tazobactam (73.0%), ceftriaxone (52.0%), cefixime (54.0%), cefotaxime (55.0%), ceftazidime (58.0%), gentamicin (42.0%), ciprofloxacin (55.0%) and sulfa-methoxazole-trimethoprim (90.0%) but relatively low resistance rates were observed with imipenem (4.0%), fosfomycin (8.0%) and amikacin (18.0%). The ESBL-producing strains accounted for 24.5% (46/187) of all uropathogenic *Enterobacteriaceae* isolates, and compared to the non-ESBL producing strains, had significantly higher resistance rates to gentamicin (p=0.018), ciprofloxacin (p=0.0003), ceftazidime (p<0.0001), ceftriaxone (p<0.0001), cefixime (p<0.0001), cefotaxime (p<0.0001), piperacillin-tazobactam (p=0.0006), and amoxicillin-clavulanate (p=0.0024).
Conclusion: Our results show high rates of *in vitro* resistance of UPEC strains to commonly used antibiotics, which potentially limits therapeutic options and therefore a real public health challenge in Congo.

Key words: Antibiotic, Uropathogenic strains, *Escherichia coli*, Antibiotic Resistance.

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Résistance aux antibiotiques des souches uropathogènes d'*Escherichia coli* au CHU de Brazzaville, Congo et conséquences thérapeutiques

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Résumé:

Contexte: Les infections des voies urinaires (IVU) constituent un motif de consultation et de prescription très fréquente d'antibiotiques dans la pratique quotidienne. L'utilisation excessive et inappropriée d'antibiotiques est responsable de l'émergence et de la propagation de bactéries uropathogènes multirésistantes (MDR). Le but de cette étude était de déterminer la fréquence d'isolement et la résistance aux antibiotiques des souches uropathogènes d'*Escherichia coli* (UPEC) isolées au laboratoire de bactériologie-virologie du Centre Hospitalier Universitaire (CHU) de Brazzaville au Congo.

Méthodologie: Il s'agissait d'une étude rétrospective descriptive sur une période de 6 mois (du 1er avril au 31 septembre 2022) ayant inclus toutes les souches d'UPEC non redondantes isolées à partir d'échantillons d'urine de patients atteints d'infections urinaires adressés au laboratoire de bactériologie-virologie du CHU de Brazzaville, au Congo. Les souches ont été isolées à partir d'échantillons d'urine après inoculation sur une gélose cystine lactose déficiente en électrolytes (CLED) et incubés en aérobiose à 37°C pendant 24 heures. L'identification a été réalisée à l'aide des galeries API 20 E de BioMérieux et des tests de sensibilité aux antibiotiques ont été réalisés sur milieu gélosé Mueller Hinton à l'aide de disques d'antibiotiques sélectionnés. La production de β-lactamases à spectre étendu (BLSE) par les isolats a été confirmée par un test de synergie à double disque. Les données ont été analysées à l'aide de Microsoft Office Excel 2013.

Résultats: Parmi les 187 Entérobactéries uropathogènes non redondantes isolées à partir d'échantillons d'urine de 187 patients présentant des signes cliniques d'infections urinaires, 81 étaient des souches d'UPEC, ce qui donne une fréquence globale d'isolement d'UPEC de 43,0%. L'âge modal des patients chez lesquels les souches d'UPEC ont été isolées était de 57 ans (tranche d'âge de 2 à 86 ans), dont 49 chez les femmes et 32 chez les hommes (rapport F: M de 1,5). Les souches d'UPEC ont montré des taux élevés de résistance à l'amoxicilline (94,0%), à l'amoxicilline-acide clavulanique (84,0%), à la pipéracilline-tazobactam (73,0%), à la ceftriaxone (52,0%), au céfixime (54,0%), au céfotaxime (55,0%), à la ceftazidime (58,0%), à la gentamicine (42,0%), à la ciprofloxacine (55,0%) et au sulfaméthoxazole-triméthroprime (90,0%). Des taux de résistance relativement faibles ont été observés avec l'imipénème (4,0%), la fosfomycine (8,0%) et l'amikacine (18,0%). Les souches productrices de BLSE représentaient 24,5% (46/187) de tous les isolats d'Entérobactéries uropathogènes. Comparées aux souches d'UPEC non productrices de BLSE, les souches d'UPEC productrices de BLSE présentaient des taux de résistance significativement plus élevés à la gentamicine (p=0,018), à la ciprofloxacine (p=0,0003), à la ceftazidime (p<0,0001), à la ceftriaxone (p<0,0001), au céfixime (p<0,0001), au céfotaxime (p<0,0001), à la pipéracilline-tazobactam (p=0,0006) et à l'amoxicilline-acide clavulanique (p=0,0024).

Conclusion: Nos résultats montrent des taux élevés de résistance in vitro des souches UPEC aux antibiotiques couramment utilisés, ce qui limite potentiellement les options thérapeutiques et donc un véritable défi de santé publique au Congo.

Mots clés: Antibiotique, Souches uropathogènes, *Escherichia coli*, Résistance aux antibiotiques

Introduction:

Urinary tract infections (UTIs) refer to inflammatory response in the urinary system resulting from invasion of the urinary tract by microbial pathogens (1,2). Urinary tract infections are a very frequent reason for consultations and medical prescriptions in everyday practice (3). Worldwide, 150 million cases of UTIs are diagnosed each year, and it is estimated that 20-40% of women over the age of 18 years will suffer at least once from UTI in their lifetime (4). Over 90% of UTIs are of monomicrobial aetiology, with *Escherichia coli* being the most common uropathogen, responsible for 75-90% of cases (5,6).

The overuse and misuse of antibiotics such as fluoroquinolones and third-generation cephalosporins in the treatment of UTIs was rapidly followed by the emergence of multidrug-resistant (MDR) strains. One of the factors responsible for the variation in antibiotic sensitivity of uropathogens is the acquisition of resistance mechanisms such as *Enterobacteriaceae* producing the enzymes extended-spectrum beta-lactamases (ESBL), which are capable of hydrolyzing the beta-lactam antibiotics. In many situations, these resistance events have compromised the use of these antimicrobial molecules of choice in the treatment of MDR infection, resulting in therapeutic failures and increased treatment costs (7,8).

Urinary tract infections caused by *E. coli* are a priority for antibiotic resistance surveillance, given their high frequency of occurrence and sometimes, severity of infection. The aim of this study was to determine the frequency of isolation and antibiotic susceptibility of UPEC strains from clinical *Enterobacteriaceae* isolates in the bacteriology-virology laboratory of the University Hospital Centre (CHU) in Brazzaville, Congo

Materials and method:

Study design and setting:

This was a descriptive retrospective study of patients with confirmed *Escherichia coli* UTIs over a period of 6 months (April 1 September 31, 2022) at the bacteriology-virology laboratory of Brazzaville University Hospital, Congo.

Sample collection and culture isolation

Voided midstream urine samples were obtained from a total of 187 hospitalized patients referred to the bacteriology-virology lab-

oratory at Brazzaville University Hospital for microbiological culture. About 10 microlitres of the urine samples were inoculated onto Cystine Lactose Electrolyte Deficient agar (CLED) and incubated at 37°C for 24 hours. Yellow, opaque colonies with a slightly darker center observed on CLED agar were preliminarily identified as *E. coli*. Urine samples with significant bacteriuria (≥10^5 CFU/ml) in the presence of leukocyturia (≥10^4 leukocytes/ml) were included for further analysis.

In culture plates where more than 2 different colony types were isolated, further analysis was discontinued except in special situations and in consultation with the clinicians. Urine sample with bacteriuria threshold of 10^3 CFU/ml for *E. coli* cystitis was also included.

Identification of *E. coli* isolates and antibiotic susceptibility testing:

Biochemical identification of *E. coli* isolates to species level was carried out using the Analytical Profile Index (API 20E) strips (BioMérieux), with the following biochemical characteristics; indole positive, urease negative, arginine dihydrolase negative, Simmon's citrate negative, Voges-Proskauer (VP) test negative, and fermentation of glucose to produce acid and gas.

Antibiotic susceptibility testing (AST) was performed on Mueller Hinton agar using the Kirby-Bauer disc diffusion method. The antibiotic discs tested were amoxicillin (25μg), amoxicillin-clavulanic acid (20/10μg), piperacillin-tazobactam (30/6μg), imipenem (10μg), cefixime (5μg), ceftriaxone (30μg), cefotaxime (30μg), ceftazidime (30μg), ciprofloxacin (5μg), gentamicin (10μg), amikacin (30μg), sulfamethoxazole-trimethoprim (1.25/23.75μg) and fosfomycin (50μg).

Sensitivity and resistance were determined by measuring the diameter of the zones of inhibition of bacterial growth with a calibrated ruler, then compared with the interpretive breakpoints. The criteria for performing, reading and interpreting the tests were those of the French Microbiology Society CA-SFM 2020 committee. Where *E. coli* was isolated more than once from the same patient with the same antibiotic susceptibility profile, only one was considered.

Phenotypic detection of ESBL:

The production of ESBL, which cause hydrolysis of all beta-lactam antibiotics, was confirmed by the 'double disc synergy test' (DDST) using the combination of amoxicillin and clavulanic acid and a third- or fourth-generation cephalosporin and/or aztreonam. The synergy was characterized by the appearance of a "champagne cork" image, which indicates the presence of ESBL.

Data analysis:

Data were entered and analysed using Microsoft Office Excel 2013. The Fisher's exact test was used to compare the antimicrobial resistance profiles of ESBL-producing and non-ESBL-producing *E. coli* strains. The significance level was less than 5%.

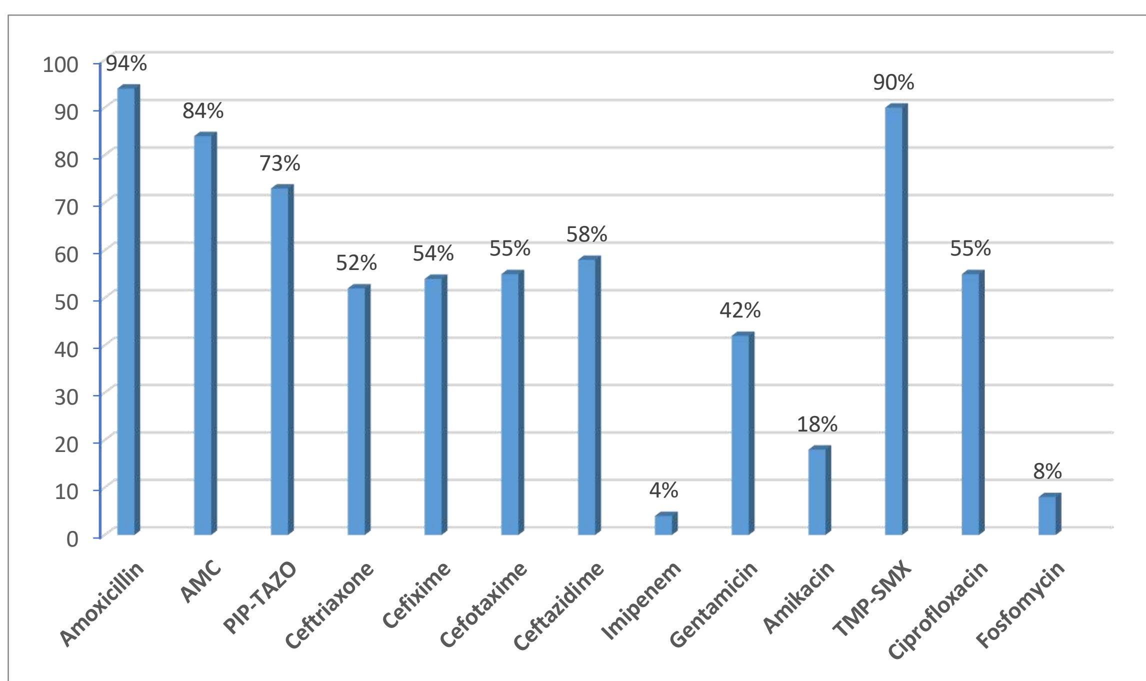
Results:

A total of 187 non-repetitive uropathogenic *Enterobacteriaceae* were isolated from 187 patients with UTI, out of which 81 were uropathogenic *E. coli* (UPEC) strains, giving an overall UPEC isolation frequency of 43.0%. Forty-nine (60.0%) the 81 patients with UPEC UTIs (60.0%) were females while 32 (40.0%) were males, with female to male ratio of 1.5. The modal age of the patients was 57 years with age range of 2 to 86 years. The distribution of the population by age group showed that 3 (3.7%) patients were under 18 years of age, 46 (56.8%) were 18-60 years of age and 32 (39.5%) were over 60 years of age

Among the ESBL-producing UPEC isolates, 23 (50.0%) were from female and 23 (50.0%) from male patients. Four (8.6%) were isolated from patients less than 18 years of age, while 21 (47.7%) each were from patients aged 18-60 years and age over 60 years. Of the non-ESBL-producing UPEC strains, 26 (74.3%) were from female and 9 (25.7%) were from male patients, while 25 (71.4%) and 10 (28.6%) were isolated from patients in the age group 18-60 years and age over 60 years respectively.

Antibiotic resistance profile of UPEC strains:

The resistance profile of the uropathogenic *E. coli* strains to the antibiotics tested showed very high rates of resistance to amoxicillin (94.0%), amoxicillin-clavulanic acid (84%), piperacillin-tazobactam (73.0%), and sulfamethoxazole-trimethoprim (90.0%) (Fig 1).



AMC = Amoxicillin-clavulanic acid; PIP-TAZO = Piperacillin-tazobactam; TMP-SMX = Sulfamethozole-trimethroprim.

Fig 1: The trend in antibiotic resistance in UPEC strains

Table 1: Comparative antibiotic resistance rates of ESBL and non-ESBL-producing UPEC strains

Antibiotics (no of isolates tested)	ESBL producing UPEC (%) (n=46)		Non-ESBL producing UPEC (%) (n=35)		OR (95% CI)	p value
	Resistant	Sensitive	Resistant	Sensitive		
Imipenem (n=59)	3 (8.8)	31 (91.2)	1 (4.0)	24 (96.0)	2.32 (0.23-23.8)	0.43*
Fosfomycin (n=74)	3 (7.5)	37 (92.5)	0	34 (100.0)	6.44 (0.32-129.3)	0.15*
Amikacin (n=50)	8 (25.0)	24 (75.0)	1 (5.6)	17 (94.4)	5.67 (0.65-49.6)	0.13*
Gentamicin (n=45)	15 (57.7)	11 (42.3)	4 (21.1)	15 (78.9)	5.11 (1.33-19.7)	0.018**
Ciprofloxacin (n=49)	23 (76.7)	7 (23.3)	4 (21.1)	15 (78.9)	12.3 (3.07-49.5)	0.0003 **
Ceftazidime (n=80)	45 (100.0)	0	2 (5.7)	33 (94.3)	1219 (56.6-26261)	<0.0001**
Ceftriaxone (n=21)	11 (100.0)	0	0	10 (100.0)	483 (8.8-26615)	<0.0001**
Cefixime (n=48)	26 (100.0)	0	0	22 (100.0)	2385 (45.4-125232)	<0.0001**
Cefotaxime (n=58)	31 (100.0)	0	1 (3.7)	26 (96.3)	1113 (43.5-28500)	<0.0001**
Piperacillin-tazobactam (n=45)	25 (92.6)	2 (7.4)	8 (44.4)	10 (55.6)	15.63 (2.8-86.8)	0.0006**
Amoxicillin-clavulanate (n=43)	23 (100.0)	0	13 (65.0)	7 (35.0)	26.11 (1.38-494.2)	0.0024**
Amoxicillin (n=49)	28 (100.0)	0	18 (85.7)	3 (14.3)	10.78 (0.53-221.2)	0.072*

* = Non-significant difference; ** = Significant difference; OR = Odds ratio; CI = Confidence interval

Comparative antibiotic resistance of ESBL and non-ESBL producing UPEC strains:

The ESBL-producing UPEC strains accounted for 24.5% (46/187) of all uropathogenic *Enterobacteriaceae* isolated. These isolates had very high resistance rates of up to 100.0% for ceftazidime, ceftriaxone, cefixime, cefotaxime, amoxicillin-clavulanic acid and amoxicillin (Table 1).

Comparative analysis of the antibiotic resistance rates between ESBL and non-ESBL-producing UPEC strains showed no significant difference for imipenem (p=0.43), fosfomycin (p=0.15), amikacin (p=0.13), and amoxicillin (p>0.072) while the resistance rates of ESBL-producing strains were significantly higher for gentamicin (p=0.018), ciprofloxacin (p=0.0003), ceftazidime (p<0.0001), ceftriaxone (p<0.0001), cefixime (p<0.0001), cefotaxime (p<0.0001), piperacillin-tazobactam (p=0.0006), and amoxicillin-clavulanate (p=0.0024), compared to non-ESBL producing UPEC strains.

Discussion:

Urinary tract infections are associated with overuse and misuse of antimicrobial agents. The latter have implications for bacterial ecology and the spread of antibiotic resistance, especially when it arises from empirical antimicrobial treatment of recurrent UTIs (9). *Escherichia coli* is a major cause of UTIs (5, 6). The evolution of resistance in *E. coli* is a testament to the effectiveness of antibiotic management policies in community and hospital medicine. These policies include recommendations for the management and empiri-

cal treatment of UTIs (10). Thus, for simple cystitis, an antibiotic will be recommended for empirical therapy if the resistance rate of the bacteria responsible is less than 20.0%. Given the possible complications, in cystitis at risk of complication, acute pyelonephritis, gestational cystitis, and male infections, this rate should be less than 10% (11).

In the present study, 60.0% of the female gender was represented, with a female to male ratio of 1.5. The majority of UTIs were seen in patients over 40 years of age, with a predominance of female patients (58.0%). Indeed, female predominance is generally reported (12,13,14) and is linked to the nature of the female urogenital tract, which is close to the anus and lacks the bacteriostatic character of male prostate secretions (15,16,17). Other host-related factors such as catheterization, pregnancy, sexual activity, urinary tract obstruction have been reported as important causes. In females over 40 years of age, recurrent UTIs could be associated with vaginal prolapse after menopause, which could increase the risk of bacteriuria as vaginal pH increases due to decreased lactobacilli in the birth canal, giving other uropathogens a chance to colonize (18). The results of this study showed a strong involvement of *E. coli* strains in 43.0% of uropathogens causing UTIs. Our findings are consistent with previous studies conducted in different geographic areas (15, 19). However, our results disagree with that of a study from southwestern Nigeria, which reported that *Klebsiella* spp was the most prevalent uropathogen involved in UTIs. This difference could be attributed to differences in study design and environmental factors.

The study of antibiotic resistance in uropathogenic *E. coli* strains showed varying rates of resistance to the antibiotics tested. Among the antibiotics tested, resistance was highest to amoxicillin (94.0%), amoxicillin-clavulanic acid (84.0%), piperacillin-tazobactam (73.0%) and trimethoprim-sulfamethoxazole (90.0%). A similar rate (94.1%) of amoxicillin resistance was reported in Antananarivo, Madagascar by Rakotovao-Ravahatra (20). High rates of resistance to amoxicillin have been reported in numerous studies (21,22,23). This resistance is acquired and is thought to be the consequence of selection pressure linked to the misuse of these antibiotics in developing countries (24). The emergence of amoxicillin-clavulanic acid resistance is a global phenomenon, reported widely at varying rates (22). This antibiotic is known to have an impact on the digestive flora. Thus, the combination of amoxicillin and clavulanic acid is not indicated for empirical treatment of UTIs but only on documentation in the few cases where the strain will be susceptible. The high resistance rates of the uropathogenic *E. coli* strains in our study to these antibiotic molecules justify that they (amoxicillin, amoxicillin–clavulanic acid) are no longer recommended for empirical treatment of UTIs (10,25).

About 73% (33/45) of the UPEC in our study were resistant to piperacillin-tazobactam. Very low rates of resistance to this antibiotic have been reported in many studies, ranging from 6.6% to 27.8% (26,27,28,29). UPEC has the ability to form biofilm (30). Bacteria in a biofilm can be 10 to 1000 times more resistant to antimicrobial agents (31,32) than the same bacteria in planktonic form. Several factors can explain this high resistance or tolerance, including the polymer matrix that acts as a barrier, reducing or preventing the spread of antimicrobial agents. Electrostatic charges on the surface of the polymer matrix can also bind some antimicrobial agents. The metabolism of bacteria in biofilm also plays a very important role. Given the low concentration of certain nutrients and the oxygen gradient, some biofilm cells will be metabolically inactive and may even be dormant. These dormant bacterial cells are likely responsible for much of the tolerance associated with biofilms (33). Ponnusamy et al., (34) reported resistance rate of 83.0% among biofilm-producing UPECs. This result is not so different from ours.

Escherichia coli is naturally susceptible to third-generation cephalosporins and resistance is mainly due to ESBL production. High rates of resistance to ceftriaxone (52.0%), cefixime (54.0%), cefotaxime (55.0%) and ceftazidime (58.0%) were seen in our study. Resistance to third-generation cephalosporins has increased significantly in some geographic areas. Resistance rates similar to that found in our study have also been reported in various studies with rates up to 97.1% for ceftriaxone and 61.8% for cefixime, 87.0% for cefotaxime and ceftazidime (27,35,36). It should also be remembered that these antibiotics are selective for the microbiota. The high frequency of resistance to third-generation cephalosporins could be related to the overuse of these antibiotics without microbiological investigations since most clinicians depend on empiric therapy as a first decision or even on self-prescription of antibiotics by patients themselves in the case of orally administered third-generation cephalosporins (cefixime) and the incomplete duration of treatment.

Low resistance rate was demonstrated to amikacin compared to gentamicin at 18.0%

and 42.0% respectively. In India, gentamicin resistance rates were 48.8% (37). Leski et al., (38) in 2016 reported high resistance rate of 72.9% to gentamicin in their study. Resistance rates of 81.3% to gentamicin and 27.0% to amikacin have been recorded in Iran (39). All of these studies differed in the resistance rates of the bacteria that cause UTIs, which could be attributed to many factors, such as the study population and differences in geographic location.

The rate of resistance to ciprofloxacin (55.0%) was also high in our study. This rate of resistance to ciprofloxacin is indicative of the high level of resistance to fluoroquinolones in our country. Fluoroquinolone resistance has become a global concern (10). Mohamed, in Somalia, reported ciprofloxacin resistance rate of 67.6%. Significant rates of resistance to ciprofloxacin have been identified in various studies (12,13,40,41,42). The fluoroquinolones induce resistance by accumulating mutations in DNA gyrase and topoisomerase genes, justifying a policy of sparing. It is now established that *E. coli* resistance to quinolones is correlated with outpatient quinolone use at the state, hospital, general practice, and community levels (43). Strict adherence to antibiotic therapy recommendations for common infections should make it possible to drastically limit the use of quinolones in UIIs by limiting the indications and durations of treatment, and by practicing therapeutic de-escalation when the susceptibility test allows it.

Antibiotic molecules with urinary specificity deserve particular attention, in this case fosfomycin. It is active against several species of *Enterobacteriaceae*, including ESBL-producing strains. These are antibiotics that are not selective for the gut microbiota. Their activity persists at a high rate in older patients. In this study, the resistance rate to this molecule was 8.0%. Resistance rates reported in the literature are highly variable but less than 15.0% (44). Their prescription should be limited to simple acute cystitis because of its activity. Trimethoprim-sulfamethoxazole is a first-line antimicrobial used in the treatment of simple cystitis. However, increasing resistance to this molecule has recently been observed in many countries. The majority of studies show resistance at or above the accepted level of 20.0% (21,22,23). A high rate of resistance to trimethoprim-sulfamethoxazole (90.0%) was found in our study. These data justify that this molecule is no longer recommended for empirical treatment of UTIs (10,25). On the other hand, relatively low resistance rates were recorded to imipenem (4.0%). These low rates of imipenem resistance are corroborated by many studies (19,23).

Among the resistance mechanisms of *E. coli* to antibiotics, the production of ESBL is the primary mechanism (45). Beta-lactamases are enzymes that hydrolyse the amide bond of the four-chain β-lactam ring of the β-lactam antibiotics (penicillins, cephalosporins, monobactams and carbapenems) (46) thus conferring resistance to β-lactams (all penicillins, cephalosporins and monobactams), with the exception of carbapenems, cephamycins and β-lactamase inhibitors (47). In our study, 56.8% (46/81) of *E. coli* strains were ESBL-producing. The frequency of ESBL-producing *E. coli* differs in different parts of the world and sometimes even in different hospitals across the country. El Bouamri (21) had reported rate of 6.0% in Marrakech. In Poland and France, rates of 8.0% and 7.6% were reported respectively (48,49). High levels of ESBL-producing *E. coli* are particularly detected in developing countries such as Iran-37.1%, Nepal-38.9%, Pakistan-40% and Jordan-50% (50,51,52). These high rates of ESBL production by UPEC strains confirm the widespread of this resistance phenomenon in hospitals (15,53).

For the comparison of antibiotic resistance rates of ESBL-producing versus non-ESBL-producing strains, the difference was not statistically significant for imipenem, fosfomycin, amikacin and amoxicillin. However, significantly higher resistance rates of up to 100% were observed among ESBL-producing compared to non-ESBL-producing *E. coli* strains for cephalosporins (ceftazidime, ceftriaxone, cefixime, cefotaxime) and aminopenicillins (amoxicillin). Also, significantly higher resistance rates to penicillins combined with inhibitors (amoxicillin-clavulanic acid-100.0%, piperacillin-tazobactam-92.6%), and to ciprofloxacin (76.7%), gentamicin (57.7%) and amikacin (25.0%) were observed. This higher resistant rate of ESBL-producing UBEC strains could be explained by the fact that ESBL genes, usually carried by plasmids, are often associated with other antibiotic resistance genes, including resistance to aminoglycosides and fluoroquinolones (42,54). A lower resistance rate was observed with imipenem (8.8%), which confirms that ESBL-producing strains of *E. coli* remain highly susceptible to the carbapenems. However, in order to preserve this class of 'last resort' antibiotic as much as possible, alternatives should be prioritized whenever possible.

One of the limitations of our study is the fact that clinical data, which are part of the case definition of the different clinical pictures of UTIs, were not collected. Also, the data obt-

ained in this study are global and do not allow us to highlight possible differences in resistance phenotypes according to clinical presentations. It would seem interesting to support the findings of our study with a larger-scale study that includes clinical data.

Conclusion:

The emergence and spread of multi-drug-resistant uropathogenic bacteria are public health problems and real challenge for medical practitioners. This prompts reflection on the management of patients with UTIs. Indeed, the treatment of UTIs should be the subject of therapeutic consensus, taking into account, the national situation of antibiotic resistance. In addition, the adoption of a policy for the proper use of antibiotics, updated by regular programmes to monitor the antibiotic susceptibility of bacteria isolates, remains one of the key tools for reducing antimicrobial resistance in bacterial uropathogens.

Contributions of authors:

MT designed the study, and contributed to the writing of the entire manuscript at its different stages (initial drafting, critical revision, editorial finalization); ONEN was involved in the study design and supervision; AA was involved in bibliographic prospecting; OIBR was involved in proofreading of the manuscript; and OAWS was involved in proofreading and validation of the manuscript. All authors approved the final version of the manuscript submitted for publication.

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References:

1. Foxman, B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med. 2002; 113 (Suppl 1A): 5S-13S. doi: 10.1016/s0002-9343(02)01054-9
2. Ronald, A. R., Nicolle, L. E., Stamm, E., et al. Urinary tract infection in adults: research priorities and strategies. Int J Antimicrob Agents. 2001; 17: 343–348. doi: 10.1016/s0924-8579(01)00303-x.
3. Hooton, T. M. Pathogenesis of urinary tract infection: an update. J Antimicrob Chemother. 2000; 46: S1-S7.
4. Gupta, K., Hooton, T. M., Naber, K. G., et al. International Clinical Practice Guidelines for the Treatment of Acute Uncomplicated Cystitis and Pyelonephritis in Women: A 2010 Update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. 2011; 52 (5): e103-e120. doi: 10.1093/cid/ciq257
5. Ochoa, S. C., Eiros, B. J. M., Pérez, M. C., et al. The etiology of urinary tract infections and the antimicrobial susceptibility of urinary pathogens. Rev Esp Quimioter. 2005; 18 (2): 124-135.
6. Passadouro, R., Fonseca, R., Figueiredo, F., et al. Evaluation of the Antimicrobial Susceptibility of Community-Acquired Urinary Tract Infection. Acta Med Port. 2014; 27 (6): 737-742.
7. Zhanel, G. G., Hisanaga, T. L., Laing, N. M., et al. Antibiotic resistance in *Escherichia coli* outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). Int J Antimicrob Agents. 2006; 27 (6): 468-475. doi: 10.1016/j.ijantimicag.2006.02.009
8. García-Tello, A., Gimbernat, H., Redondo, C., et al. Beta lactamasas de espectro extendido en las infecciones del tracto urinario causadas por enterobacterias: aproximación a su conocimiento y pautas de actuación. Actas Urol Esp. 2014; 38 (10): 678-684. https://doi.org/10.1016/j.acuro.2014.05.004
9. Kot, B. Antibiotic Resistance Among Uropathogenic *Escherichia coli*. Pol J Microbiol. 2019; 68 (4):403-415.doi:10.33073/pjm-2019-048.
10. Caron, F., Galperine, T., Flateau, C., et al. Practice guidelines for the management of adult community-acquired urinary tract infections. Med Mal Infect. 2018; 48 (5): 327-358. doi: 10.1016/j.medmal.2018.03.005.
11. Fried, L. P., Tangen, C. M., Walston, J., et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci. 2001; 56 (3): M146-M156.doi:10.1093/gerona/56.3.m146.
12. Mohamed, M. A., Abdifetah, O., Hussein, F. A., et al. Antibiotic resistance pattern of *Escherichia coli* isolates from outpatients with urinary tract infections in Somalia. J Infect Dev Ctries. 2020; 14 (3): 284-289. doi:10.3855/jidc.12189.
13. Adebola, O., Jaspreet, M., Shreya, S., et al. Phenotypic and molecular characterization of antimicrobial resistant *Escherichia coli* from urinary tract infections in Port-Harcourt, Nigeria. Pan Afr Med J. 2019; 34: 144. doi:10.11604/pamj.2019.34.144.18182.
14. Mofolorunsho, K. C., Ocheni, H. O., Aminu, F. R., et al. Prevalence and antimicrobial susceptibility of extended-spectrum beta lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated in selected hospitals of Anyigba, Nigeria. Afr Santé Sci. 2021; 21 (2): 505-512. doi:10.4314/ahs.v21i2.4.
15. Seifu, W. D., and Gebissa, A. D. Prevalence and antibiotic susceptibility of uropathogens from cases of urinary tract infections (UTI) in Shashemene referral hospital, Ethiopia. BMC Infect Dis. 2018; 18(1):30.doi:10.1186/s12879-017-2911-x.
16. Zec, S., Despotovic, A., Spurnic-Radovanovic, A., et al. Antimicrobial resistance in patients with urinary tract infections and the impact on empiric therapy in Serbia. J Infect Dev Ctries. 2016; 10 (10):1065-1072.doi:10.3855/jidc.8124.
17. Mwaka, A. D., Mayanja-Kizza, H., Kigonya, E., et al. Bacteriuria among adult non-pregnant women attending Mulago hospital assessment centre in Uganda. Afr Health Sci. 2011; 11 (2): 182-189.
18. Jung, C., and Brubaker, L. The etiology and management of recurrent urinary tract infections in postmenopausal women. Climacteric. 2019; 22 (3): 242-249. doi: 10.1080/13697137.2018.1551871.
19. Irenge, L. M., Kabego, L., Vandenberg, O., et al. Antimicrobial resistance in urinary isolates from inpatients and outpatients at a tertiary care hospital in South-Kivu Province (Democratic Republic of the Congo). BMC Res Notes. 2014; 7: 374. doi:10.1186/1756-0500-7-374.
20. Rakotovao-Ravahatra, Z. D., Randriatsarafara, F. M., Rasoanandrasana, S., et al. Phénotypes de résistance des souches d'*Escherichia coli* responsables d'infection urinaire au laboratoire du Centre Hospitalo-Universitaire de Befelatanana

Antananarivo. Pan Afr Med J. 2017; 26: 166. doi:10.11604/pamj.2017.26.166.11828.

21. El Bouamri, M. C., Arsalane, L., Kamouni, Y., et al. Current antibiotic resistance profile of uropathogenic *Escherichia coli* strains and therapeutic consequences. Prog Urol. 2014; 24 (16): 1058–1062.doi:10.1016/j.purol.2014.09.035.
22. Nadmi, H., Elotmani, F., Talmi, M., et al. Antibiotic resistance profile of community acquired uropathogenic enterobacteria in El Jadida (Morocco) Med Mal Infect. 2010; 40 (5): 303-305. doi:10.1016/j.medmal.2009.08.020.
23. Obakiro, S. B., Kiyimba, K., Paasi, G., et al. Prevalence of antibiotic-resistant bacteria among patients in two tertiary hospitals in Eastern Uganda. J Glob Antimicrob Resist. 2021; 25: 82-86.doi:10.1016/j.jgar.2021.02.021.
24. Gonsu Kamga, H., Nzengang, R., Toukam, M., Sando, Z., and Koulla Shiro, S. Phénotypes de résistance des souches d'*Escherichia coli* responsables des infections urinaires communautaires dans la ville de Yaoundé (Cameroun) Afr J Pathol Microbiol. 2014; 3: 1–4. doi:10.4303/ajpm/235891
25. AFSSAPS Practice recommendations for diagnosis and antibiotic therapy of adult community urinary tract infections. Med Mal Infect. 2008; 38 (Suppl 3): S203-S252.
26. Hoban, D. J., Nicolle, L. E., Hawser, S., Bouchillon, S., and Badal, R. Antimicrobial susceptibility of global inpatient urinary tract isolates of *Escherichia coli*: results from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program: 2009-2010. Diagn Microbiol Infect Dis. 2011; 70 (4): 507-511. doi:10.1016/j.diagmicrobio.2011.03.021.
27. Iqbal, Z., Mumtaz, M. Z., and Malik, A. Extensive drug-resistance in strains of *Escherichia coli* and *Klebsiella pneumoniae* isolated from paediatric urinary tract infections. J Taibah Univ Med Sci. 2021; 16 (4): 565-574. doi: 10.1016/j.jtumed.2021.03.004.
28. Shaaban, O. A., Mahmoud, N. A., Zeidan, A. A., Kumar, N., and Finan, A. C. Prevalence and Resistance Patterns of Pediatric Urinary Tract Infections in Bahrain. Cureus. 2021; 13 (12): e20859. doi: 10.7759/cureus.20859.
29. Abdel Gawad, A. M., Ashry, W. M. O., El-Ghannam, S., Hussein, M., and Yousef, A. Antibiotic resistance profile of common uropathogens during COVID-19 pandemic: hospital based epidemiologic study. BMC Microbiol. 2023;23(1): 28. doi:10.1186/s12866-023-02773-5.
30. Tenke, P., Kovacs, B., Jackel, M., and Nagy, E. The role of biofilm infection in urology. World J Urol. 2006; 24 (1): 13-20. doi: 10.1007/s00345-005-0050-2
31. Olson, M. E., Ceri, H., Morck, D. W., Buret, A. G., and Read, R. R. Biofilm bacteria: Formation and comparative susceptibility to antibiotics. Can J Vet Res. 2002; 66: 86–92.
32. Ceri, H., Olson, M. E., and Turner, R. J. Needed, new paradigms in antibiotic development. Expert Opin Pharmacother. 2010; 11: 1233–1237. doi: 10.1517/14656561003724747
33. Lewis, K. Multidrug tolerance of biofilms and persister cells. Curr Top Microbiol Immunol. 2008; 322: 107–131. doi: 10.1007/978-3-540-75418-3_6.
34. Ponnusamy, P., Natarajan, V., and Sevanan, M. *In vitro* biofilm formation by uropathogenic *Escherichia coli* and their antimicrobial susceptibility pattern. Asian Pac J Trop Med. 2012; 5 (3): 210-213.doi:10.1016/S1995-7645(12)60026-1.
35. Ibrahim, M. E., Bilal, N. E., and Hamid, M. E. Increased multi-drug resistant *Escherichia coli* from hospitals in Khartoum state, Sudan. Afr Health Sci. 2012; 12 (3): 368-375. doi: 10.4314/ahs.v12i3.19.
36. Shatalov, A. Prevalence and antibiotic resistance pattern of *Escherichia coli* and *Klebsiella pneumoniae* in urinary tract infections at La Paz Medical Center, Malabo, Equatorial Guinea. Open J Med Microbiol. 2005; 5(4): 177-183. doi: 10.4236/ojmm.2015.54022.
37. Manikandan, S., Ganesapandian, S., Singh, M., et al. Modèle de sensibilité aux antimicrobiens des infections des voies urinaires provoquant des bactéries pathogènes pour l'homme. Asiatique J Med Sci. 2011; 3 (2): 56-60.
38. Leski, T. A., Taitt, C. R., Bangura, U., et al. Forte prévalence d'entérobactéries multirésistantes isolées à partir d'échantillons d'urine de patients ambulatoires, mais pas dans l'environnement hospitalier de Bo, Sierra Leone. BMC Infect Dis. 2016; 16: 167.doi:10.1186/s12879-016-1495-1.
39. Azimi, L., Armin, S., Samadi Kafil, H., et al. Evaluation of phenotypic and genotypic patterns of aminoglycoside resistance in the Gram-negative bacteria isolates collected from pediatric and general hospitals. Mol Cell Pediatr. 2022; 9(1):2.doi:10.1186/s40348-022-00134-2
40. Bruyère, F., Cariou, G., Boiteux, J. P., et al. Généralités [Diagnosis, treatment and follow-up of community-acquired bacterial infections of the urinary system of men and women (acute cystitis and acute pyelonephritis) and of the genital system of men (acute prostatitis): general remarks]. Prog Urol. 2008; 18 (Suppl 1): 4-8. doi:10.1016/S1166-7087(08)70505-0.
41. El Bouamri, M. C., Arsalane, L., Kamouni, Y., et al. Évolution récente du profil épidémiologique des entérobactéries uropathogènes productrices des B-lactamases à spectre élargi à Marrakech, Maroc. Prog Urol. 2014; 24 (7): 451-455. doi: 10.1016/j.purol.2013.11.010.
42. Champs, C., Sirot, D., Chanal, C., et al. Concomitant dissemination of three extended-spectrum β-lactamases among different *Enterobacteriaceae* isolated in a French hospital. J Antimicrob Chemother. 1991; 27 (4): 441-457. doi: 10.1093/jac/27.4.441.
43. Batard, E., Montassier, E., Ballereau, F., et al. De la consommation d'antibiotiques aux résistances bactériennes: l'exemple de la résistance d'*Escherichia coli* aux quinolones. Médecine Thérapeutique. 2011; 17 (4): 294-301. doi:10.1684/MET.2011.0341.
44. Neuzillet, Y., Naber, K. G., Schito, G., et al. French results of the ARESC study: clinical aspects and epidemiology of antimicrobial resistance in female patients with cystitis. Implications for empiric therapy. Med Mal Infect. 2012; 42 (2): 66-75. doi:10.1016/j.medmal.2011.07.005.
45. Nicolas-Chanoine, N. H. Les enterobacteries productrices de bêta-lactamases à spectre élargi: où sont les dangers? Réanimation. 2012;21:260-267.doi:10.1007/s13546-012-0463-z
46. Noyal, M. J. C., Menezes, G. A., Harish, B. N., et al. Simple screening tests for detection of carbapenemases in clinical isolates of non-fermentative Gram-negative bacteria. Indian J Med Res. 2009; 129 (6): 707–712.
47. Baudry, P. J., Nichol, K., DeCorby, M., et al. Mechanisms of resistance and mobility among multidrug-resistant CTX-M-producing *Escherichia coli* from Canadian intensive care units: the 1st report of QepA in North America. Diagn Microbiol Infect Dis. 2009; 63 (3):319–326. doi:10.1016/j.diagmicrobio.2008.12.001.
48. Michno, M., Sydor, A., Wałaszek, M., et al. Microbiology and drug resistance of pathogens in patients hospitalized at the Nephrology Department in the South of Poland. Pol J Microbiol. 2018; 67 (4): 517–524. https://doi.org/10.21307/pjm-2018-061
49. Lavigne, J. P., Thibault, M., Costa, P., et al. Resistance and virulence potential of uropathogenic *Escherichia coli* strains isolated from patients hospitalized in urology departments: a French prospective multicentre study. J Med Microbiol. 2016; 65 (6): 530–533 doi: 10.1099/jmm.0.000247.

50. Ali, I., Rafaque, Z., Ahmed, S., et al. Prevalence of multidrug resistant uropathogenic *Escherichia coli* in Potohar region of Pakistan. Asian Pac J Trop Biomed. 2016; 6 (1): 60–66. https://doi.org/10.1016/j.apjtb.2015.09.022
51. Parajuli, N. P., Maharjan, P., Parajuli, H., et al. High rates of multidrug resistance among uropathogenic *Escherichia coli* in children and analyses of ESBL producers from Nepal. Antimicrob Resist Infect Contr. 2017; 6 (1): 9. https://doi.org/10.1186/s13756-016-0168-6
52. Shakhatreh, M. A. K., Swedan, S. F., Al-Odat, M. A., et al. Uropathogenic *Escherichia coli* (UPEC) in Jordan: prevalence of urovirulence genes and antibiotic resistance. JKSUS. 2018. https://doi.org/10.1016/j.jksus.2018.03.009
53. Fouquet, M., Morange, V., and Bruyère, F. Five years follow-up of infections with extended-spectrum beta-lactamase producing *Enterobacteriaceae*. Prog Urol. 2012; 22 (1): 17-21 doi: 10.1016/j.purol.2011.07.003.
54. Jacoby, G. A., and Sutton, L. Properties of plasmids responsible for production of extended-spectrum beta-lactamases. Antimicrob Agents Chemother. 1991; 35 (1): 164-169. doi: 10.1128/AAC.35.1.164.

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Original Article **Open Access**

Bacteriological profiles of urinary tract infections in patients admitted to the nephrology-haemodialysis department of the Bogodogo University Teaching Hospital (CHU B), Ouagadougou, Burkina Faso

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Abstract:

Background: Urinary tract infections (UTI) constitute a major public health problem, especially in developing countries such as Burkina Faso. They are commonly encountered in hospitals, particularly in patients suffering from chronic kidney disease whose management requires special measures to avoid treatment failures which are frequent. The objective of this study is to determine the microbial profiles of urinary tract infections (UTIs) in these patients.
Methodology: This was a cross-sectional study of hospitalized patients with UTIs in the nephrology-haemodialysis department of the CHUB from August 1 to November 31, 2020. Socio-demographic and clinical data of selected patients were collected by a well-designed data collection form. Cytobacteriological analysis of urine (CBAU) was carried out on voided or catheter-urine sample of each patient using standard microbiological technique. The disc diffusion method in agar medium modified according to the recommendations of the 2020 CA-SFM-EUCAST was used to determine the antibiotic susceptibility of each isolate. Data were processed and analyzed using Excel 2013, IBM SPSS Statistics 25.0 and CSpro 7.5 software.
Results: Urine samples were collected from a total of 77 eligible participants, 49 (63.6%) of which were CBAU positive, with 56 microbial pathogens isolated. Enterobacterales represented 58.9% (n=33), including 39.4% *Escherichia coli* (n=13) and 36.4% *Klebsiella* spp (n=12). Non-fermentative Gram-negative bacilli represented 7.1% (n=4) including *Acinetobacter baumannii* (n=3) and *Pseudomonas aeruginosa* (n=1). *Staphylococcus aureus* was isolated in 5.4% (n=3) and *Candida* spp in 28.6% (n=16). The most active antimicrobials *in vitro* against the bacterial pathogens were amikacin and imipenem, and clotrimazole and nystatin against the *Candida* spp. A total 35.7% (n=20) were multi-drug resistant bacteria with 32.1% by ESBL in Gram-negative bacteria and 66.7% (2/3) by MRSA in Gram-positive bacteria.
Conclusion: The high resistance of pathogens to antimicrobials, resulting in therapeutic failures, constitutes a significant challenge in the management of urinary tract infection, especially in people with chronic kidney disease. It is therefore necessary to put in place urgent measures aimed at the rational use of antimicrobials and strict compliance with good hospital hygiene practices.

Keywords: Bacteriological profile; UTI; Nephrology-Haemodialysis; Antimicrobial resistance

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Profils bactériologiques des infections urinaires chez les patients admis au service de néphrologie-hémodialyse du CHU de Bogodogo (CHU B), Ouagadougou, Burkina Faso

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Résumé:

Contexte: Les infections des voies urinaires (IVU) constituent un problème de santé publique majeur, notamment dans les pays en développement comme le Burkina Faso. Ils sont fréquemment rencontrés en milieu hospitalier, notamment chez les patients souffrant d'insuffisance rénale chronique dont la prise en charge nécessite des mesures particulières pour éviter les échecs thérapeutiques qui sont fréquents. L'objectif de cette étude est de déterminer les profils microbiens des infections des voies urinaires (IVU) chez ces patients.
Méthodologie: Il s'agit d'une étude transversale portant sur des patients hospitalisés atteints d'infections urinaires dans le service de néphrologie-hémodialyse du CHUB du 1er août au 31 novembre 2020. Les données sociodémographiques et cliniques des patients sélectionnés ont été collectées par un recueil de données bien conçu. formulaire. L'analyse cytobactériologique de l'urine (CBAU) a été réalisée sur un échantillon d'urine purgé ou cathéter de chaque patient en utilisant une technique microbiologique standard. La méthode de diffusion sur disque en milieu gélose modifiée selon les recommandations du CA-SFM-EUCAST 2020 a été utilisée pour déterminer la sensibilité aux antibiotiques de chaque isolat. Les données ont été traitées et analysées à l'aide des logiciels Excel 2013, IBM SPSS Statistics 25.0 et CSpro 7.5.
Résultats: Des échantillons d'urine ont été collectés auprès d'un total de 77 participants éligibles, dont 49 (63,6%) étaient positifs au CBAU, avec 56 agents pathogènes microbiens isolés. Les Enterobacterales représentaient 58,9% (n=33), dont 39,4% d'*Escherichia coli* (n=13) et 36,4% de *Klebsiella* spp (n=12). Les bacilles Gram négatif non fermentaires représentaient 7,1% (n=4) dont *Acinetobacter baumannii* (n=3) et *Pseudomonas aeruginosa* (n=1). *Staphylococcus aureus* a été isolé dans 5,4% (n=3) et *Candida* spp dans 28,6% (n=16). Les antimicrobiens les plus actifs *in vitro* contre les agents pathogènes bactériens étaient l'amikacine et l'imipénème, ainsi que le clotrimazole et la nystatine contre *Candida* spp. Au total, 35,7% (n=20) étaient des bactéries multirésistantes, dont 32,1% étaient des BLSE chez les bactéries Gram-négatives et 66,7% (2/3) des SARM chez les bactéries Gram-positives.
Conclusion: La forte résistance des pathogènes aux antimicrobiens, entraînant des échecs thérapeutiques, constitue un défi important dans la prise en charge des infections urinaires, notamment chez les personnes atteintes d'insuffisance rénale chronique. Il est donc nécessaire de mettre en place des mesures urgentes visant l'usage rationnel des antimicrobiens et le strict respect des bonnes pratiques d'hygiène hospitalière.

Mots clés: Profil bactériologique; infection urinaire; Néphrologie-Hémodialyse; Résistance aux antimicrobiens

Introduction:

Healthcare-associated infections are at the forefront of healthcare-related adverse events (1). According to the World Health Organization, the prevalence of healthcare-associated infections in Africa varies at hospital level between 2.5% and 14.8% and the risk of contracting an infection during healthcare is 2 to 20 times higher in developing countries than in developed countries (2). Nosocomial infections contracted in a healthcare structure are an integral part of healthcare-associated infections, according to the conclusions of the Committee on Nosocomial Infections and Healthcare-Associated Infections in France, established in 2006 (3).

Urinary tract infections (UTIs) are the most common infections encountered in hospital settings. Indeed, in almost 40% of cases, they are acquired in the hospital (4). Their frequency varies depending on the type of establishment and service. Studies carried out respectively in the urology and nephrology-haemodialysis departments of the Yalgado Ouedraogo University Teaching Hospital (CHUYO), reported prevalence of 65.3% in 2011, 26.8% in 2015 and 43.4% in 2019. These high frequencies were associated in nearly 80% of cases with chronic kidney failure (CKD) which was the first reason for consultation in 54.3% of cases (5).

UTI in people with renal insufficiency is a complicated infection requiring specific diagnostic and therapeutic management (6). This is explained by the fact that most of the patients suffering from CKD have an immunosuppression status which requires particular attention to aseptic conditions during when providing them cares. The numerous invasive maneuvers (such as bladder catheterization, central venous catheterization and urinary tract interventions), and long hospital stay that is often necessary for the care of the patient, constitute factors of infection aggravation. They either facilitate introduction of bacteria into the urinary tract or facilitate their developments. These infections can progress to bacteremia (7).

The high frequency of UTIs occurs in the context of increasing bacterial resistance to antibiotics (8), and this constitutes a major public health challenge, especially in developing countries such as Burkina Faso (9). A study conducted by the Pasteur Institute reported a significant increase in the proportions of extended spectrum β-lactamases (ESBL) for *Escherichia coli*, from 28.9% in 2012 to 48.2% in 2015 (10). This increase in antibiotic resistance could be the cause of numerous therapeutic failures and constitute a problem for the management of UTI in patients suffering from renal failure. The present study initiated in this context could contribute to better management of UTIs in patients hospitalized in nephrology department, through development of guidelines for antibiotic

treatment and prevention of complications through early diagnosis.

Materials and method:

Study setting:

This study was conducted at the nephrology and haemodialysis department of Bogodogo University Teaching Hospital (CHU B) Ouagadougou, Burkina Faso, where urine collection took place and the laboratory department of the hospital where bacteriological analysis was performed.

Study design and period of study:

This was a descriptive cross-sectional study of hospitalized patients whose urine analysis were carried out over a period of four months (August 1, 2020 to November 31, 2020)

Study participants, data and sample collection:

The study participants were non-anuric hospitalized patients in the nephrology-haemodialysis department of the CHUB during the study period, who consented to participate in the study. A well-designed collection form was developed for this purpose to collect information on the demographic characteristics of the participants, clinical data and microbiological results of the laboratory investigation.

The clinical records of the patients served as the data sources. Written instruction on the urine collection technique was provided in advance to the staff of the nephrology-haemodialysis department. Voided urine sample was collected by the "fly or mid-stream technique", and by catheter specimen from those on urinary catheter. Strict aseptic precautions were taken during urine collection.

Ethical considerations:

Ethical approval to conduct the study was granted by the CHUB. Approval to collect urine samples from participants and to perform laboratory analysis were given by the managers of the nephrology and haemodialysis department and the CHUB laboratory respectively. Informed consent was obtained from each patient participant. Confidentiality and anonymity were strictly adhered to.

Urine microscopy and culture:

The cytobacteriological analysis of urine (CBAU) was carried out according to the routine procedures applicable in Burkina Faso, and the interpretation of the results was made according to the recommendations of the "European guidelines for urine analysis" on uropathogenic groups distinguishing microorganisms into four groups according to their involvement in the aetiology of UTI (11).

Each urine sample was systematically inoculated on Cystine Lactose Electrolyte Deficient (CLED), Bromocresol Purple (BCP) and Eosin Methylene Blue (EMB) agar media, and incubated aerobically at 37ºC for 24 hours. Following observation of the colonies on the positive culture media and further microscopic examination, additional media were used according to the morphology of the bacteria such as Chapman agar for Gram-positive cocci in clusters and Sabouraud Dextrose-Chloramphenicol agar for yeasts.

Identification of microbial isolates:

Biochemical identification of Gram-positive cocci in clusters was done by performing catalase and coagulase tests and identified to species level using API Staph gallery. Gram-negative bacilli were identified to species level using the API 20E gallery. The BD Phoenix TM M50 was used for confirmation during repeat testing when two organisms were isolated in the same sample. *Candida* species were identified by microscopic observation of refractile ovoid cells with clear contents and confirmed by API *Candida* gallery that is standardized for identification of yeasts.

Antimicrobial susceptibility testing of microbial isolates:

Antimicrobial susceptibility testing of bacterial isolates (antibiogram) was performed using the modified Kirby-Bauer disk diffusion method in agar medium against selected antibiotics as described by the French Society of Microbiology (CASFM2020_Octobre2020_V1.2). Briefly, microbial inoculum from a 24-hour culture was prepared in a tube containing sterile saline solution (5ml of 0.9% NaCl) and standardized to 0.5 MacFarland turbidity standard (equivalent of 10^6-10^8 colony forming unit/ml). The suspension was then inoculated using a sterile swab on sterile Mueller-Hinton (MH) agar plate to obtain a homogeneous distribution of bacteria over the entire surface of the agar. Antibiotic discs were placed on the surface of the agar plate (maximum of six discs for a 90 mm Petri dish) and incubated at 37°C for 24 hours.

The diameter of zone of inhibition of bacterial growth was measured in millimeters and interpreted as sensitive or resistant using the interpretative tables of the 2019 and 2020 CASFM benchmarks. For antibiotic susceptibility test for fungi (antifungogram), the same disc diffusion method was used, with antifungal discs on Sabouraud Dextrose-Chloramphenicol agar.

Phenotypic detection of ESBLs and MRSA:

The test to detect extended-spectrum β-lactamases (ESBLs) production in the isolates

was carried out using the 'double disk synergy test' (DDST) which consists of placing an antibiotic associated with a β-lactamase inhibitor (amoxicillin-clavulanic acid) in the middle of an inoculated Mueller-Hinton (MH) agar plate with two 3rd or 4th generation cephalosporins, placed side-by-side at a distance of 20mm. The presence of a 'champagne cork' image indicates the production of ESBL. For the detection of methicillin-resistant *Staphylococcus aureus* (MRSA), cefoxitin (30µg) disc was used and inhibition zone diameter less than 27 mm indicate MRSA.

Data analysis:

The data were processed and analyzed using Excel 2013, IBM SPSS Statistics 25.0 and CSpro 7.5 software.

Results:

Socio-demographic and clinical characteristics of the study participants:

During the study period, 127 patients were hospitalized in the nephrology and haemodialysis department of CHU B but only 77 (37 females and 40 males) were eligible and selected for cytobacteriological examination of their urine. The sociodemographic characteristics of the 77 participants is shown in Table 1. Of the 77 patient participants, 49 (63.6%) were positive for cytobacteriological analysis of their urine (CBAU) out of which 21 (27.3%) had chronic kidney disease (CKD), 12 (15.6%) had end-stage kidney disease (ESKD) and 10 (13.0%) had acute kidney failure (AKF). UTI was confirmed in 95.9% (47/49) of the CBAU positive patients (or 61.0% of the 77 cases) while 2 patients (2.6%, 2/77) had significant bacteriuria without leukocyturia (no UTI), and 44 (93.6%) of the 47 patients had urinary catheter.

Risk factors for UTI among the study participants:

The only risk factors significantly associated with UTI (CBAU positive) among the participants were age >75 years (OR=0.02, 95% CI=0.0047-0.1186, p<0.0001) and age >65 years with at least three frailty criteria (OR= 0.29, 95%CI = 0.0918 - 0.9452, p=0.0417). Glomerular filtration rate (GFR) <30ml/min/ 1.73m^2 (p=0.0966), pregnancy (p=0.7003) and gender (0.6359) were not significantly associated with UTI (Table 2).

Table 1: Socio-demographic and clinical characteristics of hospitalized study participants at the nephrology and haemodialysis department of Bogodogo University Teaching Hospital Ouagadougou, Burkina Faso

Characteristics	CBAU positive n (%)	CBAU negative n (%)	Total number n (%)	x^2	OR (95% CI)	*p* value
Gender						
Female	25 (67.6)	12 (32.4)	37 (48.1)	0.2049	1.389 (0.5452-3538)	0.6508
Male	24 (60.0)	16 (40.0)	40 (51.9)			
Place of résidence						
Urban	28 (58.3)	20 (41.7)	48 (62.3)	1.562	-	0.4579
Semi-urban	18 (72.0)	7 (28.0)	25 (32.5)			
Rural	3 (75.0)	1 (25.0)	4 (5.2)			
Bladder catheter						
Yes	44 (67.7)	21 (32.3)	65 (84.4)	1.947	2.933 (0.8319-10.343)	0.1629
No	**5 (41.7)	7 (58.3)	12 (15.6)			
Current antibiotic therapy						
Yes	31 (53.4)	27 (46.6)	58 (75.3)	8.835	0.06379 (0.008-0.5102)	0.003*
No	18 (94.7)	1 (5.3)	19 (24.7)			

CBAU = cytobacteriological analysis of urine; OR=Odds ratio; CI=Confidence interval; * = statistically significant at p<0.05; ** = Two patients had significant bacteriuria but no leukocyturia (no UTI)

Table 2: Analysis of risk factors for urinary tract infections among hospitalized study participants at the nephrology and haemodialysis department of Bogodogo University Teaching Hospital Ouagadougou, Burkina Faso

Risk factors	CBAU positive n (%)	CBAU négative n (%)	Total n (%)	OR (95% CI)	*p* value
Male gender	24 (60.0)	16 (40.0)	40 (52)	0.72 (0.2826-1.834)	0.6359
Pregnancy	4 (57.1)	3 (42.9)	7 (9.09)	0.74 (0.1533-3.579)	0.7003
GFR<30mL/min/1.73m2	25 (55.6)	20 (44.4)	45 (58.4)	0.42 (0.1543-1.125)	0.0966
Age>75 years	2 (10.0)	18 (90.0)	20 (25.9)	0.02 (0.0047-0.1186)	<0.0001*
Age>65 years with at least three frailty criteria	6 (40.0)	9 (60.0)	15 (19.4)	0.29 (0.0918-0.9452)	0.0417*

CBAU = cytobacteriological examination of urine; OR=Odds ratio; CI=Confidence interval; * = statistically significant at $p<0.05$

Frequency of isolated microbial pathogens:

Of the urine samples analyzed for the 77 participants, 56 microbial pathogens were isolated from 49 participants with positive CBAU. Of the 49 CBAU positive participants, 9 (18.4%) had two microbial pathogens isolated from their urine samples. The *Enterobacterales* representted 58.9% (n=33), including 39.4% *Escherichia coli* (n=13), 36.4% *Klebsiella* spp (n=12) and 9.0% *Serratia* spp (n=3). Non-fermentative Gram-negative bacilli represented 7.1% (n=4), with 3 cases (5.4%) of *Acinetobacter baumannii* and 1 (1.8%) of *Pseudomonas aeruginosa*. *Staphylococcus aureus* was isolated in 3 (5.4%) samples. *Candida* was isolated in 16 (28.6%) cases.

Table 3: Frequency of isolated microbial pathogens of urinary tract infections among hospitalized study participants at the nephrology and haemodialysis department of Bogodogo University Teaching Hospital Ouagadougou, Burkina Faso

Order/Family	Species	Number (%)
Saccharomycetaceae	*Candida* spp	16 (28.6)
Staphylococcaceae	*Staphylococcus aureus*	3 (5.4)
Enterobacterales	*Escherichia coli*	13 (23.2)
	Klebsiella pneumoniae	11 (19.6)
	Klebsiella oxytoca	1 (1.8)
	Proteus mirabilis	1 (1.8)
	Serratia odorifera	3 (5.4)
	Enterobacter cloacae	1 (1.8)
	Citrobacter koseri	2 (3.6)
	Cedecea lapagei	1 (1.8)
Pseudomonadales	*Acinetobacter baumannii*	3 (5.4)
	Pseudomonas aeruginosa	1 (1.8)

Table 4: Antimicrobial resistance of isolated bacterial pathogens of urinary tract infections among hospitalized study participants at the nephrology and haemodialysis department of Bogodogo University Teaching Hospital Ouagadougou, Burkina Faso

Antimicrobials/Bacteria	*E. coli* (%) (n=13)	*Klebsiella* spp (%) n=12)	*Serratia* spp (%) (n=3)	*Citrobacter koseri* (%) (n=2)	*Enterobacter cloacae* (%) (n=1)	*Proteus mirabilis* (%) (n=1)	*Cedecea lapagei* (%) (n=1)
Ampicillin	13 (100)	12 (100.0)	3 (100.0)	2 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
Amoxicillin-Clavulanic acid	12 (92.3)	11 (91.6)	3 (100.0)	2 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
Piperacillin-Tazobactam	5 (38.4)	6 (0.5)	3 (100.0)	1 (50.0)	1 (100.0)	0	1 (100.0)
Cefadroxil	12 (92.3)	12 (100.0)	3 (100.0)	2 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
Cefoxitin	11 (84.6)	10 (83.3)	3 (100.0)	2 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
Ceftazidime	10 (76.9)	12 (100.0)	3 (100.0)	2 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
Cefotaxime	10 (76.9)	10 (83.3)	3 (100.0)	2 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
Cefepime	10 (76.9)	9 (75.0)	3 (100.0)	2 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
Imipenem	0	4 (33.3)	0	0	1 (100.0)	0	1 (100.0)
Ciprofloxacin	9 (69.2)	8 (66.7)	3 (100.0)	2 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
Levofloxacin	9 (69.2)	8 (66.7)	3 (100.0)	2 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
Gentamycin	3 (23.0)	9 (75.0)	2 (66.6)	2 (100.0)	1 (100.0)	0	1 (100.0)
Amikacin	0	3 (25.0)	2 (66.7)	0	0	0	0
Tobramycin	3 (23.0)	9 (75.0)	3 (100.0)	2 (100.0)	1 (100.0)	0	1 (100.0)
Sulfamethoxazole-trimethoprim	9 (69.2)	11 (91.6)	3 (100.0)	2 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)

All the 3 (100.0%) *S. aureus* isolates were resistant to penicillin, 2 (66.7%) of the 3 were resistant to cefoxitin (i. e. MRSA) and 1 (33.3%) was resistant to imipenem. Amikacin and gentamicin were inactive on all 3 *S. aureus* isolates. The susceptibility profile of the *Enterobacterales* isolates to amikacin was 84.8% (28/33), with 40.0% (13/33) for *E. coli,* 27.2% (9/33) for *Klebsiella* spp and 3.0% (1/33) for *Serratia* spp. Amikacin was active on 100% of *E. coli*, 75% (9/12) of *Klebsiella* spp and 33.3% (1/3) of *Serratia* spp. The susceptibility of the *Enterobacterales* to imipenem was 81.8% (27/33) with 40.0% for *E. coli* and 24.2% (8/33) for *Klebsiella* spp. Imipenem was active on 100% of *E. coli* and *Serratia* spp and 66.7% (8/12) of *Klebsiella* spp. The 3 (100%) strains of *Serratia* spp were sensitive to this antibiotic.

Resistance of the *Enterobacterales* to ciprofloxacin and levofloxacin was 75.7% (25/33) for each of these antibiotics. *Escherichia coli* and *Klebsiella spp* isolates were resistant to these 2 antibiotics with 76.9% (10/13) and 66.7% (8/12) respectively. All isolated strains (100%) of *Serratia* spp, *Citrobacter koseri, Enterobacter cloacae, Proteus mirabilis* and *Cedecea lapagei* were resistant to these 2 antibiotics. For cefotaxime and ceftazidime, the Enterobacterales were resistant to them in 84.9% and 90.9% respectively.

Of all the strains isolated, 20 (35.7%) were multi-resistant bacteria. Among the 18 (32.1%) bacteria producing extended spectrum β-lactamases (ESBL), there were 17 *Enterobacterales* (i. e. 30.3% of isolated strains and 51.5% *Enterobacterales*) including 61.5% (8/13) *E. coli*, 58.3% (7/12) *Klebsiella* spp, and 66.6% (2/3) *Serratia* spp. Regarding non-fermentative Gram-negative bacilli, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were isolated in four patients, i.e. in 7.1% of cases. All 3 (100.0%) *A. baumannii* isolates and 1 (100.0%) *P. aeruginosa* strain were susceptible to amikacin and gentamicin. The 3 strains of *A. baumannii* isolated were all resistant to cefixime and ceftazidime. Among the yeasts isolated, resistance to antifungals was 93.8% for amphotericin B and 62.5% for miconazole but the strains were sensitive to clotrimazole (75.0%) and nystatin (62.5%).

Discussion:

In this study, the male gender was predominant (51.9%), the majority of patients resided in urban areas (62.34%) and impaired renal function was the first reason for consultation (55.8%). The study reported a high frequency of UTI in hospitalized patients i. e. 61.0% of patients in whom cytobacteriological analysis (CBAU) was performed. This observation could be linked to the presence of high representation (more than 84.4%) of patients hospitalized with permanent urinary catheters. The presence of this device in the patient is the primary risk factor for UTI. It is indicated in situations of urinary disorders or for monitoring diuresis (12,13). During hospitalization, its presence promotes colonization of the urinary tract by pathogens from the hospital environment. The prevalence of positive CBAU was slightly higher in the female participants (67.7%) compared to the male participants (60.0%). Although the prevalence difference was not statistically significant (p=0.6508), it has been reported in the literature that women are at higher risk of UTI and that 50.0% of them experience a UTI episode during their life time (14).

Our study reported 27.3% of UTI in patients with CKD. The high frequency of infections in these fragile patients subjected to numerous invasive maneuvers such as extrarenal purification by hemodialysis (6) was also reported in the study by Some (15) in 2016 in the nephrology and hemodialysis department of CHU-YO, where 42.5% of patients with kidney failure had had at least one hemodialysis session. This is a purification process that takes place outside the body using an artificial membrane and using a central catheter which is very often the starting point of the infection

Among the pathogens isolated, *Entero bacterales* were the most incriminated in 58.9%, and *E. coli* and *Klebsiella* spp were the predominant species. These results are similar to the literature data which reported that these two bacteria are the main pathogens involved in UTI (7,11,16,17). Indeed, *E. coli* is an intestinal bacteria flora of mammals, that is very common in humans, and constitutes approximately 80% of the aerobic intestinal flora (18). *Klebsiella* are ubiquitous bacteria, present in the digestive tract and in the respiratory system of mammals, including humans, as commensal bacteria. They are called opportunistic because they very often cause infections when certain conditions are met, such as weakened immune system, surgical procedures, catheterization and the presence of a permanent urinary catheter. This leads to invasion of organs which could lead to sepsis, infection of the urinary tract or respiratory system (19). Ascending contamination is most common in UTIs. Indeed, pathogens of faecal origin coming from the perineal region get into the bladder, especially in women. This origin could explain the frequency of *Enterobacterales* in episodes of UTI.

Concerning the resistance profiles of the

isolated pathogens, imipenem, amikacin and gentamicin were the most active antibiotics *invitro*. Kafando (20) and Habou (21) reported 97.0% and 65.0% *in vitro* activity respectively for imipenem and amikacin on isolated pathogens. High resistance of the *Enterobacterales* to the fluoroquinolones (75.7%) and sulfonamides (84.8%) was noted in the present study. Other researchers such as Habou (21) reported in 2019 a resistance of 59.4% to fluoroquinolones and 62.1% to sulfonamides. The emergence of resistance of pathogens to these commonly used antibiotics, due to their easy accessibility, could be linked to their current abusive and inappropriate consumption, and to their being prescri bed as empirical antibiotic therapy most of the time. The most used antibiotic molecules were ciprofloxacin, cotrimoxazole, amoxicillin-clavulanic acid, ceftriaxone, cefotaxime, cefixime, lincomycin and metronidazole. Our study found a significant association between prevalence of UTI and antibiotic use, with frequency of positive CBAU lower in patients on antibiotics (53.4%) compared to patients who were not on antibiotics (94.7%) (OR=0.064, 95% CI=0.0008-0.5102, p=0.003). Majority of the study participants in our study were patients with chronic kidney disease, and therefore immunocompromised. Appropriate intake of antibiotics could reduce infections in this vulnerable population.

Of the 56 pathogens isolated, 20 (35.7%) were multi-drug resistant bacteria, 10.0% of which were due to methicillin-resistant *Staphylococcus aureus* (MRSA), and 90.0% with extended-spectrum β-lactamase (ESBL)-producing bacteria. This observation is in line with data from the literature, which reports the involvement of multi-drug resistant bacteria in 35.8% of UTIs in West Africa (22). This high prevalence of ESBL in *E. coli* and *Klebsiella* agrees with data from an observational study carried out by the University of Versailles Saint-Quentin-en Yvelines and the Pasteur Institute of Paris and which reported that 90.0% of cases of transmission of ESBL-producing *K. pneumoniae* to new patients could be explained by direct or indirect contact with infected patients compared to less than 60.0% for ESBL-producing *E. coli* (23). These results indicate that prevention strategies primarily focused on hand hygiene can effectively limit the transmission of these ESBL-producing bacteria. However, other measures such as environmental decontamination, and rational/documented use of antibiotics, may be necessary to prevent their emergence and spread.

High resistance of *Candida* spp to amphotericin B and miconazole will greatly hamper patient care because there are only a few antifungal drugs available (24). Exposure to drugs in the form of prophylaxis, repeated or long-term treatment, the presence of a permanent urinary catheter or central catheter, poor compliance with treatment regimens, could be associated with the emergence of this resistance (41, 70,72). Free access to antifungals, particularly imidazoles, within our pharmaceutical stores, facilitates the routine use of self-medication by patients for the management of vaginal candidiasis and skin mycoses.

Conclusion:

Urinary tract infection was identified in more than half of patients hospitalized in the nephrology-haemodialysis department of CHUB. These patients, mainly suffering from CKD, are subject to numerous invasive maneuvers and a long hospitalization stay, favoring their infection by pathogens from the hospital environment. The emergence of multi-drug resistant bacteria to antibiotics constitutes a cause of real therapeutic impasse, especially since it occurs in a hospital environment in which these bacteria are involved in most healthcare-associated UTIs. Faced with this situation, it is important to strengthen hygiene measures in healthcare environments and promote strict rules of antibiotics prescription, distribution and consumption.

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Contributions of authors:

KBA and IT conceived the study idea and led the conduct of the study and editing of the manuscript; KBA, DEA and TJR were responsible for carrying out the bacteriological diagnostic activities; KAB and KAY were responsible for English translation activities; and KAB, IT, DEA, TJR, KAY, SM, and SI were responsible for the final editing of the manuscript. All authors approved the final manuscript submitted for publication.

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References:

1. Diallo, M., Barry, H., Baldé, C. M., and Sow, L.

Situation des évènements indésirables graves liés aux soins dans les établissements de santé en milieu rural Guinéen. Conférence Internationale sur la Prévention et le Contrôle de l'Infection (ICPIC2011) Genève. 2011; 10. www.icpic2011.com
2. Bagheri, N. S., Allegranzi, B., Syed, S. B., Ellis, B., and Pittet, D. Health-care associated infection in Africa: a systematic review. Bull World Health Organ, 2011; 89(10): 757-765. doi:10.2471/BLT.11.088179
3. Quenon, J. L. Guide de définition des infections nosocomiales, C-CLIN Paris-Nord. 1995: 1-120
4. Pavese, P. Infections urinaires nosocomiales: définition, diagnostic, physiopathologie, prévention, traitement. Médecine et Maladies infectieuses. 2003; 33 (l4): 266-274. doi:10.1016/S0399-077X(03)00159-8
5. Zoehinga, P. Les infections nosocomiales dans le service de néphrologie et hémodialyse du Centre Hospitalier Universitaire Yalgado-Ouedraogo (CHU-YO). Thèse Médicine, Université Joseph Ki-Zerbo, 2015
6. Chemlal, A., Ismaili, F. A., Karimi, I., et al. Les infections urinaires chez les patients insuffisants rénaux chroniques hospitalisés au service de néphrologie: profil bactériologique et facteurs de risque. Pan Afr Med J. 2015; 1-7. doi:10.11604/pamj.2015.20.100.4356
7. Sobotova, D. Urinary tract infections and chronic renal failure. Vnitr Lek. 2011; 57 (7-8): 626-630.
8. Ministère de la Santé et des Sports de France. Infections nosocomiales: nouvelles mesures de lutte et classement des établissements de santé & Mise en place des indicateurs de sécurité du patient et de qualité des soins. 2009; 1-51
9. Organisation Mondiale de la Santé. Résistance aux antimicrobiens, 2021. https://www.who.int/fr/news-room/fact-sheets/detail/antimicrobial-resistance
10. Institut Pasteur. Forte augmentation de la résistance aux antibiotiques au Cambodge, Le journal de la Recherche 2017. https://www.pasteur.fr/fr/institut-pasteur/institut-pasteur-monde/actualites/forte-augmentation-resistance-aux-antibiotiques-au-cambodge
11. Société Française de Microbiologie. Référentiel en microbiologie médicale (bactériologie et mycologie), 6ème édition Tome I; 2018
12. Zahar, J. R, Prévention du risque infectieux chez les patients atteints d'insuffisance rénale chronique. Néphrologie & Thérapeutique. 2019; 1 (15): 21-26 https://doi.org/10.1016/j.nephro.2019.03.004
13. Boulard, G., Ravussin, P., and Humayou J. Prévention de l'infection urinaire nosocomiale au cours du sondage vésical. Annales Françaises d'Anesthésie et de Réanimation, 1992; 11 (6): 720-723. https://doi.org/10.1016/S0750-7658(05)80797-X
14. Caron, F., Galperine, T., Flateau, C., et al. Practice guidelines for the management of adult community-acquired urinary tract infections. Med Mal Infect. 2018; 48 (5): 327-358 doi: 10.1016/j.medmal.2018.03.005
15. Some, N. B. Aspects cliniques, paracliniques et devenir à court et moyen termes des patients hospitalisés et suivis pour insuffisance rénale aigue dans le service de Néphrologie et Hémodialyse du CHU-YO, Thèse Médecine, Université Joseph Ki-Zerbo, 2017.
16. Isnard, C. Infections du tractus urinaire à pathogènes émergents. Journal des Anti-infectieux, 2015; 17 (4): 152-161. https://doi.org/10.1016/j.antinf.2015.10.002
17. Öztürk, R., and Murt, A. Epidemiology of urological infections: a global burden. World J Urol. 2020; 38 (11): 2669-2679. doi: 10.1007/s00345-019-03071-4
18. Institut Pasteur. *Escherichia coli* entérohémorragiques (ECEH), 2015. https://www.pasteur.fr/fr/centre-medical/fiches-maladies/escherichia-coli
19. Institut Pasteur. La population cachée des klebsielles, bactéries multirésistantes colonisatrices du tube digestif humain. 2020 https://www.pasteur.fr/fr/journal-recherche/actualites/population-cachee-klebsielles-bacteries-multiresistantes-colonisatrices-du-tube-digestif-humain
20. Kafando, H. Etiologies des infections urinaires dans les services d'Urologie et de Néphrologie et Hémodialyse du Centre Hospitalier Universitaire Yalgado Ouedraogo (CHU YO). Thèse Pharmacie, Université Joseph Ki-Zerbo, 2014.
21. Habou, B. U. Caractérisation clinique des bactériuries et profil de résistance aux antibiotiques des germes isolés sur les ECBU des patients pris en charge dans le service de Néphrologie et Hémodialyse du CHU-YO. Thèse Médecine. Université Joseph Ki-Zerbo, 2020.
22. Bernabé, K. J., Langendorf, C., Ford, N., Ronat, J. B., and Murphy, R. A. Antimicrobial resistance in West Africa: a systematic review and meta-analysis. Int J Antimicrob Agents. 2017; 50 (5): 629-639. doi:10.1016/j.ijantimicag.2017.07.002
23. Institut Pasteur. Les contacts humains jouent un rôle majeur dans la propagation de certaines infections nosocomiales. 2019. https://www.pasteur.fr/fr/journal-recherche/actualites/contacts-humains-jouent-role-majeur-propagation-certaines-infections-nosocomiales
24. Perlin, D. S., Rautemaa-Richardson, R., and Alastruey-Izquierdo, A. The global problem of antifungal resistance: prevalence, mechanisms, and management. Lancet Infect Dis. 2017; 17 (12): 383-392. doi:10.1016/S1473-3099(17)30316-X.

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Original Article **Open Access**

Phenotypic and genotypic detection of antimicrobial-resistant bacterial pathogens from patients with infectious keratitis in selected hospitals in Ilorin, Nigeria

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Abstract:

Background: Infectious keratitis is a major cause of global blindness. Standard management approaches typically involve the collection of corneal cultures and initiation of broad-spectrum antimicrobials. However, conventional microbiological techniques, based on direct visualization or cultures of microorganisms, are limited by poor sensitivity and the prolonged time required to produce actionable results. Molecular methods based on nucleic acid amplification technique aim to circumvent the challenges of culture for hours or days. The objectives of this study are to detect the bacteria agents of infectious keratitis in Ilorin, Nigeria, using phenotypic and molecular methods, and to determine their resistance profiles to selected antimicrobials.
Methodology: This was a cross-sectional study of selected patients with clinical features of infectious keratitis attending the ophthalmology clinics of the University of Ilorin Teaching Hospital, Sobi specialist eye hospital, and the Civil Service clinic, in Ilorin, Kwara State, Nigeria, from July 2015 to July 2018. Corneal scraping samples were collected from the patients for conventional and molecular microbiological assessments. Antibiotic susceptibility testing to selected antibiotics was determined on each bacterial isolate by the Kirby-Bauer disc diffusion method. Methicillin-resistance among *Staphylococcus aureus* and extended spectrum beta-lactamases (ESBLs) among Gram-negative bacilli isolates, were detected by both phenotypic and genotypic testings. Data were analyzed by the Statistical Package for the Social Sciences (SPSS) version 20.0.
Results: A total of 81 patients with infectious keratitis were selected, with 59 (72.8%) males and 22 (27.2%) females. A total of 79 corneal scrapings yielded microbial isolates with 66 bacteria and 13 fungi. Out of the 66 bacterial isolates, Gram-positive bacteria (GPB) accounted for 28 (42.4%), with *S. aureus* 14 (21.2%) and coagulase negative staphylococci 10 (15.2%), while Gram-negative bacteria (GNB) accounted for 38 (57.6%). The most resistant isolates to the selected antibiotics are *S. aureus* (50.0%, 7/14), *Escherichia coli* (50.0%, 1/2), *Klebsiella pneumoniae* (25.0%, 1/4) and *Pseudomonas aeruginosa* (25.0%, 1/4). ESBL genes were harbored by 7 isolates of *K. pneumoniae*, *Klebsiella oxytoca, E. coli,* and *Citrobacter freundii*. Three of these harbored 3 ESBL (bla_{CTX-M}, bla_{SHV}, bla_{TEM}) genes each while 4 harbored 2 ESBL (bla_{CTX-M}, bla_{TEM}) genes each. Two (14.3%) of the 14 *S. aureus* isolates harbored *mecA* gene (MRSA).
Conclusion: *Staphylococcus aureus* was the predominant bacterial pathogen of infectious keratitis in Ilorin, Nigeria, a few of which harbored *mec*A gene. *Staphylococcus, Klebsiella* and other GNB were resistant to the commonly used antibiotics tested in the study.

Keywords: infectious keratitis, antimicrobial resistance, ESBL, MRSA

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Détection phénotypique et génotypique d'agents pathogènes bactériens résistants aux antimicrobiens de patients atteints de kératite infectieuse dans des hôpitaux sélectionnés à Ilorin, au Nigeria

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Résumé:

Contexte: La kératite infectieuse est une cause majeure de cécité mondiale. Les approches de gestion standard impliquent généralement la collecte de cultures cornéennes et l'initiation d'un traitement antimicrobien à large spectre. Cependant, les techniques microbiologiques conventionnelles, basées sur la visualisation directe ou sur des cultures de micro-organismes, sont limitées par une faible sensibilité et le temps prolongé nécessaire pour produire des résultats exploitables. Les méthodes moléculaires basées sur la technique d'amplification des acides nucléiques visent à contourner les défis de la culture pendant des heures ou des jours. Les objectifs de cette étude sont de détecter les agents bactériens de la kératite infectieuse à Ilorin, au Nigéria, à l'aide de méthodes phénotypiques et moléculaires, et de déterminer leurs profils de résistance à certains antimicrobiens.
Méthodologie: Il s'agissait d'une étude transversale portant sur des patients sélectionnés présentant des caractéristiques cliniques de kératite infectieuse fréquentant les cliniques d'ophtalmologie de l'hôpital universitaire d'Ilorin, de l'hôpital ophtalmologique spécialisé de Sobi et de la clinique de la fonction publique, à Ilorin, dans l'État de Kwara, au Nigeria, de De juillet 2015 à juillet 2018. Des échantillons de grattage cornéen ont été collectés auprès des patients pour des évaluations microbiologiques conventionnelles et moléculaires. Les tests de sensibilité aux antibiotiques sélectionnés ont été déterminés sur chaque isolat bactérien par la méthode de diffusion sur disque Kirby-Bauer. La résistance à la méthicilline chez *Staphylococcus aureus* et la bêta-lactamase à spectre étendu (BLSE) parmi les isolats de bacilles à Gram négatif ont été détectées par des tests phénotypiques et génotypiques. Les données ont été analysées par le progiciel statistique pour les sciences sociales (SPSS) version 20.0.
Résultats: Au total, 81 patients atteints de kératite infectieuse ont été sélectionnés, dont 59 (72,8%) hommes et 22 (27,2%) femmes. Un total de 79 raclages cornéens ont donné des isolats microbiens comprenant 66 bactéries et 13 champignons. Sur les 66 isolats bactériens, les bactéries à Gram positif (GPB) représentaient 28 (42,4%), avec *S. aureus* 14 (21,2%) et les staphylocoques à coagulase négative 10 (15,2%), tandis que les bactéries à Gram négatif (GNB) représentaient pour 38 (57,6%). Les isolats les plus résistants aux antibiotiques sélectionnés sont *S. aureus* (50,0%, 7/14), *Escherichia coli* (50,0%, 1/2), *Klebsiella pneumoniae* (25,0%, 1/4) et *Pseudomonas aeruginosa* (25,0%, 1/2). Les gènes de BLSE étaient hébergés par 7 isolats de *K. pneumoniae, Klebsiella oxytoca, E. coli* et *Citrobacter freundii*. Trois d'entre eux hébergeaient chacun 3 gènes de BLSE (bla_{CTX-M}, bla_{SHV}, bla_{TEM}) tandis que 4 hébergeaient chacun 2 gènes de BLSE (bla_{CTX-M}, bla_{TEM}). Deux (14,3%) des 14 isolats de *S. aureus* hébergeaient le gène *mec*A (SARM).
Conclusion: *Staphylococcus aureus* était le pathogène bactérien prédominant de la kératite infectieuse à Ilorin, au Nigeria, dont quelques-uns abritaient le gène *mec*A. *Staphylococcus, Klebsiella* et autres GNB étaient résistants aux antibiotiques couramment utilisés testés dans l'étude.

Mots clés: kératite infectieuse, résistance aux antimicrobiens, BLSE, SARM

Introduction:

Infectious keratitis is also known as corneal infection and the fifth leading cause of vision impairment and blindness globally (1). The incidence of infectious keratitis is estimated at 2.5-799 per 100,000 population-year, with a significantly higher incidence reported in low-and-middle-income-countries (LMICs). It has resulted in about 5 million cases of blindness and/or significant vision impairment and is estimated to account for 1.5-2.0 million cases of monocular blindness per year (2). The predisposing factors of infectious keratitis vary with geographical location. The leading predisposing factors to infectious keratitis in Iraq include cornea abrasions and ocular surface disorders such as dry eye, trichiasis and old scars after a healed wound or ulcer (3). In Nigeria, there has been variation in the pattern of infectious keratitis as reported by the previous researchers (4,5).

Infectious keratitis is a painful and potentially sight-threatening condition that often requires medical intervention (2,6). A timely and accurate diagnosis is often the key to a successful clinical outcome. The cornea scrapings for laboratory diagnosis are obtained in minute quantity from the patient's eye and is usually insufficient for the conventional microbiological diagnosis of the disease. Therefore, there is need for a fast and accurate diagnostic method for infectious keratitis. Advances in molecular biology have produced culture independent diagnostic tests. Molecular methods such as nucleic acid amplification like polymerase chain reaction (PCR), and hybridization techniques, aim to circumvent the challenges of culturing for hours or days in the case of bacteria and weeks in the case of moulds (7).

Proper treatment of infectious keratitis requires antimicrobials that target the organism responsible for a patient corneal ulcer (8). Antibacterial susceptibility testing provides a quantitative measurement of susceptibility, which can be used to monitor emergence and prevalence of antibacterial resistance in microbial population (9-12). The development of resistance to antibacterial agents by microorganisms could be due to production of enzymes such as beta-lactamases including the extended spectrum beta-lactamases (ESBLs), which are carried by genes on extra-chromosomal DNAs called resistant plasmids (R-plasmids) (13,14). Most ESBL genes belong to class A beta-lactamases which can be divided into genotypes; TEM, SHV, CTX-M and OXA (15). Resistance in methicillin-resistant *Staphyloco-*

ccus aureus (MRSA) is due to production of mutant penicillin-binding protein 2a (PBP2a or PBP2') encoded by *mec*A gene which is within the particular chromosomal region called Staphylococcus Cassette Chromosome (SCC*mec*) (16). Amplification of *mec*A gene can be done by PCR, which is the 'gold standard' method for detection of MRSA.

There is need to conduct research that will enable rapid detection of the increasingly emerging and re-emerging antibiotic-resistant bacterial infections, which have become great challenge and threat to public health in both the developed and developing nations of the world (8). The objectives of this study therefore are to detect the bacteria agents of infectious keratitis in Ilorin, Nigeria, using phenotypic and molecular methods, and to determine their antimicrobial resistance profiles to commonly used antimicrobial agents.

Materials and method:

Study setting:

This study was conducted at the Ophthalmology clinic of the University of Ilorin Teaching Hospital (UITH), Sobi Specialist Hospital, and Civil Service Clinic, which serve as referral hospitals in Kwara State, northcentral Nigeria, between July 2015 and July 2018.

Ethical consideration:

Ethical approval for the study was obtained from the Ministry of Health, Ilorin, Kwara State with approval code: MOH/KS/EC/777/88/24. Informed consent was also obtained from each participant.

Study participants and selection criteria:

A complete history was taken from each participant with regards to eye pain, photophobia, watering, and redness. Duration of symptoms and history of predisposing factors such as trauma, contact lens wear, dry eye, and surgery were noted. Ocular examination included visual acuity (VA) of both eyes, and slit lamp examination of the cornea for size, site, and depth of the ulcer, presence or absence of perforation. Fluorescein staining of the corneal ulcer for epithelial defect measurements and the presence or absence of hypopyon was also determined. The inclusion criteria was patient having corneal disease but with no immunosuppressive disease.

The exclusion criteria include patients with previous history of corneal disease, patients on antimicrobial medication prior to presentation, patients who declined to participate in the study and eyes with clinically suspected viral and parasitic corneal ulcers.

Cornea sample collection:

After explaining the procedure to the patient, corneal scrapings were collected from selected participants with infected cornea by the Ophthalmologist using 23G sterile needle under Slit Lamp Biomicroscope after instillation of non-preservative topical anaesthesia into the infected eye. During the corneal debridement, two corneal scrapings were directly inoculated into Brain Heart Infusion (BHI) broth and Tris EDTA buffer (which was stored at -80°C).

Other corneal scrapings were smeared directly on two separate glass microscope slides for Gram staining and a final scraping was directly placed into a sterile centrifuge tube and immediately transported to the medical microbiology and parasitology laboratory of the University of Ilorin Teaching Hospital, where it was stored at -4°C until transported to the molecular research laboratory of Obafemi Awolowo University, Ile-Ife, Nigeria, for analysis by PCR.

Culture isolation and identification of isolates:

The smears of the samples made on the slide was Gram stained for cellular morphology in accordance with Sagar (17), while the remaining sample was streaked directly onto Blood, Chocolate and MacConkey agar plates, which were incubated for 24 hours at 37°C. Identification of microbial isolates was done by conventional biochemical test schemes.

Antibiotic susceptibility test of isolates:

Antibiotic susceptibility test (AST) was performed on each isolate against selected commonly used antibiotics by the Kirby-Bauer disc diffusion method (18) on Muller Hinton agar (Oxoid, UK). The antibiotic discs used include ceftazidime (30μg), ceftriaxone (30μg), cefuroxime (30μg), ciprofloxacin (5μg), amoxicillin-clavulanic acid (30μg), erythromycin (15μg), and gentamicin (10μg).

Inoculum of pure colonies of each isolate, standardized to 0.5 McFarland turbidity standards, was spread on Mueller-Hinton (MH) agar plates and allowed to air-dry at room temperature. The antibiotic discs were placed on the inoculated agar plates and incubated at 37°C for 18 to 24 hours. The diameter of inhibition zone of each antibiotic tested against each isolate was measured using graduated meter rule and interpreted as sensitive, intermediate or resistant, according to the CLSI guidelines (19).

Phenotypic detection of extended spectrum beta-lactamase (ESBL):

Gram-negative bacteria isolate resistant to at least two third generation cephalosporin antibiotics in the AST were presumptively identified as ESBL-producers. Phenotypic confirmation of ESBL production was done by the combination disc diffusion method using the combination of cefotaxime (30μg) disc and cefotaxime/clavulanic acid (30/10μg) disc or the combination of ceftazidime (30μg) disc and ceftazidime/clavulanic acid (30/10μg)

disc (Mast, UK) according to the CLSI guidelines for non-fastidious bacteria (19).

A 0.5 McFarland standard suspension of the isolates was made in sterile saline and the bacterial suspension was evenly spread unto the surface of MH agar plate, using a sterile swab stick to rim the edge of the plate. Cefotaxime (30µg) disc alone and cefotaxime/clavulanic acid (30/10 µg) disc or ceftazidime (30µg) disc alone and ceftazidime/clavulanic acid (30/10µg) disc, were placed 25 mm apart on the MH agar plate and incubated aerobically overnight at 37°C.

Zone diameters were manually measured manually with a graduated meter ruler to the nearest millimetre. A difference of greater or equal to 5mm between the inhibition zones of cefotaxime (30µg) and cefotaxime/clavulanic acid (30/10µg) discs or ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10µg) discs was confirmed to indicate ESBL production in line with CLSI recommendation (19). *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 70603, were used as controls strains.

Phenotypic detection of methicillin resistance in *Staphylococcus aureus*:

A 0.5 McFarland turbidity standard suspension of the bacteria was spread on MH agar and allowed to air-dry at room temperature. Cefoxitin disc (30µg) was placed on the inoculated agar and incubated at 37°C for 18 to 24 hours. The inhibition zone was measured using graduated meter and interpreted as sensitive or resistant according to the CLSI guideline (20). The inhibition zone of *S. aureus* <22 mm for cefoxitin was taken to be methicillin-resistant *S. aureus* (MRSA) and *S. aureus* with the zone of inhibition greater than or equal to 22 mm was regarded as methicillin-sensitive *S. aureus* (MSSA).

Molecular detection of bacterial isolates and antibiotic resistance genes by PCR:

DNA extraction:

Bacterial DNA was extracted from all phenotypically identified bacterial isolates by the boiling method according to Grupta (20). Isolates were first grown on nutrient agar for 24 hours. A single colony growth was picked, transferred to 0.1 ml sterile water, and boiled for 10 minutes in a water bath, and then centrifuged for 5 minutes at 1000 rpm. About 5µL of the supernatant was used as the template DNA for PCR.

***PCR amplification of bacterial DNA*:**

PCR amplification of the extracted DNA was carried out in a T-3000 thermocycler (Biometra, Germany) using the universal bacterial primers and specific primers as shown in Table 1. All primers were prepared by Iqaba Biotechnology, West Africa Limited. They were used in 2 sets of PCR reactions as follows; the first set was standardized using the universal bacterial primers (27-F, 1525-R). The reaction mixture contained 12.5µL of 'go tag green' master mix, 1µM of each of upstream and downstream primers, 5µL of template DNA, and nuclease free water to complete the volume to 25µL. The PCR reaction involved 20 cycles of denaturation at 90°C for 60 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 2 mins, and a final extension step at 72°C for 5 minutes.

Table 1: Oligonucleotide primers used for amplification of bacteria DNA in the study

Primer name	Primer sequence	Uses	Product size
27-F 1525-R	5-AGCTAAATTCATAGCAGAAAGC-3 5-AAGGAGGTGATCCARCC-3	Universal bacteria	1,500
PA-GS-F PA-GS-R	GACGGGTGAGTAATGCCTA CACTGGTGTTCCTTCCTATA	*Pseudomonas* species	618
PA-SS-F PA-SS-R	GGGGGATCTTCGGACCTCA TCCTTAGAGTGCCCACCCG	*Pseudomonas aeruginosa*	956
SA-F SA-R	GACAACTAGAGATAGAGCCTTCC AGTCGAGTTGCAGACTAC	*Staphylococcus aureus*	324
91E-F 13E-R	GGAATTCAAATGAATTGACGGGGGC CGGGATCCCAGGCCCGGGAACGTATTCAC	16S rRNA *Staphylococcus epidermidis*	478

Table 2: Oligonucleotide primers used for amplification of ESBL genes and *mec*A gene in the study

Gene	Primer	Sequence (5'-3')	Expected size (bp)	Annealing Temperature (^{0}C)
SHV	SHV-F SHV-R	CGCCTGTGTATTATCTCCCT CGAGTAGTCCACCAGATCCT	293	60
TEM	TEM-F TEM-R	TTTCGTGTCGCCCTTATTCC ATCGTTGTCAGAAGTAAGTTGG	403	60
CTX-M	CTX-M-F CTX-M-R	CGCTGTTGTTAGGAAGTGTG GGCTGGGTGAAGTAAGTGAC	874	60
*mec*A	*mec*A-F *mec*A-R	AAAATCGATGGTAAAGGTTGGC AGTTCTGGAGTACCGGATTTGC	533	53

The second set of PCR amplification was carried out using species-specific primers [*Pseudomonas aeruginosa* (PA-SS-F, PA-SS-R), *Staphylococcus aureus* (SA-F, SA-R) and *Staphylococcus epidermidis* (91E-F, 13E-R)] on the samples that gave positive result in the first set of PCR reactions. The reaction mixture was of the same composition but conditions of each set of primer were different. For *P. aeruginosa,* after an initial denaturation for 2 min at 95°C, 25 cycles were completed, each consisting of 20 seconds at 94°C, 20 seconds at 58°C, and 40 seconds at 72°C. A final extension of 1-minute at 72°C was applied. For *S. aureus*, 35 cycles of amplification consisted of 94°C for 15 seconds, 54°C for 1-minute, 72°C for 2 minutes, and a final extension step at 72°C for 7 minutes. For *S. epidermidis*, an initial denaturation at 94°C for 2 minutes, followed by 30 cycles of amplification (denaturation at 94^{0}C for 1-minute, annealing at 55°C for 1-minute and extension at 72°C for 2 min), and a final extension at 72°C for 5 minutes.

PCR amplification of ESBL and mecA genes:

Primers used for PCR amplification detection of resistance ESBL genes and *mec*A gene are shown in Table 2. After a hot start at 94°C for 3 minutes, amplification of the ESBL genes followed 30 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 1-minute and a final extension at 72°C for 7 minutes. For *mec*A gene amplification, the thermal cycling protocol comprised 95°C for 3 minutes, followed by 33 cycles of 94°C for 1-minute, 53°C for 30 seconds and 72°C for 1-minute, with a final extension at 72°C for 6 minutes.

Gel electrophoresis of DNA amplicons:

The PCR amplicons were examined following electrophoresis at 120 volts for 30 minutes on 2% agarose gel stained with ethidium bromide and the images of the amplicon band sizes were visualized under ultraviolet illumination in a Gel Documentation System.

Statistical analysis:

The data were presented as frequency tables, charts and figures. Data analysis was done using the Statistical Package for the Social Sciences (SPSS) version 20.0 software (Chicago, USA). Numerical variables were described by percentages, mean and standard deviation. The means of two or more than two independent variables were compared by the Student '*t*' test and analysis of variance (ANO VA) respectively. Independent categorical variables were compared using the Chi-square test. Confidence interval was set at 95% and for all statistical test, $p<0.05$ was considered significant.

Results:

A total of 81 patients with infectious keratitis were recruited with 59 (72.8%) males and 22 (27.2%) females. A total of 79 corneal scrapings yielded microbial isolates, with 66 bacteria and 13 fungi. Out of the 66 bacterial isolates, Gram-positive bacteria (GPB) accounted for 28 (42.4%), with *S. aureus* 14 (21.2%) and coagulase negative staphylococci 10 (15. 2%), while Gram-negative bacteria (GNB) accounted for 38 (57.6%) (Table 3).

Staphylococcus aureus isolates exhibited high resistance to amoxicillin-clavulanic acid (52.4%), cefuroxime (51.7%), ceftriaxone (47.6%) and ciprofloxacin (40.0%), but low resistance rate to gentamicin (1.0%). Coagulase negative staphylococci exhibited low resistance to amoxicillin-clavulanic acid (14.2%) and cefuroxime (14.1%) while other isolates exhibited lower resistance (<10.6%) to all the tested antibiotics. *Klebsiella* spp exhibited low resistance to ceftazidime (15.9%), ceftriaxone (14.2%) and amoxicillin-clavulanic acid (14.2%) and much lower resistance rate to ceftazidime (<10.0%).

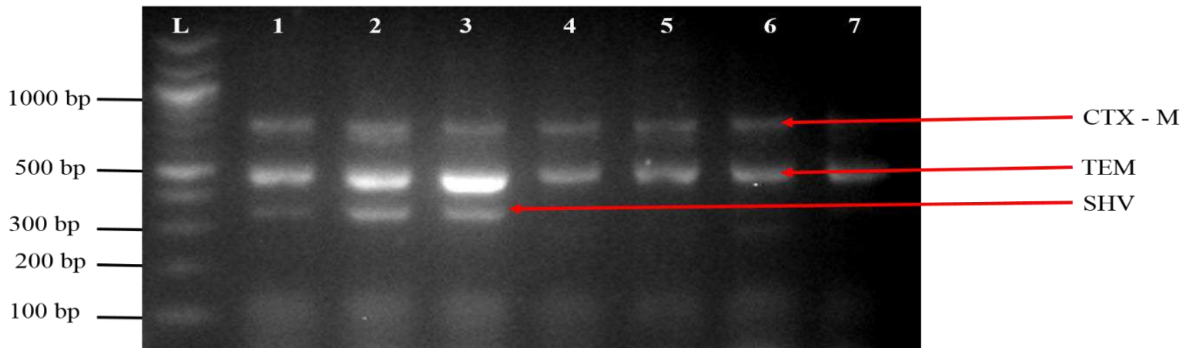
Molecular analysis of the ESBL producing GNB by PCR shows that *K. pneumoniae, K. oxytoca, E. coli* and *C. freundii* harbored ESBL genes (Fig 1). Three isolates harbored 3 ESBL

genes (*bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{TEM}$) each while 4 isolates harbored 2 genes (*bla*$_{CTX-M}$, *bla*$_{TEM}$) each as indicated in Table 4. Only 2 *S. aureus* isolates harbored *mec*A gene (Fig 2 & Table 5)

Table 3: Antibiotic resistance of bacteria isolates from patients with infectious keratitis from selected hospitals in Ilorin, Nigeria

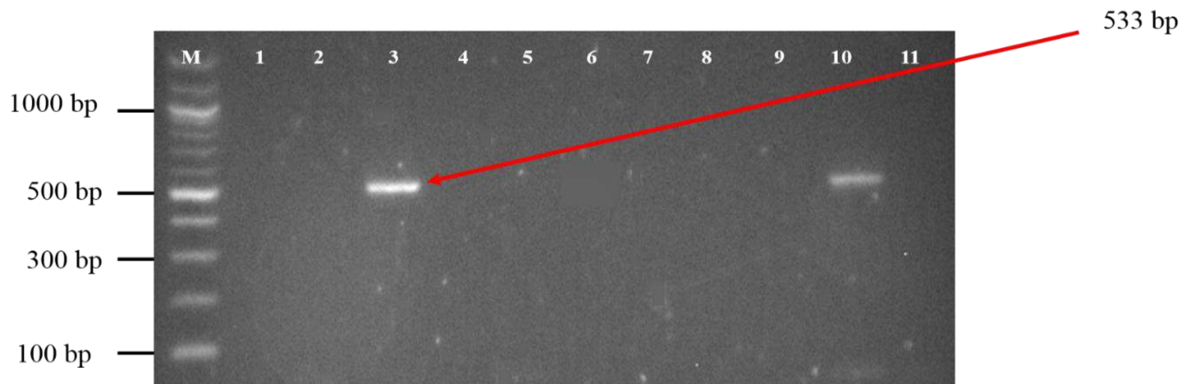
Bacteria isolates	Number of bacteria isolates	Number of resistant isolates	% of resistant strains	Antibiotic resistance in %					
				GN	CIP	CAZ	CRO	CXM	AUG
Gram positive bacteria									
Staphylococcus aureus	14	7	50.0	16.0	40.0	-	47.6	51.7	52.4
Staphylococcus auricularis	5	1	20.0	4.0	0	-	9.8	14.1	14.2
Staphylococcus xylosus	3	0	0	4.0	4.0	-	4.8	14.1	14.2
Staphylococcus epidermidis	2	0	0	0	0	-	0	4.7	0
Micrococcus spp	1	0	0	4.0	0	-	0	9.5	0
Enterococcus faecalis	1	0	0	0	0	0	0	0	0
Aeromonas salmonicida	1	0	0	8.0	4.0	5.3	4.8	4.7	0
Sub total	**28**	**8**	**28.6**						
Gram negative bacteria									
Klebsiella pneumoniae	4	1	25.0	8.0	4.0	14.2	15.9	9.5	14.2
Klebsiella oxytoca	1	0	0	0	4.0	4.8	5.3	4.7	4.8
Pseudomonas aeruginosa	4	1	25.0	8	8.0	-	15.9	-	-
Pseudomonas luteola	5	0	0	4.0	0	-	0	-	-
Pseudomonas oryzihabitus	1	0	0	0	0	-	5.3	-	-
Pseudomonas fluorescein	1	0	0	4.0	0	-	5.3	-	-
Escherichia coli	2	1	50.0	0	8.0	9.5	10.6	9.5	9.5
Citrobacter freundii	2	0	0	0	0	0	0	0	4.8
Proteus mirabilis	1	0	0	4.0	0	4.8	5.3	4.7	4.8
Enterobacter cloacae	1	0	0	8.0	4.0	0	0	4.7	9.5
Serratia marcescen	1	0	0	4.0	0	4.8	5.3	4.7	4.8
Other GNB	8	0	0	0	0	0	0	-	0
Subtotal	**38**	**3**	**7.9**						

GN- Gentamycin (10µg), CAZ- Ceftazidime (30µg), CRO- Ceftriaxone (30µg), AUG- Augmentin/Amoxicillin-clavulanic acid (30µg), CIP- Ciprofloxacin (5µg), CXM- Cefuroxime (30µg); GNB-Gram negative bacteria



Lane L: 100bp ladder; Lane 1: Isolate 1 (CTX-M, TEM, SHV); Lane 2: Isolate 2 (CTX-M, TEM, SHV); Lane 3: Isolate 3 (CTX-M, TEM, SHV); Lane 4: Isolate 4 (CTX-M, TEM); Lane 5: Isolate 5 (CTX-M, TEM): Lane 6: Isolate 6 (CTX-M, TEM); Lane 7: Isolate 7(CTX-M, TEM)

Fig 1: Gel electrophoresis of ESBL gene PCR amplicons



Lane M: 100bp ladder; Lane 1: CS 1: Lane 2: CS 8; Lane 3: CS 7; Lane 4: CS 2; Lane 5: CS 3; Lane 6: CS 4; Lane 7: CS 5; Lane 8: CS 6; Lane 9: CS 9; Lane 10: CS 10

Fig 2: Gel electrophoresis of the PCR amplification of *mecA* gene (533bp)

Table 4: ESBL genes detected among the GNB isolates

Isolates	ESBL genes detected
Klebsiella pneumoniae	bla_{CTX-M}, bla_{SHV}, bla_{TEM}
Klebsiella oxytoca	bla_{CTX-M}, bla_{SHV}, bla_{TEM}
Escherichia coli	bla_{CTX-M}, bla_{SHV}, bla_{TEM}
Klebsiella pneumoniae	bla_{CTX-M}, bla_{TEM}
Citrobacter freundii	bla_{CTX-M}, bla_{TEM}
Escherichia coli	bla_{CTX-M}, bla_{TEM}
Klebsiella pneumoniae	bla_{CTX-M}, bla_{TEM}

GNB = Gram-negative bacteria, ESBL = extended-spectrum beta-lactamase

Table 5: *Staphylococcus aureus* isolates with *mec*A gene

Isolates	*mec*A gene (MRSA)
CS1	-
CS2	-
CS3	+
CS4	-
CS5	-
CS6	-
CS7	-
CS8	+

+ = Detected, - = Not detected, CS = Cornea scrapings

Discussion:

In this study, resistance of bacteria isolates of infectious keratitis to commonly used antibiotics was evaluated. There was relatively high resistance rate of *S. aureus* to commonly used antibiotics such as amoxicillin-clavulanic acid (augmentin) (52.4%), cefuroxime (51.7%), ceftriaxone (47.6%), ciprofloxacin (40.0%), and *K. pneumoniae* to ceftazidime (15.9%), ceftriaxone (14,2%), and amoxicillin-clavulanic acid (14.2%). However, the coagulase negative staphylococci showed low resistance to amoxicillin-clavulanic acid (14.2 %) and cefuroxime (14.1%) while other bacterial isolates showed much lower resistance (<10.6%) to the tested antibiotics. These findings indicate that *S. aureus*, *Klebsiella* spp and other Gram-negative bacilli were resistant to commonly used antibiotics and are similar with the findings of a retrospective study conducted on patients with suspected ocular infections in Harbin, China (21). There are reports of increasing resistance rates in bacteria keratitis as noted in our study. This could be caused by mis-use of antibiotic eye drop, use of counterfeit or expired drugs, and non-compliance with drug regimen for eye care, leading to poor treatment outcome.

In a study by Egrilmez and Yildirim-Theveny (14) among the coagulase-negative staphylococci (n=1475 isolates), moxifloxacin resistance was observed in 31.1% overall and in 51.5% among methicillin-resistant strains. The study emphasized that the high *in vitro* resistance rate in coagulase-negative staphylococci should be considered when treating patients with ocular infections. In a 20-year follow-up study (1991-2012) conducted in the USA, Chang et al., (13) reported an MRSA rate of 30.7% and increasing resistance to fourth-generation fluoroquinolones. This is similar to the finding of our study that showed high resistance to the commonly used antibiotics tested, with more than 40.0% resistance to amoxicillin-clavulanic acid, cefuroxime, ceftriaxone and ciprofloxacin among some GNB isolates. The study by Peng et al., (16) in the United States between 1996 and 2001 reported an increase in MRSA rate from bacterial keratitis. Similarly, in a 20-year follow-up study conducted in Taiwan, Liu et al., (22) compared susceptibility data from the years 1992-2001 with those from 2007 to 2016 and reported rising rates of antibiotic resistance among Gram-positive bacteria as well as significant increase in oxacillin resistance. Although MRSA is frequently detected in ocular infections worldwide, it has been reported that methicillin-resistant *Staphylococcus epidermidis* can also cause ophthalmic infections and blindness (23, 24). It is therefore important to have good knowledge of the antimicrobial susceptibility pattern of the microbial pathogens in a given locality, which would guide the choice of appropriate antibiotics in the management of infectious keratitis.

In this study, the predominant organism was *S. aureus* (n=14, 21.2%) and Gram-positive bacteria constituted 42.4% (n=28). In the study by Sarkar et al., (24), Gram-positive bacteria were reported in 78.5%. The lower rate of Gram-positive bacteria isolates in our study may be due to the low sample size. Getahun et al., (25) and Nithya et al., (26) reported significant variation in the prevalence of MRSA ocular infections geographically at different times. The absence of the gene-coding methicillin-resistance (*mec*A) in *Staphylococcus* species is a reliable predictor of phenotypic oxacillin susceptibility in clinical isolates and may be used to narrow therapy (27). In a study of *mec*A-positive isolates (n=234), 27 (11.5%) were phenotypically oxacillin susceptible, 7 of which had oxacillin resistant co-pathogen that explained the detection of *mec*A gene (28,29). Methicillin resistance was also reported by Das et al., (30) in 26 of 173 *S. aureus* isolates, given 15.0% of MRSA by disc testing with cefoxitin. Keratitis was the most common ocular diagnosis in Taiwan, with MR SA accounting for 36.1% (31). These studies reported higher rates than the 20.0% (2 of 10 phenotypic MRSA isolates) reported in our study. The low rate in our study may be due to small sample size as well as geographical variations in the incidence of methicillin-resistant *S. aureus*.

The ESBLs of clinical significance are the CTX-M and TEM β-lactamases families. In our study, 7 Enterobacteriacaeae isolates harbored ESBL genes, with 3 isolates harboring 3 genes (bla_{CTX-M}, bla_{SHV} and bla_{TEM}) each and 4 isolates harboring 2 genes (bla_{CTX-M} and bla_{TEM}) each. The CTX-M-type ESBL enzymes have become the most prevalent among clinical isola-

tes (mostly *E. coli*) in Asia, Europe and South America (32). Since the first recognition of the CTX-M enzyme in the clinical settings in the 1990s, over 130 variants have been identified and genetically classified based on amino acid differences into 5 major groups; CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25, mostly identified in *E. coli* and *K. pneumoniae* isolates from different geographical locations (32). ESBLs have predominantly been detected among *K. pneumoniae* and *E. coli* in almost all parts of the world. In Europe and North America, the class A ESBL gene, $bla_{CTX-M-15}$, was the most prevalent (reported in >90% of *E. coli* isolates) and in 35-65.5% of *K. pneumoniae* isolates, although bla_{SHV} and bla_{TEM}-type genes were also common (33). Available data on ESBLs show considerable geographical differences in prevalence.

Conclusion:

Our study showed that *S. aureus, Klebsiella* spp and other GNB isolated from cases of infectious keratitis were resistant to the commonly used antibiotics in Ilorin, Nigeria. *Staphylococcus aureus* was the single most common bacterial pathogen, some of which harbored the *mecA* gene that confer resistance to methicillin and other beta-lactamase resistant penicillins. The absence of *mecA* gene is a reliable predictor of phenotypic susceptibility in *S. aureus* and may be used to narrow therapy of both *S. aureus* and coagulase-negative staphylococcal ocular infections.

The fact that 7 Enterobacteriaceae isolates of infectious keratitis in our study harbored ESBL genes, support the reports of increasing antimicrobial resistance to the commonly used antibiotics for therapy of ocular infections in low-income resource countries like Nigeria.

Contributions of authors:

OOJ and OJM were involved in study conceptualization; OOJ, OJM, AA, OB, OV and TT were involved in the study methodology; OOJ, OJM, OB and OP were involved in software use and formal analysis of data; OOJ, OJM, and OB were involved in writing and review of the manuscript; OOJ was involved in project administration. All authors approved the manuscript submitted for publication.

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No conflict of interest is declared

References:

1. Flaxman, S. R., Bourne, R. R. A., Resnikoff, S., et al. Global causes of blindness and distance vision impairment 1990-2020: a systemic review and meta-analysis. Lancet Glob Health. 2020; 5: e1221-e34. doi: 10.1016/S2214-109X.
2. Ting, D. S. J., Ho, C. S., Deshmukh, R., Said, D. G., and Dua, H. S. Infectious keratitis: an update on epidemiology, causative microorganisms, risk factors, and antimicrobial resistance. Eye. 2021a; 35: 1084-1101. doi: 10.1038/s41433-020-01339-3.
3. Al-Shakarchi, F. I. Initial therapy for suppurative microbial keratitis in Iraq. Br J Ophthalmol. 2017; 95: 1583-1587. doi: 10.1136/bjo.2007.123208
4. Apakama, A. I., Onwuegbuna, A. A., Uzozie, C. C., Okoye, A. E., and Ikegbue, H. C. Suppurative corneal ulcers at a tertiary hospital Southeast Nigeria: A 5-year review. Trop J Med Res. 2022; 21 (2): 65-74. https://doi.org/10.5281/zenodo.7794910
5. Saka, S. E., Ademola-Popoola, D. S., Mahmoud, A. O., and Fadeyi, A. Presentation and outcome of microbial keratitis in Ilorin, Nigeria. Br J Med Med Res. 2015; 6: 795-803. doi:10.9734/BJMMR/2015/9889
6. Khor, W. B., Prajna, V. N., Garg, P., et al. The Asia Cornea Society Infectious: A Prospective Multicenter Study of Infectious Keratitis in Asia. 2018; 195: 161-170. doi: 10.1016/j.ajo.2018.07.040
7. Zhang, S., Li, X., Wu, J., et al. Molecular Methods for Pathogenic Bacteria Detection and Recent Advances in Waste Water Analysis. Water. 2021; 13: 3551. https://doi.org/10.3390/w13243551
8. Cheng, G., Dai, M., Ahmed, S., Hao, H., Wang, X., and Yuan, Z. Antimicrobial drugs in fighting against antimicrobial resistance. Front Microbiol. 2016; 7: 470. doi: 10.3389/fmicb.2016.00470
9. Lin, L., Duan, F., Yang, Y., Lou, B., Liang, L., and Lin, X. Nine-year analysis of isolated pathogens and antibiotic susceptibilities of microbial keratitis from a large referral eye center in southern China. Infect Drug Resist. 2019b; 12: 1295-1302. doi: 10.2147/IDR.S206831
10. Lalitha, P., Manoharan, G., Karpagam, R., et al. Trends in antibiotic resistance in bacterial keratitis isolates from South India. Br J Ophthalmol. 2017; 101: 108-113. doi:10.1136/bjophthalmol-2016-308487
11. Jin, H., Parker, W. T., Law, N. W., et al. Evolving risks factors and antibiotic sensitivity patterns for microbial keratitis at a large county hospital. Br J Ophthalmol. 2017; 101: 1483-1487. doi:10.1136/bjophthalmol-2016-310026
12. Penesyan, A., Gilling, M., and Paulsen, I. T. Antibiotic discovery: combating bacterial resistance in cells and in biofilm communities. Molecules. 2015; 20: 5286-5298. doi: 10.3390/molecules20045286
13. Chang, V. S., Dhaliwal, D. K., Raju, L., and Kowalski, R. P. Antibiotic resistance in the treatment of *Staphylococcus aureus* keratitis: a 20-year review. Cornea. 2015; 34: 698-703. doi: 10.1097/ICO.0000000000000431
14. Egrilmez, S., and Yildririm-Theveny, S. Treatment-Resistant Bacterial Keratitis: Challenges and Solutions. Clin Ophthalmol. 2020; 14: 287-297. doi: 10.2147/OPTH.S181997
15. Falgenhauer, L, Waezsada, S. E., Yao, Y., et al and RESET Consortium. Colistin resistance gene *mcr-1* in extended-spectrum beta lactamase-producing and carbapenemase-producing bacteria in Germany. Lancet Infect Dis. 2016; 47: 457-465.doi:10.1016/S1473-3099(16)00009-8
16. Peng, M. Y., Cevallos, V., McLeod, S. D., Lietman, T. M., and Rose-Nussbaumer, J. Bacterial keratitis: isolated organisms and antibiotic resistance patterns in San Francisco. Cornea. 2018; 37: 84-87.doi:10.1097/ICO.0000000000001417
17. Sagar, A. Gram Staining: Principle, Procedure, Interpretation, Examples and Animation.

(Accessed February 9, 2022).

18. Wang, L., Han, L., and Yin, W. Study of Pathogens of Fungal Keratitis and the Sensitivity of Pathogenic Fungi to Therapeutic Agents with the Disk Diffusion Method. Curr Eye Res. 2015; 40: 1095-101.doi:10.3109/02713683.2015.1056802
19. Weinstein, M. P., Patel, J. B., Campeau, S., et al. Performance standards for antimicrobial susceptibility testing, M-100. Wayne, PA. Clinical and Laboratory Standards Institute; 2018.
20. Gupta, N. DNA extraction and polymerase chain reaction. J Cytol. 2019; 36: 116-117. doi: 10.4103/JOC.JOC_110_18.
21. Xu, S., Guo, D., Liu, X., et al. Ocular pathogens and antibiotic resistance in microbial keratitis over three years in Harbin, Northeast, China. Acta Ophthalmol. 2021; 99 (8): 909-915 doi: 10.1111/aos.14789
22. Liu, H. Y., Chu, H. S., Wang, I. J., Chen, W. L., and Hu, F. R. Microbial keratitis in Taiwan: a 20-year update. Am J Ophthalmol. 2019; 205: 74-81. doi: 10.1016/j.ajo.2019.03.023
23. Nazari-Alam, A., Badie, F., Sheri, M., Moniri, R., Akbari, H., and Monsoori, M. The Bacteria Profile and Microbial Susceptibility of Acute and Chronic Dacryocystitis in Martini Hospital, Kashan, Iran. Jundishapur J Microbiol. 2021; 14: e115547. doi: 10.5812/jjm.115547
24. Sarkar, I., Choudhury, S. K., Bandyopadhyay, M., and Sarkar, K. B. A clinico-bacteriological profile of chronic dacryocystitis in Rural India. Int J Health Sci Res. 2015; 5 (7): 82-87
25. Getahun, E., Gelaw, B., Assefa, A., Assefa, Y., and Amsalu, A. Bacterial pathogens associated with externa ocular infections alongside eminent proportion of multidrug resistant isolates at the University of Gondar Hospital, Northwest Ethiopia. BMC Ophthalmol. 2017; 17: 151. doi: 10.1186/s12886-017-0548-6
26. Nithya, V., Rathinam, S., Karthikeyan, R., S. G., and Lalitha, P. A ten-year study of prevalence, antimicrobial susceptibility pattern, and genotypic characterization of methicillin resistant *Staphylococcus aureus* causing ocular infections in a tertiary eye care hospital in South India. Infect Genet Evol. 2019; 69: 203-210. doi: 10.1016/j.meegid.2019.01.031.
27. Williams, M. C., Domingue, S. R., Prinzi, A., and Leek Parker, S. K. Reliability of *mec*A gene in predicting phenotypic susceptibilities of coagulase negative staphylococci and *Staphylococcus aureus.* Open Forum Infect Dis. 2020; 7 (12) : ofaa553. doi: 10.1093/ofid/ofaa553
28. Garcia, A., Martinez, C., Juarez, R. I., et al. Methicillin resistant *Staphylococcus aureus* and coagulase-negative staphylococcus in Mexico. Biomedica. 2019; 39: 513-23. doi: 10.7705/biomedica.4131
29. Lee, J., Park, Y. J., et al. Evaluation of BD MAX Staph SR Assay for differentiating between *Staphylococcus aureus*, and coagulase-negative staphylococci and determining methicillin resistance directly from positive blood cultures. Ann Lab Med. 2017; 37: 39-44. doi: 10.3343/alm.2017.37.1.39.
30. Das, S., Samantaray, R., Mallick, A., Sahu, S. K., and Sharma, S. Types of organisms and in-vitro susceptibility of bacterial isolates from patients with microbial keratitis: A trend analysis of 8 - years. Indian J Ophthalmol. 2019; 67 (1): 49-53. doi: 10.4103/ijo.IJO_500_18
31. Petrillo, F., Pignataro, D., Lella, F. M. D., et al. Antimicrobial Susceptibility Patterns and Resistance Trends of *Staphylococcus aureus* and Coagulase-Negative Staphylococci Strains Isolated from Ocular Infections. Antibiotics. 2021; 10: 527 doi: 10.3390/antibiotics10050527
32. Rameshkumar, G., Ramakrishnan, R., Shivkumar C., et al. Prevalence and antibacterial resistance patterns of extended-spectrum beta-lactamase producing Gram-negative bacteria isolated from ocular infections. Indian J Ophthalmol. 2016; 64 (4): 303-311. doi: 10.4103/0301-4738.182943
33. Mathers, A. J., Peirano, G., and Pitout, J. D. *Escherichia coli* ST 131: the quintessential example of an international multi-resistant high-risk clone. Adv Appl Microbiol. 2015; 90: doi: 10.1016/bs.aambs.2014.09.002

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Original Article **Open Access**

Phenotypic and molecular identification of antimicrobial resistance in *Escherichia coli* and *Salmonella* species isolated from apparently healthy broilers and zoo birds in Cameroon

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Abstract:

Background: Knowledge of antimicrobial resistance patterns of bacteria in food and pet birds in our environment is a prerequisite to effective control of bacterial diseases in humans and other food animals. Particularly, there is a dearth of information on the prevalence of resistant bacteria in pet and zoo birds in Cameroon. This study was carried out to determine the antibiotic resistance profiles of *Escherichia coli* and *Salmonella* spp isolates in apparently healthy poultry and zoo birds in Cameroon and to phenotypically and genotypically identify extended-spectrum β-lactamases (ESBLs) isolates in the poultry and aviary birds.
Methodology: This was a cross-sectional study of 320 randomly selected birds, which included 172 poultry and 148 zoo birds over a period of nine months, from which a total of 320 different non-repetitive samples were collected. The specimens were processed by standard microbiological culture methods at the National Veterinary Laboratory (LANAVET), Yaoundé annex, Cameroon. All isolated bacteria from cultures were identified as *E. coli* and *Salmonella* spp by conventional biochemical test scheme and confirmed with API®20E gallery. Antibiotic susceptibility test (AST) of confirmed isolates was done using the Kirby–Bauer disc diffusion technique, with AST results interpreted according to CLSI guidelines. Isolates with phenotypic characteristics of extended-spectrum beta-lactamase were subjected to molecular identification for bla_{CTX-M}, bla_{TEM} and bla_{SHV} genes. Data obtained were analyzed using descriptive statistics.
Results: Out of the 320 samples, a total of 88 *E. coli* and 17 *Salmonella* species were isolated from both broilers and zoo birds with an overall isolation prevalence of 27.5% and 5.3% respectively. High resistance of *E. coli* was observed among isolates from broiler, especially to trimethoprim-sulfamethoxazole (96.7%), ampicillin and ticarcillin (88.3%), norfloxacin (81.7%), piperacillin (78.3%) and ceftriaxone (63.3%). However, the resistance pattern among isolates from aviary birds was low with the highest resistance observed for imipenem (39.28%). The isolates had multiple antibiotic resistance (MAR) indices between 0.18-0.94 with an average of 0.3. A striking MAR index of 0.94 was observed in an ESBL isolate. Detection of β-lactamase genes in 16 phenotypic ESBL-producing *E. coli* and *Salmonella* isolates showed the presence of 75.0%, 6.3% and 12.5% for bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes respectively.
Conclusion: ESBL isolates were widespread among apparently healthy broilers in live-bird markets in Cameroon with ESBL-producing *E. coli* and *Salmonella* species showing high resistance to penicillin, quinolones and sulphonamides. In addition, there is evidence of antibiotic-resistant bacteria in wild birds which can be transmitted to humans through fecal droppings or by being in close contact with them.

Keywords: Aviary birds, *Escherichia coli*, *Salmonella* spp, ESBL, Cameroon

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Identification phénotypique et moléculaire de la résistance aux antimicrobiens chez les espèces d'*Escherichia coli* et de

Salmonella isolées en portage chez des poulets de chair et d'oiseaux de zoo au Cameroun

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Resumé:

Contexte: La résistance aux antimicrobiens est un problème croissant dans le monde entier, avec des implications majeures pour la santé humaine et animale. Au Cameroun, l'étude de la prévalence des bactéries résistantes chez les oiseaux d'abattages et les oiseaux de zoo revêt une importance capitale pour comprendre et combattre efficacement les maladies bactériennes. Cependant, il existe un manque d'informations sur ce sujet dans ce pays. C'est dans ce contexte qu'une étude a été menée pour évaluer les profils de résistance aux antibiotiques des souches *d'Escherichia coli* et de *Salmonella* spp chez les volailles et les oiseaux de zoo sains et la caractérisation moléculaire des gènes de résistance chez les isolats de phénotype BLSE positifs.

Méthodologie: Il s'agissait d'une étude transversale portant sur 320 espèces aviaires sélectionnées au hasard qui comprenait 172 volailles et 148 oiseaux de zoo sur une période de 9 mois. Les écouvillons cloacaux ont été effectués chez ses espèces aviaires selon les procédures standards. La culture a été faite selon les techniques usuelles au Laboratoire vétérinaire national (LANAVET) annexe de Yaoundé, au Cameroun. Les isolats d'*E. coli* et *Salmonella* spp ont été confirmées a l'aide de la gallérie API20E, l'antibiogramme par la méthode de diffusion en milieu gélosé de Bauer-Kirby et la présence des gènes bla_{CTX-M}, bla_{TEM} et bla_{SHV} par PCR.

Résultats: Sur les 320 échantillons, un total de 88 souches d'*E. coli* et 17 souches d'espèces de *Salmonella* ont été isolées chez des poulets de chair et des oiseaux de zoo, avec une prévalence globale d'isolement de 27,5% et 5,3% respectivement. Une résistance élevée à *E. coli* a été observée parmi les isolats de poulets de chair, en particulier au triméthoprime-sulfaméthoxazole (96,7%), à l'ampicilline et à la ticarcilline (88,3%), à la norfloxacine (81,7%), à la pipéracilline (78,3%) et à la ceftriaxone (63,3%). Cependant, le profil de résistance parmi les isolats d'oiseaux de zoo était faible, la résistance la plus élevée ayant été observée pour l'imipénème, dont la résistance était de 39,28%. Les isolats présentaient de multiples indices de résistance aux antibiotiques (MAR) compris entre 0,18 et 0,94, avec une moyenne de 0,3. Un indice MAR frappant de 0,94 a été observé dans un isolat de BLSE. La détection de gènes de BLSE chez 15 isolats d'*E. coli* et un isolat de *Salmonella* producteurs de phénotypes positifs a montré la présence de 75.0%, 6,3% et 12,5% des gènes bla_{CTX-M}, bla_{TEM} et bla_{SHV} respectivement.

Conclusion: Les isolats de BLSE étaient répandus parmi les poulets de chair commercialise dans les marches au Cameroun. Les espèces *d'E. coli* et de *Salmonella* productrices de BLSE présentaient une résistance élevée à la pénicilline, aux quinolones et aux sulfamides. De plus, il existe des preuves de bactéries résistantes aux antibiotiques chez les oiseaux sauvages qui peuvent être transmises à l'homme par les excréments fécaux ou par contact étroit avec eux. Le séquençage des génomes complets permettra de mieux étudier les différents gènes de résistance circulant chez la volaille domestique et les oiseaux de la faune sauvage afin de faire une comparaison avec les isolats humains.

Mots clés: Volailles, *Escherichia coli*, *Salmonella* spp, BLSE, Cameroun

Introduction:

Antimicrobial resistance (AMR) is an international danger to development and health. To fulfill the objectives of the Sustainable Development Goals (SDGs), urgent multi-sectoral actions are needed. According to the WHO, among the top ten global public health hazards to humanity is AMR (1). In effect, a wide range of microorganisms exists in nature, both pathogens and commensals. They include bacteria, fungi, archaea, and protists. Commensal and pathogenic bacteria include *Escherichia coli* and *Salmonella* species (2). Salmonellosis and avian colibacillosis are regarded as the two most common bacteria diseases in the poultry industry globally and they are the most prevalent avian illnesses that can infect humans (3,4).

Antibiotics are widely used in poultry production to control infectious diseases, which consequently enhances high growth rate. This practice is reported to have caused high resistance to antibiotics by pathogenic microorganisms in poultry (5). Concerns about harmful bacteria developing high levels of antibiotic resistance due to overuse, misuse and abse of antibiotics in chicken production are spr-

eading around the globe (6). Sub-therapeutic application of antibiotics in the form of feed additives has been cited as one of the selective forces for emergence of antibiotic resistance (2,7). Resistant genes find their way into the environment hence may be transferred to other livestock, human and even get into wild animals which end up serving as reservoirs of such resistant genes (8).

Zoo birds are wild/captive birds that are kept in conservational centers, which serve as a protected habitat to protect them or preserve the endangered species from extinction. Environment is one of the most significant owner factors on the health of a zoo bird (9). Wild birds are important with regard to antibiotic resistance because they can move across great distances quickly. They may act as potential carriers of antibiotic resistance and they can also act as a store house and melting pot for genes and microorganisms resistant to antibiotics (10). These resistant bacteria may serve as a potential source of resistant genes that are subsequently transmitted to human pathogens by the process of conjugation (8,11). This study was carried out to identify antibiotic resistance in microbial reservoirs in birds so as to be able to address the increasing problem of antibiotic resistance in human, wildlife and livestock pathogens.

Materials and method:

Study area:

This study was carried out in the Center, Southwest and North regions of Cameroon. The choice of study area was based on the fact that these regions represent the hub of poultry production in Cameroon and hosts the three zoological centers in the country.

Samples from live bird markets were collected in poultry markets in Yaoundé in the Center region while samples from zoo birds were collected in Mvog-betsi botanical and zoological garden in the Center region, Limbe wildlife center in the Southwest region and Garoua zoological garden in the North region.

Study design and period:

This study was a cross-sectional design involving 320 avian species; 172 broilers and 148 zoo birds. The study was conducted over a period of nine months (14 January 2023 to 19 September 2023).

Ethical approval and authorization for study:

The Regional delegation of livestock, Fisheries and Animal industries (DREPIA) for the Centre region, authorized the collection of samples from poultry markets (N^0 54/2023/L /MINEPIA/DREPIA-CE/DDEPIA-MFD) and the Ministry of Forestry and Wildlife gave authorization for samples to be collected from the three zoos in Cameroon (N^0 1783/L/MINFOF/ SETAT/SG/DFAP/SDVEF/SC/ENJ).

Sample size:

The sample size was calculated using the Thrusfield formula (12), which gave a calculated minimum sample size of 257. Nevertheless, a total number of 320 avian bird species were sampled in our study.

Data and sample collection:

Rectal swabs were taken from the selected avian species by holding the bird with its head down and its posterior end facing up for easy location of the cloaca. The swab was then removed carefully and placed in sterile tubes containing 0.5ml Normal Saline to keep the content moist. The tubes were placed in cooler containing ice packs and then transported to National Veterinary Laboratory (LANAVET) for microbiological analysis.

Culture and isolation of *Escherichia coli* and *Salmonella* species:

Samples were pre-enriched in peptone water broth and the solution was spread using a sterile bacteriological loop onto MacConkey and *Salmonella-Shigella* (SS) agar plates, and incubated at 37^{0}C for 18 to 24 hours. *E. coli* grew on MacConkey agar as non-lactose fermenter (pink color colonies) while *Salmonella* spp grew on *Salmonella-Shigella* agar as transparent colonies with dark centers.

Colonies were identified as Gram-negative bacilli or coccobacilli on Gram staining while preliminary identification was done by conventional biochemical tests (13). Presumptively identified isolates were confirmed using API®20 E gallery and the identification validated by its analytic catalogue. *Escherichia coli* ATCC 352218 and *Salmonella* spp ATCC 14028 were used as controls for each test protocol.

Antimicrobial sensitivity testing:

The antimicrobial susceptibility (AST) of each isolate was done by the disk diffusion method of Kirby-Bauer (14). Overnight colony suspension of each isolate was prepared in nutritional broth and compared to the turbidity of 0.5 McFarland standards. Mueller-Hinton agar plates were inoculated with the organism suspension using a sterile swab stick, and pre-diffusion was allowed to occur for 30 minutes. The following antibiotics were placed on the inoculated MH agar surface; ampicillin (AMP 10µg), ticarcillin (TIC 75µg), piperacillin (PIP 30µg), piperacillin-tazobactam (TZP 10-100µg, ceftazidime (CAZ 10µg), amoxicillin-clavulanic acid (AMC 30µg), cefotaxime (CTX 5µg) aztreonam (ATM 30 µg), cefepime (FEP 30µg), imipenem (IPM 10µg), ceftriaxone (CRO 30µg), norfloxacin (NOR 10µg), cefoxitin (FOX 30µg), ciprofloxacin (CIP 5µg) and sulfamethoxazole-trimethoprim (SXT 23.75/1.25µg). The isola-

tes were categorized as susceptible or resistant in accordance with the Clinical and Laboratory Standards Institute guidelines after the inhibition zone diameters were measured with a vernier caliper (15).

Multi-drug resistance (MDR) of *E. coli* and *Salmonella* spp was taken as simultaneous resistance to three or more classes of antibiotics (16). The formula for calculating and interpreting the multiple antibiotic resistance index (MARI) was MARI=a/b (17), where 'a' represents the number of antibiotics to which a specific isolate was resistant to, and 'b' represents the total number of antibiotics tested against the isolate.

Double disk synergy test:

ESBL-producing isolate was identified by the double disk synergy test using a combination of amoxicillin-clavulanic acid disc and a third or fourth generation cephalosporin (15). A standardized inoculum of each isolate was used to inoculate MH agar plate. Amoxicillin-clavulanic acid disc was placed at the center and ceftriaxone (30μg) or ceftazidime (30μg) or cefotaxime (30μg) or aztreonam (30μg) disc was placed around the disc. The plate was incubated for 16 to 24 hours at 35± 2°C (18). The result was considered positive if the zone of inhibition of the cephalosporin disc increased towards the amoxicillin-clavulanic acid disc, producing a characteristic "Champagne cork" or "keyhole" effect.

Molecular identification of ESBL isolates

Molecular identification of phenotypically positive ESBL isolates was done by the quantitative real time PCR assay. The DNA of the isolates was first extracted using commercial extraction kit (QIAGEN©, Germany) at the molecular biology unit of LANAVET in accordance with the manufacturer guideline. The different reagents were reconstituted before the extraction process using 95% alcohol. After extraction, the presence of DNA in the samples was verified by electrophoretic migration on 1% agarose gel.

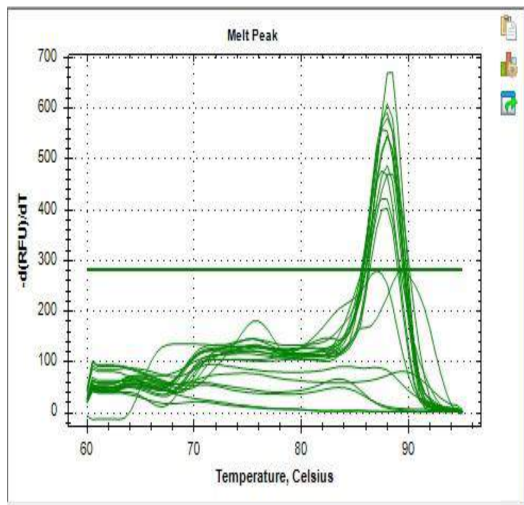
Amplification by Real time PCR assy

Simplex real time PCR (rt PCR) was used to amplify the genes encoding extended spectrum beta lactamases (*bla*$_{CTX-M}$, *bla*$_{TEM}$, *bla*$_{SHV}$) using the primers shown in Table 1. With the use of readily accessible commercial qPCR mixes and SYBR Green universal master mix, we created a simplex real-time qPCR test combined with melt curve analysis in a thermo cycler connected to a computer. About 4 μL of the extracted DNA, 10 μL of Luna universal qPCR Mix, 1 μL of primer pairs, and 5 μL of nuclease-free water made up the PCR reaction mixture, which had a total volume of 20 μl. All the genes were amplified using the following PCR conditions; initial denaturation for one minute at 95°C, 40 cycles of denaturation for fifteen seconds at 95°C, extension for thirty seconds at 60°C, and a melt curve (Tm) for 60°C, with Tm separation of $> 2^{\circ}$C deemed adequate (19).

Prior to the assay, research has produced high resolution melting curve tests for the typing and subtyping of microbiological species and beta lactamases which were typically isolated from specific medical environments (20). The amplicons with similar melting temperatures (Tm) clustered in a successive manner with each other. The melt curves successfully detected the genes from isolates containing ESBL genes, with *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ showing melting temperatures at 87.5-88^{0}c, 85^{0}c and 75^{0}c respectively (Figs 1, 2 & 3).

Table 1: Specific primers used for PCR assy

Target gene	Primer sequence (5'-3")	Amplicon size	Hybridization temperature	Reference
bla$_{SHV}$	F: TCGCCTGTGTATTATCTCCC R: CGCAGATAAATCACCACAATG	768	58	19
bla$_{TEM}$	F: GCGGAACCCCTATTTG R: ACCAATGCTTAATCAGTGAG	964	55	19
bla$_{CTX-M}$	F: ATGTGCAGYACCAGAARGTKTGC R: TGGGTRAARTARGTSACCAGAAYSAGCGG	592	55	19
bla$_{CTX-M-1}$	F: GGTTAAAAAATCACTGCGTC R: TTGGTGACGATTTTAGCCGC	863	55	19
bla$_{CTX-M-2}$	F: GATGAGACCTTCCGTCTGGACAG AAA R: CCGTGGGTTACGAT	397	55	19



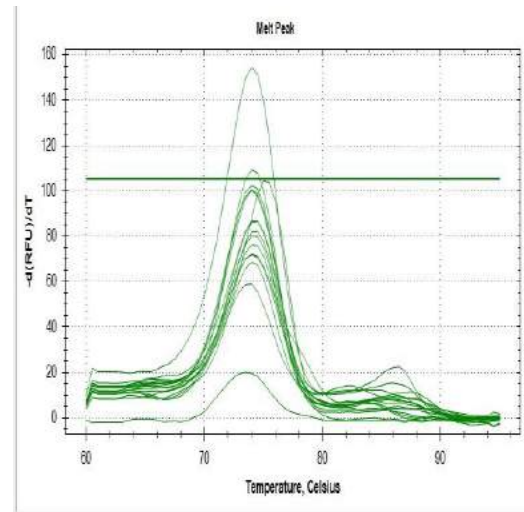


Fig 1: qRT-PCR result (melt curve analysis) of *bla*$_{CTX-M}$ (12 positive samples) Fig 2: qRT-PCR result (melt curve analysis) of *bla*$_{SHV}$ (1 positive sample)

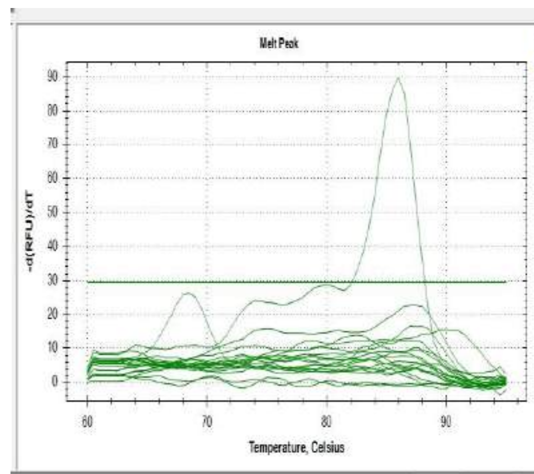


Fig 3: qRT-PCR result (melt curve analysis) of *bla*$_{TEM}$ (2 positive samples)

Results:

Out of the 320 avian species sampled, a total of 88 *E. coli* and 17 *Salmonella* spp were isolated from broilers and zoo birds, with an overall isolation prevalence of 27.5% and 5.3% respectively. Of the 88 *E. coli* isolates, 60 (68.2%) were from live bird markets (broilers) while 28 (31.8%) were isolated from the zoo birds. Of the 17 *Salmonella* spp isolates, 13 (76.5%) were from broilers while 4 (23.5 %) were from zoo birds.

The resistance profile of *E. coli* isolates with respect to animal species showed higher resistance rates among isolates from broilers, especially to trimethoprim-sulfamethoxazole (96.7%), ampicillin and ticarcillin (88.3%), norfloxacin (81.7%), piperacillin (78.3%) and ceftriaxone (63.3%), compared *to E. coli* isolates from aviary birds, in which resistance rates were low to these respective antibiotics (Fig 4), with the highest resistance rates observed against imipenem (39.28%) and piperacillin-tazobactam (38.0%). Isolates from zoo birds showed 100.0% susceptibility to ciprofloxacin, aztreonam and cefotaxime.

As shown in Fig 5, of the fifteen antibiotics tested, *Salmonella* spp from broilers were most resistant to trimethoprim-sulfamethoxazole and ciprofloxacin (84.6%), ticarcillin (69.2%), ampicillin (69.2%) and norfloxacin (69.2%) whereas in zoo birds, the isolates were 100.0% sensitive to most of the cephalosporins (ceftazidime, cefotaxime, cefoxitin), as well as to piperacillin, piperacillin-tazobactam, amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole.

The prevalence of MDR in the isolates was 77.1% (81/105). The predominant MARI phenotype was in the following antibiotic classes; penicillin, quinolones and sulphonamides. Of the total of 81 MDR isolates, 11 (34.4%) were from zoo birds, while 70 (95.9%) were from broilers. Sixty-five (80.24%) *E. coli* isolates and 16 (19.75%) *Salmonella* species were MDR. The isolates had MAR indices between 0.18-0.94. A striking MAR index of 0.94 was observed in an ESBL isolate**.**

The phenotypic characteristics of the MDR ESBL revealed that 16 isolates were ESBL producers, out of which 15 were *E. coli* and 1 was *Salmonella* species. There were 75.0% (n=12), 6.3% (n=1) and 12.5% (n=2) of $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV} genes, respectively from the 16 phenotypic ESBL producing *E. coli* and *Salmonella* species isolates (Table 2).

The simultaneous presence of two (bla_{TEM}, bla_{SHV}) and three ($bla_{CTX\text{-}M}$, bla_{CTXM1}, $bla_{CTX\text{-}M2}$) ESBL genes in a single isolate occurred in 12.5% (n=2) and 62.5% (n=10) isolates, respectively (Table 2). One of the phenotypically ESBL positive isolate (6.3%) was negative for bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ genes.

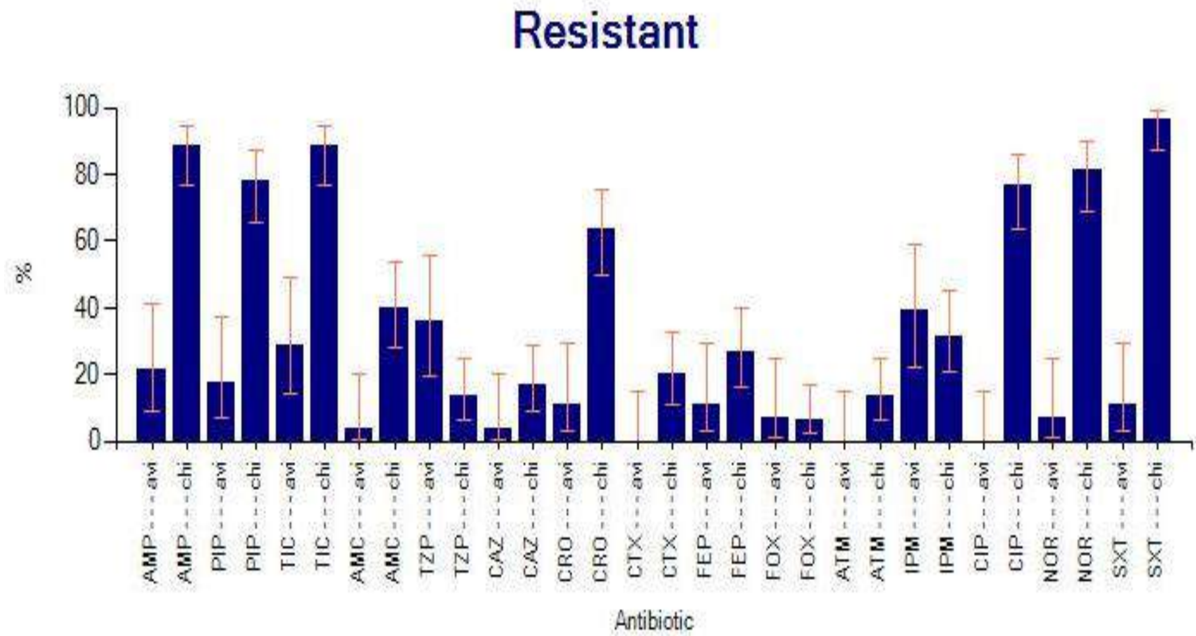


Fig 4: Comparative antibiotic resistance of *Escherichia coli* isolates from broilers (chi) and zoo (avi) birds

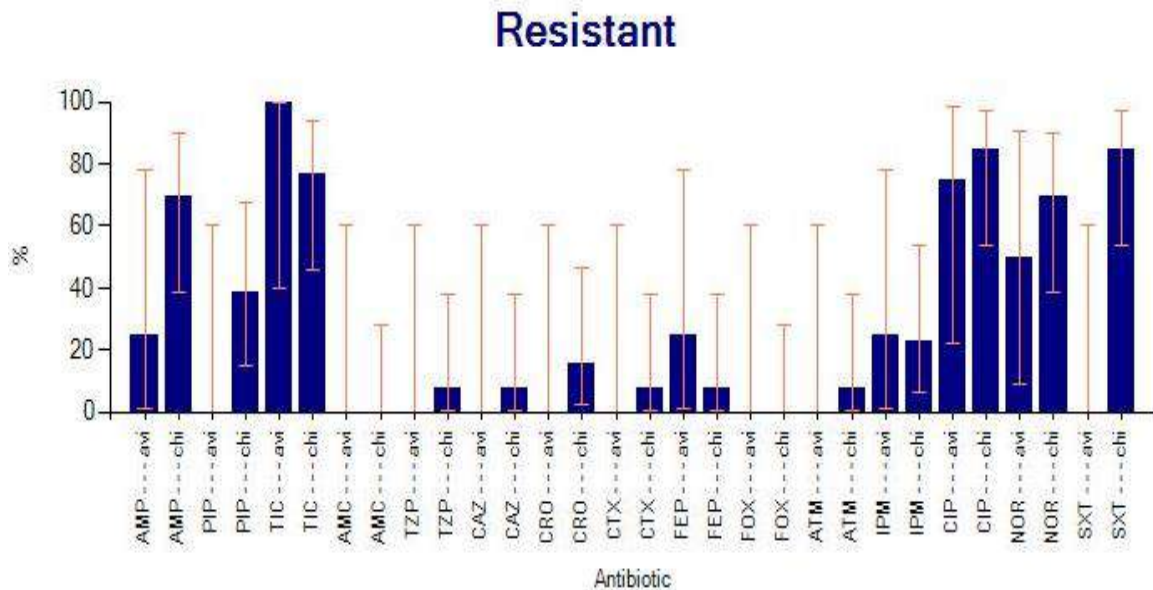


Fig 5: Comparative antibiotic resistance of *Salmonella* spp isolates from broilers (chi) and zoo (avi) birds

Table 2: Beta-lactamase gene pattern in the ESBL isolates

Beta lactamase gene pattern	No of isolates (n=16, %)
bla$_{CTX-M}$	12 (75.0)
bla$_{CTX-M1}$	12 (75.0)
bla$_{CTX-M2}$	12 (75.0)
bla$_{TEM}$	1 (6.3)
bla$_{SHV}$	2 (12.5)
bla$_{CTX-M, CTXM1, CTXM2}$	10 (62.5)
bla$_{TEM, SHV}$	2 (12.5)
No *bla*$_{CTX-M}$, *bla*$_{TEM}$, *bla*$_{SHV}$	1 (6.3)

Discussion:

Most of the antimicrobial agents tested in our study are frequently used in the poultry industry in Cameroon. *E. coli* isolates from chickens clearly demonstrated high resistance rates to almost all tested antibiotics used, in contrast to zoo birds, where relatively lower resistance rates were observed. This suggests that the degree of resistance to an antibiotic relates to the extent of its use. Isolation prevalence of 27.5% for *E. coli* and 5.3% for *Salmonella* spp in chickens in our study is lower than the prevalence reported in a study in Bangladesh (21) where the overall prevalence of *Salmonella* spp was 31.25%. However, 27.5% prevalence of *E. coli* in our study is similar to that of Leinyuy et al., (22) who reported *E. coli* isolation rate of 20.56%, followed by *Salmonella* isolation rate of 18.78% in Western region of Cameroon. The lower prevalence of *Salmonella* spp (5.3%) observed in our study could be due to differences in the types of samples collected, sampling period, locations, and types of production systems used.

In aviary birds, 31.8% of *E. coli* and 23.5% of *Salmonella* species were isolated from zoo birds. Isolation prevalence of *E. coli* (31.8%) in zoo birds in this study is similar to the findings in Messina in Italy, where 36.1% of *E. coli* was isolated from wild and zoo birds (23). The source of transmission of resistant bacteria of human and veterinary origin to wild birds seems to be via food acquisition and intake of water polluted with feces or human waste. However, it is important to conduct further epidemiological studies to understand the transmission of resistant bacteria to wild birds and back to the environment.

The susceptibility results of our study showed that *E. coli* isolates from commercial broilers were sensitive to cefoxitin (93.3%) and imipenem (63.3%). The high sensitivity to cefoxitin could be because this drug is not commonly used in chicken breeding in Cameroon. Nevertheless, a study conducted on broilers in the West region of Cameroon by Moffo et al., (7) in 2022 revealed a 100.0% sensitivity to imipenem. The low sensitivity to imipenem (63.3%) reported in our study may be an indication that farmers are resulting to the usage of this antibiotic, inspite of the fact this is an antibiotic of 'last resort' that must be used sparingly.

The results of our study showed that *E. coli* isolates from commercial chickens were resistant to trimethoprim-sulphamethoxazole, ampicillin, norfloxacin, piperacillin and ceftriaxone at the rate of 96.7%, 88.3%, 81.7%, 78.3%, and 73.3%, respectively. Higher resistance rates were seen to antibiotics of the penicillin, quinolone, and sulfonamide classes. Since these are the antibiotics reported to be frequently used by livestock caretakers in a study conducted in Cameroon (7), therefore, high resistance to antibiotics of these classes is not surprising. The result of our study is consistent with those of previous researchers, which raised concerns about the possibility that the use of antibiotics in food animals for growth or therapeutic purposes could select for antibiotic-resistant zoonotic enteric pathogens, which could subsequently be spread to humans through contaminated food or direct animal contact.

The most resisted antibiotics by *Salmonella* species isolated from broilers were trimethoprim-sulfamethoxazole (84.6%) and ciprofloxacin (84.6%), similar to the report in Bangladesh by Paul et al., (24), where 80.0% of *Salmonella* species isolates from broilers was resistant to ciprofloxacin. This high resistance rate is a major public health concern since fluoroquinolones are important antimicrobial compounds in the treatment of salmonellosis in humans.

The MDR prevalence in *E. coli* isola-

tes was 80.24% which is similar to 86.3% reported in broilers in Tanzania (25) but lower than 89.2% reported in China (26). Since MDR isolates could have a chance to contaminate food products and subsequently spread to humans, the high incidence of MDR in our study, particularly regarding isolates obtained from broiler chicken, is extremely substantial and needs to be considered a severe public health risk.

The information regarding the antibiotic resistance of the isolates from zoo birds particularly to carbapenems, will require the education of wildlife workers about the significance of using appropriate sanitation measures when handling zoo birds. As shown by a recent work, the role of wild birds as a reservoir for carbapenemase-encoding genes should be taken into account (27). Imipenem is a carbapenem, which is an antimicrobial agent that is used to treat a variety of serious infections when a microorganism is resistant to the primary agent of choice. Resistance to these antimicrobial agents is rare and limits therapeutic options. In our study resistance to imipenem occurred in 39.28% of isolates from zoo birds. It is unclear how microorganisms from these birds acquired imipenem resistance and, it is reason for concern.

Of 16 phenotypic ESBL identified, 15 were *E. coli* isolates while 1 was *Salmonella* specie. To the best of our knowledge, this is the first report of an ESBL in a *Salmonella* species isolate in Cameroon, similar to that of Mulvey et al., (28) where the first ESBL producing *Salmonella* species was discovered in an isolate in Canada. No ESBL producing bacteria was isolated in zoo birds in this study, which agrees with findings of studies carried out in other parts of the world (24).

The molecular analysis in our study showed that phenotypic ESBL *E. coli* isolates harbored various ESBL gene types. The frequency of β-lactamase genes has been reported to vary among nations, cities, and geographical areas (29). The prevalence of bla_{CTX-M}, bla_{TEM}, and bla_{SHV} in the current study was 75.0%, 6.3% and 12.5%, respectively. This finding is similar to those reported by de Jong et al., (30) in 2014 and Saliu et al., (31) in 2017, who reported that bla_{CTX} was the most predominant ESBL gene. Interestingly, 1 (6.25%) of the phenotypically positive ESBL isolate was negative for bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes. This can be explained by the possible presence of other ESBL genes, which we did not investigate in this study. In other similar studies (29,32), the coexistence of different β-lactamase genes within the same isolates have been reported. Our results showed that 12.5% of the ESBL-producing isolates carry two β-lactamase genes. Many other researchers (33,34) have expressed significant concerns regarding the possibility of ESBL transmission from poultry to humans in Africa through zoonotic agents, based primarily on the existence of the same variants of the bla_{CTX-M} ESBL genes in birds and humans.

Conclusion:

In our study, ESBL isolates were wide spread among poultry that appeared to be apparently in good health. These isolates showed high resistance rates to penicillin, quinolones and sulphonamides antimicrobial groups that are commonly used on poultry farms in Cameroon. Our research identified major ESBL genes (bla_{CTX-M}, bla_{TEM} and bla_{SHV}) as the genetic basis for resistance in the ESBL isolates, with predominance of bla_{CTX-M}. There is ample evidence to support the idea that wild birds carry antibiotic-resistant bacteria and can possibly transmit it to humans through their droppings or by being in close contact with them.

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Contributions of authors:

ZZN, KDAN, OAO, and MMMM were involved in study conceptualization, methodology, data collection, curation, resource mobilization, original manuscript draft writing, review and editing; ZZN, KDAN, ID, OAO, MMMM and NMJC were involved in formal data analysis and software; KDAN, ID and OAO were involved in study supervision; ZZN and KDAN were involved in laboratory investigation; ZN and OAO were involved in funding acquisition; and ZN, DANK, OAO and MMMM were involved in data validation. All authors approved the submitted version of the manuscript.

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Conflict of interest:

The authors declare that the research was conducted in the absence of any

commercial or financial relationships that could be construed as a potential conflict of interest.

References:

1. Ampaire, L., Muhindo, A., Orikiriza, P. Mwanga-Amumpaire, J. Bebell, L. and Boum, Y. A review of antimicrobial resistance in East Africa. Afr J Lab Med. 2016; 5(1): 432. doi: 10.4102/ajlm.v5i1.432
2. Geornaras, I., Hastings J.W., and von Holy A. Genotypic Analysis of Escherichia coli Strains from Poultry Carcasses and Their Susceptibilities to Antimicrobial Agents. Appl Environ Microbiol. 2001; 67 (4): 1940–1944. doi: 10.1128/AEM.67.4.1940-1944.2001
3. Kimura, A., C., Reddy V., Marcus, R., et al. Chicken consumption is a newly identified risk factor for sporadic *Salmonella enterica* serotype *Enteritidis* infections in the United States: a case-control study in FoodNet sites. Clin Infect Dis Off Publ Infect Dis. 2004; 38Suppl3:S244-252.doi:10.1086/381576.
4. Guabiraba, R., and Schouler, C., Avian colibacillosis: still many black holes. FEMS Microbiol Lett. 2015; 362 (15): fnv118. doi: 10.1093/femsle/fnv118.
5. Tebug, S. F., Mouiche, M. M. M., Abia, W., A., et al. Antimicrobial use and practices by animal health professionals in 20 sub-Saharan African countries. Prev Vet Med. 2021; 186: 105212. doi:10.1016/j.prevetmed.2020.105212.
6. Sapkota, A., Lefferts, L., Mckenzie, S., and Walker, P. What Do We Feed to Food-Production Animals? A Review of Animal Feed Ingredients and Their Potential Impacts on Human Health. Environ Health Perspect. 2007; 115: 663–670. doi: 10.1289/ehp.9760
7. Moffo, F., Mouiche, M. M. M., Djomgang, H. K., et al. Associations between antimicrobial use and antimicrobial resistance of *Escherichia coli* isolated from poultry litter under field conditions in Cameroon. Prev Vet Med. 2022; 204:105668 doi: 10.1016/j.prevetmed.2022.105668.
8. Hedman, H. D., Vasco, K. A., and Zhang, L. A. Review of Antimicrobial Resistance in Poultry Farming within Low-Resource Settings. Animals. 2020; 10 (8): 1264. doi:10.3390/ani10081264
9. Tully, T. N. Manual of Exotic Pet Practice 250–298. https://www.sciencedirect.com/science/article/pii/B9781416001195500135
10. Bonnedahl, J., and Järhult, J. Antibiotic resistance in wild birds. Ups J Med Sci. 2014: 119: 113-116. doi: 10.3109/03009734.2014.905663.
11. Antimicrobial resistance in livestock - Catry - 2003 - J Vet Pharmacol and Therap. Wiley Online Library https://doi.org/10.1046/j.1365-2885.2003.00463.x
12. Thrusfield, M. 3-Some general epidemiological concepts Vet Epidemiol. 1986: 20-29 doi: 10.1016/B978-0-7506-1496-2.50006-9
13. Quinn, P. J., Markey, B. K., Carter, M. E., Donnelly, W. J., and Leonard, F. C. Veterinary Microbiology and Microbial Disease. Wiley; 2001: 544
14. Qin, X., Weissman, S. J., Chesnut, M. F., Zhang, B., and Shen, L. Kirby-Bauer disc approximation to detect inducible third-generation cephalosporin resistance in Enterobacteriaceae. Ann Clin Microbiol Antimicrob. 2004; 3: 13. https://doi.org/10.1186/1476-0711-3-13
15. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing, 31st Edition: https://clsi.org/about/press-releases/clsi-publishes-m100-performance-standards-for-antimicrobial-susceptibility-testing-31st-edition/
16. Krumperman, P. H. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol. 1983; 46 (1): 165–170. doi:10.1128/aem.46.1.165-170.1983
17. Ayandele, A. A., Oladipo, E. K., Oyebisi, O., and Kaka, M. O. Prevalence of Multi-Antibiotic Resistant *Escherichia coli* and *Klebsiella* species obtained from a Tertiary Medical Institution in Oyo State, Nigeria. Qatar Med J. 2020; 2020 (1): 9. doi: 10.5339/qmj.2020.9
18. Maïworé, J., Baane, M. P., Youssouf, D., Bole, J., and Nango, F. Y. N. Portage Digestif des Bêta-Lactamases à Spectre Élargi Chez Quelques Patients de l'hôpital de la Caisse Nationale de Prévoyance Sociale de Maroua (Cameroun). 2021; 17 (10): https://eujournal.org/index.php/esj/article/view/14129
19. Aijuka, M., and Buys, E. M. Detection of extended-spectrum beta-lactamase cefotaxime resistance and virulence genes in *Escherichia coli* by duplex quantitative real-time PCR and melt curve analysis. Lett Appl Microbiol. 2020; 71 (1): 54–60 doi: 10.1111/lam.13274
20. Njage, P. M. K., and Buys, E. A. High Resolution DNA Melting Curve Analysis for the Rapid and Efficient Molecular Diagnostics of Extended Spectrum β-Lactamase Determinants from Foodborne *Escherichia coli*. Microorganisms. 2020; 8 (1): 90. doi: 10.3390/microorganisms8010090
21. Mridha, D., Uddin, MdN., Alam, B., et al. Identification and characterization of *Salmonella* spp from samples of broiler farms in selected districts of Bangladesh. Vet World. 2020;13 (2): 275–283. doi: 10.14202/vetworld.2020.275-283
22. Leinyuy, J. F., Ali, I. M., Karimo, O., and Tume, C. B. Patterns of Antibiotic Resistance in Enterobacteriaceae Isolates from Broiler Chicken in the West Region of Cameroon: A Cross-Sectional Study. Can J Infect Dis Med Microbiol J Can Mal Infect Microbiol Medicale. 2022; 2022: 4180336. doi:10.1155/2022/4180336.
23. Giacopello, C., Foti, M., Mascetti, A., et al. Antimicrobial resistance patterns of Enterobacteriaceae in European wild bird species admitted in a wildlife rescue centre. Vet Ital. 2016; 52: 139–144. doi: 10.12834/VetIt.327.1374.2
24. Paul, P., Akther, S., Ali, Z., Banu, H., Khan, S. R., and Khatun, M. M. Isolation, Identification and Antibiogram Study of *Salmonella* spp from Poultry Farm Environment. 2017; 3 (2): 298–306.
25. Mtemisika, C. I., Nyawale, H., Benju, R. J., et al. Epidemiological Cut-Off Values and Multidrug Resistance of *Escherichia coli* Isolated from Domesticated Poultry and Pigs Reared in Mwanza, Tanzania: A Cross-Section Study. Anim Open Access J MDPI. 2022; 12 (7): 835. doi: 10.3390/ani12070835
26. Yassin, A. K., Gong, J., Kelly, P., et al. Antimicrobial resistance in clinical *Escherichia coli* isolates from poultry and livestock, China. PLoS One. 2017; 12 (9): e0185326. doi:10.1371/journal.pone.0185326
27. Fischer, J., Schmoger, S., Jahn, S., Helmuth, R., and Guerra, B. NDM-1 carbapenemase-producing *Salmonella enterica* sub spp *enterica* serovar Corvallis isolated from a wild bird in Germany. J Antimicrob Chemother. 2013 ;68 (12): 2954–2956 doi:10.1093/jac/dkt260.
28. Mulvey, M. R., Soule, G., Boyd, D., Demczuk, W., and Ahmed, R. Multi-provincial *Salmonella* Typhimurium Case Control Study Group. Characterization of the first extended-spectrum beta-lactamase-producing *Salmonella* isolate identified in Canada. J Clin Microbiol. 2003; 41(1): 460–462. doi: 10.1128/JCM.41.1.460-462.2003
29. Pokhrel, R. H., Thapa, B., Kafle, R., Shah, P. K., and Tribuddharat, C. Co-existence of beta-lactamases in clinical isolates of *Escherichia coli* from Kathmandu, Nepal. BMC Res Notes. 2014; 7 (1): 694. doi: 10.1186/1756-0500-7-694
30. de Jong, A., Smet, A., Ludwig, C., et al. Antimicrobial susceptibility of *Salmonella* isolates from healthy pigs and chickens (2008–2011). Spec Issue ARAE 2013 Antimicrob Resist Bact Anim Environ. 2014; 171(3): 298–306.doi:10.1016/j.vetmic.2014.01.030.
31. Saliu, E. M., Vahjen, W., and Zentek, J. Types and prevalence of extended–spectrum beta–lactamase producing Enterobacteriaceae in poultry. Anim Health Res Rev. 2017; 18 (1): 46–57. doi: 10.1017/S1466252317000020

32. Mirkalantari, S., Masjedian, F., Irajian, G., Siddig, E. E., and Fattahi, A. Determination of the frequency of β-lactamase genes (*bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{CTX-M}$) and phylo-genetic groups among ESBL-producing uropathogenic *Escherichia coli* isolated from outpatients. J Lab Med. 2020; 44 (1): 27–33. doi:10.1515/labmed-2018-0136
33. Falgenhauer, L., Imirzalioglu, C., Oppong, K. et al. Detection and Characterization of ESBL-Producing *Escherichia coli* From Humans and Poultry in Ghana. Front Microbiol. 2019: 9: 3358 doi: 10.3389/fmicb.2018.03358
34. Aworh, M. K., Kwaga, J., Okolocha, E., et al. Extended-spectrum ß-lactamase-producing *Escherichia coli* among humans, chickens and poultry environments in Abuja, Nigeria. One Health Outlook. 2020; 2: 8. doi: 10.1186/s42522-020-00014-7

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Original Article **Open Access**

Prevalence and antimicrobial resistance profiles of faecal *Escherichia coli* isolates from local chickens in Plateau State, Nigeria

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Abstract:

Background: Poultry is a profitable business in Nigeria, with economic benefits to families and communities involved in this type of agriculture. However, infection of poultry birds by *Escherichia coli* can, in addition to causing mortality, results in reduction of egg production, with depletion of protein (egg and meat) and subsequent reduction in market value, consumer supply, cost of veterinary care, and medicines. The objectives of this study are to determine the prevalence and antimicrobial resistance profiles of faecal *E. coli* isolates from local chickens (*Gallus domesticus*) in Plateau State, northcentral Nigeria.

Methodology: This was a descriptive cross-sectional study of 540 local chickens for faecal carriage of *E. coli*, randomly selected from 9 local government areas (LGAs) (60 per LGA) in the 3 senatorial districts (180 per senatorial district) of Plateau State, Nigeria. Faecal samples were collected from the chickens for culture isolation and identification of *E. coli* using conventional microbiological methods. The isolates were confirmed by Vitek® 2 compact machine and PCR amplification of 16S rRNA gene. Antibiotic susceptibility test of 37 distinct *E. coli* strains to selected antibiotics (ampicillin, ampicillin/sulbactam, piperacillin, cefazolin, cefepime, ceftriaxone, ceftazidime, cefoxitin, ertapenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, nitrofurantoin and trimethoprim/sulfamethoxazole) was performed by the Vitek® 2 and read using the web system application. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 and R Commander version 2.9-1.

Results: The overall prevalence of faecal *E. coli* carriage among the chickens was 65.0% (351/540), with highest prevalence in Central Plateau senatorial district (76.1%, 137/180), which was significantly higher than Northern Plateau (62.2%, 112/180) and Southern Plateau (56.7%, 102/180) (x^2=15.873, p=0.0004). The prevalence of *E. coli* was highest in Pankshin (86.7%, 52/60) and Mangu (85.0%, 51/60) LGAs and this was significantly higher than in Bokkos (56.7%, 34/60) (x^2=18.761, p<0.000) and other LGAs. The antibiotic susceptibility of 37 distinct strains of *E. coli* showed that 64.9% (n=24) were resistant to at least one antibiotic, with the highest resistance rate being to trimethoprim-sulfamethoxazole (51.4%, n=19), ampicillin (48.7%, n=18), and piperacillin (43.2%, n=15). Multi-drug resistance (resistance to three or more antibiotic classes) was observed in 35.1% (n=13) of the *E. coli* strains. The multiple antibiotic resistance (MAR) index ranged from 0.06 (resistance to one antibiotic) to 0.76 (resistance to 13 antibiotics tested).

Conclusion: The results of this study provide evidence that resistance to multiple antibiotics is widespread among faecal *E. coli* isolates from local chickens in Plateau State, Nigeria, and thus poses potential risks for human infections with MDR *E. coli*.

Keywords: local chicken; faecal carriage; *Escherichia coli,* multi-drug resistance; zoonosis

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Profils de prévalence et de résistance aux antimicrobiens des isolats fécaux d'Escherichia coli provenant de poulets locaux dans l'État du Plateau, Nigeria

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Contexte: La volaille est une activité rentable au Nigeria, avec des avantages économiques pour les familles et les communautés impliquées dans ce type d'agriculture. Cependant, l'infection des volailles par Escherichia coli peut, en plus de provoquer la mortalité, entraîner une réduction de la production d'œufs, avec un appauvrissement des protéines (œufs et viande) et une réduction ultérieure de la valeur marchande, de l'approvisionnement des consommateurs, du coût des soins vétérinaires et des médicaments. Les objectifs de cette étude sont de déterminer la prévalence et les profils de résistance aux antimicrobiens des isolats fécaux d'*E. coli* provenant de poulets locaux (*Gallus domesticus*) dans l'État du Plateau, au centre-nord du Nigéria.
Méthodologie: Il s'agissait d'une étude transversale descriptive portant sur 540 poulets locaux pour le transport fécal d'E. coli, sélectionnés au hasard dans 9 zones de gouvernement local (LGA) (60 par LGA) dans les 3 districts sénatoriaux (180 par district sénatorial) du Plateau État, Nigéria. Des échantillons de matières fécales ont été prélevés sur les poulets pour l'isolement des cultures et l'identification d'*E. coli* à l'aide de méthodes microbiologiques conventionnelles. Les isolats ont été confirmés par la machine compacte Vitek® 2 et par amplification PCR du gène de l'ARNr 16S. Test de sensibilité aux antibiotiques de 37 souches distinctes d'*E. coli* à des antibiotiques sélectionnés (ampicilline, ampicilline-sulbactam, pipéracilline, céfazoline, céfépime, ceftriaxone, ceftazidime, céfoxitine, ertapénème, méropénem, amikacine, gentamicine, tobramycine, ciprofloxacine, lévofloxacine, nitrofurantoïne et triméthoprime-sulfaméthoxazole) a été réalisée par le Vitek® 2 et lue à l'aide de l'application du système Web. L'analyse des données a été réalisée à l'aide du progiciel statistique pour les sciences sociales (SPSS) version 20.0 et de R Commander version 2.9-1.
Résultats: La prévalence globale du portage fécal d'*E. coli* parmi les poulets était de 65,0% (351/540), avec une prévalence plus élevée dans le district sénatorial du Plateau Central (76,1%, 137/180), qui était significativement plus élevée que dans le Plateau Nord (62,2%, 112/180) et Plateau Sud (56,7%, 102/180) (x^2=15,873, p=0,0004). La prévalence d'*E. coli* était la plus élevée dans les LGA de Pankshin (86,7%, 52/60) et de Mangu (85,0%, 51/60), et elle était significativement plus élevée qu'à Bokkos (56,7%, 34/60) (x^2=18,761, p<0,000) et autres LGA. La sensibilité aux antibiotiques de 37 souches distinctes d'*E. coli* a montré que 64,9% (n=24) étaient résistantes à au moins un antibiotique, le taux de résistance le plus élevé étant celui du triméthoprime-sulfaméthoxazole (51,4%, n=19), de l'ampicilline (48,7%, n=18) et pipéracilline (43,2%, n=15). Une multi-résistance aux médicaments (résistance à trois classes d'antibiotiques ou plus) a été observée chez 35,1% (n=13) des souches d'*E. coli*. L'indice de résistance multiple aux antibiotiques (MAR) variait de 0,06 (résistance à un antibiotique) à 0,76 (résistance à 13 antibiotiques testés).
Conclusion: Les résultats de cette étude prouvent que la résistance à plusieurs antibiotiques est répandue parmi les isolats fécaux d'*E. coli* provenant de poulets locaux dans l'État du Plateau, au Nigeria, et présente donc des risques potentiels d'infections humaines par *E. coli* MDR.

Mots-clés: poulet local; transport fécal; *Escherichia coli*; la multirésistance aux médicaments; zoonose

Introduction:

Escherichia coli belongs to the *Enterobacteriaceae* family, which cause infection that poses or constitutes great hazard to the poultry industry causing loss of weight, reduction of egg production and high mortality (1). All over the world, *E. coli* infection is one of the serious problems that cause great threat to the profitability of avian enterprises. *E. coli* in poultry intestine is a member of the normal microbiota but the colonization of the respiratory tract by pathogenic *E. coli* strains is associated with extraintestinal disease which results in morbidity and mortality of poultry by causing septicaemia (2). Although *E. coli* is a normal flora inhabiting the intestinal tract of birds, under the influence of predisposing factors such as inadequate and faulty ventilation, overcrowding, hunger, thirst, extremes of temperatures and low vitality, high mortality during rearing, reduced weight gain and condemnation of birds at the time of slaughter (1).

Avian colibacillosis is a complex syndrome characterized by multiple organ lesions with air sacculitis and associated pericarditis, perihepatitis and peritonitis being most typical (1). Naturally, *E. coli* infected chicks present with clinical signs such as loss of appetite, cyanosis, tendency to huddle respiratory distress, depression, reduction of weight gain, dropped wings, closed eyes, and labored breathing (1). Certain strains of *E. coli* have virulence properties associated with host tissue colonization, production of toxins, iron uptake systems, defensins and serum resistance, though the pathogenesis of colibacillosis is not completely understood (2).

Local chickens are free range birds which may serve as a reservoir or carrier of pathogenic *E. coli* to the environments, poultry and households. Due to increasing demand for egg and meat products in the poultry sector which is among the fastest growing agro-based industries worldwide, disease burden has however, remained a great challenge in poultry production (3). The majority of these bacteria do not cause disease because they are environmental and normal flora. However, with the estimated bacteria species reaching about 10^{30} worldwide, it is important to identify and differentiate those species that are pathogenic, particularly from a medical and public health perspective (4). However, contamination of food with pathogenic *E. coli* species can cause serious foodborne illness in humans (4).

Antibiotic resistance is a growing global health concern with the huge societal risk

of reverting to pre-antibiotic era if not addressed. By 2050, it is estimated that death from from an antibiotic-resistant infection will occur in every three seconds (5). Between the rise in antibiotic-resistant infections and the 90 billion tons of chicken meats that are produced worldwide annually, there is a well-documented connection and due to this connection, the World Health Organization (WHO) adopted the 'One Health' approach in 2017, which states that the health of people, animals, and the environment are inextricably related to one another. According to WHO, it is relevant to refer to 'One Health' approach when discussing antibiotic resistance, food safety, and the control of zoonoses (5). Infections caused by antibiotic-resistant bacteria have resulted in increased hospitalization rate and longer hospital stay for infected individuals (5).

The use of antibiotic as growth promotion has increased and has been linked to the development of antibiotic resistance among bacterial strains (4). The practice of administering sub-therapeutic doses of antibiotics to livestock in preventing disease did not only increase resistance in bacteria found in animals, but also in humans. As a result of farming practices, workers and consumers may be exposed to antibiotic-resistant bacteria. Poultry farm workers and families living on farms using antibiotics in the feed including neighboring families, have an elevated risk of exposure to antibiotic-resistant *E. coli*. Consumers that purchase poultry products which utilize antibiotics in production may also be exposed through cross-contamination from raw meat on surfaces and consumption of undercooked meat. Antibiotic resistance genes are able to move horizontally, especially through conjugative gene transfer, to other bacteria (6). It has been suggested in several studies that *E. coli*, specifically, to human health has particular relevance because is able to transmit from retail meat to people and ultimately be source of urinary tract infections (7).

The emergence of resistance has the potential to impact on the treatment and management of infectious diseases in both animals and humans. This increasing resistance has received considerable national and international attention (1). This research aimed to determine the occurrence and antimicrobial resistance profiles of pathogenic *E. coli* in cloacal swabs of local chickens (*Gallus domesticus*) in Plateau State, Nigeria.

Materials and method:

Study area:

This study was carried out at the Antimicrobial Resistance Laboratory and Biotechnology Division, National Veterinary Research Institute, Vom, Plateau State. Plateau is a State in northcentral Nigeria with Jos as the administrative capital. It has an area of 26,899 km^2 is located between latitude 80°24'N and longitude 80°32' and 100°38' East. The State is bordered to the north by Kaduna and Bauchi States, with Benue State on the southern border and flanked on the West and East sides by Nasarawa and Taraba States respectively. Presently, the State has 17 Local Government Areas (LGAs) which include; Jos-North, Jos-East, Jos-South, Bassa, Riyom, Barkin Ladi, Bokkos, Mangu, Pankshin, Kanke, Kanam, Langtang-North, Langtang-South, Wase, Mikang, Shendam and Quanpan.

Plateau State derives its name from the geographical landscape that predominates this part of the country which is often referred to as the Jos Plateau. The economic potential of the State is mainly agrarian with over 70% of its population engaging in agriculture or agricultural related areas. The study was carried out in 3 Senatorial Zones of Plateau State, Nigeria (Fig 1).

Study design and sample size determination:

This was a descriptive cross-sectional study of poultry birds for faecal carriage of *E. coli*. The sample size for the study was determined using the formula, $N=Z^2pq/L^2$, where N is the sample size, Z is the level of confidence for two-tailed tests at 95% (=1.96), p is the reported prevalence of *E coli* which was 13.4% from Mude et al., (9), and L is the allowable error (precision) of 5% (=0.05). This gave the sample size of 178, which was adjusted to 180.

Source: (8)

Fig 1: Map of Plateau State showing the study sites in the three Senatorial Districts

Method of poultry sampling and cloacal sample collection:

Three local government areas (LGAs) in each of the 3 senatorial districts in Plateau State were selected by systematic random sampling technique. Sixty local chickens were randomly selected from each LGA, giving an overall total of 540 chickens, with 180 from each senatorial district. Cloacal swabs samples were collected from each local chicken using a sterile swab stick in buffered peptone water and transported in cold chain to the laboratory for analysis.

Culture of *Escherichia coli* from sample:

The procedure Feng et al., (10) was used for culture isolation of *E. coli*. The swabs were dipped into 10 ml buffered peptone water and incubated at 37°C for 24 hours after which 0.5 mL of the aliquots were dispensed into Ec broth and incubated at 37°C for 24 h, then a loop full of the aliquots were streaked on MacConkey agar plate (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 hours. The growth was sub-cultured on Levine's Eosin-Methylene Blue Agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 hours. Presumptive identification of *E. coli* was based on colonial morphology and cultural appearance (appeared as dark centered and flat, with or without the presence of green metallic sheen) and Gram-staining reaction.

Biochemical identification of *E. coli* isolates:

Suspected *E. coli* isolates were confirmed using conventional biochemical tests such as indole, methyl red, Voges-Proskauer (VP), citrate, and sugar fermentation reactions. Confirmed *E. coli* isolates were kept at 4°C in nutrient agar slants and skimmed milk for determination of antimicrobial susceptibility tests and further studies.

Molecular detection of *E. coli* by polymerase chain reaction (PCR) assay:

DNA extraction:

DNA extraction of phenotypically identified *E. coli* isolates was done using DNA extraction mini kit (Qiagen, USA). In brief, the pooled culture was placed into 1.5 Eppendorf tubes and centrifuged, the cell pellet was resuspended in phosphate buffer saline to a final volume of 200 µL. 20 µL of proteinase K and 200 µL buffer AL were added to the suspension and mixed by pulse-vortexing for 15 seconds. The tube containing the suspension was incubated at 56^0C for 10 mins and then centrifuged to remove drops from the inside of the lid. About 200 µL of ethanol (96-100%) was added to the suspension and mixed by pulse-vortexing, and then briefly centrifuged to remove drops from the inside of the lid.

The mixture was carefully applied to the QIAamp Mini spin column (in a 2mL collection tube) without wetting the rim. The cap

was closed and centrifuged at 6000 x g (8000 rpm) for 1min. The QIAamp Mini spin column was placed in a clean 2 mL collection tube and the tube containing the filtrate was discarded. The QIAamp Mini spin column was then carefully opened and 500 µL buffer AW1 was added without wetting the rim. The cap was closed and centrifuged at 600 x g (8000 rpm) for 1min. The QIAamp Mini column was placed in a clean 2 mL collection tube and the collection tube containing the filtrate was discarded. Again, the QIAamp Mini spin column was carefully opened and 500 µL buffer AW2 was added without wetting the rim. The cap was closed and centrifuge at full speed of 20,000 x g (14,000 rpm) for 3 mins. QIAamp Mini spin column was placed in a new 2ml collection tube and the old collection tube was discarded with the filtrate. It was then centrifuged at full speed for 1 min. The QIAamp Mini column was placed in another clean 1.5 mL microcentrifuge tube, the collection tube containing the filtrate was discarded. The QIAamp Mini column was carefully opened and 200 µL buffer AE was added and then incubated at room temperature (15-25^{0}C) for 1min and centrifuged at 6000 x g (8000 rpm) for 1 min, after which the QIAamp Mini column was discarded, and the filtrate is the DNA extract.

PCR amplification E. coli 16S rRNA gene:

About 5 µL of the DNA extract from each *E. coli* isolate were added to 20 µL of the master-mix which contains green Taq DNA polymerase, 2 x MM (12.5), nuclear free water (5.5), including specific target primers (forward primer ID *E. coli* 16S 11-F-5′-ATCAACC GAGATTCCCCCAGT-3′and reverse primer ID *E. coli* 16S 1338-R-5′-CACTATCGGTCAGTCAG GAG-3′ with expected amplicon size of 401 bp. GenBank accession number NCTC-13846 was used as control.

The PCR reaction was caried in a thermal cycler with initial denaturation at 95°C for 5 mins for one cycle and the second step involved denaturation for 35 cycles at 95°C for 1 min, annealing at 50°C for 50 seconds, and extension at 72°C for 1 min, with a final extension at 72°C for 10 minutes.

Agarose gel electrophoresis of PCR amplicons:

Approximately 15µL of the PCR amplicons of each isolate along with 5µL of the base pair DNA marker (New England Biolabs®) used as a molecular size marker, were loaded into different wells of prepared agarose gel into which 5µL ethidium bromide has been added. This was then carefully placed in electrophoresis tank and totally submerged in TBE buffer solution. The electrophoresis tank was closed, and the positive and negative terminals of the electrophoresis tank was connected to power source and run for 40 minutes at 80 volts. After the electrophoresis run, the amplicon bands were visualized and photographed using the Gel Documentation System (Bio Rad).

Antimicrobial susceptibility test of *E. coli* isolates by Vitek 2 system

Antibiotic susceptibility of confirmed *E. coli* isolates was performed with the VITEK® 2 compact machine (BioMerieux, USA). First, pure culture of each isolate was inoculated on Columbia sheep blood agar and incubated at 37°C for 24 hours. The Vitek® 2 compact cassette, Densicheck plus, saline dispenser, sterile swabs, polystyrene tubes on racks, lint-free wipes, pure culture plates of isolates were placed on a clean flat bench and all configuration options were set correctly. Inoculum suspension of the isolate was prepared and standardized to 0.5-0.63 McFarland standards using the Densicheck. About 145 µL of the suspension was transferred into 3 ml of saline, the test card (Gram-negative) was placed in the appropriate slots on the cassette and the cassette information was entered into FLEX prep view in the Vitek® 2 system web application. The cassette was then placed into the chamber and fill door closed to begin the filling process.

After completion of the fill cycle, the cassette was removed from the filler station and loaded into the load/unload station. Once loaded, the instrument bar code reader scans the test cards and cassette bar code. During the test card processing, the instrument unloads the test cards from the cassette and placed them into the carousel (in the incubator). After scanning, the cards are removed, and results read as susceptible (S), intermediate (I) or resistant (R) using the Vitek® 2 system web application.

Statistical analysis:

The prevalence data for *E. coli* were presented as percentage frequency and analysed using the Statistical Package for the Social Sciences (SPSS) Version 20.0 and R Commander version 2.9-1.

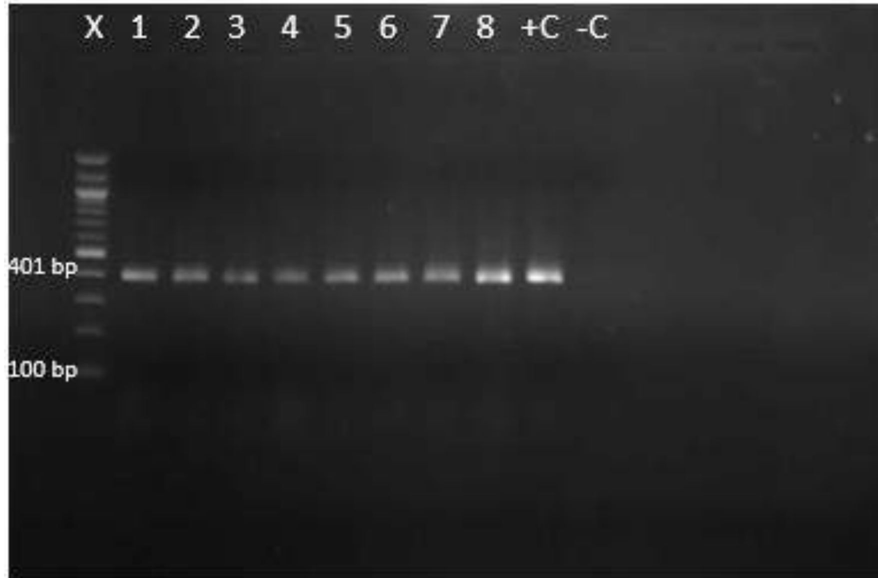
Results:

Prevalence of faecal carriage of *E. coli* in the chickens:

Of the 540 cloacal swab samples collected from 540 local chickens, *E. coli* was isolated and confirmed in 351 samples (Fig 1), giving an overall prevalence of *E. coli* carriage of 65.0% in the study. The prevalence of *E. coli* based on senatorial zones shows that Central Plateau has the highest prevalence rate of 76.1% (137/180), followed by Northern Plateau (62.2%, 112/180) and Southern Plateau, with the lowest prevalence of 56.7% (102/180). The statistics show the prevalence of faecal *E. coli* in the chickens to be significantly higher in Central Plateau than Southern

and Northern Plateau senatorial districts (x^2= 15.873, p=0.0004) (Table 1).

The prevalence of *E. coli* based on LGAs shows that Pankshin with 86.7% (52/60) had the highest prevalence, followed by Mangu with 85.0% (51/60), Riyom with 65.0% (39/60), Shandam and Barkin Ladi with 63.3% (38/60) each, Jos North with 58.3% (35/60), Mikang and Bokkos with 56.7% (34/60) each, and Qua'anpan with the least occurrence of 50.0% (30/60) (Table 1). In Central Plateau district, the prevalence of faecal *E. coli* was significantly higher in Pankshin and Mangu compared to Bokkos LGA (x^2=18.761, p<0.000) and to other LGAs in Northern and Southern Plateau senatorial districts.



Lane X: Molecular size marker 100 bp DNA Ladder, Lane 1 - 8: Positive samples, Lane +C: Positive control (reference strains of NCTC-11954) and Lane -C: Negative control (Nuclease free water).
All the selected isolates showed molecular relatedness with the positive control as seen in the bands produced after electrophoresis. All the samples ran equal distances with the control showing that the lanes 1-8 contained similar DNA sequence as the positive control (Lane +C).

Fig 1: Agarose gel electrophoresis of 16S rDNA amplicon of representative *Escherichia coli* isolates

Table 1: Prevalence of *E. coli* isolates in chickens based on Senatorial Districts and LGA of Plateau State, Nigeria

Senatorial districts	+Number positive by Senatorial district (%)	LGAs	Number positive By LGA (%)	X^2	p-value
Central Plateau (n=180)	137 (76.1)	Bokkos (n-60)	34 (56.7)	18.761	<0.000***
		Mangu (n=60)	51 (85.0)		
		Pankshin (n=60)	52 (86.7)		
Southern Plateau (n=180)	102 (56.7)	Qua'anpan (n=60)	30 (50.0)	2.172	0.338
		Mikang (n=60)	34 (56.7)		
		Shandam (n=60)	38 (63.31)		
Northern Plateau (n=180)	112 (62.2)	Jos North (n=60)	35 (58.3)	0.615	0.735
		Barkin Ladi (n=60)	38 (63.31)		
		Riyom (n=60)	39 (65.0)		
Total (n=540)	**351 (65.0)**	**Total (n=540)**	**351 (65.0)**		

+ = statistically significant difference in prevalence with respect to senatorial district (x^2=15.873, p=0.0004); *** = statistically significant difference in prevalence with respect to LGA in Central Plateau

Antimicrobial resistance profiles of *E. coli* isolates:

The phenotypic antimicrobial resistance of 37 distinct *E. coli* strains is as shown in Table 2 and Fig 1. The *E. coli* strains were resistant to trimethoprim-sulfamethoxazole (51.4%, n=19), ampicillin (48.7%, n=18), and and piperacillin (43.2%, n=15) but highly sus-

ceptible to cefoxitin, ertapenem, meropenem and amikacin (100.0%, n=37); ciprofloxacin, levofloxacin and nitrofurantoin (91.9%, n=34); cefazolin, ceftazidime, ceftriaxone, cefepime, gentamicin, and tobramycin (89.2%, n=33); and ampicillin-sulbactam (75.7%, n=28). Intermediate resistance was observed for few of the antibiotics tested, ranging from 2.7% (n=1) to 5.4% (n=2), except for ampicillin-sulbactam with 43.2% (n=6).

Table 2: Antibiotic susceptibility of 37 *Escherichia coli* strains isolated from cloacal of local chickens in Plateau State, Nigeria

Antimicrobial	Resistance (%)	Intermediate (%)	Susceptible (%)
Ampicillin	18 (48.7)	1 (2.7)	18 (48.7)
Ampicillin-sulbactam	3 (8.1)	6 (43.2)	28 (75.7)
Piperacillin	16 (43.2)	2 (5.4)	19 (51.4)
Cefazolin	4 (10.8)	0	33 (89.2)
Cefoxitin	0	0	37 (100.0)
Ceftazidime	4 (10.8)	0	33 (89.2)
Ceftriaxone	4 (10.8)	0	33 (89.2)
Cefepime	4 (10.8)	0	33 (89.2)
Ertapenem	0	0	37 (100.0)
Meropenem	0	0	37 (100.0)
Amikacin	0	0	37 (100.0)
Gentamicin	4 (10.8)	0	33 (89.2)
Tobramycin	2 (5.4)	2 (5.4)	33 (89.2)
Ciprofloxacin	3 (8.1)	0	34 (91.9)
Levofloxacin	2 (5.4)	1 (2.7)	34 (91.9)
Nitrofurantoin	1 (2.7)	2 (5.4)	34 (91.9)
Trimethoprim-sulfamethoxazole	19 (51.4)	0	18 (48.7)

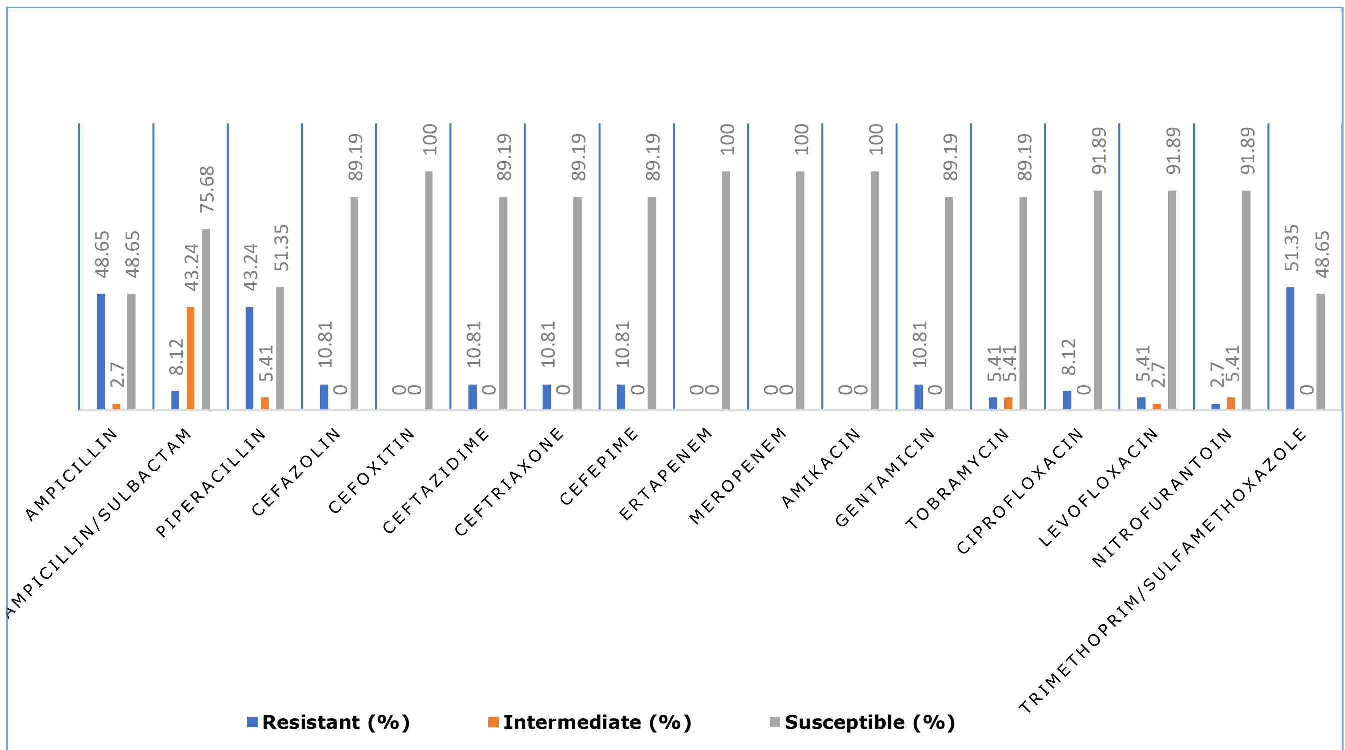


Fig 1: Antibiotics susceptibility of 37 *Escherichia coli* strains from local chickens in Plateau State, Nigeria

Multi-drug resistance and multiple antibiotic resistance index of *Escherichia coli* strains: The multiple antibiotics resistance (MAR) index, as shown in Table 3 and Fig 2, ranged from 0.06 (resistant to one antibiotic) to 0.76 (resistant to thirteen antibiotics) and the overall prevalence of multi-drug resistant (MDR) (resistance to two or more different classes of antimicrobials) with 35.1% (13/37).

Table 3: Antibiotic resistance profile and multiple antibiotic resistance index of 37 *Escherichia coli* strains isolates from cloacal of local chickens in Plateau State, Nigeria

S/NO	*Escherichia coli* code	Antibiotics resistant profile	Number of antibiotics	MARI
1	ASTGN9-1		0	0
2	ASTGN130-1		0	0
3	ASTGN156-1		0	0
4	ASTGN148-1		0	0
5	ASTGN292-1		0	0
6	ASTGN253-1		0	0
7	ASTGN379-1		0	0
8	ASTGN41-1		0	0
9	ASTGN113-1		0	0
10	ASTGN215-1		0	0
11	ASTGN211-1		0	0
12	ASTGN128-1		0	0
13	ASTGN249-1		0	0
14	ASTNG181-1	TrS	1	0.06
15	ASTGN90-1	TrS	1	0.06
16	ASTGN78-1	TrS	1	0.06
17	ASTGN2-1	TrS	1	0.06
18	ASTGN76-1	TrS	1	0.06
19	ASTGN395-1	Amp, Pip	2	0.12
20	ASRGN334-1	Amp, Pip	2	0.12
21	ASTGN122-1	Amp, Pip	2	0.12
22	ASTGN391-1	Amp, Cip	2	0.12
23	ASTGN31-1	Ntf, TrS	2	0.12
24	ASTGN387-1	Amp, TrS	2	0.12
25	ASTGN97-1	Amp, Pip, TrS	3	0.18
26	ASTNG136-1	Amp, Pip, TrS	3	0.18
27	ASTNG275-1	Amp, Pip, TrS	3	0.18
28	ASTGN30-1	Amp, Pip, TrS	3	0.18
29	ASTGN46-1	Amp, Pip, TrS	3	0.18
30	ASTGN58-1	Amp, Pip, TrS	3	0.18
31	ASTNG316-1	Amp, AmpS, Pip, TrS	4	0.24
32	ASTGN359-1	Amp, Pip, Gn, TrS	4	0.24
33	ASTGN384-1	AmpS, Pip, Gn, TrS	4	0.24
34	ASTGN195-1	Amp, Pip, Cef, Cefz, Cefx, Cep	6	0.35
35	ASTGN236-1	Amp, Pip, Cef, Cefz, Cefx, Cep,	6	0.35
36	ASTGN149-1	Amp, AmpS, Pip, Cf, Cefz, Cefx, Cep, Gn, Tbr, Cip, Levf, TrS	12	0.71
37	ASTGN154-1	Amp, AmpS, Pip, Cf, Cefz, Cefx, Cep, Gn, Tbr, Cip, Levf, Ntf, TrS	13	0.76

Amp: ampicillin; AmpS: Ampicillin/Sulbactam; Pip: Piperacillin; Cip: ciprofloxacin; Cefx: ceftriaxone; Cef: Cefazolin; Cefz: Ceftazidime; Cep: Cefepime; Gn: Gentamicin; TrS: Trimethoprim/ sulfamethoxazole; Tbr: Tobramycin; Levf: Levofloxacin; Ntf: Nitrofurantoin

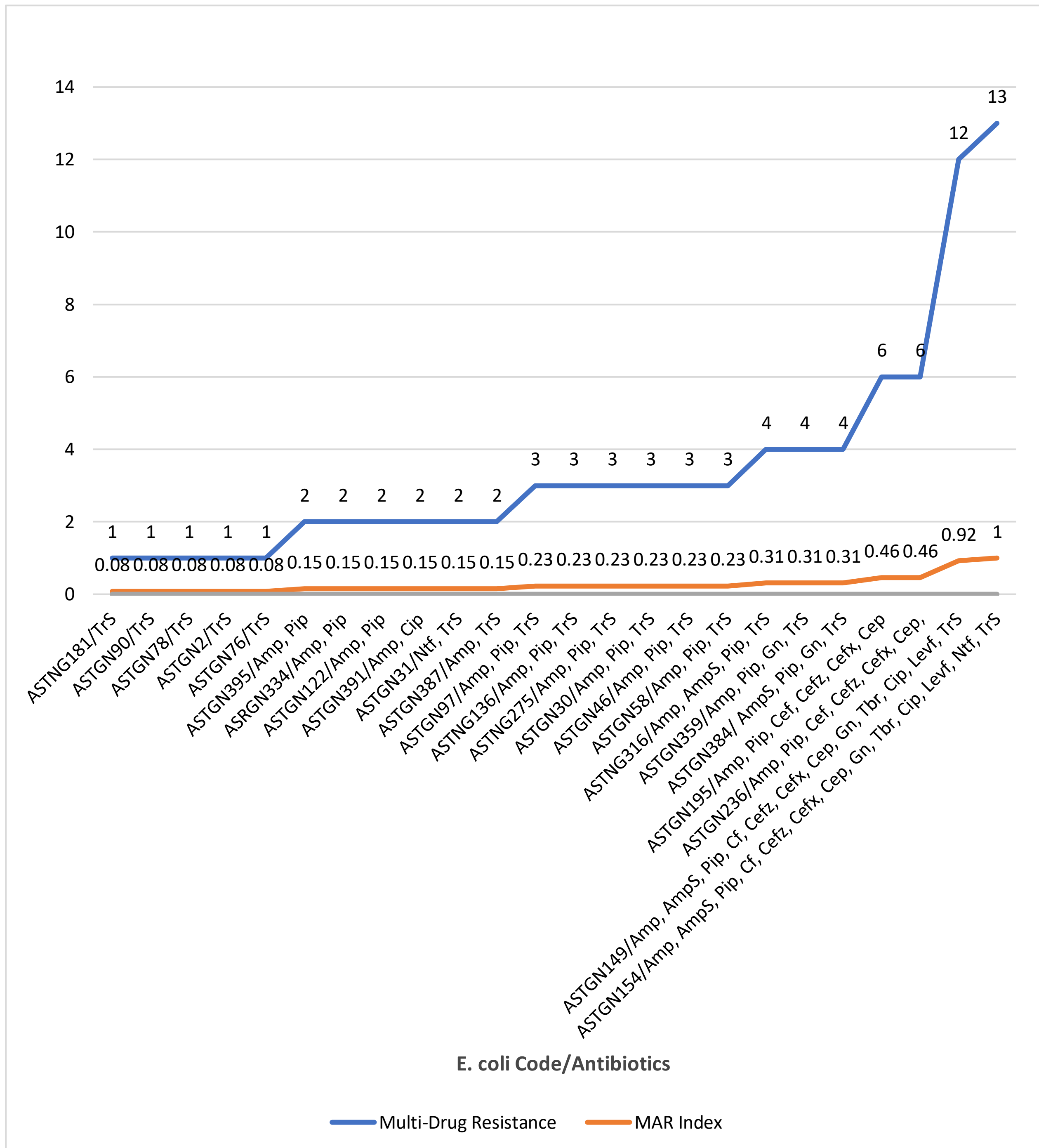


Fig 2: Multi-drug resistance pattern of 37 *Escherichia coli* strains

Discussion:

The early identification of carrier animals and information on the shedding state are crucial to prevent the spread of infection to animals and humans. It is a generally accepted opinion that the food chain has been recognized as one of the main passages for the transfer of antibiotic resistant bacteria between human and animal populations (11). The prevalence of 65.0% faecal carriage of *E. coli* in local chickens in our study is higher than the 50.0% reported in Zaria, Nigeria (12), but lower than the prevalence of 80.0% reported in a study conducted in Ghana by Adzitey et al., (13). Local chickens in Nigeria are frequently raised under free-range conditions with little care, exposing them to environmental contaminants and increasing the risk of bacterial infections (14). This may explain why local chickens in this study had a high prevalence of

faecal *E. coli* carriage. Local chickens in Nigeria are fed on food scraps, grass, maggots from cow dung, and other environmental waste, which could expose them to pathogenic bacteria like *E. coli* (15). They are also not routinely vaccinated or put on antibiotic medications.

This high prevalence (65.0%) of *E. coli* in this study is significant because of its pervasiveness and highly promiscuous nature for antimicrobial resistomes (16), which makes it a tool for the spread of antimicrobial resistant genes in the food chain and the environment. Due to the prevalence of resistant strains or residues in humans, the overall effect is that humans do not respond to antimicrobial treatments. Given that the local chickens are free range and in close proximity to other animals and the environment, the high rate of antibiotic resistance seen in this study may be the result of the indiscriminate use of antibiotics. According to reports, rather than being utilized to encourage growth in animals, antibiotics are primarily employed as preventative medicine and for the treatment of sick animals. Most farmers often fail to observe periods of withdrawal when giving antibiotics or other medications to their livestock and they are more likely to become resistant to antibiotics and to accumulate antimicrobials in their muscle tissues as an outcome.

The *E. coli* isolates were highly resistant to trimethoprim-sulfamethoxazole (51.4%) followed by ampicillin (48.7%), and piperacillin (43.2%) followed by low resistance to cefazolin, ceftazidime, ceftriaxone, cefepime and gentamicin (10.1%); ampicillin-sulbactam and ciprofloxacin (8.1%); tobramycin and levofloxacin (5.4%), with the least resistance to nitrofurantoin (2.7%), but susceptible to cefoxitin, ertapenem, meropenem and amikacin (100%); ciprofloxacin, levofloxacin and nitrofurantoin (91.9%); cefazolin, ceftazidime, ceftriaxone, cefepime, gentamicin and tobramycin (89.2%); and ampicillin-sulbactam (75.7%). Intermediate resistance was observed for few of the antibiotics examined, ranging from 2.7% to 5.4%, except for ampicillin-sulbactam with 43.2%. These findings are similar to those of the study by Vranic et al., (17) which showed highest resistance to trimethoprim-sulfamethoxazole (40.9%) and ampicillin (82.8%).

In this study, the multiple antibiotics resistance (MAR) index ranged from 0.06 (resistant to one antibiotic) to 0.76 (resistant to 13 antibiotics) and the study recorded multidrug resistance (MDR), that is resistance to 2 or more different classes of antimicrobials (18) among the isolates. Ampicillin, ampicillin-sulbactam, piperacillin, ciprofloxacin, ceftriaxone, cefazolin, ceftazidime, cefepime, gentamicin, trimethoprim-sulfamethoxazole, tobramycin, levofloxacin, and nitrofurantoin were all completely ineffective invitro against *E. coli* isolates in the study. This level of antibiotic resistance may have been brought on by unchecked and excessive use of antibiotics in the poultry (18-20). Availability and accessibility of antibiotics in poultry contribute to their overuse, and according to the research finding by Salihu (21), the resistance seen in *E. coli* isolated from local chickens was caused by the transfer of resistance gene(s) from another host in the same production setting. Due to instances of comparable antibiotic resistance genes being simultaneously recovered from human and poultry samples, the proximity of these poultry to home could constitute a serious threat to biosecurity (7). The absorption of resistant genes discovered in single and multiple size plasmids in *E. coli* isolates was the likely mechanism by which these resistances were acquired.

The World Health Organization (WHO) recognized antibiotic resistance as one of the greatest threats to public health because of its potential impact on health outcomes globally as more bacteria develop antibiotic resistance (22). According to a United Kingdom (UK) assessment, bacterial infections that are resistant to antibiotics could kill over 10 million people by the year 2050 (23). It is worrisome that most of these resistant bacteria are zoonotic, and infections triggered by these pathogens can be difficult to treat because previously potent drugs become less efficacious against the same pathogen. Environmental contamination by antibiotic residue and AMR genes has been shown to be a major driver of AMR in livestock because up to 70% of antimicrobials administered to livestock has been shown to be released as unmetabolized agents (24).

Conclusion:

Since many isolates in this study were resistant to two or more drugs, it is likely that the local chicken population is home to *E. coli* strains that are multidrug resistant. Therefore, local poultry might act as a conduit for MDR transmission to humans. Furthermore, the environment, farms, and live bird markets in areas with low levels of biosecurity may have a significant impact on the spread of MDR *E. coli* among animals and humans through their network.

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Contributions of authors:

CGA was the principal investigator who conceived the idea of the study; CGA, AC

and JFN designed the study; CGA and ECSO carried out sampling and bacteriological assays; CGA, KNA, BJA and DC carried out the microbiological analysis on PCR and Vitek-2; CGA, AC, JFN, MM and KNA wrote the manuscript and were responsible for the final editing of the manuscript. All authors approved the final manuscript submitted for publication.

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Conflict of interest:

Authors declare no conflict of interest

References:

1. Abd El Tawab, A., Ammar, A., Nasef, S., and Reda, R. Prevalence of *E. coli* in diseased chickens with its antibiogram pattern. Benha Vet Med J. 2015; 28 (2): 224–230. https://doi.org/10.21608/bvmj.2015.32507
2. Coura, F. M., Diniz, S. A., Silva, M. X., et al. Phylogenetic Group of *Escherichia coli* Isolates from Broilers in Brazilian Poultry Slaughterhouse. Sci World J. 2017; https://doi.org/10.1155/2017/5898701
3. Abdu, H. U., and Ibrahim, A. Isolation and Characterization of Multidrug-resistant *Escherichia coli* from Poultry Litter samples from Selected Farms in Kano metropolis, Nigeria. Nig J Microbiol. 2021 35 (1): 5623- 5629.
4. Spitzer, H. An Analysis of Bacterial Contamination of Chicken Eggs and Antimicrobial Resistance. College of Saint Benedict and Saint John's University Digital Com. 2015
5. Sanchez, H. M., Whitener, V. A., Thulsiraj, V., et al. Antibiotic resistance of *Escherichia coli* isolated from conventional, no antibiotics, and humane family owned retail broiler chicken meat. Animals. 2020; 10 (12): 1–17. https://doi.org/10.3390/ani10122217
6. McInnes, R. S., McCallum, G. E., Lamberte, L. E., and van Schaik, W. Horizontal transfer of antibiotic resistance genes in the human gut microbiome. Curr Opin Microbiol. 2020; 53: 35–43. doi:10.1016/j.mib.2020.02.002.
7. Johnson, J. R., Porter, S. B., Johnston, B., et al. Extraintestinal pathogenic and antimicrobial resistant *Escherichia coli*, including sequence type 131 (ST131), from retail chicken breasts in the United States in 2013. J Appl Environ Microbiol. 2017; 83 (6): e02956-16 doi:10.1128/AEM.02956-16
8. Chollom, C. S., Osawe, S., Lar, P., Egah, D. Z., Mamman, I., and Abimiku, A. Analysis of Reported Cases of Lassa Fever in Plateau State and the Need for Strategic Action Plan. Int J Trop Dis Health. 2016; 13 (2): 1-7. doi:10.9734/IJTDH/2016/20568
9. Mude, S., Thomas, N., Kemal, J., and Muktar, Y. Cloacael Carriage and Multidrug Resistance *Escherichia coli* O157:H7 from Poultry Farms, Eastern Ethiopia. J Vet Med. 2017; 2: 1-9. https://doi.org/10.1155/2017/8264583
10. Feng, P., Weagant, S. D., Grant, M. A., and Burkhardt, W. Enumeration of *Escherichia coli* and the coliform bacteria. Bacteriological Analytical Manual (BAM), Chapter 4: 2017. https://www.fda.gov/food/laboratory-methods-food/bam-4-enumeration-escherichia-coli-and-coliform-bacteria.
11. Rega, M., Andriani, L., Poeta, A., Bonardi, S., Conter, M., and Bacci, C. The Pork Food Chain as a Route of Transmission of Antimicrobial Resistant *Escherichia coli*: A Farm-to-Fork Perspective. Antibiotics. 2023. doi: 10.3390/antibiotics12020376
12. Ejeh, F. E., Lawan, F. A., Abdulsalam, H., Mamman, P. H., and Kwanashie, C. N. Multiple antimicrobial resistance of *Escherichia coli* and Salmonella species isolated from broilers and local chickens retailed along the roadside in Zaria, Nigeria. Sokoto J Vet Sci. 2017; 15 (3): 45 – 53 doi:10.4314/sokjvs.v15i3.7
13. Adzitey, F., Assoah-Peprah, P., Teye, G. A., Somboro, A. M., Kumalo, H. M., and Amoako, D. G. Prevalence and Antimicrobial Resistance of *Escherichia coli* Isolated from Various Meat Types in the Tamale Metropolis of Ghana. Int J Food Sci. 2020; 12: 8877196 doi:10.1155/2020/8877196.
14. Geidam, Y. A., Ambali, A. G., and Onyeyili, P. A. Detection and antibiotic sensitivity pattern of avian pathogenic *Escherichia coli* strains among rural chickens in the arid region of north-eastern Nigeria. Vet World, 2012; 5 (6): 325–329.
15. Amadi, V. A., Watson, N., Onyegbule, O. A., et al. Antimicrobial resistance profiles of *Escherichia coli* recovered from feces of healthy free-range chickens in Grenada, West Indies. Int J Curr Microbiol Appl Sci. 2015; 4 (6): 168175
16. Nhung, N. T., Cuong, N. V., Thwaites, G., and Carrique-Mas, J. Antimicrobial Usage and Antimicrobial Resistance in Animal Production in Southeast Asia: A Review. Antibiotics (Basel). 2016; 5 (4): 37 doi:10.3390/antibiotics5040037
17. Vranic, S. M., and Uzunovic, A. Antimicrobial resistance of *Escherichia coli* strains isolated from urine at outpatient population: a single laboratory experience. Mater Sociomed. 2006; 28 (2): 121-124. doi:10.5455/msm.2016.28.121-124.
18. Velge, P., Cloeckaert, A., and Barrow, P. Emergence of *Salmonella* epidemics: The problem related to *Salmonella enterica* serotype *enteritidis* and multiple antibiotic resistance in other major serotypes. J Vet Res. 2005; 36 (3): 267–288. doi:10.1051/vetres:2005005.
19. Nsofor, C. A., Iroegbu, C. U., Call, D. R., and Davies, M. A. The genetic relatedness of drug resistant *E. coli* isolates of human and animal origin in Nigeria. Int J Genet Mol Biol. 2013; 5 (3): 37–41. doi:10.5897/IJGMB2013.0077
20. Adeyanju, G. T., and Ishola, O. Salmonella and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo state, Nigeria. Springer plus. 2014. doi: 10.1186/2193-1801-3- 139.
21. Salihu, A. E., Onwuliri, F. C., and Mawak, J. O. Antimicrobial resistance profiles of *Salmonella gallinarum* isolates from free range chickens in Nasarawa state, Nigeria. Int J Bacteriol Res. 2014 2 (1): 19 - 27.
22. World Health Organization. Global Action Plan on Antimicrobial Resistance. Geneva: WHO. 2017.
23. O'Neill, J. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. 2014; 1–16.
24. Anand Kumar, P. Antimicrobial Resistance (AMR) Surveillance Under One Health. In: Mothadaka, M. P., Vaiyapuri, M., Rao Badireddy, M., Nagarajrao Ravishankar, C., Bhatia, R., Jena, J. (eds). Handbook on Antimicrobial Resistance. Springer, Singapore. 2023: 1-15 doi:10.1007/978-981-16-9723-4_33-1

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Knowledge, attitude and prevention practice against hospital-acquired infections among healthcare workers in National Hospital Abuja, Federal Capital Territory, Nigeria

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Abstract:

Background: Hospital-acquired infections (HAIs) pose serious challenges to safe and high-quality healthcare delivery. They are associated with prolonged hospital stays, disability, economic burden, and mortality, and are usually consequences of poor infection prevention and control practices. The objective of this study was to assess the level of knowledge, attitude and practice of healthcare workers on infection prevention, and the determining factors at the National Hospital Abuja, Nigeria.
Methodology: This was a descriptive cross-sectional study of 300 participants selected by multi-stage and systematic random sampling techniques at the National Hospital Abuja. Data on knowledge of HAIs, attitude toward HAI prevention, and practice of HAI prevention were collected from each participant using self-administered structured questionnaires. Data were analyzed using the Statistical Package for the Social Science, version 25.0. Chi-square test was used to determine the association between categorical variables, and the level of significance was set at $p<0.05$.
Results: Of the 300 questionnaires administered, 286 were duly filled and returned, resulting in a response rate of 95.3%. One hundred and three (53.0%) respondents were within the age group 31-40 years, over half of the respondents were females (58.7%) and 57.0% had work experience of less than 5 years. Based on the cut-off scores of 15.7, 32.2 and 8.5 that characterized respondents' knowledge, attitude and practice of infection prevention respectively as good or poor, 50.4% of the respondents had good knowledge of HAIs, 71.0% had good attitude towards HAIs prevention and 55.5% had good infection prevention practices. However, good knowledge of HAIs was significantly associated with poor infection prevention practices ($p=0.002$). Female gender ($p=0.029$), work experience of less than 5 years ($p=0.036$), laboratory scientist profession ($p=0.010$), and no previous training on HAIs ($p=0.005$) were factors significantly associated with good infection prevention practices among the respondents.
Conclusion: In this study, good knowledge of HAIs, and infection prevention practices among the respondents were average, although good attitude towards HAIs prevention was high. These findings highlight the need to continue intensive and in-service trainings of healthcare workers toward HAIs prevention, including behavioral change, using innovative approaches.

Keywords: Hospital-acquired infection, health care workers, knowledge, attitude, practice

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Connaissances, attitudes et pratiques de prévention contre les infections nosocomiales chez les agents de santé de l'hôpital National d'Abuja, Territoire de la Capitale Fédérale, Nigéria

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Résumé:

Contexte: Les infections nosocomiales (IN) posent de sérieux problèmes pour la prestation de soins de santé sûrs et de qualité. Elles sont associées à des séjours hospitaliers prolongés, à l'invalidité, à un fardeau économique et à la mortalité, et sont généralement les conséquences de mauvaises pratiques de prévention et de contrôle des infections. L'objectif de cette étude était d'évaluer le niveau de connaissances, l'attitude et la pratique des professionnels de santé en matière de prévention des infections, ainsi que les facteurs déterminants à l'hôpital national d'Abuja, au Nigéria.
Méthodologie: Il s'agissait d'une étude transversale descriptive portant sur 300 participants sélectionnés par des techniques d'échantillonnage aléatoire systématique à plusieurs degrés à l'hôpital national d'Abuja. Les données sur la connaissance des IN, l'attitude envers la prévention des IN et la pratique de la prévention des IN ont été collectées auprès de chaque participant à l'aide de questionnaires structurés auto-administrés. Les données ont été analysées à l'aide du progiciel statistique pour les sciences sociales, version 25.0. Le test du chi carré a été utilisé pour déterminer l'association entre les variables catégorielles, et le niveau de signification a été fixé à $p<0,05$.
Résultats: Sur les 300 questionnaires administrés, 286 ont été dûment remplis et renvoyés, ce qui donne un taux de réponse de 95,3%. Français Cent trois (53,0%) répondants étaient dans la tranche d'âge de 31 à 40 ans, plus de la moitié des répondants étaient des femmes (58,7%) et 57,0% avaient une expérience professionnelle de moins de 5 ans. Sur la base des scores limites de 15,7, 32,2 et 8,5 qui caractérisaient respectivement les connaissances, l'attitude et la pratique des répondants en matière de prévention des infections comme bonnes ou mauvaises, 50,4% des répondants avaient une bonne connaissance des IASS, 71,0% avaient une bonne attitude à l'égard de la prévention des IASS et 55,5% avaient de bonnes pratiques de prévention des infections. Cependant, une bonne connaissance des IASS était significativement associée à de mauvaises pratiques de prévention des infections ($p=0,002$). Le sexe féminin ($p=0,029$), une expérience professionnelle de moins de 5 ans ($p=0,036$), la profession de scientifique de laboratoire ($p=0,010$) et l'absence de formation antérieure sur les IASS ($p=0,005$) étaient des facteurs significativement associés à de bonnes pratiques de prévention des infections parmi les répondants.
Conclusion: Dans cette étude, la bonne connaissance des IAS et les pratiques de prévention des infections parmi les répondants étaient moyennes, bien que la bonne attitude envers la prévention des IAS soit élevée. Ces résultats soulignent la nécessité de poursuivre les formations intensives et continues des agents de santé en matière de prévention des IAS, y compris le changement de comportement, en utilisant des approches innovantes.

Mots clés: infection nosocomiale, agents de santé, connaissances, attitude, pratique

Introduction:

According to the World Health Organization (WHO), hospital-acquired infections (HAIs), also known as nosocomial infections, are defined as infections occurring in patients during the process of care within a healthcare facility that was not present or incubating at the time of admission (1–4). HAIs are a major public health challenge globally and the incidence is increasing despite efforts at hospital infection control measure, and this contributes significantly to morbidity and mortality (5,6).

HAIs are the most frequent adverse events in healthcare facilities worldwide, and impose major detrimental effects on the quality of clinical services (1). The prevalence of HAIs ranging from 25% to 40% is high globally (4) and varies between countries and within healthcare facilities. However, according to the estimates from the WHO, hundreds of millions of patients are affected yearly by HAIs worldwide with significant proportion of these occurring in the low-and-middle-income-countries (4,7,8). In Europe, HAIs account for 16 million additional hospital stays with estimated total costs of €7 billion. HAIs also cost the United States healthcare system an estimated $30-45 million (9).

The risk of contracting HAIs has been reported to be up to 20 times more in developing compared with the developed countries (10). Thus, the spread of infection serves as a major source of worry for healthcare practice, particularly in the developing countries where the healthcare system is already overstrained. In this regard, HAIs affect patients, healthcare workers (HCWs), support staff, medical students, and patient attendants (9). HCWs are especially at increased risk of acquiring HAIs through occupational exposure (4).

It has been widely reported in the literature that there are variations in healthcare workers' knowledge, attitude, and practice regarding prevention of HAIs. Different studies have shown that most healthcare providers have good knowledge of HAIs with percentages ranging from 45.5% to 94.1% (10–15). The attitude of HCWs toward prevention of HAIs reported from different countries varies, with 55.6% in Ethiopia (9,16), 33.0% in Iran (17), and 82.9% in Nigeria (18). Several studies conducted in different parts of the world on HCWs practices of HAI prevention reported good practice, ranging from 50.8% to 84.6% (5,11,12,14,18,19). Age, gender, work experience, and previous training on infection prevention measures are some of the estab-

lished factors influencing HCWs knowledge and practice concerning HAIs (12,20). The objectives of this study are to assess HCWs knowledge, attitude, and prevention practices and determine factors affecting these parameters at the National Hospital, Abuja, Nigeria.

Materials and method:

Study area:

The study was conducted at the National Hospital, Abuja, Nigeria. Abuja is the Federal Capital Territory (FCT) which came into existence in 1976. It spreads over a land mass area of approximately 7,315 km^2, of which the definite city occupies 275.3 km^2. The population of Abuja is estimated to be approximately 2 million. The National Hospital Abuja (NHA) is one of the tertiary hospitals in the FCT and is a 450-bed tertiary public hospital located in the cosmopolitan city of Abuja.

Study design and participants:

The study is a descriptive cross-sectional survey carried out on healthcare workers at the National Hospital, Abuja. The study participants are consenting healthcare workers who have worked for a minimum of one year and are potentially at high risk of HAIs. Health care workers on any form of leave were excluded from the study.

Ethical consideration:

Ethical approval was obtained from the Ethical Review Board of the National Hospital, Abuja. Informed consent was obtained from the respondents after the objective of the study was duly explained to them.

Sample size calculation:

The Leslie Fisher's formula was used to calculate the sample size which gave a calculated sample size of 248, which was adjusted for 10% non-response rate, to give adjusted sample size of 273. However, a total of 300 questionnaires were distributed to increase the power of the study.

Sampling techniques:

The multistage and systematic random sampling techniques were employed in the selection of the 300 respondents. Healthcare workers were stratified into different cadres such as nurses, doctors, laboratory scientists and others. The list of different cadres was generated, and respondents were selected by systematic random sampling method based on proportional allocation of number of staffs per cadre. Sampling continued until the desired sample size of 300 was obtained.

Data collection instrument and administration:

A semi-structured interviewer administered questionnaire was administered to the participants to collect information on their knowledge of nosocomial infection, attitude toward nosocomial infection prevention and practice of nosocomial infection prevention. The questionnaire was adapted from previous studies. The procedure for filling the questionnaire was explained to the participants by trained research assistants and were manually collected after being successfully filled.

Data analysis:

The questionnaires were checked for completeness and clarity. The data were analysed using IBM Statistical Packages for Social Sciences (SPSS) Version 25.0. Frequency distributions, percentages, and mean scores of variables were computed. Chi-square test was used for bivariate analysis of categorical variables at 5% level of significance.

Results:

Socio-demographic characteristics of respondents:

Of the 300 questionnaires administerred, 286 were duly filled and returned, given a response rate of 95.3%. Table 1 presents the socio-demographic characteristics of the respondents. The largest number of the respondents were in the age group 31-40 years (36.0%, n=103), females (58.7%, n=168), and first-degree holder (67.5%, n=193). More respondents (57%, n=163) had less than 5 years of work experience.

Table 1: Sociodemographic characteristics of the respondents at the National Hospital, Abuja, Nigeria

Characteristics	Frequency	Percentage
Age group (years)		
<20	4	1.4
21-30	93	32.5
31-40	103	36.0
41-50	60	21.0
51-60	24	8.4
>61	2	0.7
Gender		
Male	118	41.3
Female	168	58.7
Tribe		
Yoruba	85	29.7
Igbo	123	43.0
Hausa	78	27.3
Religion		
Christianity	202	70.6
Islam	82	28.7
Traditionalist	2	0.7
Education		
Diploma	33	11.5
Bachelor degree	193	67.5
Master degree	60	21.0
Length of work experience		
<5years	163	57.0
>5years	123	43.0
Profession		
Medical doctor	32	11.2
Dentist	19	6.6
CHEW	79	27.6
Nurse	49	17.1
Radiographers	14	4.9
Pharmacist	15	5.2
Laboratory Scientist technician	30	10.5
Optometrist	12	4.2
Others	36	12.6
Any seminar/training on nosocomial infection		
Yes	161	56.3
No	125	43.7

CHEW: community health extension worker

Table 2: Knowledge of hospital-acquired infections among the respondents at National Hospital, Abuja, Nigeria

Variables	Frequency	Percentage
Definition of nosocomial infection		
Right answer	270	94.5
Wrong answer	16	5.5
Source of information**		
Television	128	44.8
Training and seminar	170	59.4
Family and friends	21	7.3
Others	26	9.1
Source of nosocomial infection**		
Hospital	240	83.9
Patients bring from home	12	4.2
Could either be from hospital or home	41	14.3
Ways of preventing nosocomial infection**		
Face mask use by patient	240	84.2
Good waste management	269	94.4
Aseptic care of puncture wound	145	50.9
Segregation of clinical and non- clinical waste	280	98.6
Blood-stained linen should be thrown in red linen bag	268	94.0
Use of surgical face mask by health worker	282	98.9
Regular hand washing	283	99.3
Wearing of apron and gown to prevent splash	280	98.2

** Multiple responses allowed.

Respondents' knowledge of nosocomial infection:

Table 2 shows that almost all the respondents (94.5%, n=270) were correct about the definition of nosocomial infection. The main source of respondents' information on nosocomial infection was from training and seminars (59.4%). Most of the respondents (83.5%) believe that nosocomial infections are contracted from the hospital with most implycated organisms are from urinary tract infection (72.4%) followed by hepatitis C virus infection (58.0%).

Respondents' attitude towards nosocomial infection prevention:

Tables 3 shows that almost all the respondents agreed to the categorizing of hospital waste before disposal (93.3%, n=264), that health worker's hand is a potential source of infection (93.3%, n=264), that washing of hands after removing gloves is helpful (96.5 %, n=276) and that antiseptic is necessary (94.0% n=266). About 67.5% (n=191) of the respondents agreed that changing mask before moving to another patient is helpful, 86.6% (n= 245) responded that invasive procedures are risk factors for multidrug resistant (MDR) infections and 87.2% (n=246) responded that patient's history determines their use of personal protective equipment (PPE).

Respondents' practice of nosocomial infection prevention and control:

Table 4 shows that more than two third (66.8%, n=189) of respondents always perform hand washing before starting work while almost all (92.2%, n=261) perform hand washing before handling new patients. Almost all the respondents change gloves before handling new patients (92.9%, n=263), wear masks while handling TB-suspected patients (93.6%, n=264) and disinfect infectious materials or leftover samples (91.1%, n=257).

Table 3: Attitude of respondents towards prevention of hospital-acquired infections in National Hospital, Abuja, Nigeria

Variables	Frequency	Percentage
Categorizing hospital waste before disposal		
Agree	264	93.3
Indifferent	14	4.9
Disagree	8	1.8
Health care workers' hand is a potential source of infection		
Agree	264	93.3
Indifferent	12	4.2
Disagree	10	2.5
Changing mask before moving to another patient is helpful		
Agree	191	67.5
Indifferent	80	28.3
Disagree	15	4.2
Invasive procedure is a risk factor for multidrug resistance		
Agree	245	86.6
Indifferent	35	12.4
Disagree	6	1.1
Washing of hands after removing glove is helpful		
Agree	276	96.5
Indifferent	7	2.4
Disagree	3	1.1
Patient's history determines my use of PPE		
Agree	246	87.2
Indifferent	22	7.8
Disagree	18	5.0
Antiseptic is necessary		
Agree	266	94.0
Indifferent	14	4.9
Disagree	6	1.1

Table 4: Respondents prevention practices towards hospital-acquired infections in National Hospital, Abuja, Nigeria

Variables	Frequency	Percentage
Hand washing before starting work		
Always	189	66.8
Often	76	26.9
Sometimes	12	4.2
Not at all	9	2.1
Hand washing before handling new patient		
Always	261	92.2
Often	16	5.7
Not at all	9	2.1
Changing gloves before start handling new patients		
Always	263	92.9
Often	13	4.6
Sometimes	1	0.4
Not at all	9	2.1
Wearing mask during handling TB suspected patient		
Always	264	93.6
Often	10	3.5
Sometimes	2	0.7
Not at all	6	2.1
Disinfection of infectious materials and left over sample		
Always	257	91.1
Often	15	5.3
Sometimes	4	1.4
Not at all	6	2.1

Cut-off scores for respondents' knowledge, attitude and practice:

The cut-off scores that characterized respondents' knowledge of nosocomial infection, attitude towards infection prevention, and practice of prevention measures as good or poor, were 15.7, 32.2 and 8.5 respectively. Based on these scores, half of respondents (50.4%, n=144) have good knowledge of nosocomial infection, more than two third (71%, n=204) have good attitude towards prevention of nosocomial infection, and just over half (55.5%, n=158) practice preventive measures effectively (Table 5).

Bivariate analysis of respondents' knowledge and practice of nosocomial infection prevention with socio-demographic characteristics:

Table 6 shows the associations between socio-demographic characteristics of the respondents with knowledge and practice of infection prevention. Knowledge of nosocomial infection was statistically associated with prevention practices, but it was good knowledge that was significantly associated with poor prevention practices (p=0.002).

Table 7 shows that female gender (60.9%, 100/164, p=0.029), work experience of <5 years (60.9%, 98/161, p=0.036), laboratory scientist profession (60.0%, 18/30, p= 0.010), and no previous training on HAIs (65.0%, 80/123, p=0.005) were factors significantly associated with good infection prevention practices among the respondents.

Table 5: Respondents knowledge, attitude and practice scores of hospital-acquired infections in National Hospital, Abuja

Parameter	Number of respondents		
	Poor (%)	Good (%)	Total (%)
Knowledge	142 (49.6)	144 (50.4)	286 (100.0)
Attitude	82 (29.0)	204 (71.0)	286 (100.0)
Practice	128 (45.5)	158 (55.5)	286(100.0)

Table 6: Bivariate analysis of respondents' knowledge of hospital-acquired infections and infection prevention practice

Variables	Practice		Total (%)	x^2 statistics	OR (95% CI)	*p* value
	Poor (%)	Good (%)				
Knowledge						
Poor (%)	49 (35.3)	90 (64.7)	139 (49.5)	9.493	0.4728 (0.2927-0.7637)	0.002*
Good (%)	66 (46.5)	76 (53.5)	142 (50.5)			
Total (%)	125 (44.5)	156 (55.5)	281 (100.0)			

OR=Odds Ratio; CI=Confidence Interval

Table 7: Bivariate analysis of sociodemographic characteristics of respondents with nosocomial infection prevention practice

Variables	Practice		Total (%)	x^2 statistics	*p* value
	Poor (%)	Good (%)			
Gender					
Male	61 (52.1)	56 (47.9)	117 (41.6)	4.754	0.029*
Female	64 (30.1)	100 (60.9)	164 (58.4)		
Tribe					
Yoruba	38 (45.2)	46 (54.8)	84 (29.9)	0.029	0.986
Igbo	54 (44. 3)	68 (55.7)	122 (43.4)		
Hausa	33 (44.0)	42 (56.0)	75 (26.7)		
Religion					
Christianity	93 (47.0)	105 (53.0)	198 (70.5)	2.911	0.233
Islam	32 (39.5)	49 (60.5)	81 (28.8)		
Traditionalist	0	2 (100.0)	2 (0.7)		
Educational status					
Diploma	13 (40.6)	19 (59.4)	32 (11.4)	2.901	0.234
Bachelor degree	80 (42.1)	110 (57.9)	190 (67.6)		
Master's degree	32 (54.2)	27 (45.8)	59 (21.0)		
Work experience					
<5 years	63 (39.1)	98 (60.9)	161 (57.3)	4.375	0.036*
>5years	62 (51.7)	58 (48.3)	120 (42.7)		
Profession					
Medical Doctor	16 (50.0)	16 (50.0)	32 (11.4)	20.176	0.010*
Dentist	9 (50.0)	9 (50.0)	18 (6.4)		
CHEWs	42 (54.5)	35 (45.5)	77 (27.4)		
Nurses	24 (50.0)	24 (50.0)	48 (17.1)		
Radiographers	6 (42.9)	8 (57.1)	14 (5.0)		
Pharmacist	7 (46.7)	8 (53.3)	15 (5.3)		
Laboratory scientist	12 (40.0)	18 (60.0)	30 (10.7)		
Optometrist	5 (41.7)	7 (58.3)	12 (4.3)		
Others	4 (11.4)	31 (88.6)	35 (12.5)		
Any training on hospital-acquired infection					
Yes	82 (51.9)	76 (48.1)	158 (56.2)	8.036	0.005*
No	43 (35.0)	80 (65.0)	123 (43.8)		

* = statistically significant at $p<0.05$

Discussion:

Nosocomial infections among healthcare workers and the patients they take care more often than not, occurs from breach in the guidelines on hospital infection control. To reduce this, there is a dire need to orientate and re-orientate healthcare givers on infection control measures. To this end, it is imperative to understand the gaps in knowledge as well as practice of infection control among healthcare workers. In this study, the proportion of healthcare workers who had good knowledge of HAIs is consistent with the report of the study conducted in Ethiopia (11). However, the knowledge score in our study was lower than many other studies conducted in Nigeria and some other parts of the world where the knowledge score was much higher (10,13–15). This finding could be explained by the fact that only a little above half of the respondents have had seminar on the nosocomial infection.

The finding of 71.0% of respondents with good attitude toward prevention of nosocomial infection in our study is lower than the rate reported in a study from Addis Ababa,

Ethiopia (9) but higher than those of other studies (17,18). The difference in rates might be due to differences in the experience of healthcare workers and training exposure. The level of practice of preventive measures against nosocomial infection among the health workers in our study is comparably similar to that of Asfwa et al., (11) in Ethiopia, but slightly higher than that of a previous study conducted in two tertiary hospitals in Nigeria (19). However, the score in our study is lower than those reported from similar studies in Nigeria and other parts of the world (12,18, 21). This discrepancy could be due to differences in sample size and study participants since those previous studies were conducted among only a group of professionals, while our study was conducted across many professional groups. This may also imply that efforts are needed to increase knowledge of HAIs and practice of infection prevention, that will lead to reduction in the incidence of hospital-acquired infections.

In our study, the number of laboratory scientists with good infection prevention practice was significantly higher than other group of healthcare workers. This may be explained by the fact that laboratory scientists handle microorganisms directly in the laboratories and may therefore be more conscious of infection control practices that prevent them from acquiring HAIs in the laboratories. In this study, there was a significant relationship between respondent healthcare workers knowledge of HAIs and infection prevention practices, which is similar to those of other studies that reported significant relationship between knowledge of HAIs and infection prevention practices (5,22). However, the relationship in our study was opposite, with good knowledge of HAIs significantly associated with poor infection prevention practices among the respondents ($p=0.002$). This is at variance with the finding of the two studies (5,22) which reported that good knowledge of HAIs is a predictor of good infection prevention practices.

With regards to sociodemographic characteristics of the respondents and infection prevention practices, there was a significant relationship between years of experience of healthcare workers and infection prevention practice ($p=0.036$) in our study, which agrees with those of previous studies (10,23). These previous studies showed that longer length of work experience was significantly associated with infection prevention practices, which may be attributed to increased knowledge of the use of preventive equipment and the amount of in-service training that healthcare workers may have received. However, surprisingly in our study, significantly higher number of respondent healthcare workers with less than 5-year work experience had good infection prevention practice compared to those with more than 5-year work experience, which contradicts the findings of these previous studies.

Surprisingly still, significantly higher number of respondent healthcare workers who had no training on HAIs had good infection prevention practice compared with those who have had any training on HAIs ($p=0.005$). This contrasts the finding of a previous study conducted in northern Nigeria which reported that HAI training contributed to improved knowledge and compliance with standard infection control precautions (21). These contrasting findings of our study may be due to over-familiarity with standard infection prevention practices among older healthcare workers and those who have had trainings on HAIs, which may lead to complacency in adhering to prevention practices. This indicates that training of healthcare workers on HAIs should, in addition to education and awareness, incorporate behavior change program and periodic monitoring and evaluation, that will ensure compliance with HAIs prevention practice among new and old healthcare workers.

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Contributions of authors:

IOR was involved in the study conceptualization, literature review, data analysis and project supervision; AUC was involved in study conceptualization, data collection, and writeup of the manuscript; OSI and AVT were involved in literature review and methodology; and ANE was involved in literature review and search. All authors approved the final manuscript submitted for publication.

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References:

1. Hassan, K., Islam, S., Islam, Z., et al. Healthcare providers infection prevention practices and associated factors in community clinics in

Bangladesh: A cross-sectional study. PLoS Glob Publ Health. 2022; 2 (6): e0000574. doi:10.1371/journal.pgph.0000574
2. Khan, H. A., Ahmad, A., and Mehboob, R. Nosocomial infections and their control Strategies. Asian Pac J Trop Biomed. 2015; 5 (7): 509–514. https://doi.org/10.1016/j.apjtb.2015.05.001
3. Yasmeen, S., Ali, T. S., Khalid, W., Kurji, Z., Hazara, S. M., and Bashir, S. Knowledge and Practices Regarding Standard Precautions for Infection Control Among Nurses Working at a Public, Tertiary Care Hospital Islamabad, Pakistan. Int J Curr Res Rev. 2022; 14 (07): 32–39. doi:10.31782/IJCRR.2022.14702
4. World Health Organization. Report on the burden of endemic health care-associated infection worldwide: A systemic review of the literature. World Health Organization; Geneva, Switzerland. 2011.
5. Maitanmi, J. O., Anise, I., Maitanmi, O. T., Okwuipo, M. I., and Leslie, T. A. Preventive practices of nosocomial infections among health workers in two selected tertiary hospitals in Ogun State, Nigeria. Bayero Journal of Nursing and Healthcare. 2021; 3 (1): 737-747.
6. Mbim, E. N., Mboto, C. I., and Agbo, B. E. A review of nosocomial infections in sub-Saharan African. Br Microbiol Res J. 2016; 15 (1): 1–11. doi:10.9734/BMRJ/2016/25895
7. Nwankwo, E. Isolation of pathogenic bacteria from fomites in the operating room of a specialist hospital in kano North-western Nigeria. Pan Afr Med J. 2012; 12: 90.
8. Geberemariyam, B. S., Donka, G. M., and Wordofa, B. Assessment of knowledge and practices of healthcare workers towards infection prevention and associated factors in healthcare facilities of West Arsi District, Southeast Ethiopia: A facility-based cross-sectional study. Arch Publ Health. 2018; 76 (1): doi: 10.1186/s13690-018-0314-0.
9. Sahiledengle, B., Gebresilassie, A., Getahun, T., and Hiko, D. Infection Prevention Practices and Associated Factors among Healthcare Workers in Governmental Healthcare Facilities in Addis Ababa. Ethiop J Health Sci. 2018; 28 (2): 177–186. doi:10.4314/ejhs.v28i2.9
10. Maitanmi J. O., and Anise, I. Knowledge and Preventive Practices of Nosocomial Infections among Health Workers in Two Selected Tertiary Hospitals in Ogun State. Int J Caring Sci. 2021: 14 (1): 174-184
11. Asfaw, N. Knowledge and practice of nurses towards prevention of hospital acquired infections and its associated factors. Int J Afr Nurs Sci. 2021; 15: https://doi.org/10.1016/j.ijans.2021.100333
12. Sebro, S. F., Birhanu, M., Bilal, A., and Sahle, T. Knowledge and practice toward hospital-acquired infections prevention and associated factors among nurses working at university referral hospitals in Southern Nations, Nationalities, and Peoples' Region, Ethiopia 2021. SAGE Open Med. 2023; 11: 20503121221149362. doi:10.1177/20503121221149362
13. Bayleyegn, B., Mehari, A., Damtie, D., and Negash, M. Knowledge, attitude and practice on hospital-acquired infection prevention and associated factors among healthcare workers at university of Gondar comprehensive specialized hospital, northwest Ethiopia. Infect Drug Resist. 2021; 14: 259–266. doi: 10.2147/IDR.S290992
14. Alrubaiee, G., Baharom, A., Shahar, H. K., Daud, S. M., and Basaleem, H. O. Knowledge and practices of nurses regarding nosocomial infection control measures in private hospitals in Sana'a City, Yemen. Saf Health. 2017; 3: 16 https://doi.org/10.1186/s40886-017-0067-4
15. Arinze-Onyia, S. U., Ndu, A. C., Aguwa, E. N., Modebe, I., and Nwamoh, U. N. Knowledge and practice of standard precautions by health-care workers in a tertiary health institution in Enugu, Nigeria. Niger J Clin Pract. 2018; 21 (2): 149–155. doi: 10.4103/njcp.njcp_69_17.
16. Gulilat, K. Assessment of Knowledge, Attitude and Practice of Health Care Workers on Infection Prevention in Health Institution Bahir Dar City Administration. Sci J Publ Health. 2014; 2 (5): 384-393.doi:10.11648/j.sjph.20140205.13
17. Sarani, H., Balouchi, A., Masinaeinezhad, N., and Ebrahimitabas, E. Knowledge, Attitude and Practice of Nurses about Standard Precautions for Hospital-Acquired Infection in Teaching Hospitals Affiliated to Zabol University of Medical Sciences Glob J Health Sci. 2015; 8 (3): 193–198. doi: 10.5539/gjhs.v8n3p193
18. Ojo, O., Adeniran, A., Goodman, O., et al. Infection Control Practices among Laboratory Healthcare Workers in an Urban Local Government Area of Lagos State, Nigeria. Babcock University Medical Journal (BUMJ). 2021; 4 (2): 86–96.https://doi.org/10.38029/bumj.v4i2.82
19. Ogoina, D., Pondei, K., Adetunji, B., Chima, G., Isichei, C., and Gidado, S. Knowledge, attitude and practice of standard precautions of infection control by hospital workers in two tertiary hospitals in Nigeria. J Infect Prev. 2015; 16 (1): 16–22.doi: 10.1177/1757177414558957
20. Kolade, O. A., Abubakar, S., Adejumoke, S. R., Hanson, V. F., and Tijani, A. Knowledge, attitude and practice of surgical site infection prevention among post-operative nurses in a tertiary health institution in north-central Nigeria. International Journal for Nursing and Midwifery. 2017; 9 (6): 65–69. doi: 10.5897/IJNM2017.0262
21. Iliyasu, G., Dayyab, F. M., Habib, Z. G., et al. Knowledge and practices of infection control among healthcare workers in a Tertiary Referral Center in North-Western Nigeria. Ann Afr Med. 2016; 15 (1): 34–40. doi: 10.4103/1596-3519.161724
22. Oli, A. N., Okoli, K. C., Ujam, N. T., Adje, D. U., and Ezeobi, I. Health professional's knowledge about relative prevalence of hospital-acquired infections in Delta State of Nigeria. Pan Afr Med J. 2016; 24 (1): 148–153. doi: 10.11604/pamj.2016.24.148.9270
23. Desta, M., Ayenew, T., Sitotaw, N., Tegegne, N., Dires, M., and Getie, M. Knowledge, practice and associated factors of infection prevention among healthcare workers in Debre Markos referral hospital, Northwest Ethiopia. BMC Health Serv Res. 2018; 18 (1): 465. doi: 10.1186/s12913-018-3277-5.

Nwafia et al. *Afr. J. Clin. Exper. Microbiol.* 2024; 25 (3): 342 – 349 https://www.afrjcem.org

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Knowledge of antibiotic resistance and habits of antibiotic use among medical students of University of Nigeria Enugu: a descriptive cross-sectional survey

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Abstract:

Background: The adverse effects of antimicrobial resistance (AMR) are manifesting worldwide. A major contributing factor to AMR is the inappropriate use of antimicrobials in humans and animals. It is imperative to raise awareness among medical students of the current public health challenges of AMR and make them antibiotic guardians because they are the future medical doctors. This survey was designed to assess the knowledge of AMR and habits of antimicrobial use (AMU) among medical students as a means to guide them in their future practice.
Methodology: This descriptive cross-sectional survey was conducted among medical students of the College of Medicine, University of Nigeria (UNN), Enugu State, Nigeria, from July to September 2021. The sample size of 602 was calculated using an online Raosoft sample size calculator. Pretested structured questionnaires, designed to collect information on students' knowledge of antimicrobials and factors responsible for emergence of AMR as well as the students' habits of antimicrobial use, were self-administered to consenting students. The data were analyzed using descriptive and inferential statistics.
Results: Of the 602 questionnaires administered to the students, 550 were filled out, giving a response rate of 91.4%. Out of the 550 respondents, 60.4% were females, majority (51.1%) of the respondents were between the ages of 21 and 25 years. Regarding knowledge, 97.2%, 62.5%, and 54.2% have heard of the terms 'antibiotic resistance', 'multi-drug drug resistance' and 'antimicrobial stewardship' respectively. About 97.3% knew that AMR was a global problem, however only 64.7% felt that it was a problem for medical students. Surprisingly, 20.4% indicated that viruses were sensitive to antibiotics. Regarding the students' habit of antimicrobial use, only 22.2% always consult a doctor before starting an antibiotic, 13.1% go for laboratory tests, and 90.5% always take antibiotics anytime they have a fever. Above half of the participants (56.5%) do not complete the dosage of the antibiotics while 63.5% keep leftover antibiotics for future use. In assessing the factors responsible for AMR emergence, 88.8% responded not adhering to a doctor's prescription and 92.0% responded poor quality of drugs, while only 42.8% responded that overuse of antibiotics in livestock is a factor.
Conclusion: Our study gave an insight into the knowledge gap and the need to increase awareness and education on AMR and AMS among the medical students, especially in the early phase of their academic and professional training.

Keywords: Antimicrobial use; Antimicrobial resistance; Knowledge: Habits; Medical students

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Connaissance de la résistance aux antibiotiques et des habitudes d'utilisation des antibiotiques parmi les étudiants en médecine de l'Université du Nigéria à Enugu: une enquête transversale descriptive

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Résumé:

Contexte: Les effets néfastes de la résistance aux antimicrobiens (RAM) se manifestent dans le monde entier. L'un des principaux facteurs contribuant à la RAM est l'utilisation inappropriée d'antimicrobiens chez les humains et les animaux. Il est impératif de sensibiliser les étudiants en médecine aux enjeux de santé publique actuels liés à la RAM et d'en faire les gardiens des antibiotiques car ils sont les futurs médecins. Cette enquête a été conçue pour évaluer les connaissances sur la RAM et les habitudes d'utilisation des antimicrobiens (UMA) parmi les étudiants en médecine afin de les guider dans leur pratique future.
Méthodologie: Cette enquête transversale descriptive a été menée auprès d'étudiants en médecine de la Faculté de médecine de l'Université du Nigéria (UNN), dans l'État d'Enugu, au Nigéria, de juillet à septembre 2021. La taille de l'échantillon de 602 a été calculée à l'aide d'un échantillon en ligne de Raosoft calculatrice. Des questionnaires structurés prétestés, conçus pour recueillir des informations sur les connaissances des étudiants en matière d'antimicrobiens et de facteurs responsables de l'émergence de la RAM, ainsi que sur leurs habitudes d'utilisation des antimicrobiens, ont été auto-administrés aux étudiants consentants. Les données ont été analysées à l'aide de statistiques descriptives et inférentielles.
Résultats: Sur les 602 questionnaires administrés aux étudiants, 550 ont été remplis, soit un taux de réponse de 91,4%. Sur les 550 répondants, 60,4% étaient des femmes, la majorité (51,1%) des répondants étaient âgés de 21 à 25 ans. Concernant les connaissances, 97,2%, 62,5% et 54,2% ont respectivement entendu parler des termes «résistance aux antibiotiques», «résistance multidrogue» et «gestion des antimicrobiens». Environ 97,3% savaient que la RAM était un problème mondial, mais seulement 64,7% estimaient qu'il s'agissait d'un problème pour les étudiants en médecine. Étonnamment, 20,4% ont indiqué que les virus étaient sensibles aux antibiotiques. Concernant l'habitude des étudiants en matière d'utilisation des antimicrobiens, seuls 22,2% consultent toujours un médecin avant de commencer un antibiotique, 13,1% passent des analyses de laboratoire et 90,5% prennent toujours des antibiotiques chaque fois qu'ils ont de la fièvre. Plus de la moitié des participants (56,5%) ne terminent pas le dosage des antibiotiques tandis que 63,5% conservent les antibiotiques restants pour une utilisation future. En évaluant les facteurs responsables de l'émergence de la RAM, 88,8% ont répondu le non-respect des prescriptions médicales et 92,0% ont répondu que les médicaments étaient de mauvaise qualité, tandis que seulement 42,8% ont répondu que la surutilisation d'antibiotiques chez le bétail était un facteur.
Conclusion: Notre étude a donné un aperçu du manque de connaissances et de la nécessité d'accroître la sensibilisation et l'éducation sur la RAM et l'AMS parmi les étudiants en médecine, en particulier dans la phase précoce de leur formation académique et professionnelle.

Mots clés: Utilisation d'antimicrobiens; Résistance aux antimicrobiens; Connaissances: Habitudes; Étudiants en médecine

Introduction:

It has been said that the discovery of antibiotics represents the single biggest historical gain in healthcare. After antibiotics were developed and widely used, the mortality linked to several previously lethal illnesses significantly decreased. Antimicrobial drugs are the bedrock of modern medicine and improve average life expectancy and quality of life generally. However, the emergence and spread of antimicrobial resistance (AMR) are challenging the ability to treat and contain infectious diseases (1). The irresponsible use of antimicrobial agents is the main factor speeding up the process of emergence of AMR (2), although this can happen naturally over time through genetic changes.

It has been reported that about 60% of antimicrobial drugs in both therapy and prophylaxis are considered inappropriate (3). The adverse effects of AMR are manifesting worldwide and the World Health Organization has rated AMR among the top ten public health threats (4), with the burden being higher in middle-and-low-income countries, including Nigeria. Many cases of AMR have been reported in Nigeria and beyond. As AMR raises healthcare costs and prolongs disability (1), it continues to pose a danger to the effective prevention and treatment of a wide range of illnesses.

Medical doctors have a major role to play in reversing the scourge of AMR because they prescribe antibiotics during routine clinical practice, promote health education, and encourage patients to strictly adhere to antimicrobial therapy and avoid self-medication (5). Moongtui and colleagues (6) observed that intake of antimicrobials without prescription and improper physician prescriptions, among others, are some of the variables that contribute to the rise in AMR. Thus, any change in prescribing behaviour promoting responsible antibiotic use through interventions in education may help to tackle AMR.

Medical students are future doctors therefore, it is imperative to make them aware of current public health issues and integrate them into appropriate prescribing prac-

tices. Previous researchers have recommended that AMR interventions targeting responsible antimicrobial use should be introduced early in the medical career and continued until the postgraduate level (7,8). According to a Nigerian study, medical students only had mediocre understanding of antibiotic resistance, which emphasizes the need for improved education and awareness (9). Mudenda et al., (10), observed that undergraduate medical students from Zambia need to improve their practices even though they showed acceptable knowledge and attitudes regarding antibiotic use and resistance. The WHO has also highlighted the importance of adequate training for medical students in responsible antimicrobial prescribing (11). The Global Action Plan (GAP) on AMR stated education, knowledge and training as part of their objectives, emphasizing their importance in the fight against AMR (12). Thus, medical students play a major role in combating the spread of AMR because the knowledge they acquired during their training will help promote responsible antimicrobial prescribing and antimicrobial use.

Realizing the importance of medical students in combating AMR, we designed this study with the aim of assessing the knowledge of AMR and habits of antibiotic use among medical students of University of Nigeria, Enugu, Nigeria. This will help provide advice on the target area for intervention in order to combat the rising incidence of AMR.

Materials and method:

Study setting:

The study was conducted among medical students at the College of Medicine, University of Nigeria (UNN). UNN is a federal university and the first indigenous university in Nigeria. The university has three campuses for medical students; the first campus is located in Nsukka for the first-year pre-clinical students, the second campus is in Enugu for the second- and third-year pre-clinical students, and the third campus is in Ituku-Ozalla for the fourth-, fifth- and sixth-year clinical students. The college has over 1000 students across the six study levels.

Ethical consideration:

The study protocol was approved by the Research and Health Ethics Committee of the University of Nigeria Teaching Hospital (UNTH) Enugu. Informed consent of each study participant was obtained.

Study design, participants & sampling:

This was a descriptive cross-sectional survey conducted from July to September 2021 among medical students at different levels of study using a self-administered questionnaire. The sample size for the study was calculated using an online Raosoft calculator with a 5% error margin and 95% confidence interval, which gave a sample size of 602. The study participants were recruited by the simple random sampling technique.

Data collection:

The questionnaire was created after intense literature review and contained four sections; the first section collected information on demographic characteristics of the participants, the second section assessed the knowledge of antimicrobials and AMR, the third assessed the habits of antimicrobial use and the fourth section assessed knowledge of the factors responsible for emergence of AMR. The questionnaire was first pilot-tested among 20 medical students who were randomly selected from different years of study, before being self-administered to the study participants.

Data analysis:

Data were entered in Microsoft Excel, 2016 version and analysed using the Statistical Package for the Social Sciences (SPSS). Descriptive analysis for categorical analysis was done using frequencies and proportions.

Results:

Sociodemographic characteristics of the study participants:

A total of 602 questionnaires were ad ministered to the study participants but 550 questionnaires were completely filled, given a response rate of 91.36%. Out of the 550 study participants, 56.0% were females, with majority (40.2%) being within the ages of 21-25 years, followed by 26-30 years (32.9 %) and the least (1.5%) being 31-35 years. With regards to the years (level) of study, 22.0% were in 400 level (4th year), 21.6% in 200 level (2nd year), 18.9% in 500 level and 18.4% in 300 level. Majority (91.3%) of the participants were single (Table 1).

Table 1: Socio-demographic characteristics of the study participants

Characteristics	Frequency	Percentage
Age group (years)		
15-20	140	25.5
21-25	221	40.2
26-30	181	32.9
31-35	8	1.5
Gender		
Male	242	44.0
Female	308	56.0
Marital status		
Single	502	91.3
Married	48	8.7
Year (level) of study		
100	50	9.1
200	119	21.6
300	101	18.4
400	121	22.0
500	104	18.9
600	55	10.0
Ethnicity		
Igbo	527	95.8
Hausa	8	1.5
Yoruba	11	2.0
Others	4	1.0

Table 2: Knowledge of medical students on antibiotics use and resistance

Question	Frequency of response	Percentage
Have you heard about antibiotics resistance?		
Yes	535	97.3
No	15	2.7
Have you heard about multidrug resistance organisms?		
Yes	344	62.5
No	206	37.5
Have you heard about antimicrobial stewardship?		
Yes	298	54.2
No	252	45.8
Antimicrobial resistance is a global problem		
Yes	535	97.3
No	0	0
I don't know	15	2.7
Antimicrobial resistance is a problem for medical students		
Yes	355	64.7
No	135	24.5
I don't know	60	10.9
Which microorganism is sensitive to antibiotics?		
Bacteria	392	71.2
Viruses	112	20.4
Fungi	54	9.8
Antimicrobial resistance is the ability of the organisms o grow in presence of drugs that will normally kill or inhibit their growth		
Yes	386	70.2
No	52	9.4
I don't know	112	20.4
How long should a patient take antibiotics?		
When symptoms stop	120	21.8
As prescribed by the physician	430	78.2

Knowledge of antibiotic use and resistance:

Participants were required to respond to questions that assessed their knowledge about antibiotic use and resistance. About 97.3%, 62.5%, and 54.2% indicated that they have heard of the terms 'antibiotic resis-

tance', 'multidrug drug resistance' and 'antimicrobial stewardship' respectively. Among the study participants, 97.3% agreed that it was a global problem. However, only 64.7% felt that it is a problem for medical students. Surprisingly, 20.4% indicated that viruses are sensitive to antibiotics. Among the study participants, 70.2% knew that antimicrobial resistance is the ability of the organisms to grow in the presence of drugs that would normally kill or inhibit their growth. The results are reflected in Table 2.

When comparing the responses across the years of study, the percentage of students with knowledge of AMU and AMR increased progressively with the years of study, 39.0% of students in the 1st and 2nd year, 58.0% in the 3rd year, 85.0% in the 4th year, 95.0% in the 5th year and 99.0% in the 6th year, had knowledge of AMR and AMS (Fig 1).

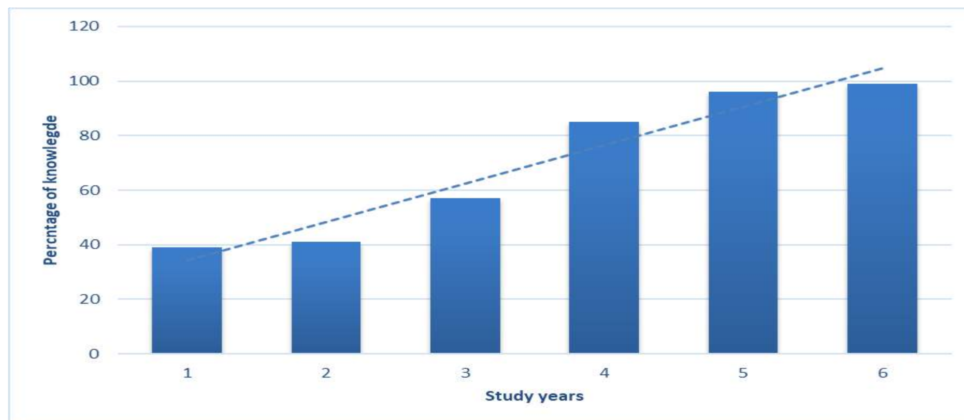

Fig 1: The trend in knowledge of AMR and AMS according to year of study

Table 3: Habits of the medical students to antibiotics use

Question	Frequency of response	Percentage
I consult a doctor before starting antibiotics		
Always	122	22.2
Sometimes	391	71.1
Never	54	6.7
I go for laboratory test before commencing		
Always	32	17.1
Sometimes	230	41.8
Never	248	45.1
I take antibiotics anytime I have fever		
Always	498	90.5
Sometimes	52	9.5
Never	0	0
I stop antibiotics when I feel better or the symptoms stop		
Always	311	56.5
Sometimes	130	23.6
Never	109	19.8
I keep the remaining/leftover antibiotics for future use after recovery		
Always	349	63.5
Sometimes	142	25.8
Never	59	10.7
I give the remaining/leftover antibiotics to a friend when he/she falls sick		
Always	39	7.1
Sometimes	111	20.2
Never	400	72.2
I take antibiotics when I have catarrh and/or cough		
Always	327	59.5
Sometimes	192	34.9
Never	31	5.6

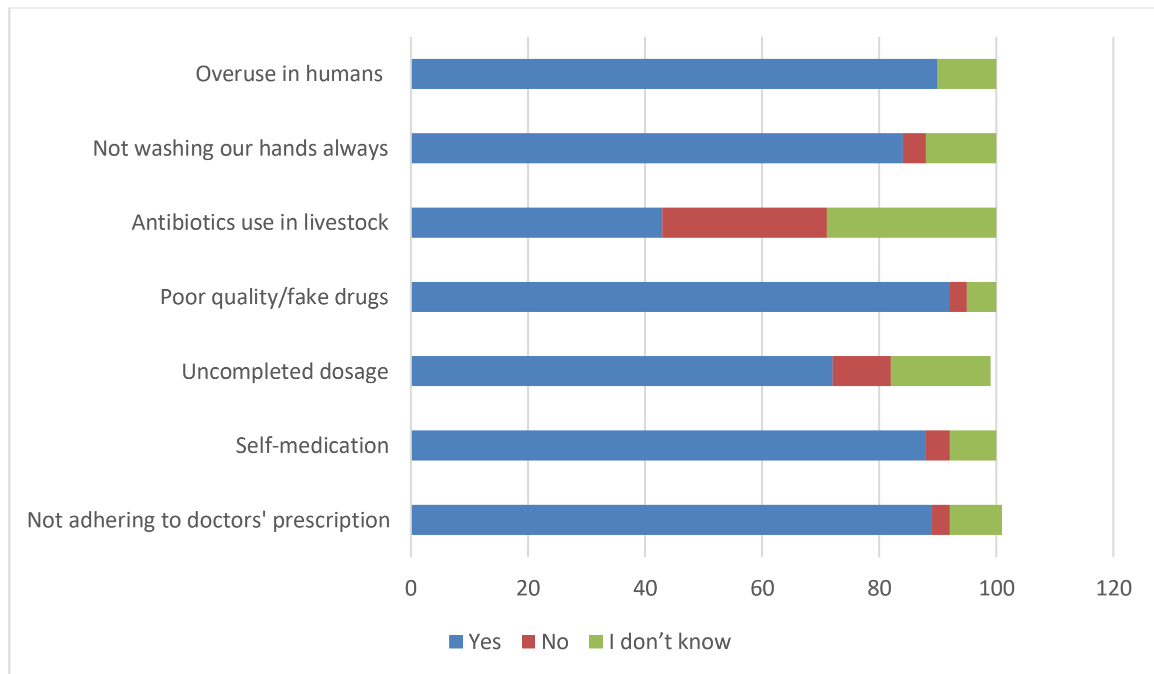

Fig 2: Knowledge of factors responsible for emergence of antimicrobial resistance

Habits of students to antimicrobial use:

Regarding the habits of antimicrobial use by the respondents, only 22.2% always consult a doctor before starting an antibiotic, 13.1% go for laboratory tests, and 90.5% always take antibiotics anytime they have a fever. Above half of the respondents (56.5%) do not complete the dosage of the antibiotics while 63.5% keep leftover antibiotics for future use. Surprisingly, above half (59.5%) of the respondents always take antibiotics when they have cough and or catarrh (Table 3).

Knowledge of factors responsible for emergence of antimicrobial resistance:

In assessing the factors responsible for emergence of AMR, 88.8% indicated not adhering to a doctor's prescription, 92.0% indicated that poor quality of drugs could cause resistance, 88.4% noted self-medication, and 89.6% indicated that overuse of antimicrobials in human can cause AMR. However, only 42.8% of students knew that over use of antibiotics in livestock as the cause of AMR (Fig 2).

Discussion:

Antimicrobial resistance has become a greater concern globally (13), with the bulk of the burden occurring in sub-Saharan Africa, including Nigeria (14). Promoting responsible antimicrobial use has become a crucial step in reducing antimicrobial resistance, and healthcare professionals are involved in antibiotic prescribing, dispensing, administering, and usage. This study provides valuable insights into the knowledge of AMR and habit of antibiotic use among medical students of the University of Nigeria Enugu. A high response rate (91.36%) was obtained from this study, which may be because the researchers were mainly medical students who are passionate about AMR, who therefore facilitated wide distribution and collection of questionnaires, despite the different locations of the three UNN campuses.

In this study, majority of the study participants (97.3%) have heard about AMR, but only slightly more than half of them (54.2%) have heard about the term 'antimicrobial stewardship'. Antimicrobial steward ship is rapidly evolving and involves a set of interventions that promote responsible antimicrobial use. Surprisingly, a study done in three medical schools in the United States reported that only 40% (115 of 288) of the respondents were familiar with AMS (15). It is believed that knowledge of AMR in developing countries should be vast, but this report has clearly shown that the lack of knowledge in AMS spans across countries. The majority (97.3%) of the study participants responded that AMR is a global problem. This agrees with previous studies conducted among medical students in Nigeria (16) and Peru (17), where 98.4 % and 98.0% of the respondents, respectively stated that AMR was a global problem. Although, majority of the participants responded that AMR is a global problem, 64.7% of them seem to underestimate that this problem can affect them. In a study

by Dyar et al (14), and Brink et al (18), a good number of students indicated that it is only a national problem. Similarly, a study conducted in France and Scotland among junior doctors reported that 95% indicated that it is a national problem, but only 63.0% believed that it could affect their practice (7).

About 71.3% knew that bacteria are sensitive to antibiotics and 70.2% indicated that AMR is the ability of the organisms to grow in the presence of drugs that would normally kill or inhibit their growth. More so, 21.8% indicated that antibiotics should be stopped when the clinical symptoms subside while 78.2% responded that they should be guided by doctors' prescriptions. When antibiotics are wrongly used, it induces 'selection pressure' among the bacteria and promotes AMR. We observed that the overall knowledge level of the study participants increased with the year of study. This is in accordance with previous studies that observed that students who were in the final years of study had better knowledge than others (19,20). The plausible explanation is that AMS has been included in the curriculum of older students. Additionally, these students have completed their clinical rotations with improved knowledge of prescription habits and antimicrobial use (19,20). This finding underscores the significance of education and awareness in fostering responsible antibiotic use in the early stages of training of medical students.

Although the knowledge of AMR was high among the participants in our study, it was evident that this did not reflect in the habits of antibiotic use in their regular lives. This is shown by the proportion of students (90.5%) who always use antibiotics whenever they have a fever and 59.5% use them for the management of cough and catarrh. This habit may be because of the lack of knowledge of the students concerning rational antibiotic use and this is disastrous because it will reflect in their antibiotic prescription patterns during practice, affecting the general well-being of the population (21,22). This is in agreement with studies by Efthymiou et al., (23), and Nisabwe et al., (20), who reported that the majority of their participants claimed that antibiotics are used for treating viral infections and common flu. A systematic review of antibiotic use for common cold and acute purulent rhinitis reported there is no benefit in taking antibiotics for common cold, sore throat, and cough (24). This is because these infections are mostly caused by viruses, and antibiotics do not affect them

Only 22.2% of the students in our study always seek doctors' advice before taking antibiotics. In a study conducted in Nigeria, antibiotics were among the top three medications used for self-medication among undergraduate healthcare students (9). A higher rate was obtained in a survey conducted among students in Ghana (25) and India (5), where 79.2% and 71.6% of students respectively, used antibiotics prescribed by doctors. Self-medication is a major contributor to AMR and it is worse when it is not guided by laboratory results. Only 13.1% of the students indicated that they always go for laboratory tests before commencing antibiotics.

About 56.5% of the students reported not always completing the dose of the antibiotics and stopping therapy when symptoms subside. Also, 63.5% always keep the leftover drugs for future use, among whom 7.1% give leftover to their friends when they fall sick. These are bad habits that promote inappropriate antimicrobial use (wrong drug, wrong dose, non-adherence or unnecessary antimicrobials) that contributes to the emergence and spread of AMR. Understanding the factors that promote emergence and spread of AMR, participants identified poor hand hygiene practices, not adhering to prescriptions (88.8%), poor drug quality (92.0%), and self-medication (88.4%). Surprisingly, the proportion that indicated overuse of antibiotics in livestock was the least (42.8%). This suggests that training and awareness of the contributors from other sectors (animal and environment) in AMR are imperative. The WHO has highlighted the importance of an integrated and holistic multisectoral 'One Health' approach in combating AMR (26).

This study presents several strengths. Firstly, the large sample size used in the study shows the accuracy of the result. Secondly, the study cuts across students in different years of study, opening up the gap in the training of the younger medical students. Thirdly, the voluntary and anonymous nature of the survey reduced the possibility of the study participants providing "socially desirable" answers. However, a major limitation of the study is its unicentric nature, necessitating a survey that will involve more centres.

Conclusion:

This survey has identified knowledge gaps in AMR/AMS education and training which underscores the importance of targeted interventions to bridge the knowledge gaps among medical students, especially in the early phase of their academic and professional training. Improving adherence to proper antibiotic use and addressing misconceptions, such as antibiotics' effectiveness against viruses, among others, are crucial steps in combating AMR. Education and awareness campaigns tailored to specific cohorts can contribute to more responsible antibiotic use and a better understanding of the global challenge

of antibiotic resistance.

Contributions of authors:

NI, NS, IP, AK, NS, ES and OO conceptualized and designed the study; NI, IF, NL, NS, OO, ER and NL were involved in data collection; PG, ES, IF, ON, NS, ER, NL and AK performed data analysis; IP, NS, ES, NI, PG, IP and ON wrote the manuscript. All authors approved the final manuscript submitted for publication.

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Authors declare no conflict of interest

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References:

1. World Health Organization. Antimicrobial resistance Media Centre; 2018. http://www.who.int/mediacentre/factsheets/fs194/en/. (Accessed December 1, 2023).
2. Rice, L. B. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. J Infect Dis. 2008; 197 (8): 1079–1108. doi:10.1086/533452.
3. Cusini, A., Rampini, S. K., Bansal, V., et al Different patterns of inappropriate antimicrobial use in surgical and medical units at a tertiary care hospital in Switzerland: a prevalence survey. PLoS One. 2010; 5 (11): e1401. doi:10.1371/journal.pone.0014011
4. World Health Organization. Antimicrobial Resistance. 2020. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance. (Accessed December 1, 2023).
5. Khan, A. K., Banu, G., and Reshma, K. K. Antibiotic resistance and usage-a survey on the knowledge, attitude, perceptions and practices among the medical students of a southern Indian teaching hospital. J Clin Diagn Res. 2013; 7: 1613–1616. doi:10.7860/JCDR/2013/6290.3230.
6. Moongtui, W., Picheansathian, W., and Senaratana, W. Role of nurses in prevention of antimicrobial resistance. Reg Health Forum. 2011; 15 (1): 104–111.
7. Pulcini, C., Williams, F., Molinari, N., Daveyc, P., and Nathwanie, D. Junior doctors' knowledge and perceptions of antibiotic resistance and prescribing: a survey in France and Scotland. Clin Microbiol Infect. 2011; 17 (1): 80–87. doi:10.1111/j.1469-0691.2010.03179.x.
8. Heaton, A., Webb, D. J., and Maxwell, S. R. Undergraduate preparation for prescribing: the views of 2413 UK medical students and recent graduates. Br J Clin Pharmacol. 2008; 66 (1): 128–134.doi:10.1111/j.1365-2125.2008.03197.x
9. Akande-Sholabi, W., and Ajamu, A. T. Antimicrobial stewardship: Assessment of knowledge, awareness of antimicrobial resistance and appropriate antibiotic use among healthcare students in a Nigerian University. BMC Med Educ. 2021; 21: 488. doi:10.1186/s12909-021-02912-4.
10. Mudenda, S., Mukela, M., Matafwali, S., et al. Knowledge, Attitudes, and Practices towards Antibiotic Use and Antimicrobial Resistance among Pharmacy Students at the University of Zambia: Implications for Antimicrobial Stewardship Programmes. Sch Acad J Pharm. 2022; 11: 117–124. doi:10.36347/sajp.2022.v11i08.002
11. Dyar, O. J., Pulcini, C., Howard, P., and Nathwani, D. ESGAP (ESCMID Study Group for Antibiotic Policies). European medical students: A first multicentre study of knowledge, attitudes and perceptions of antibiotic prescribing and antibiotic resistance. J Antimicrob Chemother. 2014; 69: 842–846. doi:10.1093/jac/dkt440.
12. World Health Organization. Global Action Plan on Antimicrobial Resistance. World Health Organization; Geneva, Switzerland: 2015.
13. Spellberg, B., Guidos, R., and Gilbert, D. The epidemic of antibiotic resistant infections: A call to action for the medical community from the Infectious Diseases Society of America. Clin Infect Dis. 2008; 46 (2): 155–164. doi:10.1086/524891.
14. Dyar, O., Howard, P., Nathwani, D., and Pulcini, C. Knowledge, attitudes, and beliefs of French medical students about antibiotic prescribing and resistance, Médecine et Maladies Infectieuses. 2013; 43 (10): 423-430. doi:10.1016/j.medmal.2013.07.010
15. Abbo, L. M., Cosgrove, S. E., Pottinger, P. S., et al. Medical Students' Perceptions and Knowledge About Antimicrobial Stewardship: How Are We Educating Our Future Prescribers? Clin Infect Dis. 2013; 57 (5): 631–638. https://doi.org/10.1093/cid/cit370
16. Okedo-Alex, I., Madubueze, U. C., Umeokonkwo, C. D., Oka, O. U., Adeke, A. S., and Okeke, K. C. Knowledge of antibiotic use and resistance among students of a medical school in Nigeria. Malawi Med J. 2019; 31(2): 133-137. doi:10.4314/mmj.v31i2.5
17. García, C, Llamocca, L. P., García, K., et al. Knowledge, attitudes and practice survey about antimicrobial resistance, and prescribing among physicians in a hospital setting in Lima, Peru. BMC Clin Pharmacol. 2011; 11 (1): 18. doi:10.1186/1472-6904-11-18.
18. Brink, A., Schoeman, J., and Muntingh, G. Undergraduate antibiotic stewardship training: Are we leaving our future prescribers 'flapping in the wind'? S Afr Med J. 2017; 107: 357–358. doi:10.7196/SAMJ.2017.v107i5.12496.
19. Hagiya, H., Ino, H., Tokumasu, K., et al. Anti- biotic literacy among Japanese medical students. J Infect Chemother. 2020; 26: 1107–1109. doi:10.1016/j.jiac.2020.06.021
20. Nisabwe, L., Brice, H., Umuhire, M. C., et al. Knowledge and attitudes towards antibiotic use and resistance among undergraduate healthcare students at University of Rwanda. J Pharm Policy Pract. 2020; 13: 7 https://doi.org/10.1186/s40545-020-00207-5
21. Haenssgen, M. J., Xayavong, T., Charoenboon, N., Warapikuptanun, P., and Khine, Y. The con- sequences of AMR education and awareness raising: outputs, outcomes, and behavioural impacts of an antibiotic-related educational activity in Lao PDR. Antibiotics. 2018; 7 (4): 95. doi:10.3390/antibiotics7040095.
22. Ghiga, I. No time to wait: securing the future from drug-resistant infections. Report to the Secretary-General of the United Nations. 2019.
23. Efthymiou, P., Gkentzi, D., and Dimitriou, G. Knowledge, Attitudes and Perceptions of Medical Students on Antimicrobial Stewardship. Antibiotics (Basel). 2020; 9 (11): 821. doi:10.3390/antibiotics9110821.
24. Kenealy, T., and Arroll, B. Antibiotics for the common cold and acute purulent rhinitis. Cochrane Database of Syst Rev. 2013; 2013 (6): CD000247. doi:10.1002/14651858.CD000247.pub3.
25. Owusu-Ofori, A. K., Darko, E., Danquah, C. A., Agyarko-Poku, T., and Buabeng, K. O. Self-Medi- cation and Antimicrobial Resistance: A Survey of Students Studying Healthcare Programmes at a Tertiary Institution in Ghana. Front Publ Health. 2021; 9: 706290. doi:10.3389/fpubh.2021.706290.
26. Robinson, T., D., Bu, J., Carrique-Mas, E., et al. Antibiotic resistance is the quintessential One Health issue. Trans Roy Soc Trop Med Hyg. 2016; 110: 377-380. doi:10.1093/trstmh/trw048

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Original Article **Open Access**

Blood culture contamination in Babcock University Teaching Hospital, Nigeria: A five-year retrospective study

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Abstract:

Background: Bloodstream infections are a leading cause of morbidity and mortality among in-patients globally. Blood culture is the 'gold standard' test for the diagnosis of bloodstream infections. The value of this valuable investigation in the diagnosis of infections however may be affected when an organism of questionable evidence is isolated, which occurs mainly due to contamination during the pre-analytical phase. Blood culture contamination can lead to the administration of unnecessary antibiotics, wastage of hospital resources, and risks to patient life. Hence, this study aimed to analyse the blood culture contamination rate in a private tertiary hospital in southwest Nigeria.

Methodology: This was a retrospective observational study of patients with clinical features of bloodstream infections at Babcock University Teaching Hospital, Ilishan-Remo, Ogun State, Nigeria between January 2018 and December 2022. Blood culture results of patients in the wards or units of the hospital were reviewed, and contamination rates and organisms isolated from positive blood cultures were documented. Data were analysed using SPSS version 22.0

Results: A total of 1,612 non-repetitive blood cultures were obtained from 1,612 patients (910 males and 702 females) during the study period, out of which 397 (24.6%) were positive, 1215 (75.4%) were negative, and 124 (7.7%) were deemed as contaminants. The contamination rate was higher in females (8.7%) than in males (6.9%), although the difference was not statistically significant (x^2=1.501, OR=0.7816, 95% CI=0.5416-1.128, p=0.2204). The contamination rate was higher in adults (8.1%) than children (7.3%) with the highest contamination occurring in the age group 35-39 years (9.0%), although the difference was not statistically significant (x^2=0.3227, OR=0.8835, 95% CI=0.6120-1.276, p=0.5700). The female surgical ward (11.9%) had the highest contamination rate while the accident and emergency had the lowest contamination rate (1.3%) but the difference was not statistically significant (x^2=11.825, p=0.2970). Coagulase-negative staphylococci were the predominant blood culture contaminants. The contamination rate increased during the 5 years from 4.8% in 2018 to 9.4% in 2022

Conclusion: The rate of blood culture contamination in our study is higher than the acceptable international rate, and mainly due to normal skin microbiota, suggesting challenges during sample collection. There is a need for a multidimensional approach to minimize blood culture contamination and hence avoid unnecessary antibiotic use.

Keywords: Blood culture contamination, false-positive cultures, Coagulase-negative staphylococcus

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Contamination des hémocultures au Hôpital Universitaire de Babcock, Nigeria: une étude rétrospective sur cinq ans

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Résumé:

Contexte: Les bactériémies sont l'une des principales causes de morbidité et de mortalité chez les patients

hospitalisés dans le monde. L'hémoculture est le test de référence pour le diagnostic des infections sanguines. La valeur de cette enquête précieuse dans le diagnostic des infections peut cependant être affectée lorsqu'un organisme dont les preuves sont douteuses est isolé, ce qui se produit principalement en raison d'une contamination au cours de la phase pré-analytique. La contamination des hémocultures peut entraîner l'administration d'antibiotiques inutiles, un gaspillage des ressources hospitalières et des risques pour la vie des patients. Par conséquent, cette étude visait à analyser le taux de contamination des hémocultures dans un hôpital tertiaire privé du sud-ouest du Nigeria.
Méthodologie: Il s'agit d'une étude observationnelle rétrospective portant sur des patients présentant des caractéristiques cliniques d'infections du sang à l'hôpital universitaire Babcock, Ilishan-Remo, dans l'État d'Ogun, au Nigeria, entre janvier 2018 et décembre 2022. Résultats des hémocultures des patients dans les services ou unités de l'hôpital ont été examinés et les taux de contamination et les organismes isolés à partir d'hémocultures positives ont été documentés. Les données ont été analysées à l'aide de SPSS version 22.0
Résultats: Au total, 1612 hémocultures non répétitives ont été obtenues chez 1612 patients (910 hommes et 702 femmes) au cours de la période d'étude, parmi lesquelles 397 (24,6%) étaient positives, 1215 (75,4%) étaient négatives et 124 (7,7%) ont été considérés comme des contaminants. Le taux de contamination était plus élevé chez les femmes (8,7%) que chez les hommes (6,9%), bien que la différence ne soit pas statistiquement significative (x^2=1,501, OR=0,7816, IC 95%=0,5416-1,128, p=0,2204). Le taux de contamination était plus élevé chez les adultes (8,1%) que chez les enfants (7,3%), la contamination la plus élevée se produisant dans la tranche d'âge de 35 à 39 ans (9,0%), bien que la différence ne soit pas statistiquement significative (x^2=0,3227, OR= 0,8835, 95% IC=0,6120-1,276, p=0,5700). Le service de chirurgie féminin (11,9%) avait le taux de contamination le plus élevé tandis que le service d'accident et d'urgence avait le taux de contamination le plus faible (1,3%) mais la différence n'était pas statistiquement significative (x^2=11,825, p=0,2970). Les staphylocoques à coagulase négative étaient les principaux contaminants des hémocultures. Le taux de contamination a augmenté au cours des 5 années passant de 4,8% en 2018 à 9,4% en 2022.
Conclusion: Le taux de contamination des hémocultures dans notre étude est supérieur au taux international acceptable, et principalement dû au microbiote cutané normal, suggérant des difficultés lors du prélèvement des échantillons. Il est nécessaire d'adopter une approche multidimensionnelle pour minimiser la contamination des hémocultures et ainsi éviter l'utilisation inutile d'antibiotiques.

Mots-clés: Contamination des hémocultures, cultures faussement positives, staphylocoque à coagulase négative

Introduction:

Bloodstream infections are the leading cause of morbidity and mortality in hospitalized patients globally (1–4). Blood culture is the 'gold standard' in the diagnosis of patients with bloodstream infections and sepsis (3,5–7). The aim of blood culture is to isolate the pathogen and determine the appropriate antimicrobial agents for effective therapy. However, blood culture contamination results in a false positive, thereby limiting the utility of this valuable test (5,8). Blood culture contamination remains a source of frustration for clinical microbiologists and clinicians. Due to difficulties in the interpretation of a false positive blood culture, especially in a solitary culture, unnecessary administration of antibiotics can result in the emergence of drug resistant strains, drug interactions, increased cost of healthcare, prolonged hospital stay and increased morbidity and mortality (3,4,8,9).

Generally, blood culture contamination occurs during the collection and handling of blood specimens, before laboratory analysis (2, 5). A potential blood culture contaminant is an organism which commonly inhabits the human skin, which accounts for contamination in 50% of cases. The sources of contamination of blood culture include the skin of the patient, contaminated hands of healthcare providers and the equipment used for blood sampling or transfer. The common factors associated with contamination include inappropriate sampling, non-compliance with aseptic techniques, non-use of dedicated phlebotomists or personnel in blood collection, collection of blood from indwelling central venous catheters and difficulty in collecting blood from children and elderly patients (3–5,8,9). The common microorganisms that have been implicated as contaminants are the coagulase-negative staphylococci (CoNS), *Micrococcus* spp, *Propionibacterium* spp, and *Bacillus* spp (2,4,8,9).

Blood culture contamination rate is commonly used as a key indicator of the quality of laboratory performance and patient care (3,8,9). The Clinical and Laboratory Standards Institute (CLSI) recommends less than 3% as the acceptable rate of blood culture contamination in a health facility (4,10). This study aimed to determine the blood culture contamination rate in a private tertiary teaching hospital in southwest Nigeria over a 5-year period.

Materials and method:

Study design and setting:

This was a retrospective study of blood culture results of patients with clinical features of bloodstream infections at the Medical Microbiology Laboratory of Babcock University Teaching Hospital, Ilishan-Remo, Ogun State, Nigeria between January 2018 and December 2022. This healthcare facility is a 200-bed, private tertiary hospital owned by the Seventh Day Adventist Church. It provides medical and surgical services to the host community, Ogun State as well as neighbouring Lagos, Oyo, Osun, Ondo and Ekiti States.

Ethical consideration:

Ethical approval was obtained from the Babcock University Health Research and Ethics Committee (BUHREC). Informed consent of patients was not required.

Blood culture collection and processing:

Routinely in the hospital, solitary blood cultures are usually collected from patients into BACTEC culture bottles (Becton Dickinson). All cultures are incubated in BACTEC blood culture machine at 35-37ºC and atmospheric pressure for a maximum duration of 5 days. Cultures indicating growth are routinely sub-cultured on solid media and incubated for 24-48 hours for isolation and subsequent identification of the microorganism by conventional microbiological identification tests.

Criteria to determine contamination:

The records of all blood cultures were reviewed, and information was sought from the managing physicians on the technique of blood collection. As there is no "gold standard" in the determination of contaminants, clues and criteria were followed to distinguish contamination from true infection (11). These included; (i) identity of the isolated microorganism such as coagulase-negative staphylococci, *Corynebacterium* spp and (ii) clinical status of the patient, history and laboratory findings such as presence of fever, leukocytosis or leukopenia and high C-reactive protein (CRP), or presence of foreign device. In summary, the contaminants were determined based on the isolation of a skin commensal and the patient's clinical scenario.

Data analysis:

Data were analysed using IBM SPSS for Windows, version 22 (IBM Corp., Armonk, N.Y., USA). Descriptive analysis was carried out for demographic, clinical characteristics and causative microorganisms. The blood culture contamination rate was calculated by dividing the number of contaminated blood cultures by the total number of routine blood cultures obtained and multiplying by a factor of 100.

Categorical variables were presented as frequencies and percentages. Mean and standard deviation were calculated for continuous variables. Categorical variables were compared using Fisher Exact or Chi-square test as appropriate. A p-value of less than 0.05 was considered statistically significant for all analyses.

Results:

A total of 1,612 blood cultures were obtained during the study period; of which 800 (49.6%) were blood specimens from paediatric patients while 812 (50.4%) were from adults. There were 910 (56.5%) males and 702 (43.5%) females. Of the total 1,612 cultures, 397 (24.6%) were positive and 1,215 (75.4%) were negative. From the 397 positive cultures, 124 (31.2%) were deemed as contaminants while 273 (68.8%) were true pathogens (Table 1). The overall contamination rate was 7.7%. Contamination rate was higher in females (8.7%, 61/702) than in males (6.9%, 63/910); although, the difference was not statistically significant (x^2=1.501, OR=0.7816, 95% CI= 0.5416-1.128, p=0.2204).

Blood culture contamination rates in different age groups and wards/units:

Blood culture contamination rates varied between children (<18 years) and adults (≥18 years), with children having lower contamination rate (7.3%, 58/800) while adults had higher rate (8.1%, 66/812). However, the difference was not statistically significant (x^2= 0.3227, OR=0.8835, 95% CI=0.6120-1.276, p=0.5700). With respect to age, age group 35-39 years had the highest contamination rate (9.0%, 16/177) while age group 5–17 years (1.6%, 3/183) had the lowest contamination rate. The difference is statistically significant (x^2=11.567, p=0.041) (Table 2).

Comparing the blood culture contamination rates of the wards/units, the female surgical ward had the highest contamination rate (11.9%, 10/84), followed by the intensive care unit (10.4%, 29/279) while the accident and emergency had the lowest contamination rate (1.3%, 1/80) (Table 3). However, the difference was not statistically significant (x^2=11.825, p=0.2970).

Table 1: Blood culture contamination rates by gender

Gender	Number of blood cultures	Number of blood culture contaminated (%)	x^2	OR (95% CI)	p value
Male	910	63 (6.9)	1.501	0.7816 (0.5416-1.128)	0.2204
Female	702	61 (8.7)			
Total	**1612**	**124 (7.7)**			

OR = Odd ratio; x^2 = Chi-square, CI = Confidence interval

Table 2: Blood culture contamination rates by age groups

Age group (years)	Number of blood cultures	Number of blood culture contaminated (%)	x^2	*p* value
<5	617	55 (8.9)	11.567	0.041*
5-17	183	3 (1.6)		
18-34	204	16 (7.8)		
35-49	177	16 (9.0)		
50-65	198	14 (7.1)		
>65	233	20 (8.6)		
Total	**1612**	**124 (7.7)**		

x^2 = Chi-square; * = statistically significant

Table 3: Blood culture contamination and total blood cultures from the various wards/units

Wards/units	Frequency of blood culture contamination (%)	Total blood cultures	x^2	*p* value
Intensive Care	29 (10.4)	279	11.825	0.2970
Female Medical	28 (8.1)	344		
Children Emergency	21 (6.3)	333		
Neonatal Intensive Care	15 (7.4)	203		
Female Surgical	10 (11.9)	84		
Male Medical	7 (6.4)	110		
Paediatrics	6 (7.4)	81		
Male Surgical	3 (8.6)	35		
General Outpatient Department	3 (8.8)	34		
Accident and Emergency	1 (1.3)	80		
Obstetrics and Gynaecology	1 (3.4)	29		
Total	**124 (7.7)**	**1612**		

x^2 = Chi-square

Table 4: Blood culture contamination rates by years

Year	Total number of blood cultures	Number of positive cultures (%)	True positive (%)	False positive (%)	x^2	*p* value
2022	480	126 (26.3)	81 (16.9)	45 (9.4)	4.246	0.2630
2021	219	62 (28.3)	42 (19.2)	20 (9.1)		
2020	493	134 (27.2)	99 (20.1)	35 (7.1)		
2019	336	62 (18.5)	42 (12.5)	20 (6.0)		
2018	84	13 (15.5)	9 (10.7)	4 (4.8)		
Total	**1612**	**397 (24.6)**	**273 (16.9)**	**124 (7.7)**		

x^2 = Chi-square

Microorganisms of blood culture contamination:

The contaminants isolated were coagulase-negative staphylococci (93.5%, n=116), *Bacillus* spp (4.0%, n=5) and *Corynebacterium* spp (2.4%, n=3).

Distribution of contamination rates by year:

The contamination rates increased progressively over the 5 years from 2018 to 2022. As shown in Table 4, the highest annual rate of blood culture contamination rate was in 2022 (9.4%) followed by 2021 (9.1%) and the lowest in 2018 (4.8%). The blood culture contamination rate increased over the 5 years, however, this increment was not statistically significant (x^2=4.246, p=0.2630).

Discussion:

Determination of blood culture contamination is critical for proper management of patients with bloodstream infections and judicious utilization of hospital resources. A decrease in blood culture contamination will reduce the unnecessary use of antimicrobial agents and their potential adverse effects such as increased risk of development of AMR, toxic side effects of the drugs, increased cost etc. In this study, the overall blood culture contamination rate was 7.7%, with the annual blood culture contamination rate increasing over the 5 years, reaching its highest level in 2022 with 9.4%, although this increase was not statistically significant. These contamination rates are above the global benchmark of <3% (11). Contamination rates reported in the literature vary between institutions, ranging from 0.9%-56% (9, 12). The rate observed in our study is lower than that reported in a previous study in north-central Nigeria (8).

The high rate in our study could be attributed to inadequate aseptic practices such as not waiting for the recommended contact or drying time of the antiseptic solution, re-palpation of the disinfected area before phlebotomy and the use of non-dedicated phlebotomists. Blood culture contamination has been linked to several factors including improper aseptic techniques used when collecting blood, especially by poorly trained staff. Studies have also reported that the use of dedicated phlebotomists with competency training and assessment focu-

sed on aseptic techniques is associated with a reduction in the blood culture contamination rate (4,13,14). Some recommendations to achieve a low rate of blood culture contamination should be followed, including the use of well-trained and dedicated phlebotomists and use of effective antiseptic agents and adherence to the protocol on standard collection and aseptic techniques (6,15). There will also be a need for regular calculation and analysis of the blood culture contamination rate to maintain a low rate. The observed progressive increase in contamination rate over the 5-year period may be attributable to rapid staff turnover, lack of ongoing training in the teaching hospital setting and blood culture contamination rate not being a targeted performance indicator in our institution.

The age group 35-49 years had the highest contamination rate (9.0%). This is contrary to the observation in other studies, which reported that the highest rate of contamination was among patients <5 years old and the elderly (1,4,15). Determination of the department with the highest rate of blood culture contamination is essential in reducing the rate of blood culture contamination. The female surgical ward (11.9%) had the highest contamination rate in our study. This is similar to studies conducted in Saudi Arabia and South Africa which reported the highest contamination rates in the surgical units (1,16). Other studies have reported the highest rate of contamination in the emergency and dialysis units (3,4,7). We are not able to speculate on the possible reasons for high rate of contamination in the female surgical ward due to the retrospective nature of our study as well as inability to examine possible factors that may contribute to this observation. The intensive care unit (10.4%) accounted for the second-highest rate of contamination. The high rate observed in the intensive care unit might be attributable to the critical condition of the patients, who may be hypovolemic or hypotensive thereby resulting in multiple needle sticks to obtain blood from fragile and less prominent veins. A study in the United States reported admission into intensive care unit as a risk factor for blood culture contamination (2).

Coagulase-negative staphylococci were the most common contaminant observed in this study, which is similar to reports of other studies (1,4,6,8,15). *Bacillus* spp and *Corynebacterium* spp were other contaminants observed in this study which is similar to other studies. The microorganisms observed in this study are normal skin microbiota indicating that contamination occurred during the process of specimen collection. Other studies have reported *Micrococcus*, viridians group of streptococci and *Propionibacterium* spp as possible blood culture contaminants (3,4,7).

There are some limitations in this study. Firstly, only one blood culture sample was used in the study which may not be sufficient to differentiate contaminants from true pathogens in some cases. Repeated isolation of the same organism in different blood culture bottles from a patient supports the organism as being a true pathogen as some studies have reported CoNS as true pathogens in bloodstream infections especially in children and patients on intravenous catheters if repeatedly isolated in different blood culture bottles or isolated from blood and primary focus. Secondly, the retrospective nature of our study limits the ability to examine possible factors responsible for contamination and to provide outcome data. A prospective study is preferred.

Conclusion:

The overall blood culture contamination rate of 7.7% in this study is higher than <3% recommended and all the contaminants were normal skin flora. There is the need to adopt multi-dimensional strategies such as the establishment of disinfection policies and protocols, the use of phlebotomists, continuous monitoring and feedback to reduce blood culture contamination rate and avoid unnecessary use of antibiotics.

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Contributions of authors:

TOO, IIO and CJE conceptualized and designed the study, HNO analyzed and interpreted the data, TOO, IIO, and AOO contributed to drafting the manuscript. All authors contributed equally to the development and critical review of the manuscript, and approved the final version submitted for publication.

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Authors have no conflict of interest

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Oral presentation of this research work was made at 3rd Annual Scientific Conference and General Meeting of the Clinical Microbiology and Infectious Diseases Society of Nigeria (CLIMIDSON) on November 23-24, 2023, and the abstract was published in the conference brochure.

References:

1. Alnami, A. Y., Aljasser, A. A., Almousa, R. M., et al. Rate of blood culture contamination in a teaching hospital: A single center study. J Taibah Univ Med Sci. 2015; 10 (4): 432–436. doi:10.1016/j.jtumed.2015.08.002
2. Liaquat, S., Baccaglini, L., Haynatzki, G., Medcalf, S. J., and Rupp, M. E. Patient-specific risk factors contributing to blood culture contamination. Antimicrob Steward Healthc Epidemiol. 2022; 2 (1): e46. doi:10.1017/ash.2022.22
3. Hemeg, H. A., Almutairi, A. Z., Alharbi, N. L., et al. Blood culture contamination in a tertiary care hospital of Saudi Arabia. Saudi Med J. 2020; 41 (5): 508–515. doi:10.15537/smj.2020.5.25052
4. Aiesh, B. M., Daraghmeh, D., Abu-Shamleh, N., Joudallah, A., Sabateen A., and Al Ramahi, R. Blood culture contamination in a tertiary care hospital: a retrospective three-year study. BMC Infect Dis. 2023; 23 (1): 448. doi:10.1186/s12879-023-08428-0
5. Snyder, S. R., Favoretto, A. M., Baetz, R. A., et al. Effectiveness of practices to reduce blood culture contamination: A Laboratory Medicine Best Practices systematic review and meta-analysis. Clin Biochem. 2012; 45 (13): 999–1011. doi:10.1016/j.clinbiochem.2012.06.007
6. Dargère, S., Cormier, H., and Verdon, R. Contaminants in blood cultures: importance, implications, interpretation and prevention. Clin Microbiol Infect. 2018; 24 (9): 964–969. doi:10.1016/j.cmi.2018.03.030
7. Gunvanti, R., Lakshmi, J. T., Ariyanachi, K., et al. Blood Culture Contamination Rate as a Quality Indicator-a Prospective Observational Study. Mædica. 2022 17 (2): 311–316. doi:10.26574/maedica.2022.17.2.311
8. Iregbu, K. C., and Yakubu, S. Quality assurance in blood culture: A retrospective study of blood culture contamination rate in a tertiary hospital in Nigeria. Niger Med J. 2014; 55 (3): 201–203. doi:10.4103/0300-1652.132038
9. Dempsey, C., Skoglund, E., Muldrew, K. L., and Garey, K. W. Economic health care costs of blood culture contamination: A systematic review. Am J Infect Control. 2019; 47 (8): 963–967. doi:10.1016/j.ajic.2018.12.020
10. Clinical and Laboratory Standards Institute (CLSI). Principles and Procedures for Blood Cultures; Approved Guideline. Clinical and Laboratory Standards Institute document M47-A, 2007.
11. Roth, A., Wiklund, A. E., Pålsson, A. S., et al. Reducing Blood Culture Contamination by a Simple Informational Intervention. J Clin Microbiol. 2010; 48 (12): 4552–4558. doi:10.1128/jcm.00877-10
12. Doern, G. V., Carroll, K. C., Diekema, D. J., et al. Practical Guidance for Clinical Microbiology Laboratories: A Comprehensive Update on the Problem of Blood Culture Contamination and a Discussion of Methods for Addressing the Problem. Clin Microbiol Rev. 2019; 33 (1): e00009-19. doi:10.1128/CMR.00009-19
13. Bekeris, L. G., Tworek, J. A., Walsh, M. K., and Valenstein, P. N. Trends in Blood Culture Contamination: A College of American Pathologists Q-Tracks Study of 356 Institutions. Arch Pathol Lab Med. 2005; 129 (10): 1222–1225. doi:10.5858/2005-129-1222-TIBCCA
14. Dawson, S. Blood culture contaminants. J Hosp Infect. 2014; 87 (1): 1–10. doi:10.1016/j.jhin.2014.02.009
15. Yunus, N., Batool, A., Yaqoob, A., Khawaja, A., Lone, D., and Ahmed, Q. Rate of Blood Culture Contamination as an Indicator of Quality of patient care - a retrospective study. Pakistan J Med Health Sci. 2021; 15 (4): 729-731.
16. Opperman, C. J., Baloyi, B., Dlamini, S., and Samodien, N. Blood culture contamination rates at different level healthcare institutions in the Western Cape, South Africa. South Afr J Infect Dis. 2020; 35 (1): 5. doi: 10.4102/sajid.v35i1.222

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Original Article **Open Access**

Candida bloodstream infection among immunocompromised paediatric patients admitted to the University College Hospital, Ibadan, Nigeria

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Abstract:

Background: Invasive candidiasis is a major hospital acquired fungal infection in Nigeria. Despite advances in support of critically ill patients, candidaemia is still associated with high morbidity and mortality. Data on *Candida* bloodstream infection among paediatric patients is limited in Nigeria and this informed this study, which was undertaken to investigate the prevalence, species distribution, antifungal susceptibility pattern for blood stream infections due to *Candida* species in University College Hospital, Ibadan, Nigeria.
Methodology: This was a descriptive study which recruited 322 immunocompromised paediatric patients. All *Candida* isolates obtained from their blood samples through blood culture were identified to species level by germ tube test and PCR-Restriction Fragment Length Polymorphism (RFLP) analysis of 16S rRNA genes with M*sp*I. Antifungal susceptibility test was performed on the isolates using the Vitek 2 system
Results: Eighteen (5.6%) of the 322 patients had candidaemia, with *Candida albicans* accounting for 14 (77.0%), and *Candida glabrata* and *Candida tropicalis* accounting for 2 (11.0%) of the isolates each. Fourteen (77.0%) isolates were susceptible to fluconazole and voriconazole, 8 (44.0%) were susceptible to caspofungin and micafungin, 10 (55.0%) were susceptible to amphotericin B and 17 (94.0%) were susceptible to flucytosine.
Conclusion: This study highlights the reality of candidaemia in hospitalized immunocompromised children, mostly caused by *Candida albicans* and other *Candida* species, exhibiting resistance to echinocandins, azoles and amphotericin B. It is important to have a high index of suspicion and efforts should be made to rightly identify the concerned *Candida* species and perform susceptibility testing before initiating antifungal treatment. This will ensure better outcome for the patients.

Keywords: Candidaemia, prevalence, paediatric, immunocompromised, PCR-RFLP

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Infection sanguine à *Candida* chez des patients pédiatriques immunodéprimés admis à l'Hôpital Universitaire, d'Ibadan, au Nigeria

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Résumé:

Contexte: La candidose invasive est une infection fongique nosocomiale majeure au Nigeria. Malgré les progrès réalisés dans la prise en charge des patients gravement malades, la candidémie reste associée à une morbidité et une mortalité élevées. Les données sur les infections sanguines à *Candida* chez les patients pédiatriques sont limitées au Nigéria et cela a éclairé cette étude, qui a été entreprise pour étudier la prévalence, la répartition des espèces et le profil de sensibilité aux antifongiques pour les infections sanguines dues aux espèces de *Candida* à l'Hôpital Universitaire d'Ibadan, au Nigeria.
Méthodologie: Il s'agissait d'une étude descriptive qui a recruté 322 patients pédiatriques immunodéprimés. Tous les isolats de *Candida* obtenus à partir de leurs échantillons de sang par hémoculture ont été identifiés au niveau de l'espèce par un test en tube germinal et une analyse PCR-Polymorphisme de Longueur des Fragments de Restriction (RFLP) des gènes de l'ARNr 16S avec M*sp*I. Un test de sensibilité aux antifongiques a été réalisé sur les isolats à l'aide du système Vitek 2.
Résultats: Dix-huit (5,6%) des 322 patients présentaient une candidémie, *Candida albicans* représentant 14 (77,0%) et *Candida glabrata* et *Candida tropicalis* représentant 2 (11,0%) des isolats chacun. Quatorze (77,0%) isolats étaient sensibles au fluconazole et au voriconazole, 8 (44,0%) étaient sensibles à la caspofungine et à la micafungine, 10 (55,0%) étaient sensibles à l'amphotéricine B et 17 (94,0%) étaient sensibles à la flucytosine.
Conclusion: Cette étude met en évidence la réalité des candidémies chez les enfants immunodéprimés hospitalisés, principalement causées par *Candida albicans* et d'autres espèces de *Candida*, présentant une résistance aux échinocandines, aux azoles et à l'amphotéricine B. Il est important d'avoir un indice de suspicion élevé et des efforts doivent être faits pour identifier correctement les espèces de Candida concernées et effectuer des tests de sensibilité avant de commencer un traitement antifongique. Cela garantira de meilleurs résultats pour les patients.

Mots clés: Candidémie, prévalence, pédiatrique, immunodéprimé, PCR-RFLP

Introduction:

Candidiasis refers to fungal infection caused by a yeast from the genus *Candida* (1). In the past 40-50 years, *Candida* species have changed from occasionally encountered pathogens that were initially regarded as mere contaminants to pathogens that cause myriad of superficial and invasive diseases (2). Superficial infections tend to be community-acquired, and cause considerable morbidity. On the other hand, invasive and systemic *Candida* infections tend to be acquired in hospitals or healthcare settings (1,2). Superficial candidiasis has different clinical manifestations, ranging from oropharyngeal and cutaneous, to vaginal candidiasis, while systemic candidiasis is made up of two syndromes; candidaemia, which refers to bloodstream infection by *Candida*, and disseminated candidiasis, which refers to organ infection by *Candida* species.

The genus *Candida* contains 150 to 200 species but only about 20 of them are known to cause disease in humans. From among these, the main pathogens that cause infections in man are *Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida krusei, Candida guilliermondii, Candida kefyr, Candida lusitaniae, Candida dubliniensis,* and *Candida pseudotropicalis,* with the first five *Candida* species causing the majority of systemic human infections (3,4).

Candida species are found everywhere in nature and are known to inhabit the human alimentary canal, the skin and the genital tract of females. Cases of candidiasis in debilitated patients have been known for decades but the emergence of *Candida* as an important cause of human infection started with the introduction and use of new drugs that inhibit normal human host defence mechanisms, especially the use of potent broad-spectrum antibiotics. These agents disrupt the normal microbiota of the human body and enable non-bacterial organisms to flourish (5,6).

Following the introduction and increasing use of antifungal drugs, the causes of *Candida* infections changed from primarily *C. albicans* to the other non-albicans species, and these are now responsible for about 50% of all cases of candidaemia and other forms of systemic disseminated candidiasis (7). This is a clinically important fact worth noting because the various *Candida* species differ in their susceptibility to antifungal agents, especially the newer agents. In the developed countries, where medical therapeutics are commonly used, *Candida* species are now among the most common nosocomial pathogens (8,9). *Candida* causes serious infection especially when it gets into the bloodstream. Globally, there are five species primarily involved in these bloodstream infections in both adult and paediatric patients; *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis* and *C. krusei* but *C. albicans* is still the most common isolate in *Candida* bloodstream infection globally (10-12).

The diagnosis of *Candida* bloodstream infection is based on isolation of the offending organism from blood culture; however, the sensitivity remains low and it takes an average of 4-5 days to complete the process. This has led to the development of other complementary serological tests and even

molecular tests that detect fungal DNA directly in the blood sample, greatly speeding up the diagnostic process. There is still need to perform antifungal susceptibility testing of the isolate in selected cases to properly guide the therapy (13). Treatment is based on the local fungal epidemiology and the antifungal susceptibility pattern but generally, because of the changing landscape of fungal isolates, the recently updated guidelines from the Infectious Disease Society of America (IDSA) recommends echinocandin or fluconazole as the empirical treatment in most paediatric and adult patients (5).

The management of invasive candidiasis is negatively affected by the delays in prompt diagnosis and the dearth of reliable diagnostic methods that enable detection of both *Candida* bloodstream infection and tissue invasion by *Candida* species (1,7). This study was undertaken to investigate the prevalence, species distribution, antifungal susceptibility pattern of bloodstream infections caused by *Candida* species at the University College Hospital, Ibadan, Nigeria.

Materials and method:

Study setting:

This study was conducted in the department of paediatrics of the University College Hospital (UCH), a tertiary health facility situated in Ibadan, the second largest city in southwestern Nigeria. The hospital is an 820-bed facility and attends to patients from all over the country, especially southwest Nigeria.

The department of paediatrics is one of the core foundation departments of the hospital whose primary activity is the provision of specialized and patient-friendly services for children. It has 7 wards and an out-patients clinic which runs every weekday. Paediatric patients, when indicated, are admitted to the general intensive care unit (ICU) and burns unit.

Study design:

This was a cross-sectional study in which immunocompromised children at risk of invasive *Candida* infection in UCH were recruited for the study. The entire study was done between February 2019 and February 2020

Sampling technique/selection of study participants:

A purposive sampling method was adopted. Every week, the first 10 at-risk patients in the wards whose caregivers consented were recruited into the study. The hospital has a total of 7 wards involved in this study and these were grouped into two. Patients were recruited from alternate groups on alternate weeks which allowed for proportionate selection of patients.

Study population:

This comprised of immunocompromised children admitted into any of the paediatric wards of the department of paediatrics, ICU and burn unit of the hospital. An immunocompromised host is a patient who has a weakened immune system, and such patients have reduced ability to fight infections and other diseases. This may be due to certain diseases such as AIDS, cancers, diabetes, chronic renal diseases, severe malnutrition, prematurity, extremes of age and certain genetic disorders. Other causes include certain medicines or treatments such as anticancer drugs, radiation therapy, and stem cell or organ transplant.

Inclusion criteria:

Immunocompromised paediatric patients (0-18 years of age), whose caregivers consented to the study and who were admitted to UCH Ibadan with signs of bloodstream infection and sepsis with at least two of the following; fever (core temperature >38ºC) or hypothermia (core temperature <36ºC), abnormal leucocyte profile with counts either elevated or depressed for age or > 10% immature neutrophils, tachypnoea (RR>2SD above normal for age, and tachycardia (HR>2SD above normal for age) or bradycardia (HR< 10th percentile for age).

Ethical consideration:

Ethical approval was obtained from the Joint Ethics Committee of the University of Ibadan and University College Hospital, Ibadan prior to commencement of the study. Informed consent of parents or guardians of participants and informed assent of the older participants were obtained before recruitment into this study.

Specimen collection and handling:

About 2ml of venous blood was collected from septic neonates, infants and children under 5 years of age into to BD BACTEC –BD Peds Plus-Blood culture vials, while 8-10 mls of venous blood was collected from older children into BD BACTEC-Mycosis IC/F Medium culture vials, which was used for selective culture and recovery of fungi from blood. The sample vials were transported to the laboratory for culture.

Laboratory culture of samples:

The vials were incubated at 37ºC in the automated BACTEC FX40 blood culture system (Becton Dickinson, Inc., Sparks, MD, USA). The vials flagged positive for growth were removed from the incubator and inoculated onto SDA, Blood, Chocolate, and MacConkey agar plates. These were incubated

overnight at 37⁰C under aerobic condition, after which the colonies were Gram stained.

Phenotypic identification of *Candida* from cultures:

Isolates identified from culture plate as *Candida* on Gram stain was tested by germ tube test (GTT) to presumptively identify *C. albicans* and non-albicans *Candida*.

Molecular identification of *Candida* from cultures by PCR and RFLP analysis:

Candida isolates were identified to species level by conventional PCR and Restriction Fragment Length Polymorphism (RFLP) analysis. The DNA was extracted from each *Candida* isolate using Yeast DNA preparation kit (Jena Bioscience) in accordance with the manufacturer's instructions, and amplified by PCR using primers that targets the internal transcribed spacer regions of the ribosomal DNA (ITS1 5'-TCC GTA GGT GAA CCT GCG G-3' and ITS4 5'-TCC TCC GCT TAT TGA TAT GC-3') (14).

The PCR reaction was performed in a thermal cycler (Applied Biosystems, ABS 7000) programmed as follows; initial denaturation of 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C and then a final elongation of 72°C for 7 minutes. PCR grade water as the negative controls were included in each test. The amplified PCR products were electrophoresed in 2% agarose gel and photographed by a camera.

The different strains of the *Candida* species were detected by RFLP analysis with M*sp*I and H*ae*II enzymes (Jena Biosciences, Jena Germany) following the manufacturers instruction. Briefly, 10µl of the PCR amplicon was added to 2µl of universal buffer, with 2µl of either of the restriction enzymes, and incubated at 38⁰C for 30 minutes. The products were then run on agarose gel electrophoresis and the species determined based on the cutting pattern of the M*sp*I enzyme (15).

Antifungal susceptibility test:

This was done with Vitek 2 antifungal susceptibility system (BioMérieux) against the following antifungals; voriconazole, fluconazole, amphotericin B, flucytosine, micafungin and caspofungin**.** Quality control strain used was *C. parapsilosis* ATCC 22019. The test was done in accordance with CLSI document M27-A3, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard, 3rd edition.

Data analysis:

Data were analysed using the SPSS version 25.0 software and presented as descriptive and inferential statistics. Means (± SD) were derived for quantitative variables, while qualitative variables were summarized as proportions. Chi-square test was used to determine the association between variables. The multivariate analysis was performed by binary logistic regression to ascertain the association between the risk variables and prevalence of candidaemia. P values ≤ 0.05 was considered significant.

Results:

Demographic and clinical characteristics of the study participants:

A total of 322 participants were recruited into this study. The mean age was 2.4 years, with 129 (40.1%) patients being less than 1 month old and 3 (0.9%) patients more than 15 years of age. The male to female ratio was 1.28:1. All recruited patients were admitted into the different wards and the majority 222 (70.5%) were on admission for between 1-10 days, 26 (8.1%) patients were admitted for between 21-30 days while 3 patients (0.9%) were on admission for more than 30 days. About 80% of the patients had sepsis as the underlying pathology, 18 (5.6%) had malignancies, 45 (14.0%) had very low birth weight, 8 (2.5%) had HIV and 23 (7.1%) had sickle cell disease.

Majority of the patients received intravenous broad-spectrum antibiotics while on admission, and the average number of days of antibiotics administration was 7.3 days (Table 1). One hundred and eleven (34.0%) patients had antibiotics for a number of days ranging between 8-14 days, while 3 (0.9%) received antibiotics for more than 21 days. All (100.0%) the patients had intravenous lines, 16 (5.0%) had surgery, 8 (2.4%) had urethral catheter, 88 (27.3%) had total parenteral nutrition, 5 (1.6%) had haemo/peritoneal dialysis while 3 (0.9%) had mechanical ventilation in the course of hospital admission (Table 1).

Table 1: Demographic and clinical characteristics of the study participants

Variable	Frequency	Percent
Age group (days)		
0-28 days	129	40.1
> 28 days	193	59.9
Gender		
Male	181	56.2
Female	141	43.8
Days on admission		
1 -10	227	70.5
11- 20	66	20.5
21- 30	26	8.1
30 +	3	0.9
Underlying disease		
Sepsis	189	58.7
Systemic infection	31	9.6
Solid tumours	10	3.1
Haematological malignancy	8	2.5
Very low birth weight with sepsis	45	14.0
Human immunodeficiency virus infection	8	2.5
Renal disease	3	0.9
Severe burns	5	1.6
Sickle cell disease with sepsis	23	7.1

Prevalence of blood stream infection and distribution of *Candida* species:

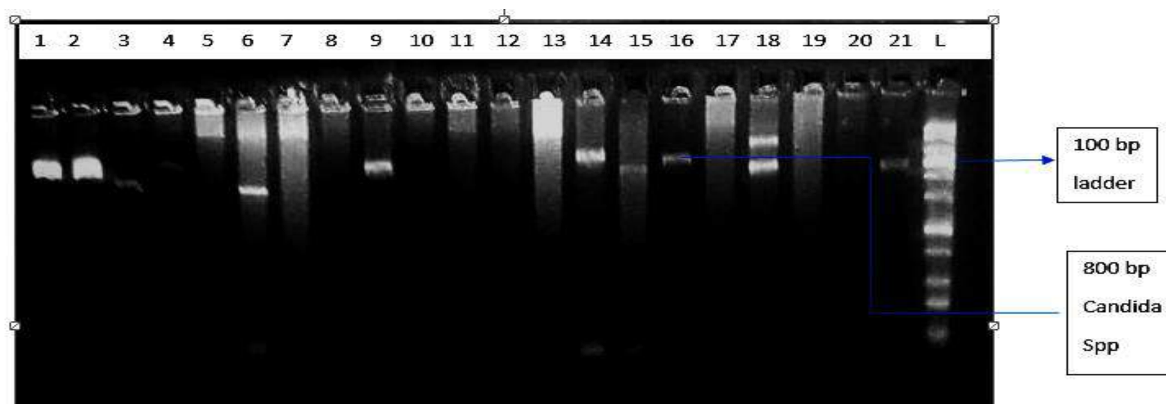
A total of 72 (22.4%) patient's blood samples yielded growth of one or more organisms while 252 (77.6%) samples yielded no growth. This gave the prevalence of microbiologically documented bloodstream infection of 22.4% in this study. Of those with positive blood cultures, 18 (24.3 %) had *Candida* bloodstream infection while 54 (75.7%) had bacterial bloodstream infection.

The distribution of the *Candida* species by PCR and RFLP analyses is as follows; *C. albicans* 14 (77.8%), *C. glabrata* 2 (11.1%) and *C. tropicalis* 2 (11.1%), while the germ tube test (GTT) identification of the *Candida* isolates is as follows; *C. albicans* 10 (55.5%) and non-albicans *Candida* 8 (44.4%).

Table 2: Distribution of *Candida* species by PCR-RFLP analysis

Isolates	Frequency	Percentage
Candida albicans	14	77 .77
Candida glabrata	2	11.11
Candida tropicalis	2	11.11
Total	**18**	**100**

Table 2 shows the species distribution of the *Candida* isolates identified using PCR while Fig 1 shows the image of agarose gel electrophoresis after PCR. Fig 2 shows the agarose gel electrophoresis after RFLP with restriction enzyme M*sp*I.



Samples in lanes 1,2,3,4,6,9, 14,15,16, 18 and 21 had the expected band sizes of 700 to 800bp.

Fig 1: Agarose gel electrophoresis picture of PCR amplicons for detection of *Candida* species using primers ITS1 and ITS4

Lanes 7-18 = *C. albicans,* Lanes 1 and 19 = *C. glabrata;* Lanes 5 and 21 = *C. tropicalis*

Fig 2: Agarose gel electrophoresis picture of PCR-RFLP with restriction enzyme M*sp*I

Antifungal susceptibility of *Candida* species: Fourteen (77%) *Candida* isolates were susceptible to fluconazole and voriconazole, 3 (16.6%) were resistant while 1 (5.5%) was of intermediate susceptibility to the antifungals respectively. Eight (44.4%) of the isolates were susceptible to caspofungin and mica fungin respectively while 10 (55.5%) were resistant to both antifungals. Ten (55.5%) of the isolates were susceptible to amphotericin B, 5 (27.7%) were resistant while 3 (16.6%) had intermediate susceptibility to the antifungal. All but one isolate was susceptible to the antifungal, flucytosine (Table 3).

Table 3: Antifungal susceptibility test result of the *Candida* species to selected antifungal agents

Antifungal/Isolates		*Candida albicans* (n = 14)	*Candida glabrata* (n = 2)	*Candida tropicalis* (n = 2)
Fluconazole	S (%)	10 (71.0)	2 (100.0)	1 (50.0)
	I (%)	1 (7.0)	-	-
	R (%)	3 (21.0)	-	1 (50.0)
Voriconazole	S (%)	10 (71.0)	2 (100.0)	2 (100.0)
	I (%)	1 (7.0)	-	-
	R (%)	3 (21.0)	-	-
Caspofungin	S (%)	7 (50.0)	-	1 (50.0)
	I (%)	-	-	-
	R (%)	7 (50.0)	2 (100.0)	1 (50.0)
Micafungin	S (%)	6 (43.0)	1 (50.0)	1 (50.0)
	I (%)	-	-	-
	R (%)	8 (57.0)	1 (50.0)	1 (50.0)
Amphotericin B	S (%)	9 (64.0)	1 (50.0)	1 (50.0)
	I (%)	3 (22.0)	-	-
	R (%)	2 (14.0)	1 (50.0)	1 (50.0)
Flucytosine	S (%)	14 (100.0)	2 (100.0)	1 (50.0)
	I (%)	-	-	-
	R (%)	-	-	1 (50.0)

S = Susceptible, I = Intermediate, R = Resistant, N = Number of isolates

Discussion:

Candidaemia is a type of common hospital acquired bloodstream infection that occurs primarily in the immunocompromised. In this study, *Candida* spp as a group was found to be the most common aetiological agent of bloodstream infection among immunocompromised paediatric patients admitted into the hospital during the period of this study. A study conducted among immunosuppressed patients in Turkey put *Candida* species as the third commonest cause of bloodstream infection while another study in South Africa among oncology paediatric patients reported *Candida* species as the fifth commonest cause of bloodstream infections. Studies from the United States and Europe however showed that *Candida* species are the third most common pathogen isolated in paediatric bloodstream infection (12,16-19). The difference could be because in our study, all the participants were immunocompromised and had signs and symptoms of bloodstream infection, together with the fact that a selective blood culture media was used. In addition, the developed world, unlike our environment, has long established protocols and guidelines which are applied consistently in diagnosis, management and prevention of invasive fungal infections.

The prevalence of candidaemia in our study was 5.6% (18/322). This is lower than 7.4% reported in a study in India (19) but higher than 3.5% reported in a study in the United States (20). Although this rate is still high, it could reflect changes made in the management of invasive candidiasis in our environment in the last couple of years, which include high index of suspicion of such infections, better and more sensitive diagnostic techniques employed in the evaluation of patients, stricter adherence to infection control measures in the management of at-risk patients and local availability of more groups of antifungal drugs employed in the management of such patients when a diagnosis is made.

The male to female ratio of the participants in our study was 1.28:1 while the ratio of patients with candidaemia was slightly less at 1.25:1. Eleven of the patients with candidaemia were neonates, buttressing the observation made by Saiman et al., (21) who reported that candidaemia is more common in neonates than in other children because of their immature immune system, prematurity, low birth weight, and more frequent level of intubation. Two of the patients with candidaemia had malignancies, one was HIV infected, and these also corroborates other studies that show a higher incidence of candidaemia in patients with immunosuppression caused by these conditions (18).

Candida albicans accounted for 77% of the *Candida* isolates. This is similar to the results of the studies by Ezenwa et al., (22) and Santolaya et al., (23) which show that *C. albicans* is still the predominant *Candida* species that cause invasive *Candida* infection, but differs from the result of the studies by Awasthi et al., (20) and Kaur et al., (24) which showed *C. tropicalis* and *C. parapsilosis* respectively to be the primary *Candida* species implicated in candidaemia. In this study, resistance rate to fluconazole was 22.2% for *Candida* spp, which is higher than the rate reported by Oladele et al., (25) in a previous study conducted in UCH Ibadan over a decade ago where no resistance to fluconazole was reported. It is however lower than the rate reported in a study by Kaur et al., (24) who reported rate of 37.8%.

Candida albicans exhibited resistance rate of 21.0% to fluconazole, *C. tropicalis* exhibited resistance rate of 50.0% while *C. glabrata* did not exhibit any resistance to fluconazole. It is important to note the increasing level of resistance to fluconazole in Ibadan and take measures aimed at countering it since fluconazole is still the primary antifungal drug used for most invasive fungal infections in Nigerian hospitals. Another study by Adhikary et al., (26) in Southern India reported higher level of resistance (47.9%) for all *Candida* isolates to fluconazole, however, these isolates were susceptible to polyenes, flucytosine and echinocandins. Fortunately, the level of fluconazole resistance is still relatively low in our study despite the fact that it is the main antifungal drug used in the treatment of candidaemia in our environment.

About 71.0% of *C. albicans* in our study were susceptible to voriconazole but the other non-albicans *Candida* spp were 100.0% susceptible to this antifungal. This is the contrary to the study by Kaur et al., (24), who reported *C. albicans* to be 100.0% susceptible to voriconazole while *C. parapsilosis* was 62.5% susceptible to the drug. It is obvious that voriconazole was an effective azole among all the *Candida* isolates tested, and it is worth noting that in our study, almost all *Candida* isolates resistant to fluconazole were susceptible to voriconazole. This beyond doubt proves that voriconazole is a very effective azole. Such finding implies that voriconazole due to its vast species coverage, can be used in the treatment of candidemia caused by fluconazole resistant strains. These observations are in accordance with previous studies done by Madhavan et al., (27).

This study demonstrated that the *Candida* spp were 44.0% susceptible to caspofungin, 77.0% susceptible to amphotericin B and 94.0% susceptible to flucytosine. Althou-

gh these antifungals are not readily available in our environment, it would be a very useful measure to make them more available, especially the flucytosine because they will come in handy when an azole resistant *Candida* spp is implicated in cases of invasive candidiasis. Unfortunately, these drugs are however very expensive and not affordable by many of the patients who might need them in our resource limited environment.

Conclusion:

Candida bloodstream infection is a very present in our environment and is also increasing in prevalence even among paediatric patients on admission, especially those who are immunocompromised. It is necessary to have a high index of suspicion when managing these patients and endeavour to correctly identify the culpable organism and perform antifungal susceptibility test as regularly as possible. This will substantially improve patients' survival and reduce the mortality of this infection.

Contribution of authors:

ACE is the principal investigator, responsible for all aspects of the study: conceptualization and design, patients' recruitment, sample collection, laboratory analysis, statistical analysis, and final manuscript write up; AO was responsible for data collation; OM was responsible for proof reading the manuscript; TA was involved in sample collection; ARM was involved in the laboratory analysis; and TF was involved in the sample collection and proof reading. All authors approved the manuscript submitted for publication.

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No conflict of interest is declared.

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Oral presentation of the findings of this study was made at 3rd Virtual Conference of CLIMIDSON that held on November 23-24, 2023 and the abstract was published in the conference brochure.

References:

1. McCarty, T. P., and Pappas, P. G. Invasive Candidiasis. Infect Dis Clin North Am. 2016; 30 (1):103-124. doi: 10.1016/j.idc.2015.10.013
2. Martins, N., Ferreira, I. C., Barros, L., et al. Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment. Mycopathologia. 2014; 177 (5-6): 223-240. doi:10.1007/s11046-014-9749-1
3. Dutta, A., Zaoutis, T, E., and Palazzi, D. L. An Update on the Epidemiology of Candidemia in Children. Curr Fungal infect Rep. 2012; 6 (4): 296-302. doi:10.1007/s12281-012-0113-y
4. Green, L., and Dolen, W. K. Chronic Candidiasis in Children. Curr Allergy and Asthma Rep. 2017; 17 (5): 31. doi:10.1007/s11882-017-0699-9.
5. Pappas, P. G., Kauffman, C. A., Andes, D. R., et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2016; 62 (4): e1-50. doi:10.1093/cid/civ933.
6. Pfaller, M. A., and Diekema, D. J. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007; 20 (1): 133-163. doi:10.1128/CMR.00029-06.
7. Prigitano, A., Cavanna, C., Passera, M., et al. CAND-LO 2014-15 study: changing epidemiology of candidemia in Lombardy (Italy). Infection. 2016; 44 (6): 765-780. doi:10.1007/s15010-016-0951-6.
8. Al-Rawahi, G. N., and Roscoe, D. L. Ten-year review of candidemia in a Canadian tertiary care centre: Predominance of non-albicans *Candida* species. Canad J Infect Dis Med Microbiol. 2013; 24 (3): e65-e68. doi:10.1155/2013/929717.
9. Caggiano, G., Coretti, C., Bartolomeo, N., et al. *Candida* Bloodstream Infections in Italy: Changing Epidemiology during 16 Years of Surveillance. BioMed Res Int. 2015; 2015: 256580. doi:10.1155/2015/256580.
10. Guinea, J., Global trends in the distribution of *Candida* species causing candidemia. Clin Microbiol Int. 2014; 20 (Suppl 6): 5-10. doi: 10.1111/1469-0691.12539
11. Quindós, G. Epidemiology of candidaemia and invasive candidiasis. A changing face. Revista iberoamericana de micologia. 2014; 31 (1): 42-48. doi:10.1016/j.riam.2013.10.001
12. Wisplinghoff, H., Ebbers, J., Geurtz, L., et al. Nosocomial bloodstream infections due to *Candida* spp in the USA: species distribution, clinical features and antifungal susceptibilities. Int J Antimicrob Agents. 2014; 43 (1): 78-81. doi:10.1016/j.ijantimicag.2013.09.005.
13. Downey, L. C., Smith, P. B., and Benjamin, D. K, Jr., Recent Advances in the Detection of Neonatal Candidiasis. Curr Fungal Infect Rep. 2010; 4 (1): 17-22. doi:10.1007/s12281-010-0002-1.
14. White, T. J., Bruns, T., and Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. J (eds). PCR Protocols: A Guide to Methods and Applications. London, UK Academic Press. 1990: 315-322
15. Mirhendi, H., Makimura, K., Khoramizadeh, M., and Yamaguchi, H. A one-enzyme PCR-RFLP assay for identification of six medically important *Candida* species. Nihon Ishinkin Gakkai Zasshi. 2006; 47: 225–229.doi:10.3314/jjmm.47.225
16. Lovero, G., De Giglio, O., Montagna, O., et al. Epidemiology of candidemia in neonatal intensive care units: a persistent public health problem. Annali di Igiene: Medicina Preventivae di Comunita. 2016; 28 (4): 282-287. doi:10.7416/ai.2016.2107.
17. Mvalo, T., Eley, B., Bamford, C., et al. Bloodstream infections in oncology patients at Red Cross War Memorial Children's Hospital, Cape Town, from 2012 to 2014. 2018; 77: 40-47. doi:10.1016/j.ijid.2018.09.012
18. Tural Kara, T., Erat, T., Yahşi, A., et al. Bloodstream infections in paediatric haemato logy/oncology patients: Six years' experience of a single center in Turkey. Turkish J Med Sci. 2019; 49 (4): 1157-1164. doi:10.3906/sag-1812-101.

19. Zaoutis, T. E., Argon, J., Chu, J., et al. The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: a propensity analysis. Clin Infect Dis. 2005; 41 (9): 1232-1239. doi:10.1086/496922
20. Awasthi, A. K., Jain, A., Awasthi, S., Ambast, A., Singh, K., and Mishra, V. Epidemiology and microbiology of nosocomial pediatric candidemia at a northern Indian tertiary care hospital. Mycopathologia. 2011; 172 (4): 269-277. doi:10.1007/s11046-011-9431-9.
21. Saiman, L., Ludington, E., Pfaller, M., et al. Risk factors for candidemia in Neonatal Intensive Care Unit patients. The National Epidemiology of Mycosis Survey study group. Paediatr Infect Dis J. 2000; 19 (4): 319-324. doi:10.1097/00006454-200004000-00011
22. Ezenwa, B. N., Oladele, R. O., Akintan, P. E., et al. Invasive candidiasis in a neonatal intensive care unit in Lagos, Nigeria. Nig Postgrad Med J. 2017; 24 (3): 150-154. doi:10.4103/npmj.npmj_104_17.
23. Santolaya, M. E., Alvarado, T., Queiroz-Telles, F., et al. Active surveillance of candidemia in children from Latin America: a key requirement for improving disease outcome. Paediatr Infect Dis J. 2014; 33 (2): e40-e44. doi:10.1097/INF.0000000000000039.
24. Kaur, R., Jaggi, S., and Dhakad, M. S. An etiological and antifungal profile of candidemia in children. Int J Comm Med. 2019; 6 (9): 3899-3904.doi:10.18203/2394-6040.ijcmph20193990
25. Oladele, R. O., Bakare, R. A., Petrou, M. A., et al. Candidaemia in a tertiary hospital in Nigeria. Afr J Lab Med. 2014; 3 (1): 89-83. doi:10.4102/ajlm.v3i1.89.
26. Adhikary, R., and Joshi, S. Species distribution and anti-fungal susceptibility of Candidaemia at a multi super-specialty center in Southern India. Indian J Med Microbiol. 2011; 29 (3): 309-311. doi:10.4103/0255-0857.83920.
27. Madhavan, P., Jamal, F., Chong, P. P, and Ng, K. P. In vitro activity of fluconazole and voriconazole against clinical isolates of *Candida* spp. by E-test method. Trop Biomed. 2010; 27 (2): 200-207.

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Short Communication **Open Access**

Draft metagenome-assembled genomes of *Pseudomonas putida* isolated from human gut microbiome in Nasarawa State, Nigeria

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Abstract:

The metagenome-assembled genome (MAG) sequences of *Pseudomonas putida* PP14A and PP20A were obtained by metagenomic sequencing from the gut microbiomes of a female and a male patient both 24 years old from the same household presenting to a health outreach laboratory with complaint of headache, and occasional diarrhoea in Mararaba, Nasarawa State, Nigeria. The phylogenetic relationship observed between the two PP MAGs with other *Pseudomonas* spp MAGs from human, points to the global spread of *Pseudomonas putida* through human activity and migration.

Keywords: *Pseudomonas putida,* metagenome-assembled genome, gut microbiome, virulence, phylogeny

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Projet de génomes assemblés par métagénome de *Pseudomonas putida* isolés du microbiome intestinal humain dans l'État de Nasarawa, Nigéria

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Résumé:

Les séquences du génome assemblé par métagénome (MAG) de *Pseudomonas putida* PP14A et PP20A ont été obtenues par séquençage métagénomique à partir des microbiomes intestinaux d'une femme et d'un homme de 24 ans, tous deux âgés de 24 ans, issus du même foyer, se présentant dans un laboratoire de santé de proximité se plaignant de maux de tête et diarrhée occasionnelle à Mararaba, État de Nasarawa, Nigeria. La relation phylogénétique observée entre les deux MAG PP avec d'autres MAG de *Pseudomonas* spp provenant de l'homme indique la propagation mondiale de *Pseudomonas putida* par l'activité humaine et la migration.

Mots clés: *Pseudomonas putida*, génome assemblé par métagénome, microbiome intestinal, virulence, phylogénie

Introduction:

Pseudomonas putida is a Gram-negative bacterium which is commonly found in the soil, water and plant surfaces (1). Its ability to degrade a wide range of organic compounds and genetic adaptability has accorded it a vast use in biotechnology as a model organism (2). Here we report non-pathogenic *Pseudomonas putida* strains with foreign virulence genes derived from other bacteria in gut microbiome of human hosts.

Methodology:

Clinical presentation and phenotypic identification methods:

Two 24-years old patients (one female and one male) from the same household pre-

sented to a health outreach laboratory in Mararaba, Nasarawa State, Nigeria, with complaints of headache, fever and occasional diarrhoea. The patients' symptoms were consistent with malaria and gastroenteritis, and therefore were screened for malaria parasite and their stool samples were screened for *Salmonella enterica* growth by first enriching the samples in Selenite F broth and then plating on *Salmonella-Shigella* agar. Colonies suspected to be *Salmonella* sp. were further purified on tryptone soy agar and used for conventional biochemical confirmatory tests (urease, and triple sugar iron test). Antibiotic susceptibility test (AST) was performed against ampicillin (10 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), and cefotaxime (30 µg) (Oxoid, UK) using the Kirby-Bauer diffusion method (3).

DNA extraction, library preparation and metagenomic sequencing:

The DNA was extracted using the ZYMO Research Quick-DNA Fungal/Bacterial Miniprep Kit according to the manufacturer's instructions. A sequencing library was constructed using the Nextera XT DNA Library Prep Kit and sequenced on an Illumina NovaSeq 6000 run at 250 bp paired-end with 60x coverage. The libraries were checked on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA chip, normalized, and pooled with the Kapa Biosystems Library Quantification Kit for Illumina. Trimmomatic v0.32 was used to trim adapter sequences as per the protocol outlined by (4).

Metagenomic sequence assembly & analysis:

A de novo assembly of the reads was performed using the St. Petersburg Genome Assembler (SPAdes) v3.15.1. (5). The reads were then remapped to the resulting contigs using BWA mem to obtain additional quality metrics. MetaBAT2 v2.15 (6) binning algorithm was used to bin the MAGs from the assembled metagenome. CheckM v1.2.2 (7) assessed the quality of the MAGs. Kraken (8) and multilocus sequence typing was applied to identify the taxonomy of the binned MAGs. FastANI v1.33 (9) was applied on sample 1 MAG and sample 2 MAG against a reference genome of *Pseudomonas putida* ATCC 12633 (GenBank ascension number GCA_024508115.1) genome to confirm their taxonomic identity as *Pseudomonas putida*, PP14A and PP20A, respectively.

The Comprehensive Antimicrobial Resistance Database (CARD) (10) resistance gene identifier was employed to identify resistance genes in PP14A and PP20A MAGs. Bakta1.8.2 (11) was used to annotate the genomes, and PathogenFinder (12) was used to investigate pathogenicity. Phigaro 1.0.1 (13) was employed to investigate prophage regions in both MAGs while the VFanalyser tool of the Virulence Factor Database (14) was utilized to identify virulence factors in PP14A and PP20A MAGs. A phylogenomic tree was inferred using IQ-TREE rapid bootstrap analysis (1000 repetitions) and concatenated alignments with all MLST genes from 46 *Pseudomonas* spp genomes, via the autoMLST interface (https://automlst.ziemertlab.com/results /9ec72497-e35d-429d-86c0-348163fd28fa/report/).

Results and Discussion:

The triple sugar iron and urease test confirmed the isolates as negative for *Salmonella enterica* growth. However, the AST of the isolate from the male patient showed antibiotic resistance to ampicillin (10 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), and cefotaxime (30 µg) while the isolate from the female patient showed sensitivity to ciprofloxacin (5 µg) and ofloxacin (5 µg), which sparked our curiosity to investigate the genome of the isolates. The PP14A and PP20A MAGs are considered high-quality metagenome-assembled genome having 98.38% completeness for both MAGs, with 0.63% (PP14A) and 0.79% (PP20A) contamination (15). PP14A and PP20A were not classified as human pathogenic strains of *Pseudomonas putida* with average nucleotide identity of 88% to *Pseudomonas putida* ATCC 12633 (Table 1). PP14A MLST sequence type was *55b3 while PP20A was *3936 sequence type.

There were no complete (100%) antibiotic resistance genes identified in both MAGs, however some incomplete genes (>70% identity) for efflux pumps (*abaQ*) were seen in both MAGs. Although, PP14A and PP20A were not classified as human pathogenic strains of *P. putida*, they both contained foreign virulence genes; *hopJ* gene for type II secretion system effector protein peculiar to *Pseudomonas syringae*; *epsE* and *epsG* for type II secretion system of *Vibrio cholerae*; and *sodCI* gene for stress adaptation in *S. enterica*, were seen in both PP14A and PP20A, while LPS-O antigen gene for *P. aeruginosa* was seen in PP20A alone. Several other virulence genes of *P. aeruginosa* LESB58 *(*GCA_000026645.1.) were also identified in both MAGs (Table 2).

Table 1: Genomic features of the two *Pseudomonas putida* (PP14A and PP20A) clinical isolates

Features	**PP14A**	**PP20A**
Genome size	5,280,125 bp	5,052,825 bp
Number of contigs	105	106
Average contig length	49812 bp	47668 bp
Largest contig length	310054bp	241253 bp
Smallest contig length	2067 bp	2294 bp
N_{50}	82,436 bp	70607 bp
G+C	62.9%	62.9%
Total number of genes	5007	4783
Number of coding regions (with protein)	4864	4651
tRNA	57	56
Non-coding RNA	41	39
Average nucleotide identity (*Pseudomonas putida* ATCC 12633)	88.0%	88.1%
Prophage regions	4	4
Horizontal gene transfer regions	168	196

RNA = ribonucleic acid

Table 2: *Pseudomonas aeruginosa* LESB58 (GCA_000026645.1.) virulence factors found in PP14A and PP20A MAGs

S/N	**Virulence Genes**	**Gene Products**
1	*fleN; fleQ; fleR* *flgC; flgD; flgE; flgF; flgG; flgH; flgI; flgJ flgK; flgL* *flhA; flhB; flhF; fliC; fliD; fliE; fliF; fliG; fliH; fliI; fliJ* *fliM; fliN; fliO; fliP; fliQ; fliR*	Flagellar synthesis regulator Flagellar basal-body rod proteins Flagellar hook-associated proteins Flagellar assembly proteins
2	*fimT, fimU, fimV,* *pilA; pilB; pilC; pilE; pilF; pilG; pilH; pilI; pilJ; pilK; pilM;* *pilN; pilO; pilP; pilQ. pilT*	Fimbrial biogenesis proteins Motility proteins
3	*chpA* *chpC*	Still frameshift probable component of chemotactic signal transduction system Probable chemotaxis protein
4	*alg44* *alg8* *algA, algQ, algR, algU, algX, algZ* *mucA; mucB; mucC*	Alginate biosynthesis protein Alginate-c5-mannuronan-epimerase GDP-mannose 6-dehydrogenase Alkaline metalloproteinase precursor
5	*fpvA* *pvdA* *pvdD, pvdE*	Ferripyoverdine receptor FpvA L-ornithine N5-oxygenase Pyoverdine synthetase
6	*clpV1, vgrG1* *hcp, icmF1*	Type VI secretion system AAA+ family ATPase Type VI secretion system substrate Hcp1

PP = *Pseudomonas putida*; MAG = Metagenome-assembled genomes

There were 168 horizontal gene transfer (HGT) regions in PP14A and 196 HGT regions from PP20A, accounting for presence of four prophage regions (from one Myoviridae and three Siphoviridae phages) (Fig 1) embedded in the bacteria genome of each of them.

The phylogenetic tree (Fig 2) shows that PP14A and PP20A shared closest clonal relationship with *Pseudomonas* spp NBRC 111 120, *Pseudomonas* spp GTC 16481, *Pseudomonas* spp NBRC 111126 and *Pseudomonas* spp NBRC 111129, all isolated from human urine from four different cities in Japan (2009-2012) (16). The closest relationship to non-human isolate was with *Pseudomonas* spp TJI-51 isolated from plant (mango orchid) collected in 2009 from Pakistan (17).

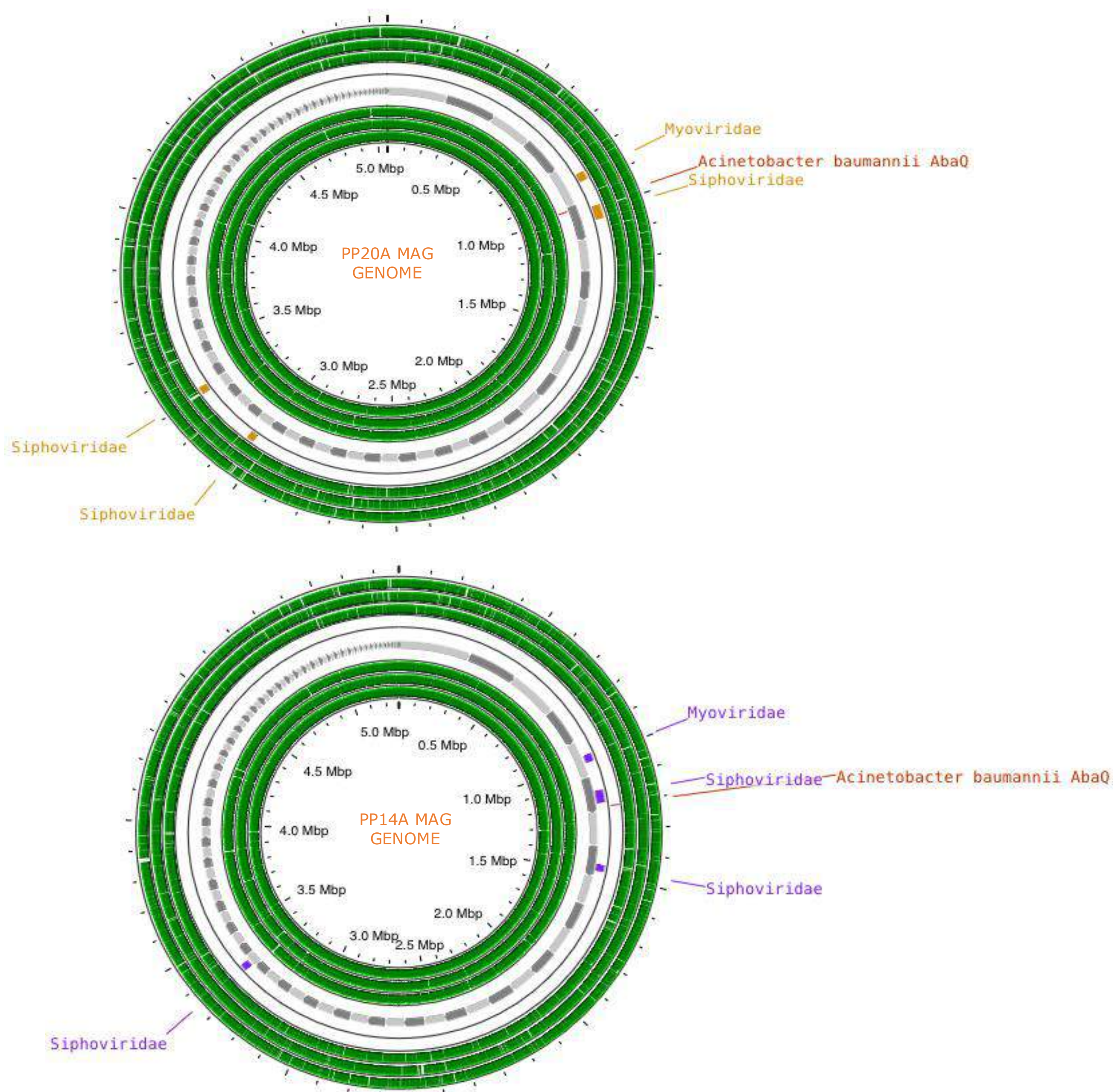

Fig 1: Circular presentation of PP14A and PP20A MAG genome highlighting prophages and antibiotic-resistant genes

Bacteria are known to exhibit plasticity and while existing in any microbiome community acquire traits that ensure its survival (18). PP14A and PP20A were isolated from gut microbiome of two different patients in the same community and although it is not yet classified as a human pathogen, its specie-inherent virulence genes as well as the foreign ones can be transferred to other pathogenic or non-pathogenic bacteria in its community promoting molecular evolution of the microbiome. The closeness of PP14A and PP20A on the phylogeny tree (Fig 1) shows that they share same ancestry and this can be seen in similar features they shared (Tables 1 and 2, Fig 2). However, the presence of *P. aeruginosa* LPS O-antigen gene in PP20A but not in PP14A may be as a result of the 12.5% strain heterogeneity in PP20A which could have been contributed by presence of *P. aeruginosa* which 16S rRNA was not appropriately captured due to limitation of the sequencing coverage (60x) in the study.

Comparing both genomes showed that although both MAGs had four prophages, and same antibiotic resistance gene (*abaQ*), they were positioned at different locations in the genome. This further proves that although the MAGs may share ancestry and were isolated from persons in the same household, they may not belong to the same generation and trans-

mission from one person to the other may have happened after a few generations. Phylogenetic relationships observed between PP14A and PP20A MAGs with other *Pseudomonas* spp (Fig 2) point to global spread of *Pseudomonas putida* through human activity and migration.

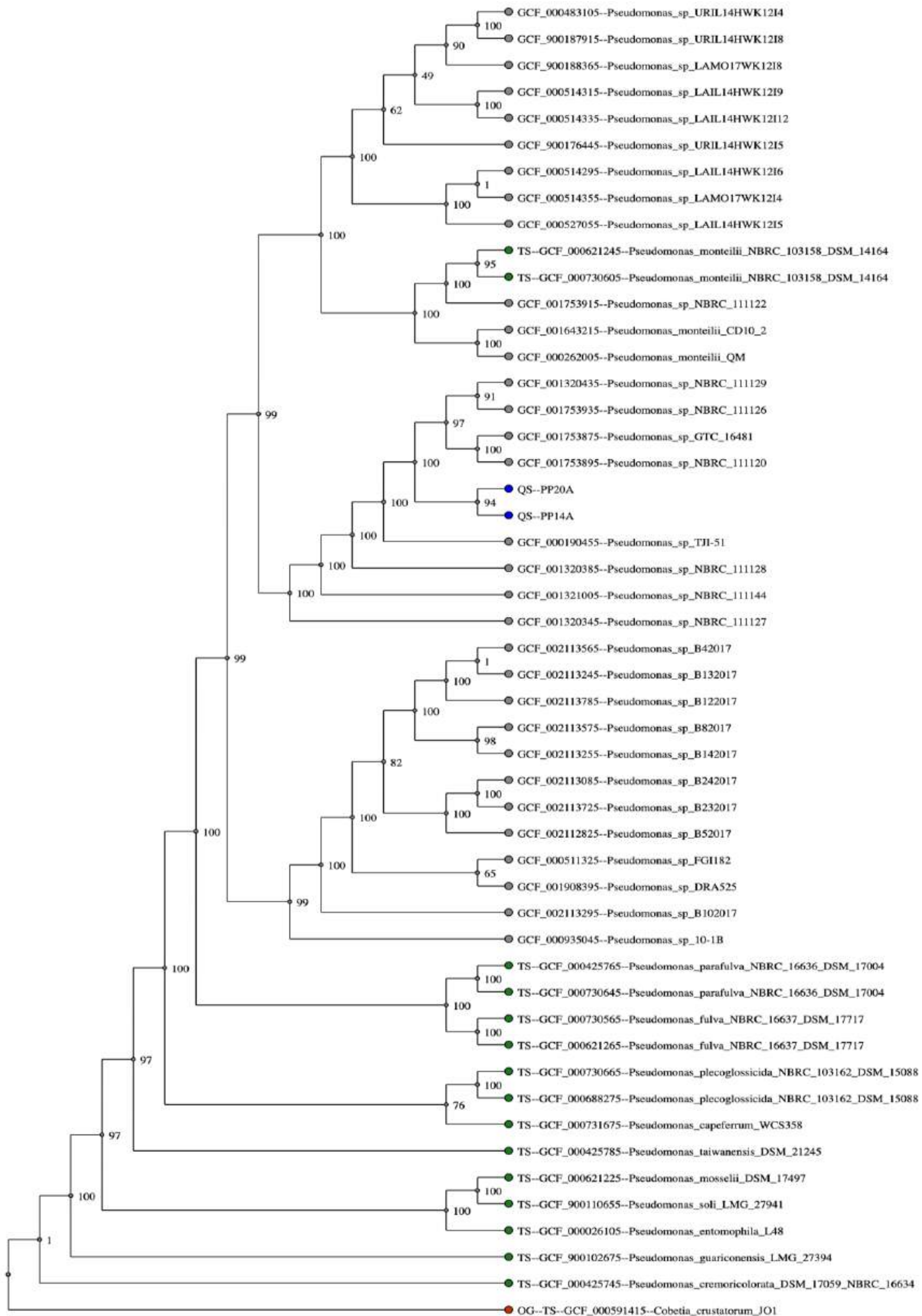

Fig 2: Phylogenetic tree of PP14A and PP20A isolates

Ethical consideration:

Ethical approval was obtained from the Nasarawa State Ministry of Health with approval number NHREC 18/06/2017 on November 16, 2020 and from the ethical review board of Faculty of Biological Science, University of Nigeria Nsukka (UNN/FBS/EC/1012). Informed consent was obtained from the study participants.

Data availability:

PP14A MAG was submitted to the NCBI database under Biosample SAMN41785133 and SRR 28820204. PP20A MAG was submitted to the NCBI database under Biosample SAMN4180 4069. Both PP14A and PP20A sequencing projects are registered on the NCBI under Project number PRJNA1075677.

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Contributions of authors:

The author conceptualized the study, analysed and interpreted the data and wrote the manuscript.

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Conflict of interest:

Author declares no conflict of interest.

References:

1. Peter, S., Oberhettinger, P., Schuele, L. et al. Genomic characterisation of clinical and environmental *Pseudomonas putida* group strains and determination of their role in the transfer of antimicrobial resistance genes to *Pseudomonas aeruginosa*. BMC Genomics. 2017; 18: 859. https://doi.org/10.1186/s12864-017-4216-2
2. Volke, D. C., Calero, P., and Nikel, P. I. *Pseudomonas putida.* Trends Microb. 2020; 28 (6): 512-513. doi:10.1016/j.tim.2020.02.015
3. Clinical and Laboratory Standards Institute. M02-A12 Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Twelfth Edition. 2015. www.clsi.org. (Accessed 6 July 2021).
4. Bolger. A.M., Lohse, M., and Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30 (15): 2114–2120. https://doi.org/10.1093/bioinformatics/btu170
5. Prjibelski, A., Antipov, D., Meleshko D., et al. Using SPAdes De Novo Assembler. Curr Protocol Bioinform. 2020; 7: 1. https://doi.org/10.1002/cpbi.102
6. Kang, D. D., Li, F., Kirton, E., et al. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from meta-genome assemblies. PeerJ. 2019; 7: e7359. doi: 10.7717/peerj.7359.
7. Parks, D. H., Imelfort, M., Skennerton, C. T., et al. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 2015; 25 (7): 1043-1055. doi: 10.1101/gr.186072.114.
8. Wood, D. E., and Salzberg, S. L. Kraken: ultrafast metagenomic sequence classification using exact alignments 2014. http://ccb.jhu.edu/software/kraken/ (Accessed 31 Jan 2022).
9. Jain, C., Rodriguez-R, L. M., Phillippy, A. M., et al. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun. 2018; 9(1): 5114. doi: 10.1038/s41467-018-07641-9.
10. Alcock, B. P., Huynh, W., Chalil, R., et al. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucl Acids Res. 2022 . https://doi.org/10.1093/nar/gkac920
11. Schwengers, O., Jelonek, L., Dieckmann, M. A., et al. Bakta: rapid and standardized annotation of bacterial genomes via alignment-free sequence identification. Microb Genom. 2021; 7 (11): 000685. doi: 10.1099/mgen.0.000685.
12. Cosentino, S., Voldby-Larsen, M., Møller-Aarestrup, F., et al. PathogenFinder – Distingui-shing Friend from Foe Using Bacterial Whole Genome Sequence Data. PLoS One. 2013; 8 (10): e77302. doi:10.1371/journal.pone.0077302
13. Starikova, E. V., Tikhonova, P. O., Prianichnikov, N. A., et al. Phigaro: high-throughput prophage sequence annotation. Bioinformatics. 2020; 36 (12): 3882-3884. doi:10.1093/bioinformatics/btaa250.
14. Liu, B., Zheng, D., Jin, Q., et al. VFDB 2019: A comparative pathogenomic platform with an interactive web interface. Nucl Acids Res. 2019; 47 (D1): 687–692. https://doi.org/10.1093/nar/gky1080
15. Bowers, R. M., Kyrpides, N. C., Stepanauskas, R., et al. Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. Nat Biotechnol. 2017; 35 (8):725-731. doi:10.1038/nbt.3893. Erratum in: Nat Biotechnol. 2018; 36 (2): 196.
16. National Institute of Technology and Evaluation. *Pseudomonas* Genome Sequencing Project 2009-2012. https://www.ncbi.nlm.nih.gov/bioproject/PRJDB3001/
17. International Center for Chemical and Biological Sciences *Pseudomonas* sp. TJI-51 whole genome shotgun project. 2009. https://www.ncbi.nlm.nih.gov/nuccore/AEWE00000000
18. Shen, J. P., and Chou, C. F. Morphological plasticity of bacteria-Open questions. Biomicrofluidics. 2016;10 (3): 031501. doi:10.1063/1.4953660.

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doi: 10.4314/ajtcam.v4i2.31207

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