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Editorial **Open Access**

COP27 Climate Change Conference: Urgent action needed for Africa and the world

Wealthy nations must step up support for Africa and vulnerable countries in addressing past, present and future impacts of climate change

The 2022 report of the Intergovernmental Panel on Climate Change (IPCC) paints a dark picture of the future of life on earth, characterised by ecosystem collapse, species extinction, and climate hazards such as heatwaves and floods (1). These are all linked to physical and mental health problems, with direct and indirect consequences of increased morbidity and mortality. To avoid these catastrophic health effects across all regions of the globe, there is broad agreement—as 231 health journals argued together in 2021—that the rise in global temperature must be limited to less than 1.5°C compared with pre-industrial levels.

While the Paris Agreement of 2015 outlines a global action framework that incorporates providing climate finance to developing countries, this support has yet to materialise (2). COP27 is the fifth Conference of the Parties (COP) to be organised in Africa since its inception in 1995. Ahead of this meeting, we—as health journal editors from across the continent—call for urgent action to ensure it is the COP that finally delivers climate justice for Africa and vulnerable countries. This is essential not just for the health of those countries, but for the health of the whole world.

Africa has suffered disproportionately although it has done little to cause the crisis

The climate crisis has had an impact on the environmental and social determinants of health across Africa, leading to devastating health effects (3). Impacts on health can result directly from environmental shocks and indirectly through socially mediated effects (4). Climate change-related risks in Africa include flooding, drought, heatwaves, reduced food production, and reduced labour productivity (5).

Droughts in sub-Saharan Africa have tripled between 1970-79 and 2010-2019 (6). In 2018, devastating cyclones impacted three million people in Malawi, Mozambique and Zimbabwe (6). In west and central Africa, severe flooding resulted in mortality and forced migration from loss of shelter, cultivated land, and livestock (7). Changes in vector ecology brought about by floods and damage to environmental hygiene has led to increases in diseases across sub-Saharan Africa, with rises in malaria, dengue fever, Lassa fever, Rift Valley fever, Lyme disease, Ebola virus, West Nile virus and other infections (8,9). Rising sea levels reduce water quality, leading to water-borne diseases, including diarrhoeal diseases, a leading cause of mortality in Africa (8). Extreme weather damages water and food supply, increasing food insecurity and malnutrition, which causes 1.7 million deaths annually in Africa (10). According to the Food and Agriculture Organization of the United Nations, malnutrition has increased by almost 50% since 2012, owing to the central role agriculture plays in African economies (11). Environmental shocks and their knock-on effects also cause severe harm to mental health (12). In all, it is estimated that the climate crisis has destroyed a fifth of the gross domestic products (GDP) of the countries most vulnerable to climate shocks (13).

The damage to Africa should be of supreme concern to all nations. This is partly for moral reasons. It is highly unjust that the most impacted nations have contributed the least to global cumulative emissions, which are driving the climate crisis and its increasingly severe effects. North America and Europe have contributed 62% of carbon dioxide emissions since the Industrial Revolution, whereas Africa has contributed only 3% (14).

The fight against the climate crisis needs all hands on deck

Yet it is not just for moral reasons that all nations should be concerned for Africa. The acute and chronic impacts of the climate crisis create problems like poverty, infectious disease, forced migration, and conflict that spread through globalised systems (6,15). These knock-on impacts affect all nations. COVID-19 served as a wake-up call to these global dynamics and it is no coincidence that health professionals have been active in identifying and responding to the consequences of growing systemic risks to health. But the lessons of the COVID-19 pandemic should not be limited to pandemic risk (16,17). Instead, it is imperative that the suffering of frontline nations, including those in Africa, be the core consideration at COP27: in an interconnected world, leaving countries to the mercy of environmental shocks creates instability that has severe consequences for all nations.

The primary focus of climate summits remains to rapidly reduce emissions so that global

temperature rises are kept to below 1.5°C. This will limit the harm. But, for Africa and other vulnerable regions, this harm is already severe. Achieving the promised target of providing $100 billion of climate finance a year is now globally critical if we are to forestall the systemic risks of leaving societies in crisis. This can be done by ensuring these resources focus on increasing resilience to the existing and inevitable future impacts of the climate crisis, as well as on supporting vulnerable nations to reduce their greenhouse gas emissions: a parity of esteem between adaptation and mitigation. These resources should come through grants not loans, and be urgently scaled up before the current review period of 2025. They must put health system resilience at the forefront, as the compounding crises caused by the climate crisis often manifest in acute health problems. Financing adaptation will be more cost-effective than relying on disaster relief.

Some progress has been made on adaptation in Africa and around the world, including early warning systems and infrastructure to defend against extremes. But frontline nations are not compensated for impacts from a crisis they did not cause. This is not only unfair, but also drives the spiral of global destabilisation, as nations pour money into responding to disasters, but can no longer afford to pay for greater resilience or to reduce the root problem through emissions reductions. A financing facility for loss and damage must now be introduced, providing additional resources beyond those given for mitigation and adaptation. This must go beyond the failures of COP26 where the suggestion of such a facility was downgraded to "a dialogue" (18).

The climate crisis is a product of global inaction, and comes at great cost not only to disproportionately impacted African countries, but to the whole world. Africa is united with other frontline regions in urging wealthy nations to finally step up, if for no other reason than that the crises in Africa will sooner rather than later spread and engulf all corners of the globe, by which time it may be too late to effectively respond. If so far they have failed to be persuaded by moral arguments, then hopefully their self-interest will now prevail.

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This Comment is being published simultaneously in multiple journals. For the full list of journals see: https://www.bmj.com/content/full-list-authors-and-signatories-climate-emergency-editorial-october-2022

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Review Article **Open Access**

A systematic review of clinical characteristics, co-morbidities and outcomes of COVID-19 in children and adolescents

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Abstract:

Background: COVID-19 is a major global health challenge that has affected all age groups and gender, with over 5 million deaths reported worldwide to date. The objective of this study is to assess available information on COVID-19 in children and adolescents with respect to clinical characteristics, co-morbidities, and outcomes, and identify gaps in the literatures for appropriate actions.
Methodology: Electronic databases including Web of Science, PubMed, Scopus, and Google Scholar were searched for observational studies such as case series, cross-sectional and cohort studies published from December 2019 to September 2021, using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guide. Data extracted included (i) patient demography (age and gender), (ii) clinical characteristics including vaccination status and presence of co-morbidities, (iii) clinical management including the use of sequential organ failure assessment (SOFA) scores, oxygen requirement, use of mechanical ventilation, and (iv) disease outcomes including length of hospital and intensive care unit (ICU) admission, recovery, complications with sequelae, or death. Data were analyzed using descriptive statistics.
Results: A total of 11 eligible studies were included with a total of 266 children and adolescents; 137 (51.5%) females and 129 (48.5%) males. The mean age of the children was 9.8 years (range of 0 - 19 years), and children ≥ 6 years were more affected (40.7%) than age groups 1 - 5 years (31.9%) and < 1 year (27.4%). The major co-morbidities were respiratory diseases including pre-existing asthma (3.4%), neurologic conditions (3.4%) and cardiac pathology (2.3%). Majority (74.8%, 199/266) of the patients were discharged without sequelae, 0.8% (2/266) were discharged with sequalae from one study, and mortality of 1.9% (5/266) was reported, also from one study. SOFA scores of patients at admission were not stated in any of the study, while only one study reported patient vaccination status.
Conclusion: It is recommended that safe vaccines for children < 1 year of age should be developed in addition to other preventive measures currently in place. SOFA scores should be used to assess risk of COVID-19 severity and monitor prognosis of the disease, and vaccination status of children should be documented as this may impact the management and prognosis of the disease.

Keywords: COVID-19; children; co-morbidity; hospital admission; ICU admission; disease outcome

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Une revue systématique des caractéristiques cliniques, des comorbidités et des résultats du COVID-19 chez les enfants et les adolescents

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Résumé:

Contexte: Le COVID-19 est un défi sanitaire mondial majeur qui a touché tous les groupes d'âge et tous les sexes, avec plus de 5 millions de décès signalés dans le monde à ce jour. L'objectif de cette étude est d'évaluer les informations disponibles sur le COVID-19 chez les enfants et les adolescents en ce qui concerne les caractéristiques cliniques, les comorbidités et les résultats, et d'identifier les lacunes dans la littérature pour des actions appropriées.
Méthodologie: Des bases de données électroniques, notamment Web of Science, PubMed, Scopus et Google Scholar, ont été recherchées pour des études d'observation telles que des séries de cas, des études transversales et de cohorte publiées de décembre 2019 à septembre 2021, en utilisant les éléments de rapport préférés pour les revues systématiques et les méta -Guide des analyses (PRISMA). Les données extraites comprenaient (i) la démographie des patients (âge et sexe), (ii) les caractéristiques cliniques, y compris le statut vaccinal et la présence de comorbidités, (iii) la prise en charge clinique, y compris l'utilisation des scores d'évaluation séquentielle des défaillances d'organes (SOFA), les besoins en oxygène, l'utilisation de la ventilation mécanique et (iv) les résultats de la maladie, y compris la durée de l'admission à l'hôpital et en unité de soins intensifs (USI), la récupération, les complications avec séquelles ou le décès. Les données ont été analysées à l'aide de statistiques descriptives.
Résultats: Un total de 11 études éligibles ont été incluses avec un total de 266 enfants et adolescents ; 137 (51,5%) femmes et 129 (48,5%) hommes. L'âge moyen des enfants était de 9,8 ans (intervalle de 0 à 19 ans), et les enfants ≥ 6 ans étaient plus touchés (40,7%) que les tranches d'âge 1-5 ans (31,9%) et < 1 an (27,4%). Les principales comorbidités étaient les maladies respiratoires, y compris l'asthme préexistant (3,4%), les troubles neurologiques (3,4 %) et la pathologie cardiaque (2,3%). La majorité (74,8%, 199/266) des patients sont sortis sans séquelles, 0,8% (2/266) sont sortis avec des séquelles d'une étude et une mortalité de 1,9% (5/266) a été rapportée, également d'une étude. Les scores SOFA des patients à l'admission n'ont été indiqués dans aucune des études, tandis qu'une seule étude a rapporté le statut vaccinal des patients.
Conclusion: Il est recommandé que des vaccins sûrs pour les enfants de < 1 an soient développés en plus des autres mesures préventives actuellement en place. Les scores SOFA doivent être utilisés pour évaluer le risque de gravité du COVID-19 et surveiller le pronostic de la maladie, et le statut vaccinal des enfants doit être documenté car cela peut avoir un impact sur la gestion et le pronostic de la maladie.

Mots clés: COVID-19; enfants; comorbidité; admission à l'hôpital; admission aux soins intensifs; issue de la maladie

Introduction:

Coronavirus disease-2019 (COVID-19) is a disease caused by severe acute respiratory syndrome-coronavirus-2 (SARS-COV-2), a newly discovered respiratory virus, which has caused over 5 million deaths globally from respiratory tract, cardiac and renal complications, and from an overwhelming cytokine storm (1). It belongs to the kingdom Orthornavirae, phylum Pisuviricota, order Nidovirales, family Coronaviridae, subfamily Orthocoronavirinae and genus Betacoronavirus (1). The highest risk has been found in elderly people, especially the ones with other co-morbidities (2). However, recent data has suggested that children are also affected but present with much milder symptoms (3). Although children do not appear to be at high risk of severe disease, they can spread the virus to others. Therefore, preventive measures should be taken (4,5).

A study by Cui et al., (5) reported a 55-day old female infant in China who was admitted after she tested positive to COVID-19 because she was severely sick with decreased arterial oxygen and elevated lactic acid. A one-year-old boy with COVID-19 was also reported from Wuhan's children's hospital with clinical presentations of diarrhoea, vomiting and shortness of breath (6). Vertical transmission of COVID-19 is yet to be confirmed from available data. However, perinatal SARS-COV-2 infection has been shown to lead to fetal distress, thrombocytopaenia accompanied by abnormal liver function and even death (7). The aim of this systematic review is to assess available information in the literature on the clinical characteristics, co-morbidities, and outcomes of COVID-19 in children and adolescents, and identify gaps in clinical case management of the disease.

Materials and method:

Literature search strategy

A two-step strategy was employed in conducting this systematic review. First, electronic (online) databases including Web of Science, PubMed, Scopus, and Google Scholar were searched by three of the authors (RRI, NM and TOM) for observational studies such as case series and cohort design, published between December 2019 and September 2021. Secondary sources were subsequently searched, including references of articles previously identified during the initial search. The keywords used for the search were; 'COVID-19' OR 'SARS-CoV-2' OR 'coronavirus' AND 'childhood' OR 'paediatric' OR 'child', 'SARS-CoV-2 infections in children' OR 'childhood SARS-CoV-2 infections', 'COVID-19 OR 'SARS-CoV-2' AND 'children'.

Identification of eligible publications

The PRISMA guide was used for the identification of eligible publications (Fig. 1). The inclusion criteria for selecting publications for the systematic review were; (i) articles on observational study design such as case series, cross-sectional and cohort studies; and (ii) articles with a study population of children <19 years of age containing information on patient demography such as age and gender, clinical characteristics including vaccination status and presence of co-morbidities, clinical management including the use of SOFA score, oxygen and/or mechanical ventilation requirement, and disease outcome including length of hospital and ICU admission, recovery, complications, or death. Articles that did not contain data on these characteristics, and single case reports, articles containing secondary data such as consensus documents, clinical trials, clinical guidelines, letters, editorials, reviews, systematic reviews and/or meta-analysis were excluded.

A total 1136 articles were retrieved from all the databases searched and after removing duplicate publications, a total of 194 articles were screened. Screening of abstracts and titles of 92 of these articles resulted in selection of 47 eligible articles for which full text assessments were subsequently conducted. Quality scores were awarded to each article using the Joanna Briggs Institute (JBI) critical appraisal list for prevalence studies (8). Article with scores of 7-9 was considered high quality, 4-6 moderate quality, and < 3 low quality. Only moderate and high-quality articles were included in the systematic review, and this yielded a total of 11 articles (Table 1). Resolution of any disagreement on eligibility of any article was achieved through discussions and consensus by third author (BA).

Data extraction and analysis

Information extracted from the 11 eligible articles includes; corresponding authors surname, publication year, number of children in the study, age at diagnosis of COVID-19, SOFA score, vaccination status, co-morbidities, length of hospital and ICU admission, requirement for oxygen and/or artificial ventilation, and treatment outcomes (Table 1). The data were entered into Excel spreadsheet and analysed using descriptive statistics.

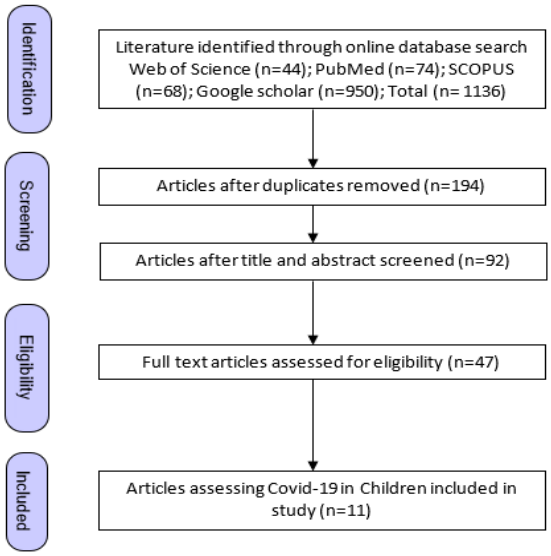

Fig. 1: Process for selection of publications (PRISMA guide) for the systematic review

Table 1: Characteristics of included studies on COVID-19 in children used for systematic review

S/N	Authors	Article type	Country of origin	Publication setting	Year of publication	Sample size	No of males	No of females	Age range (median age of onset)	Reference
1	Abdel-Mannan et al	Case series	London, UK	Hospital	2020	4	2	2	8 – 15 years (12 years)	(9)
2	Posfay-Barbe et al	Case series	Switzerland	Outpatient clinic and hospital	2020	40	18	22	0 - 16 years (11.1 years)	(10)
3	Ma et al	Single center retrospective study	Wuhan, China	Children's Hospital	2020	50	28	22	0 - 16 years (2.5 years)	(11)
4	Tang et al	Retrospective study	Shenzhen, China	Hospital	2020	26	9	17	1 – 13 years (7 years)	(13)
5	Oualha et al	Single center retrospective study	Paris, France	Hospital	2020	27	10	17	1 month - 18 years (6 years)	(14)
6	Qui et al	Observational retrospective cohort study	Zhejiang, China	Hospitals	2020	36	23	13	1 – 16 years (8.3±3.5 years)	(15)
7	Cai et al	Case series	Anhui & Quigdon, China	Hospitals	2020	10	4	6	3 months – 10 years (5.6 years)	(16)
8	Xia et al	Case series	Wuhan, China	Hospitals	2020	20	13	7	1day - 14years 7months (2 years 1.5 months)	(17)
9	Wei et al	Retrospective study	China	Hospitals	2020	9	2	7	1 – 11 months (6 months)	(18)
10	Xu et al	Observational	China	Hospital	2020	10	6	4	2 months - 15 years (7.9 years)	(19)
11	Zhang et al	Retrospective observational case series	China	Hospitals	2020	34	14	20	1 month - 12 years (2.8 years)	(11)
Total						**266**	**129**	**137**		

Results:

Characteristics of included studies on COVID-19 in children

A total of 11 studies on COVID-19 in children (9-19) met our eligibility criteria and were used for the systematic review, with 266 children; 137 (51.5%) females and 129 (48.5%) males. The mean age of the children was 9.8 years (range of 0-19 years) (Table 1).

Age of children with COVID-19 at diagnosis.

The specific age of children with COVID-19 at diagnosis were available for 113 patients (some studies had a mixture of specific ages, age ranges, and mean age). Of these 113 children, age group ≥ 6 years were more affected with COVID-19 (40.7%) than age group 1-5 years (31.9%) and children < 1 year of age (27.4 %). The mean age reported across studies where specific ages were not reported or partially reported ranged from 6-11.1 years (10, 13–15). Table 2 shows the age group distribution of the children with COVID-19 from the selected studies.

Co-morbidity in children with COVID-19

Table 3 shows the specific types of co-morbidities reported in the studies used for the review. While 60.2% (160/266) of children with COVID-19 in the review had no co-morbidity (reported in two studies), pre-existing asthma/respiratory diseases (3.4%), neurological diseases (3.4%) and cardiac pathology (2.3%) were the most frequent co-morbidities reported. The study by Oualha et al., (14) reported positive correlation between existence of co-morbidities and increased mortality.

Table 2: Age of children and adolescents with COVID-19 at diagnosis

Age at diagnosis	No of studies	No of patients	Percentage (reference)
< 1 year	5	31	27.4 (11,16–19)
1-5 years	7	36	31.9 (11,13–17,19)
≥6	8	46	40.7 (9,11,13–17,19)
Total		**113**	

Table 3: Prevalence of co-morbidity in children with COVID-19

Co-morbidity	No of studies	No of patients	Percentage (reference)
Pre-existing asthma/other respiratory condition	1	9	3.4 (10,11,14)
Cardiac pathology	2	6	2.3 (11,17)
Neurological	3	9	3.4 (11,14,17)
Obesity	2	3	1.1 (9,10)
Sickle cell disease	1	4	1.5 (14)
Diabetes mellitus	1	3	1.1 (10)
Hypertension	1	1	0.4 (10)
Malignancy	1	1	0.4 (11)
Premature birth	1	2	0.8 (10)
No co-morbidity	2	160	60.2 (9–11,13,15,17–19)
Co-morbidity not mentioned	1	68	25.6 (12,14,16)
Total		**266**	

SOFA score and vaccination status of children with COVID-19 at diagnosis

None of the studies recorded SOFA score of the patients at first diagnosis and none except one reported the vaccination status of the children.

Maximum length of hospital admission for children with COVID-19

Most studies reported the maximum duration of admission of the children with variable information about the specific days each patient stayed on admission. The range of hospital stay among those admitted and discharged in hospital was < 1 day - 6 weeks. Four studies reported that children were admitted in hospital without stating the actual number of days spent. In these four studies, some sub-sets of the patients were still on admission at the time of preparing this paper for publication. In one study, it was unclear if any of the patients were admitted into hospital (Table 4). Abdel-Manna et al., (9) reported admission days of 2-3 weeks for patients with two other patients still on admission at three and six weeks respectively at the time of this preparation.

Shorter admission durations were reported by Klara et al., (10) with median of three days and all patients discharged by seven days. Qui et al., (15) reported mean admission duration of two weeks for children with COVID-19 and all of them recovered completely by six weeks. Five studies (11,12,14,18,19) did not specify lengths of admission (Table 4).

Length of ICU admission of children with COVID-19

All except three studies (12,13,17) reported that a proportion of the children were admitted in hospital but most children (52.3%) were not managed in the ICU (Table 5). One child was in the ICU for less than two weeks and another for more than two weeks (9). The duration of ICU admission was not specified in 29 patients. (Table 5).

Requirement for artificial ventilation

Mechanical ventilation was not used for 157 (59.0%) of patients as reported in seven studies (10,11,14–16,18,19). A total of 13 children received mechanical ventilation for duration between 1-18 days in total (9,14). There was no report for 36.1% of children in the studies selected (Table 6).

Treatment outcome of children with COVID-19

A total of 74.8% of the children with COVID-19 recovered fully (9-13,15,16,18,19) and were discharged from hospital (Table 7). Mortality was reported in 1.9% of the children (14) and two children were reported to have sequelae at the time of this manuscript preparation (9).

Table 4: Length of hospital admission of children with COVID-19

Length of hospital admission (weeks)	No of studies	No of patients	Percentage (reference)
< 1	1	7	4.3 (10)
1 - < 2	1	26	16.0 (13)
< 3	1	18	11.1 (17)
2-3 - < 4	1	10	6.2 (16)
< 5	1	27	(13)
< 6	1	36	22.2 (15)
Not available	1	4	2.5 (9)
Not specified	5	61	37.7 (11,12,17,18,19)
Total		**162**	

Table 5: Length of ICU admission of children with COVID-19

Length of ICU admission (weeks)	No of studies	No of patients	Percentage (reference)
< 2	1	1	0.4 (9)
> 2	1	1	0.4 (9)
No admission	6	139	52.3 (10,11,15,16,18,19)
Not specified	2	29	10.9 (9,14)
Not available	3	96	36.1 (12,13,17)
Total		**266**	

Table 6: Requirement for and length of artificial ventilation

Requirement/length of artificial ventilation (days)	No of studies	No of patients	Percentage (reference)
1 - 7	1	4	1.5 (9)
1 - 18	1	9	3.4 (14)
No admission	6	157	59.0 (10,11,14–16,18,19)
Not reported	3	96	36.1 (12,13,17)
Total		**266**	

Table 7: Treatment outcomes of children with COVID-19

Treatment outcome	No of studies	No of patients	Percentage (reference)
Full recovery and discharged	9	199	74.8 (9–13,15,16,18,19)
Recovered with sequelae (morbidity)	1	2	0.8 (9)
Death (mortality)	1	5	1.9 (14)
Discharged with unreported sequelae	3	52	19.5 (12,14,17)
Unreported	2	8	3.0 (17,19)
Total		**266**	

Discussion:

In this systematic review, we aimed to assess current literature on COVID-19 in children. We identified 11 papers for final inclusion in the review and obtained information on age of children at diagnosis, presence of co-morbidities, duration of hospital and ICU admission, requirement for oxygen and/or mechanical ventilation, SOFA scoring, and vaccination status. A total of 266 children with COVID-19 were reviewed and the mean age of the children was 9.8 years (age range 0-19 years) with

129 males and 137 females (M:F ratio of 1:1.1). SARS-COV-2 has been reported to infect children of all ages and gender, including newborns, infants, and young children (18). When mainland China confirmed 11,791 COVID-19 cases at the onset of COVID-19, 74 (0.6%) of them were between the ages of 1.5 months and 18 years (20). In a study of 474 paediatric patients on admission in a facility at the peak of the pandemic in 2020, about 5.2% of them were found to be positive with a mean age of 5.83 years, and significantly higher than those that tested negative (21).

In a retrospective analysis of 366 hospitalized children aged ≤16 years with respiratory infections in three branches of Tongji hospital all located in central Wuhan, 6 (1.6%) patients had SARS-COV-2 infection, with median age of 3 years (age range 1-7 years) (20). The Centers for Disease Control and Prevention reported that children constituted 2% of 44,672 cases in China as at early 2020, and 4.5% of 1,663,519 cases in the US by the middle of year 2020 (22). In South Korea, about 6.4% of confirmed cases were reported to be children with no record of death (23). In a meta-analysis of 14 studies by Yudan et al., (24), the age range of children with COVID-19 was 0-17 years and mean age was 5.5± 2.2 years (95% CI 4.2–6.8).

Currently, the median age of patients with COVID-19 in the US is 11 years (age range 0-17 years) and in China, it is 7 years (age range 1-18 years)(25,26). Children under 10 years of age have been shown by the Chinese CDC to account for 1% of confirmed COVID-19 cases while the United States CDC reported that children < 18 years of age accounted for 4.5% of 2,336,615 confirmed cases (27–29). Our current systematic review showed that COVID-19 was less frequent among children < 1 year of age (27.4%) than among children ≥ 6 years of age (40.7%), who also had more severe disease. Therefore, it is advisable to develop safe vaccines for this age group in addition to other preventive measures.

The SOFA score is a validated prognostic scoring protocol with scores ranging from 0-24, where scores of 0-4 could be assigned for each of the 6 organ systems of the body (neurologic, pulmonary, cardiovascular, renal, hepatic, haematologic) depending on the evidence of organ failure. Higher scores have been found to correlate with higher likelihood of in-hospital mortality (30,31). COVID-19 patients with higher SOFA scores are at increased mortality risk (32,33). In our review, none of the study reported on the use of SOFA scores to assess the risks of COVID-19 at admission or monitor the progress of the disease on admission. It would be advantageous for paediatric physicians to assess COVID-19 progression and monitor prognosis in hospitalized patients using SOFA scoring system and clearly document these in the case files of hospitalized patients, from which the importance of the scoring system can be assessed in future studies.

Mass vaccination has been reported from recent studies to decrease population transmission of SARS-COV-2 (34). Children appear to be less susceptible to SARS-CoV-2 infection and transmission, compared to adults (35,36) however, they generally have higher rates of social contact than adults (37). Therefore, vaccinating children will help to protect the more vulnerable adults from the virus (34,38–41). The proportion of children COVID-vaccinated has been shown to vary from country to country due to differences in vaccine availability, vaccine hesitancy and efficiency of vaccination programmes (39). The vaccination status of the children with COVID-19 in our review was not reported in any the studies except one. The knowledge of childhood vaccination status might be necessary to assess whether fully vaccinated children mount a stronger immune response to the SARS-CoV-2 than those not vaccinated.

Children with COVID-19 and underlying diseases are at increased risk of developing a severe or critical illness. Respiratory neurological and cardiac co-morbidities were the most frequent underlying diseases in this systematic review. Co-morbidities commonly reported to be associated with poorer COVID-19 outcomes are developmental delays, immune suppression, obesity, diabetes, seizure disorders, congenital heart diseases, chronic pulmonary disease (including asthma), chronic kidney disease, chronic liver disease, malnutrition, and hematologic conditions such as sickle cell disease (42,43). Reassuringly, the mortality rate of children with COVID-19 remains low accounting for <1% of all deaths due to COVID-19 in the United States (44,45).

The median length of admission in children hospitalized with COVID-19 has been reported to be 7.5 days (range 5-13 days) (46). The admission length for the children with record of hospitalization in our systematic review was < 1 day - 6 weeks. Majority of children with COVID-19 reportedly have mild clinical disease, with faster recovery and therefore most did not require hospital admissions (47). In the United States, fewer children were admitted to hospital and ICU with 5.7-20.0% and 0.58%-2.0% respectively, compared to adults aged 18-64 years with 10.0-33.0% and 1.4-4.5% respectively.

With regards to requirement for artificial ventilation, a study of 220 children with COVID-19 on admission in Turkey by Yayla et al., (48) reported that only three (1.4%) children required respiratory support, and all of them had underlying co-morbidities including fulminant myocarditis, Stevens-Johnson syndrome and osteopetrosis. On the other

hand, Prata-Barbosa et al., (49) studied 79 children admitted into the ICU of a hospital in Brazil with COVID-19 and observed that 51 of them (65%) required some form of ventilatory support. This is far higher than 4.9% (13/266) of children we found requiring mechanical ventilation in our review. Hypoxic respiratory failure was the most common reason for requiring ventilatory support (50). Concerning COVID-19 mortality in children, the overall mortality has been reported to be about 0.1% compared to 2.27% in adults (37), but the mortality reported in one study (14) from our review was 1.9%.

Conclusion:

Data from our systematic review and the literature indicate that prognosis of COVID-19 in children is good, as many infected children tend to have mild disease and recovered without sequelae, and mortality is low. However, we recommend that SOFA scores should be used to assess risk of COVID-19 severity and monitor prognosis in children requiring hospitalization to reduce adverse outcomes, and vaccination status of children should be documented, as this may impact the management and prognosis of the disease.

As a result of the emergence of SARS-CoV-2 variants and children being possible source of virus transmission, it is recommended that children should be fully vaccinated and comply with other preventive measures such as wearing of face masks, observing social distancing, and avoiding crowded areas. Development of effective vaccines for children < 1 year of age is desirable for additional protection since these infants are susceptible to severe COVID-19.

Contributions of authors:

AB designed the outline of the systematic review, served as the consensus reviewer, and edited the manuscript. MTO screened and reviewed journal for articles, analyzed the data, prepared the tables and wrote portions of the manuscript. IRR screened and reviewed the articles, analyzed the results and prepared the tables. MIN wrote the literature review. ASA and BM reviewed the articles and wrote portions of the manuscript. NM screened the articles, extracted data and edited the manuscript.

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Review Article **Open Access**

A review of the role of infections in the aetiology of haemolysis in patients with sickle cell diseases: pathogenesis, management, and prevention

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Abstract:

Background: Sickle cell disease (SCD) is associated with chronic haemolysis, immuno-suppression and susceptibility to infections, which may trigger infection-associated haemolysis (IAH). SCD patients are vulnerable to anaemic effect of IAH due to vicious interaction between pre-existing 'inherited' chronic haemolysis and 'acquired' IAH. IAH in SCD manifests as febrile haemolytic crisis with clinical and laboratory features of severe anaemia or pancytopenia. Clinico-pathological perspectives of IAH in SCD are fragmented. This review presents a comprehensive but concise overview of pathogenesis, management and prevention of IAH in SCD.
Methodology and results: Online literature search using search terms such as 'sickle cell disease, viral, bacterial, parasitic, fungal, infections, hyperhaemolytic crisis, haemophagocytic syndrome, severe anaemia, pancytopenia' in various combinations was done on PubMed/Medline, Google, Google-Scholar and Bing. Overall, 112 relevant publications were retrieved, which included 109 peer reviewed journal articles, 2 World Health Organization (WHO) technical reports, and 1 edited text book. A range of bacterial (*Bartonella* spp, *Mycoplasma* spp., *Mycobacterium avium* complex), viral (Dengue, SARS-CoV-2, Parvovirus-B19, Cytomegalovirus, Epstein-Barr virus), parasitic (*Plasmodium* spp., *Babesia* spp.), and fungal (*Histoplasma* spp.) infections were associated with IAH in SCD. There are two broad types of IAH in patients with SCD; infection associated extra-medullary haemolysis (IAEMH) and infection associated intra-medullary haemolysis (IAIMH). While IAEMH is associated with severe anaemia due to intravascular haemolysis caused by red cell invasion, oxidative injury, auto-antibodies, and/or pathogen-haem interaction, IAIMH is associated with haemophagocytic tri-lineage destruction of haematopoietic precursors in the bone marrow.
Conclusion: Various microbial pathogens have been associated with IAH in SCD. SCD patients with fever, severe anaemia or pancytopenia should be investigated for early diagnosis and prompt treatment of IAH, which is a life-threatening haematological emergency for which transfusion therapy alone may not suffice. Prompt and sustainable termination of IAH may require therapeutic combination of transfusion, anti-microbial chemotherapy, and immune modulation therapy. SCD patients should also receive counselling on hygiene, barrier protection against vectors, routine chemoprophylaxis for locally endemic diseases, and immunization for vaccine-preventable infections as a long-term preventive strategy against IAH.

Keywords: sickle cell disease; infection; hyperhaemolytic crisis; haemophagocytic syndrome

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Une revue du rôle des infections dans l'étiologie de l'hémolyse chez les patients drépanocytaires: pathogenèse, prise en charge et prévention

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Résumé:

Contexte: La drépanocytose (SCD) est associée à une hémolyse chronique, à une immunosuppression et à une susceptibilité aux infections, ce qui peut déclencher une hémolyse associée à une infection (HIA). Les patients atteints de SCD sont vulnérables à l'effet anémique de l'HIA en raison de l'interaction vicieuse entre l'hémolyse chronique "héréditaire" préexistante et l'HIA "acquise". L'HIA dans la SCD se manifeste par une crise hémolytique fébrile avec des caractéristiques cliniques et de laboratoire d'anémie sévère ou de pancytopénie. Les perspectives clinico-pathologiques de l'HIA dans la SCD sont fragmentées. Cette revue présente un aperçu complet mais concis de la pathogenèse, de la gestion et de la prévention de l'HIA dans la drépanocytose.
Méthodologie et résultats: Une recherche documentaire en ligne à l'aide de termes de recherche tels que "drépanocytose, virale, bactérienne, parasitaire, fongique, infections, crise hyperhémolytique, syndrome hémophagocytaire, anémie sévère, pancytopénie" dans diverses combinaisons a été effectuée sur PubMed/Medline, Google, Google-Scholar et Bing. Au total, 112 publications pertinentes ont été récupérées, dont 109 articles de revues à comité de lecture, 2 rapports techniques de l'Organisation mondiale de la santé (OMS) et 1 manuel édité. Une gamme bactérienne (*Bartonella* spp, *Mycoplasma* spp., *Mycobacterium avium* complex), virale (Dengue, SARS-CoV-2, Parvovirus-B19, Cytomegalovirus, Epstein-Barr virus), parasitaire (*Plasmodium* spp., *Babesia* spp.), et les infections fongiques (*Histoplasma* spp) étaient associées à l'IAH dans la SCD. Il existe deux grands types d'HIA chez les patients atteints de SCD; hémolyse extra-médullaire associée à une infection (IAEMH) et hémolyse intra-médullaire associée à une infection (IAIMH). Alors que l'IAEMH est associée à une anémie sévère due à une hémolyse intravasculaire causée par l'invasion des globules rouges, une lésion oxydative, des auto-anticorps et/ou une interaction pathogène-hème, l'IAEMH est associée à la destruction tri-lignée hémophagocytaire des précurseurs hématopoïétiques dans la moelle osseuse.
Conclusion: Divers agents pathogènes microbiens ont été associés à l'IAH dans la SCD. Les patients atteints de SCD avec de la fièvre, une anémie sévère ou une pancytopénie doivent être examinés pour un diagnostic précoce et un traitement rapide de l'HIA, qui est une urgence hématologique potentiellement mortelle pour laquelle la thérapie transfusionnelle seule peut ne pas suffire. L'arrêt rapide et durable de l'HIA peut nécessiter une combinaison thérapeutique de transfusion, de chimiothérapie antimicrobienne et de thérapie de modulation immunitaire. Les patients atteints de drépanocytose devraient également recevoir des conseils sur l'hygiène, la barrière de protection contre les vecteurs, la chimioprophylaxie de routine pour les maladies endémiques locales et la vaccination contre les infections évitables par la vaccination en tant que stratégie préventive à long terme contre l'HIA.

Mots clés: drépanocytose; infection; crise hyperhémolytique; syndrome hémophagocytaire

Introduction:

Haemoglobin-S (HbS) is a variant of the normal HbA. HbS arose as a result of GAG> GTG base transition at codon-6 of the β-globin gene on chromosome-11, which corresponds to a substitution of glutamic acid (a polar amino acid) by valine (a neutral amino acid) in the sixth position of the β-globin chain (βGlu6Val) (1,2). As a result of this substitution, HbS has less anionic potential, slower electrophoretic mobility, and reduced deoxygenated solubility that leads to polymerization and red cell sickling (1,2).

The prevalence of sickle β-gene in tropical African countries is as high as 25% - 30% (3), because sickle cell trait (SCT) protects against severe malaria (3), and confers survival advantage through natural selection (4), balanced polymorphism (5), and immunological and biochemical protective mechanisms against malaria (6). There are at least five different sickle β-gene mutation haplotypes that vary in HbF levels and disease severity. The Arab-Asian and Senegal haplotypes are associated with relatively higher HbF levels and mild sickle cell disease (SCD), while the Benin, Bantu, and Cameroon haplotypes are associated with relatively lower HbF levels and severe SCD (7).

The red cells of individuals with SCT have the HbAS phenotype, thus containing both HbS (20-40%) and HbA (60-80%) (8). The relative preponderance of HbA in the red cells of SCT prevents sickling and undue haemolysis (8). Consequently, red cells of SCT have normal life span, and SCT carriers have normal life expectancy (9). HbS gene is thus genetically recessive, and SCT carriers are essentially asymptomatic except for the occasional occurrence of haematuria due to renal papillary necrosis (8), splenic infarction at high altitude (10) or bone pain upon exposure to certain haematopoietic growth factors (11).

Sickle cell disease arises from homozygous inheritance of HbS gene or double heterozygosity of HbS gene with another haemoglobinopathy gene such as HbSC, HbSD, HbSE, HbSO and HbSβthal (1). The clinical course of SCD is characterized by painless and stable periods of relative well-being referred to as the 'steady-state', which is intermittently interrupted by painful and unstable periods referred to

as 'crisis' (12). Painful vaso-occlusive crisis (VOC) due to bone necrosis is the commonest type of crisis in SCD, and it is clinically pathognomonic of SCD (12). Clinical transition from steady state to VOC results from excessive deoxygenation of HbS and red cell sickling, which is usually triggered by several factors that vary from physiological factors (e. g., menstruation) to pathological factors (e. g., infection) on the one hand, and from psychological factors (e. g., emotional stress) to physical factors (e. g., extreme weather conditions) on the other hand (12).

Red cell sickling is a pathognomonic feature of SCD. Red cells of patients with SCD go through repeated cycles of deoxygenation (in the tissues) and re-oxygenation (in the lungs) (13). This sequence of events creates a dynamic scenario of red cell sickling and unsickling until the red cell membrane sustains a significant degree of damage, which eventually leads to the formation of irreversibly sickled cells that are invariably prematurely haemolysed (13). Consequently, the red cell life span in SCD is shortened to less than 20 days (14), which cannot be completely compensated even at the maximum rate of erythroid hyperplasia of the most active marrow (15). Chronic haemolysis is therefore the fundamental aetiopathogenetic cause of anaemia in SCD patients in steady state (14).

However, apart from anaemia, haemolysis is also associated with other important and serious life-threatening consequences. This is because haemolysis has dual adverse effects on patients with SCD. First, haemolysis causes anaemia thus predisposing to transfusion with concomitant risks of iron overload, transfusion transmissible infections (TTIs), immune sensitization and reactions (16). Second, haemolysis increases the availability of cell-free Hb and haem, which support bacterial growth and sepsis (17), quenches vaso-modulatory effect of nitric oxide, and causes vasculopathy with multi-organ dysfunctions such as stroke, nephropathy, and pulmonary hypertension (18). Every patient with SCD maintains a certain degree of steady state haemolysis, which can be aggravated by infections.

Infections can cause haemolysis even in persons without SCD through several mechanisms, which include red cell invasion, oxidative damage to red cell membrane, production of haemolytic toxins (haemolysins), and/or production of red cell auto-antibodies, any or all of which can cause infection associated haemolysis (IAH) (19). However, SCD patients are particularly vulnerable to the anaemic effect of IAH due to the vicious interaction between the 'acquired' IAH and pre-existing 'inherited' SCD-associated haemolysis. There is therefore the need to understand the clinico-pathophysiologic perspectives of IAH in SCD patients because of their susceptibility to infections.

Patients with SCD are susceptible to infections for two important reasons. First, SCD patients are usually managed by recurrent or chronic blood transfusion, which predisposes to acquisition of various types of TTIs (16). Second, SCD is pathophysiologically associated with immunosuppression, which predisposes to the acquisition of any locally endemic infections (20). Therefore, SCD patients are at increased risk of acquiring infections, which could potentially lead to development of IAH. Several studies had focused on the cause-and-effect relationship between infections and VOC in SCD (21), but relatively less attention has been given to the relationship between infections and haemolysis in SCD. The risk of IAH would be especially high among SCD patients living in their native tropical African countries, which carry the heaviest dual burdens of infectious diseases (22) and SCD (23).

To the best of our knowledge from literature search, the clinico-pathological perspectives of IAH in SCD are fragmented, and have not been holistically or comprehensively appraised in the literature. Nonetheless, IAH in SCD is of triple clinical significance. First, IAH tends to be persistent or recurrent as long as the infection remains active and untreated, thereby increasing the frequency of hospital visits, which would lead to high rates of school absenteeism and poor intellectual development in children with SCD (24). Second, the persistent and/or recurrent nature of IAH invariably increases patient's transfusion requirement along with its wide range of undesirable adverse effect, which includes acquisition of TTIs that may potentially worsen IAH (16). Third, IAH cannot be effectively managed by transfusion alone. Optimal management of IAH requires detection of causative pathogens, followed by synchronized application of transfusion therapy, anti-infection chemotherapy and immune modulation therapy.

These three highlighted reasons underscore the need for SCD caregivers in general and in the tropics in particular, to have thorough understanding of the clinico-pathological perspectives of IAH in patients with SCD in order to ensure that IAH is quickly diagnosed and treated, and prevented in the future. Hence, the aim of this review is to present an updated and comprehensive but concise overview of the pathogenesis, management, and

prevention of IAH in patients with SCD as accrued from the literature.

Methodology and results:

Literature search was conducted on databases using the search terms; 'sickle cell disease, viral, bacterial, parasitic, fungal, infections, hyperhaemolytic crisis, haemophagocytic syndrome, severe anaemia, and pancytopenia' in various combinations on PubMed, Medline, Google, Google-Scholar, and Bing.

Overall, 112 relevant publications were retrieved, which included 109 peer reviewed journal articles, 2 World Health Organization (WHO) technical reports, and 1 edited text book. A range of bacterial, viral, parasitic, and fungal infections were associated with IAH in SCD. The pathogenesis, management and prevention of IAH in SCD vis-à-vis the haematological features and clinical manifestations of individual causative infections are outlined in Table 1.

Table 1: Pathogenesis, management, and prevention of infection-associated haemolysis in patients with SCD

Categories	Pathogens	Possible mechanisms for haemolysis	Haematologic manifestations	Management strategy (in addition to red cell transfusion)	Preventive and avoidance strategy
Protozoa	*Plasmodium* spp.	Red cell invasion with or without autoimmune haemolysis	Anaemia	Anti-malarial chemotherapy; Immune modulation if autoimmune haemolysis present	Protection against vectors; blood donor screening; vaccination
	Babesia spp.	Red cell invasion with or without autoimmune haemolysis	Anaemia	Anti-babesia chemotherapy; Immune modulation if autoimmune haemolysis present	Protection against vectors; blood donor screening
Bacteria	*Bartonella* spp.	Red cell invasion with or without autoimmune haemolysis	Anaemia	Anti-bartonella chemotherapy; Immune modulation if autoimmune haemolysis present	Protection against vectors; blood donor screening
	Mycoplasma spp.	Autoimmune haemolysis	Anaemia	Anti-mycoplasma chemotherapy; Immune modulation for autoimmune haemolysis	Personal and environmental sanitation
	Mycobacterium avium Complex (MAC)	Haemophagocytic Lympho Histiocytosis (HLH)	Pancytopenia	Anti-MAC chemotherapy; Immune modulation therapy for HLH	Personal and environmental sanitation
Viruses	Dengue virus	Inflammation oxidative red cell injury	Anaemia	No effective anti-viral therapy; symptomatic support; platelet transfusion if severe thrombocytopenia present	Protection against vectors; vaccination
	SARS-CoV-2	Virus-haem interaction; reduced red cell deformability with or without immune haemolysis	Anaemia	Anti-viral chemotherapy; Immune modulation if autoimmune haemolysis present	Hand sanitizers; Face masks; vaccination
	Parvovirus-B19	HLH	Pancytopenia	Anti-viral immunoglobulin therapy; Immune modulation therapy for HLH	Personal and environmental sanitation
	Cytomegalovirus	HLH	Pancytopenia	Anti-viral chemotherapy; immune modulation therapy for HLH	Personal and environmental sanitation
	Epstein Barr Virus	HLH	Pancytopenia	No effective anti-viral therapy; symptomatic support; Immune modulation therapy for HLH	Personal and environmental sanitation
Fungi	*Histoplasma* spp.	HLH	Pancytopenia	Anti-fungal chemotherapy; Immune modulation therapy for HLH	Personal and environmental sanitation

HLH = haemophagocytic lympho-histiocytosis; SARS-COV-2 = Severe acute respiratory syndrome-coronavirus-2

Discussion:

There are two broad types of IAH in patients with SCD; infection associated extra-medullary haemolysis (IAEMH), and infection associated intra-medullary haemolysis (IAIMH).

Infections associated extra-medullary haemolysis in SCD: the classical acute hyperhaemolytic crisis

SCD produces a chronic uncompensated hemolytic anaemia in the steady state. However, an acute-on-chronic hyperhaemolytic crisis can occur and cause precipitous drop in Hb concentration in patients with SCD. The classical hyperhaemolytic crisis is characterized by the occurrence of intravascular haemolysis at a rapid rate that significantly exceeds the usually tolerated slow rate of haemolysis seen in steady state. Hyperhaemolytic crisis is therefore associated with life threatening exacerbation of anaemia, which can be triggered by any of the following outlined infections.

Malaria and hyperhaemolysis in SCD

Malaria is endemic in tropical countries (25), where SCD is most prevalent (26). Five mosquito transmissible *Plasmodium* species: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*, have been associated with human infections, with the first two being the most important species (25). SCD patients in tropical countries are at double risk of acquiring malaria through mosquito bites and blood transfusions because a significant proportion of tropical blood donors have asymptomatic malaria (27,28). Interestingly, a recent study demonstrated that in comparison to blood donors with SCT, donors with HbAA were associated with higher risk of asymptomatic malarial parasitaemia, which implied that HbAA blood carries higher risk of transfusion transmitted malaria (TTM) (29,30). Therefore, patients who are selectively transfused with HbAA blood, such as SCD patients, could be at greater risks of acquiring TTM, and such patients need closer post transfusion monitoring for early detection and treatment of TTM (29,30).

The malaria parasites are both erythrocytotropic and erythrocytopathic, hence the parasites invade and replicate within the patients red cells during the erythrocytic phase of their life cycles (31). Moreover, malaria is sometimes associated with development of red cell autoantibodies (32). Malaria is thus capable of causing red cell haemolysis as a result of a dual concert between direct red cell invasion and autoimmune mediated haemolysis (31,32). Consequently, malaria is strongly associated with anaemia even in non-SCD patients (33). It is therefore conceivable that malaria is an important aetiological factor in the pathogenesis of acute hyperhaemolytic crisis and severe anaemia in patients with SCD (34,35). It is nonetheless possible to minimize the risk of malaria associated hyperhaemolysis by providing prompt treatment for acute malaria and continuous lifelong anti-malarial chemoprophylaxis in the standard of care for managing SCD in malaria endemic countries (36).

Long term protection can also be achieved by barrier protection against mosquito vectors at home, and serological screening and deferral of malaria infected prospective blood donors at donation centres (37). However, malaria vaccine remains the ultimate strategy for sustainable and cost-effective control measure against malaria in the tropical countries. Unfortunately, the RTS,S/AS01 vaccine showed only modest efficacy in preventing symptomatic *P. falciparum* malaria (38). In essence, the RTS,S/AS01 vaccine alone would not be sufficient for global or regional malaria eradication, but it can nonetheless be considered as another addition to existing list of malaria control strategies, and should not be considered as an independent malaria prevention tool (38).

Accordingly, in 2021, the RTS,S/AS01 vaccine was endorsed by the WHO for use in children in conjunction with other malaria control strategies such as the use of insecticide treated nets and environmental vector control (39). Therefore, SCD patients living in *P. falciparum* endemic countries should be encouraged to receive the RTS,S/AS01 vaccine. The vaccine is a non-live recombinant protein-based vaccine (39), hence it can be given to all SCD patients including those with pre-existing HIV infections.

Babesia infection and hyperhaemolysis in SCD

Babesiosis is a zoonotic tick-borne malaria-like febrile illness caused species of the intra-erythrocytic protozoan parasite called *Babesia* (40). Babesiosis is particularly common in mid-western and north-eastern United States, but is also seen sporadically throughout the world in parts of Europe, Asia and Africa (40). The four identified *Babesia* species that cause infection in humans are *B. microti*, *B. divergens, B. duncani,* and *B. venatorum (*40*)*. However, the life cycle of all four species within humans remain essentially the same (40).

Babesia parasites are intracellular obligate parasites that target the red blood cells (40). Besides its natural route of transmission via the infected tick vector bites, the parasite is

also transmissible by transfusion via the red blood cells of infected donors (41). Immunocompromised persons, especially those with splenectomy or hyposplenism are at increased risk of babesiosis (42). Like malaria parasites, *Babesia* parasites are both erythrocytotropic and erythrocytopathic, hence the parasites invade and replicate within the patients red cells (40). In similarity with malaria, babesiosis is associated with the development of red cell autoantibodies (43). Babesiosis is thus capable of causing red cell haemolysis as a result of dual effects of direct red cell invasion and autoimmune mediated haemolysis (40,43). Consequently, babesiosis is an important cause of morbidity and haemolytic anaemia even in non -SCD patients (40).

Patients with SCD living in areas that are endemic for babesiosis are at high risk of infection due to the effects of auto-splenectomy, immune suppression and recurrent transfusion (41,44). In accordance with expectations, babesiosis had been shown to aggravate haemolysis, cause hyperhaemolytic crisis and severe anaemia in patients with SCD (45). Nonetheless, it is possible to minimize the risk of babesiosis associated hyperhaemolysis by providing prompt diagnosis and standard anti-babesia chemotherapy for patients with SCD (40). Long term prevention strategy is achievable through personal barrier protection against tick vectors coupled with environmental vector control programs (40), while molecular donor screening methods for *Babesia* is currently being evaluated for detection and deferral of infected donors within endemic areas (46). Unlike malaria, an effective vaccine has not yet been developed against babesiosis, but there are potential candidate vaccines in the preclinical stages of development that will hopefully be available for clinical use in the near future (47).

Bartonella infection and hyperhaemolysis in SCD

Three zoonotic species of *Bartonella* (*B. henselae, B. quintana*, and *B. bacilliformis*) are known to be responsible for the vast majority of human infections (48). While the infection caused by *B. henselae* has a worldwide distribution, *B. quintana* and *B. bacilliformis* cases are more geographically restricted infection; *B. quintana* in Europe and USA, and *B. bacilliformis* in Peru, Ecuador, and Colombia (48). *Bartonella* spp are intracellular fastidious Gram-negative bacteria that cause a wide range febrile illnesses in both immuno-competent and immuno-suppressed persons, with the latter being more severely affected (48). *Bartonella* spp are spread from animals to humans by fleas, lice, sand flies, or contact with flea infested animals, and have special tropism for endothelial cells and red cells (48).

The biological ability of *Bartonella* spp to invade human red cells is of double clinical significance. First, *Bartonella* spp can be transmitted from asymptomatic blood donors to blood recipients (49). Second, *Bartonella* spp can cause significant haemolysis in persons with symptomatic infections (50,51). Moreover, *Bartonella*-induced haemolysis is sometimes aggravated by development of red cell autoantibodies (52). Therefore, in similarity with malaria and babesiosis, *Bartonella* infection can cause red cell haemolysis as a result of the combined effects of direct red cell invasion and autoimmune mediated haemolysis (50,51,52). Consequently, bartonellosis is also an important cause of morbidity and haemolytic anaemia even in non-SCD patients (50,51).

Patients with SCD living in areas that are endemic for bartonellosis are at high risk of infection due to the effects of auto-splenectomy, immunosuppression and recurrent transfusion (44,49). Because of these risk factors, several cases of bartonellosis have been reported in SCD patients in whom the infection often run severe course (due to SCD-associated immunosuppression), causing hyperhaemolytic crisis, aggravated anaemia, and eventually increasing the risk of blood transfusion (53-55). It is therefore important for clinicians to have high index of clinic suspicion and investigate all cases of fever in SCD patients living in areas endemic for bartonellosis, for early diagnosis (56), and initiation of appropriate antibiotic therapy (57), to avert the risk of hyperhaemolytic crisis.

Long term prevention strategy is achievable through the personal barrier protection against vectors coupled with environmental vector control programs (48), while serological screening and deferral of asymptomatic infected blood donors should be enshrined in the national transfusion services of endemic countries (56). An effective vaccine has not yet been developed against bartonellosis, but there are promising candidate vaccines that are in early stages of development (58).

Mycoplasma infection and hyperhaemolysis in SCD

Patients with SCD are usually immunocompromised and thus at risk of infection with atypical bacterial species (59). One of the most important atypical causative bacterial agents of respiratory tract infection and acute chest syndrome in patients with SCD is *Mycoplasma*

pneumoniae (60). *Mycoplasma* infection is often complicated by the development of complement fixing IgM anti-I cold reacting red cell auto-antibodies that can cause haemolysis in the colder peripheral parts of the body (61).

Moreover, in rare cases, *Mycoplasma* infection may also be associated with production of warm IgG anti-Rh red cell auto-antibodies that can cause haemolysis at core body temperature of 37^{o}C (62). Therefore, SCD patients who develop *Mycoplasma* infection are at risk of developing hyperhaemolytic crisis if the infection is associated with red cell auto-antibodies (61,62). Thus, it is important to ensure that SCD patients with respiratory infection or acute chest syndrome due to *Mycoplasma* are promptly diagnosed and treated with macrolides or other effective antibiotics against atypical bacteria (63).

Furthermore, patients infected with *Mycoplasma* should be serologically screened for cold and warm haemolytic autoantibodies, especially if any features of hyperhaemolysis are present (61,62). While keeping the patients in warm environment would largely mitigate the effect of cold autoantibodies (61), administration of steroidal immune modulation therapy may be necessary to abolish the production of both warm and cold autoantibodies in order to down regulate and eventually terminate any associated hyperhaemolysis (62).

Dengue haemorrhagic fever and hyperhaemolysis in SCD

Dengue haemorrhagic fever (DHF) is a mosquito vector (*Aedes aegypti and albopictus*) borne disease caused by the Dengue virus, which belongs to the family Filoviridae and genus Flavivirus (64). Thrombocytopaenia and hypofibrinogenemia are consistent findings in DHF (65). Hypofibrinogenemia is due to plasma leakage into pleural and peritoneal cavities (65). However, the dominant haemostatic abnormality in DHF is thrombocytopaenia, which is due to the dual effects of myelosuppression and immune mediated platelet destruction (65). Consequently, the haemorrhagic manifestation of DHF range from positive tourniquet test, to spontaneous ecchymoses, epistaxis, gum bleeding and/or severe gastrointestinal haemorrhages (65).

In addition to haemorrhagic complications, DHF has also been associated with severe acute intravascular haemolysis. Cases of Coombs negative hemolytic anemia complicating DHF have been previously reported even in non-SCD patients (66-68). These cases suggest that the virus is capable of causing direct or inflammation-induced oxidative red cell injury. Hence, acute intravascular haemolysis with haemoglobinuria and acute renal failure are recognized, albeit rare, complications of DHF even in non-SCD patients (68).

Because, patients with SCD are usually immuno-compromised, the WHO expert guidelines on Dengue fever has considered SCD to be a risk factor for development of severe and fatal DHF (69,70). Indeed, DHF has been reported to aggravate haemolysis in patients with SCD who are already battling with pre-existing inherited haemolytic anaemia (71-73). Although low platelet count is haematological feature of DHF, severe thrombocytopenia is not often seen in SCD patients with DHF (73). This is probably due to the fact that thrombocytosis is a common finding in patients with SCD in steady state (74). Hence, the high pre-infection steady state platelet count is thought to protect SCD patients from severe thrombocytopenia and bleeding during the course of DHF (73). However, SCD patients with DHF tend to present with severe hyperhaemolysis, which aggravates the pre-existing SCD-associated anaemia and often necessitates blood transfusion (73).

It is thus important that SCD patients in DHF endemic areas who present with triad of fever, thrombocytopenia and hyperhaemolysis should be promptly screened for the infection by both serological and antigen detection methods in order to plan for transfusion and other relevant supportive therapies as there is no specific anti-viral therapy at the moment (64). It is also paramount for DHF endemic countries to control the spread of the disease through environmental hygiene and vector control programs (64). Moreover, SCD caregivers should also counsel and encourage patients with SCD to use insecticides, bed nets and other barrier protection methods, and be vaccinated with the Dengue fever vaccine, Dengvaxia (64). Dengvaxia is a live-attenuated dengue vaccine, hence it cannot be given to HIV-infected persons, including those with SCD (64). The vaccine has been shown in clinical trials to be efficacious and safe in persons who have had a previous Dengue virus infection (64).

However, persons who experience their first natural Dengue infection after receiving Dengvaxia are paradoxically at an increased risk of developing severe DHF (64). For that reason, WHO recommends that only persons aged 9-45 years with evidence of a past Dengue virus infection should receive the vaccine (64). Accordingly, non-HIV-infected patients with SCD aged 9-45 years who live in areas endemic for DHF with a past history of

Dengue infection are eligible to receive Dengvaxia (64).

Coronavirus disease-2019 and hyperhaemolysis in SCD

Coronavirus disease 2019 (COVID-19) is a viral disease caused by the severe acute respiratory syndrome – coronavirus - 2 (SARS-CoV-2) (75). Clinical manifestations of severe COVID-19 include acute respiratory distress syndrome, systemic inflammation, sepsis, thrombosis, multi-organ failure, and death (75). Although SCD is basically an inherited chronic haemolytic anaemia, it shares certain pathophysiologic and clinical manifestations with COVID-19 such as anaemia, endothelial dysfunction, chronic inflammation, hypercoagulability, ischaemic stroke, pulmonary hypertension, and acute chest syndrome (76), all of which are potential risk factors for poor COVID-19 outcomes (77-80). The clinico-pathological similarities between the two diseases suggest that SCD patients may experience more severe COVID-19, however, there is conflicting evidence on whether patients with SCD actually experience more severe COVID-19 compared with patients without SCD (81).

A critical review of the literature on COVID-19, SCD and hypercoagulability revealed that while most studies had surprisingly reported mild to moderate COVID-19-related disease course in patients with SCD, literature review suggested that SCD was associated with increased risks of acute chest syndrome, hospitalisation and death from COVID-19 (81,82). Only little is known about the tendency of SARS-CoV-2 to cause haemolysis in infected patients. However, previous studies have suggested that SARS-CoV-2 cause haemolysis via at least three possible mechanisms. First, SARS-CoV-2 has been shown to trigger the production of both IgG and/or IgM red cell autoantibodies that can cause Coombs-positive intravascular hyperhaemolysis (83-85). Second, SARS-CoV-2-associated protein ORF8 is known to bind the porphyrin part of haemoglobin at the β1 chain, thereby causing haemolysis (86). Third, SARS-COV-2-associated inflammatory and oxidative stress significantly decreases red cell deformability (86); decreased red cell deformability is known to shorten red cell survival and is major determinant of haemolysis especially in patients with SCD (76).

The second and third mechanisms were thought to be responsible for haemolysis in a reported case of severe combined vaso-occlusive and Coombs-negative intravascular hyperhaemolytic crises in COVID-19 patient with SCD in whom the haemolysis was so severe that it necessitated urgent red blood cell exchange transfusion (87). Therefore, SCD patients with COVID-19 should have their red cells preemptively grouped and their sera saved for possible cross match in anticipation of exchange transfusion if and when they develop severe hyperhaemolytic crisis. However, as anti-viral pharmacologic therapy is becoming increasingly available for COVID-19 (88), it is possible to mitigate severe manifestations of COVID-19 in SCD patients, such as acute chest syndrome (81,82) and haemolysis (87), by prompt administration of anti-COVID-19 chemotherapy.

Because patients with SCD are immuno-suppressed, they should take preventive measures against acquiring COVID-19, which includes regular use of hand sanitizers, face masks and vaccination. However, vaccine hesitancy is a significant impediment among patients with SCD (89). The hesitancy is based on two inter-related pathophysiologic perspectives (89). First, SCD is inherently associated with thrombotic tendency (90). Second, COVID-19 vaccine is also associated with thrombotic side effects (91). Hence, SCD patients, quite logically, consider themselves to be at an increased risk of vaccine-induced immune thrombotic thrombocytopenia (VIITT) (89).

While some studies consider SCD patients to be at no greater risk of post-vaccination VIITT or VOC (89), the fear for VIITT is sustained by a few incoming case reports of SCD patients who experienced significant drop in platelets count, severe VOC, or even fatal TTP-like syndrome after taking the vaccine (92-94). However, some studies have suggested that adenoviral Covid-19 vaccines are more thrombogenic than m-RNA COVID-19 vaccines (91), which we believe should be safer for patients with SCD. It is therefore the responsibility of clinicians and vaccine providers to select a less thrombogenic vaccine and render closer post-vaccination monitoring for persons with underlying pro-thrombotic disorders such as the SCD (92-94).

Infections associated intra-medullary haemolysis in SCD: the haemophagocytic syndrome

Infection associated intra-medullary haemolysis (IAIMH) is solely caused by haemophagocytic lympho-histiocytosis (HLH), which manifests as haemophagocytic syndrome that is associated with excessive and uncontrolled tri-lineage phagocytic destruction of erythroid, myeloid, and megakaryocytic haematopoietic precursors in the bone marrow. HLH is a rare

but potentially life-threatening syndrome, caused by a hyper-inflammatory response leading to multi-organ damage (95,96). Hence, HLH is characterized by fever, hepato-splenomegaly, hyper-ferritinaemia, hyper-triglyceridaemia, intra-medullary haemophagocytosis, and peripheral pancytopenia, all of which form the diagnostic frame work for HLH (97).

HLH can be primary (inherited) or secondary. Primary HLH is generally seen in infancy and is associated with mutations that affect cytotoxic T-cell or inflammasome receptor functions (98,99). Secondary HLH is more common, and is often triggered by infections, haematologic malignancies, autoimmune disorders or drugs (95). The most common form of secondary HLH is infection-associated HLH. The spectrum of infectious triggers of HLH includes a wide range of bacteria, viruses, parasites, and fungi (100,101).

Due to their susceptibility to infections (20), SCD patients could be at increased risk of HLH. However, there may be some dilemma in diagnosing HLH in patients with SCD, which should be resolved by careful consideration of the diagnostic criteria for HLH vis-à-vis SCD (102,103). For example, while severe anaemia in SCD can be caused by hyperhaemolytic or aplastic crisis, such crises are usually not associated with pancytopenia, thus severe anaemia in conjunction with pancytopenia should raise suspicion of HLH in patients with SCD (102, 103).

However, the diagnostic strength of pancytopenia should always be weighed within the context of any concurrent hydroxyurea therapy, which might also potentially cause drug-induced pancytopenia (102,104). Another diagnostic criterion of HLH that requires careful consideration in SCD is hyper-ferritinaemia, which might as well be caused by chronic hyper-transfusion in patients with SCD (16, 102,103). Moreover, it should be noted that prominent enlargement of spleen or liver might as well be caused by sequestration crisis, which must be diligently ruled. In contradistinction to HLH, acute sequestration organomegaly is usually tender and the patient is typically in hypovolaemic shock (105). Therefore, the diagnosis of HLH in SCD requires careful interpretation because of the clinico-pathological overlap between the diagnostic criteria of HLH and clinical manifestations and/or side effects of treatment for SCD (102-105).

In spite of the fact that SCD is associated with high incidence of infection (20), the literature regarding HLH in patients with SCD is surprisingly sparse (102,106-111), which would suggest a paradoxically low incidence of HLH in SCD. It is not clear whether this paradox is due to under-reporting or under-diagnosis of HLH in patients with SCD. While it has been reported that VOC (106) and blood transfusion (107) independently trigger HLH in patients with SCD, most of the remaining few cases of HLH were reported in SCD have been associated with infections due a myriad of pathogens such as unspecified periodontal bacteria (108), Epstein-Barr virus and Cytomegalovirus (109), Parvovirus-B19 (102), *Histoplasma* spp. (110), and atypical mycobacteria (111).

Once the diagnosis of HLH is made, treatment becomes urgent. The few reported cases of HLH in SCD in the literature were treated with a combination of antimicrobials, supportive transfusion, and/or immuno-modulatory therapy with corticosteroids, immunoglobulins, etoposide or interleukin-1 receptor antagonists (103,108). Nonetheless, systemic corticosteroids must always be used judiciously in patients with SCD because of the potential risk of steroid-induced VOC (112). Documented cases of HLH in patients with SCD underscores the importance of having a high index of suspicion for HLH in patients with SCD who present with fever, pancytopenia and multi-organ dysfunction. Such patients should be promptly screened for underlying infections and bone marrow evidence of excessive haemophagocytosis in order to initiate life saving transfusion with concurrent anti-microbial chemotherapy and immune modulation therapy.

Since any infection is a potential trigger of HLH, the risk of HLH in SCD should be mitigated by ensuring that SCD patients are optimally immunized against all locally prevalent 'vaccine-preventable' infectious diseases, while the observance of good personal and environmental hygiene in conjunction with routine chemoprophylaxis should be an important defense against infectious diseases for which vaccines are not currently available.

Conclusion:

Various bacterial, viral, parasitic, and fungal infections have been aetiologically associated with IAH in SCD, which may present as classical intravascular hyperhaemolytic crisis or intra-medullary haemophagocytic syndrome. SCD caregivers should investigate all patients with fever, severe anaemia or pancytopenia for early diagnosis and prompt treatment of IAH.

IAH in SCD is a life-threatening haematological emergency for which transfusion therapy alone may not suffice. Prompt and sustainable termination of IAH may require therapeutic combination of transfusion, anti-microbial

chemotherapy, and immune modulation therapy. SCD caregivers should counsel patients on personal and environmental hygiene, barrier protection against disease spreading vectors, routine chemoprophylaxis for locally endemic diseases, and immunization for vaccine-preventable infections as a long-term preventive strategy against IAH.

Contributions of authors:

SGA was involved in conceptual design, discussion and appraisal of intellectual content; UAI is involved in the literature search, selection, harmonization, tabulation of results, and manuscript draft

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Original Article **Open Access**

A point-prevalence survey of carbapenem-resistant Enterobacteriaceae in two different cities in Kuwait and Nigeria

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Abstract:

Background: The family Enterobacteriaceae belongs to the order Enterobacterales, a large diverse group of Gram-negative, facultatively anaerobic bacteria that sometimes cause multidrug-resistant infections which treatment options are often challenging. They are the leading cause of nosocomial bloodstream infection (BSI) and urinary tract infections (UTI). The objective of the study was to carry out a point-prevalence survey of antimicrobial resistance and carbapenem-resistant Enterobacteriaceae (CRE) clinical isolates in two hospitals in Kuwait and Nigeria.
Methodology: Clinically significant bacterial isolates of patients from Kuwait and Nigeria, identified by VITEK-2 and MALDI-TOF mass spectrometry analysis were studied. Susceptibility testing of selected antibiotics was performed using E-test and broth dilution methods. Genes encoding carbapenemase, β-lactamases, and extended-spectrum β-lactamases (ESBLs) were detected by conventional PCR and sequencing, and whole genome sequencing (WGS) analyses.
Results: Of 400 isolates from Kuwait and Nigeria, 188 (47.0%) and 218 (54.5%) were *Escherichia coli* and 124 (31.0%) and 116 (29.0%) *Klebsiella pneumoniae*, respectively. The prevalence of CRE was 14.0% in Kuwait and 8.0% in Nigeria. The resistance rates of CRE isolates against colistin and tigecycline in Kuwait were 6.6% versus 25.0%, and in Nigeria were 14.2% versus 14.2%, respectively. $bla_{\text{OXA-181}}$ gene was the commonest in CRE isolates in Kuwait and $bla_{\text{NDM-7}}$ in Nigeria. The commonest ESBL gene among the CRE isolates was $bla_{\text{CTX-M-15}}$ in both countries. AmpC resistance genes were present in only Kuwait isolates and mediated by bla_{EBC}, bla_{CIT} and bla_{DHA}. WGS analysis of 12 selected CRE isolates with carbapenem MICs>32µg/ml but no detectable genes from conventional PCR, revealed the presence of multidrug efflux pump genes such as major facilitator superfamily antibiotic efflux pump and resistance-nodulation-cell division antibiotic efflux pump groups.
Conclusion: The prevalence of CRE was higher among isolates from Kuwait than Nigeria and the genes encoding resistance in CRE were different. The presence of efflux pump was a main mechanism of resistance in most of the Nigerian CRE isolates.

Keywords: CRE; point-prevalence-survey; Kuwait; Nigeria

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Une enquête sur la prévalence ponctuelle des entérobactéries résistantes aux carbapénèmes dans deux villes différentes du Koweït et du Nigeria

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Résumé:

Contexte: La famille des Entérobactéries appartient à l'ordre des Entérobactéries, un grand groupe diversifié de bactéries anaérobies facultatives à Gram négatif qui provoquent parfois des infections multirésistantes dont les options de traitement sont souvent difficiles. Ils sont la principale cause d'infections nosocomiales du sang (BSI) et d'infections des voies urinaires (UTI). L'objectif de l'étude était de mener une enquête sur la prévalence ponctuelle de la résistance aux antimicrobiens et des isolats cliniques d'entérobactéries résistantes aux carbapénèmes (CRE) dans deux hôpitaux au Koweït et au Nigeria.
Méthodologie: Des isolats bactériens cliniquement significatifs de patients du Koweït et du Nigéria, identifiés par analyse par spectrométrie de masse VITEK-2 et MALDI-TOF, ont été étudiés. Les tests de sensibilité des antibiotiques sélectionnés ont été effectués à l'aide des méthodes de test E et de dilution en bouillon. Les gènes codant pour la carbapénémase, les β-lactamases et les β-lactamases à spectre étendu (BLSE) ont été détectés par PCR et séquençage conventionnels et analyses de séquençage du génome entier (WGS).
Résultats: Sur 400 isolats du Koweït et du Nigéria, 188 (47,0%) et 218 (54,5%) étaient *Escherichia coli* et 124 (31,0%) et 116 (29,0%) *Klebsiella pneumoniae*, respectivement. La prévalence de la CRE était de 14,0% au Koweït et de 8,0% au Nigeria. Les taux de résistance des isolats CRE à la colistine et à la tigécycline au Koweït étaient de 6,6% contre 25,0%, et au Nigeria de 14,2% contre 14,2%, respectivement. Le gène *bla*$_{OXA-181}$ était le plus courant dans les isolats CRE au Koweït et *bla*$_{NDM-7}$ au Nigeria. Le gène BLSE le plus courant parmi les isolats CRE était *bla*$_{CTX-M-15}$ dans les deux pays. Les gènes de résistance à l'AmpC étaient présents uniquement dans les isolats du Koweït et médiés par *bla*$_{EBC}$, *bla*$_{CIT}$ et *bla*$_{DHA}$. L'analyse WGS de 12 isolats CRE sélectionnés avec des CMI de carbapénème >32 µg/ml mais aucun gène détectable par PCR conventionnelle, a révélé la présence de gènes de pompe d'efflux multidrogues tels que la pompe d'efflux antibiotique de la superfamille facilitatrice majeure et les groupes de pompe d'efflux antibiotique de division cellulaire de résistance-nodulation.
Conclusion: La prévalence de la CRE était plus élevée parmi les isolats du Koweït que du Nigeria et les gènes codant pour la résistance à la CRE étaient différents. La présence d'une pompe à efflux était un mécanisme principal de résistance dans la plupart des isolats CRE Nigérians.

Mots clés: CRE; enquête de prévalence ponctuelle; Koweït; Nigéria

Introduction:

Species of the family Enterobacteriaceae are members of the normal gut flora that can cause severe healthcare-associated infections such as bloodstream infections, pneumonia, urinary tract infections and intra-abdominal infections. Due to the rise in the proportion of Gram-negative resistant bacteria, for example extended-spectrum β-lactamases (ESBLs)-positive Enterobacteriaceae causing various infections in hospitalized patients, the use of carbapenems in hospital has increased exponentially in the last decade (1). This rise in consumption of carbapenems has been accompanied by the emergence of carbapenem-resistant Enterobacteriaceae (CRE) (2).

The rapid emergence of CRE in this decade has caused tremendous amount of global clinical and public health concerns. According to the Center for Disease Control and Prevention (CDC), CRE is considered as an urgent threat with high mortality and morbidity due to narrow, more toxic, and less effective therapeutic options (3-5). In 2017 for example, there were 13,100 estimated cases of CRE infections among hospitalized patients in the USA with 1,100 estimated deaths and $130 million estimated attributable healthcare costs (5).

The main mechanisms for carbapenem-resistance in Enterobacteriaceae are the production of carbapenemases or ESBLs and/or AmpC cephalosporinase (AmpC) in combination with membrane permeability and efflux pump (6). In Kuwait, the first clinical report of *bla*$_{NDM-1}$–positive *Klebsiella pneumoniae* isolate was detected in 2 patients in Mubarak hospital (7). Then, *bla*$_{VIM-4}$, *bla*$_{OXA-48}$ and *bla*$_{KPC}$ have been discovered from different Enterobacteriaceae such as *Escherichia coli*, *Enterobacter cloacae*, *Morganella morganii*, and *Proteus stuartii* (8-10) in different hospitals in Kuwait. While in Nigeria, the first phenotypic determination of CRE was reported in 2015 (11). Then, this was followed by other reports on molecular characterization of carbapenem resistance genes such as *bla*$_{VIM}$, *bla*$_{GES}$, *bla*$_{NDM}$, *bla*$_{OXA-181}$, *bla*$_{KPC}$ and *bla*$_{OXA-48}$ in different Enterobacteriaceae isolates (12-14).

In this study, we conducted point-survey studies on the prevalence of CRE in 2 hospitals in Kuwait and Nigeria, determined their antimicrobial susceptibility pattern and investigated the molecular epidemiological features of the CRE isolates.

Materials and method:

Bacterial isolates

Four hundred consecutive clinically significant non-repetitive isolates from the family Enterobacteriaceae recovered from clinical sam-

ples of patients with proven infections, attending National hospital (NH), Abuja, Nigeria and Mubarak Al Kabeer Hospital, Kuwait, over one-month period (20th January to 27th February, 2019) were studied. NH is a Federal government owned 850-bed teaching hospital with two intensive care units (ICUs), a dialysis unit and an oncology unit, and Mubarak Al Kabeer Hospital is also 850-bed government teaching hospital with two ICUs and a huge dialysis unit. All isolates were sent to the Hospital Infection Reference Laboratory, Faculty of Medicine, Kuwait University, Kuwait for further analysis.

The bacterial isolates were collected from routine laboratory work for patient care in clinical microbiology laboratories of the two hospitals. No additional clinical specimens or clinical data were collected and the isolates were not linked to patient's identity. Ethical approval for the study was therefore not required.

Bacterial identification

Bacterial strains were identified using VITEK-MS system (bioMérieux, Marcy l'Etoile, France), a matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. The strains were stored in Brain Heart Infusion Broth (Oxoid Ltd, Basingstoke, Hampshire, England) with 20% glycerol (bio-Mérieux) in -80ºC freezer for further antimicrobial susceptibility and investigation of resistance mechanisms.

Antimicrobial susceptibility testing (AST)

The minimum inhibitory concentrations (MICs) of 13 antimicrobial agents, amikacin, ampicillin, amoxicillin-clavulanic acid, cefepime, cefoxitin, cefotaxime, ceftazidime, ciprofloxacin, colistin, ertapenem, imipenem, meropenem and tigecycline against all isolates were determined using E test (bioMérieux) according to the manufacturer's instruction, except for colistin which AST was performed by the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) (15).

The following quality control strains were included in each run; *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 (for carbapenem), *E. coli* ATCC 35218 (for β-lactam-β-lactamase inhibitors combination) and colistin-resistant *E. coli,* NCTC 13846 (*mcr-1* positive). The results were interpreted according to the breakpoints and criteria of the CLSI (15). The breakpoints for tigecycline and colistin was based on US Food and Drug Administration (FDA) standards and EUCAST guidelines (16), respectively.

Screening for carbapenemase

All isolates were phenotypically screened for CRE strains using an imipenem-EDTA double disc synergy test and modified Hodge test. These tests were also used to screen and assess the ability of test isolates to produce carbapenemases according to CLSI guidelines (15).

Investigation of resistance mechanisms:

For all CRE isolates, polymerase chain reaction (PCR) was used to detect the genes that encoded the following carbapenemases: bla_{NDM}, bla_{OXA-48}, $bla_{OXA-48\ like}$, bla_{VIM}, bla_{IMP} and $bla_{KPC;}$ and ESBLs: bla_{CTX-M}, bla_{SHV}, bla_{TEM} and bla_{CMY} (17,18). PCR products were purified with a QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) and sequenced using a GenAmp PCR system 9700 by cycle sequencing with ABI BigDye® Termination Version 3.1 Kit (Applied Biosystem, Carlsbad, CA, USA). Detection of genes mediating AmpC β-lactamases (bla_{MOX}, bla_{CIT}, bla_{DHA}, bla_{ACC}, bla_{EBC} and bla_{FOX}) was carried out on CRE isolates cefoxitin-resistant and cefepime susceptible by previously described method (19).

Whole genome sequencing (WGS) and analysis

WGS was carried out on 12 selected isolates with carbapenem MICs > 32 µg/ml in which the carbapenemase genes were undetectable by conventional PCR. These isolates were 5 *M. morganii* (K137, K150, K171, N62 and N85), 4 *P. mirabilis* (K142, N18, N74 and N88), 2 *K. pneumoniae* (K120 and K14) and 1 *E. cloacae* (N40). Bacterial DNA was extracted using QIAamp DNA minikit (Qiagen) according to the manufacturer's instructions. Quantification and quality of the DNA were assessed by Nano-Drop-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and analyzed on agarose gel electrophoresis. Purified DNA samples were sent to a commercial company, Novogene Company Limited (Cambridge Science Park, Milton, Cambridge, UK) for WGS. The WGS data (Fastaq) were then uploaded into the web server of the Center for Genomic Epidemiology (http://www.genomic epidemiology.org/).

The plasmid replicon, multi-locus sequence type (MLST), and antimicrobial resistance genes were determined using the following programs; PlasmidFinder (version 2.0.1), MLST (version 2.0.4), and ResFinder (version 4.0), respectively (20-22). The presence of insertion sequences and other mobile genetic elements were determined by ISFinder (https://www-is.biotoul.fr/) to confirm their identity. Multi-drug–resistant (MDR) efflux pumps and outer

membrane porin genes were identified by the Comprehensive Antibiotic Resistant Database (CARD) analysis.

Results:

Distribution of the isolates:

Table 1 shows the distribution of the clinical isolates from Kuwait and Nigeria. The top 3 of the 200 Enterobacteriaceae isolates from Kuwait were *E. coli* 94 (47%); *Klebsiella pneumoniae* 62 (31%) and *Serratia marcescens* 11 (5.5%). Others were *Proteus mirabilis* 8 (4%); *Morganella morganii* 7 (3.5%); *Citrobacter freundii* 7 (3.5%); *Enterobacter cloacae* 6 (3%); *Raoultella planticola* 3 (1.5%) and *Providentia stuartii* 2 (1%). The top 3 isolates from Nigeria were *E. coli* 109 (54.5%); *K. pneumoniae* 58 (29%) and *P. mirabilis* 19 (9.5%). Other isolates were *E. cloacae* 5 (2.5%); *M. morganii* 3 (1.5%); *C. freundii* 3 (1.5%); *S. marcescens* 2 (1%) and *Aeromonas hydrophila* 1 (0.5%).

A total of 28 (14%) isolates from Kuwait were CRE. These were 6 (21.4%) *P. mirabilis* 5 (17.9%); *M. morganii* 4 (12.3%); *K. pneumoniae* 5 (17.9%); *E. coli* 2 (7.1%); *S. marcescens* 2 (7.1%); *C. freundii* 2 (7.1%); *P. stuartii* 1 (3.6%); *K. aerogenes* and *E. cloacae* 1 (3.6%). Their sources were 7 (25%) urinary tract infections (UTIs); 6 (21.4%) lower respiratory tract infections (LRTIs), 4 (14.3%) blood stream infections (BSIs), 3 (10.7%) wound infections, 4 (14.3%) tissues, 2 (7.1%) pus aspirates, 1 (3.6%) peritoneal fluid, and 1 (3.6%) bed sore. By contrast, only 16 (8%) of the Nigerian isolates were CRE; 3 (18.8%) *K. pneumoniae*, 2 (12.5%) *E. coli*, 2 (12.5%) *E. cloacae*, 6 (37.5%%) *P. mirabilis* and 3 (18.8%) *M. morganii*. They were isolated from 9 (56.3%) wound infections, 3 (18.8%) UTIs, 1 (6.3%) BSIs, 1 (6.3%) cerebrospinal fluid, 1 (6.3%) ventricular shunt and 1 (6.3%) from eye swab.

Table 1: Distribution of clinical bacterial isolates from two hospitals in Kuwait and Nigeria

Bacterial isolate	**Number (%) of isolates**	
	Kuwait	**Nigeria**
Escherichia coli	94 (47)	109 (54.5)
Klebsiella pneumoniae	62 (31)	58 (29)
Serratia marcescens	11 (5.5)	2 (1)
Proteus mirabilis	8 (4)	19 (9.5)
Morganella morganii	7 (3.5)	3 (1.5)
Citrobacter freundii	7 (3.5)	3 (1.5)
Enterobacter cloacae	6 (3)	5 (2.5)
Raoultella planticola	3 (1.5)	0 (0)
Providentia stuartii	2 (1)	0 (0)
Aeromonas hydrophila	0	1 (0.5)
Total	**200 (100.0)**	**200 (100.0)**

Table 2: Antimicrobial susceptibility profiles of clinical Enterobacteriaceae isolates from Kuwait and Nigeria

Antimicrobial (breakpoints in µg/ml)	**Kuwait (n=200)**				**Nigeria (n=200)**			
	MIC range	**MIC_{50}**	**MIC_{90}**	**% resistance**	**MIC range**	**MIC_{50}**	**MIC_{90}**	**% resistance**
Amikacin (16)	1 - >256	4	12	5	0.25 - >256	3	8	3
Ampicillin (8)	0.5 - >256	256	256	93	0.25 - >256	>256	>256	89
Amoxicillin-clavulanic acid (8)	0.38 - >256	6	32	31	0.38 - >256	6	32	34
Cefepime (2)	0.016 - >256	1	>256	47	0.016 - >256	6	>256	57
Cefoxitin (8)	1 - >256	3	256	29	0.75 - >256	4	>256	27
Cefotaxime (1)	0.012 - >256	256	256	59	0.006 - >256	>256	>256	59
Ceftazidime (4)	0.047 - >256	3	256	48	0.016 - >256	3	>256	47
Ciprofloxacin (1)	0.004 - >32	0.5	32	43	0.004 - >32	8	>32	58
Colistin (2)*	0.125 - >256	0.75	>256	1.7	0.094 - >256	0.5	>256	2.3
Ertapenem (0.5)	0.008 - >32	0.094	0.5	7	0.002 - 3	0.032	0.25	1
Imipenem (1)	0.094 - >32	0.25	2	13	0.032 - 12	0.25	1	8
Meropenem (1)	0.064 - >32	0.125	0.25	3	0.012 - 6	0.094	0.19	1
Tigecycline* (2)	0.125 - >256	1	4	9.8	0.094 - >256	0.5	3	4.5

*Excluding the following organisms from the calculation: *Proteus mirabilis*, *Morganella morganii*, *Providentia stuartii* and *Serratia marcescens*
MIC=minimum inhibitory concentration; n=number

Antimicrobial susceptibility test results:

Antimicrobial susceptibility results including MIC range, MIC_{50}, MIC_{90} and percentage of resistance for all the 400 isolates from Kuwait and Nigeria are shown in Table 2. Among the isolates from Kuwait, colistin, meropenem, amikacin, ertapenem and tigecycline demonstrated excellent activities with low resistance rates of 1.7%, 3%, 5%, 7%, 9.8%, respectively. Similarly, meropenem, ertapenem, colistin, amikacin, tigecycline and imipenem exhibited excellent activities on the isolates from Nigeria with low resistance rates of 1%, 1%, 2.3%, 3%, 4.5% and 8%, respectively. The majority of the isolates from Kuwait (93%) and Nigeria (89%) were resistant to ampicillin.

The resistance rates to cefepime, cefotaxime and ceftazidime were relatively high in both Kuwait (47%, 59%, and 48%, respectively) and Nigeria (57%, 59% and 47%, respectively). Ciprofloxacin also demonstrated very poor activities against isolates from Kuwait and Nigeria with resistance rates of 43% and 58%, respectively.

Antimicrobial susceptibility of the CRE isolates:

Antimicrobial susceptibility rates of the CRE isolates are shown in Table 3. The most active antimicrobial agents against CRE isolates from Kuwait were colistin to which only 6.6% were resistant, followed by meropenem (17. 8%), tigecycline (25%) and amikacin (28%). The most active antibiotics against the Nigerian isolates were amikacin (0% resistance), ertapenem and meropenem (12.5% each), colistin (14.2%) and tigecycline (14.2%). The majority of the isolates in Kuwait and Nigeria showed high resistance to amoxicillin-clavulanic acid with rates of 75% and 68.7%, respectively.

Resistance to other β-lactam antibiotics in Kuwaiti and Nigerian CRE isolates were relatively high; respective resistance rates to cefepime were 42.8% and 56.2%, ceftazidime 39.2% and 37.5%, cefotaxime 57.1% and 56.2% and cefoxitin 64.2% and 62.5%. Half (50%) of the isolates from Kuwaiti were resistant to ciprofloxacin compared to 31% from Nigeria.

Table 3: Antimicrobial susceptibility of clinical carbapenem-resistant Enterobacteriaceae isolates from Kuwait and Nigeria

Antimicrobial (breakpoints in µg/ml)	**Kuwait (n=28)**				**Nigeria (n=16)**			
	MIC range	**MIC_{50}**	**MIC_{90}**	**% resistance**	**MIC range**	**MIC_{50}**	**MIC_{90}**	**% resistance**
Amikacin (16)	0.5 - >256	3	24	28	1.5 - 6	2	3	0
Ampicillin (8)	1.5 - >256	>256	>256	96.4	0.5 - >256	>256	>256	87.5
Amoxicillin-clavulanic acid (8)	0.38 - >256	24	>256	75	0.5 - >256	24	>256	68.7
Cefepime (2)	0.032 - >256	0.38	>256	42.8	0.094 - 48	3	32	56.2
Cefoxitin (8)	2 - >256	24	>256	64.2	2 - >256	16	>256	62.5
Cefotaxime (1)	0.012 - >256	8	>256	57.1	0.012 - >256	>256	>256	56.2
Ceftazidime (4)	0.047 - >256	0.5	>256	39.2	0.047 - >256	0.19	>256	37.5
Ciprofloxacin (1)	0.008 - >32	1	>32	50	0.016 - >32	0.25	16	31.2
Colistin* (2)	0.38 - 32	32	32	6.6	0.13 - 8	8	8	14.2
Ertapenem (0.5)	0.016 - >32	0.38	>32	46.4	0.012 - 3	0.032	3	12.5
Imipenem (1)	0.19 - >32	3	>32	89.2	1.5 - 12	2	8	100
Meropenem (1)	0.125 - >32	0.25	4	17.8	0.064 - 6	0.19	4	12.5
Tigecycline* (2)	0.25 - >256	12	12	25	0.094 - 3	3	3	14.2

*Excluding the following organisms from the calculation: *Proteus mirabilis*, *Morganella morganii*, *Providentia stuartii*, and *Serratia marcescens*
MIC=minimum inhibitory concentration; n=number

Table 4: Carbapenemase resistant genes harbored by CRE strains in Kuwait and Nigeria

Detected genes	**Number of CRE isolates harboring specific genes**					
	Kuwait (n=10)					**Nigeria (n=2)***
	K. pneumoniae	***E. coli***	***P. mirabilis***	***M. morganii***	***S. marcescens***	***E. coli***
$bla_{OXA-181}$	1	2	0	1	0	0
$bla_{OXA-833}$	1	0	0	0	0	0
bla_{OXA-48}	0	0	1	0	0	0
$bla_{OXA-244}$	0	1	0	0	0	0
$bla_{OXA-233}$	0	0	0	0	0	0
bla_{KPC-2}	0	0	0	0	1	0
bla_{NDM-7}	0	0	0	0	0	2
$bla_{OXA-232}$ + bla_{NDM-5}	2	0	0	0	0	0
Total	**4**	**3**	**1**	**1**	**1**	**2**

*Only *E. coli* harbored a carbapenemase gene among the Nigerian isolates

Carbapenemase genes

A total of 10 (35.7%) out of 28 CRE isolates from Kuwait harbored 1 or 2 genes mediating carbapenemase production as shown in Table 4. Four (40%) of the 10 isolates harbored *bla*$_{OXA-181}$. Other genes detected were *bla*$_{OXA-48}$, *bla*$_{OXA-244}$, *bla*$_{OXA-833}$, and *bla*$_{KPC-2}$. Two *K. pneumoniae* isolates co-harbored both *bla*$_{OXA-232}$ and *bla*$_{NDM-5}$. Only 2 (12.5%) of the 16 Nigerian CRE isolates harbored carbapenemase gene, *bla*$_{NDM-7}$.

Other mechanisms associated with carbapenem resistance:

The conventional PCR failed to detect the usual genes mediating carbapenemases production in 18 (64.2%) out of the 28 CRE isolates from Kuwait and 14 (87.5%) of the 16 isolates from Nigeria. From Kuwait, these isolates were; 5 *M. morganii*, 5 *P. mirabilis*, 2 *E. coli*, 2 *P. stuartii*, 1 *S. marcescens*, 1 *E. cloacae*, 1 *K. aerogenes* and 1 C. *freundii*. Those from Nigeria included 6 *P. mirabilis*, 3 *K. pneumoniae*, 3 *M. morganii* and 2 *E. cloacae*.

However, ESBL encoding genes (*bla*$_{CTX-M}$, *bla*$_{SHV}$ and *bla*$_{TEM)}$ and *bla*$_{CMY}$ mediating AmpC were detected by conventional PCR in 24 and 14 isolates, respectively as shown in Tables 5 and 6. Among the ESBL-positive isolates, *bla*$_{CTX-M-15}$ were detected in 6 isolates from Kuwait, *bla*$_{CTX-M-65}$ in 2 and *bla*$_{CTX-M-14}$ in 1, and *bla*$_{SHV-1}$ gene was detected in 4 isolates. Four isolates from Nigeria carried *bla*$_{CTX-M-15}$ and 3 *bla*$_{CTX-M-3}$. None of the Nigerian isolates harbored any AmpC gene.

Results of WGS analysis:

As shown in Table 7, there were several antimicrobial resistance genes that mediated resistance to several antimicrobial agents among the randomly selected 12 non-carbapenemase-producing (NCP) CRE isolates. These genes were located either on chromosome or plasmid. All 12 NCP CRE isolates harbored homologues of *KpnE*, *KpnF*, *KpnG*, or *KpnH* genes alone or in combinations, which were associated with major facilitator superfamily (MFS) antibiotic efflux pump and its regulations (CRP gene).

Five *M. morganii* NCP CRE isolates (K137, K150, K171, N62 and N85) harbored AmpC β-lactamase gene, *bla*$_{DHA}$. Six NCP CRE isolates (K142 *P. mirabilis*, K150 *M. morganii*, N18 *P. mirabilis*, N62 *M*. *morganii*, N85 *M*. *morganii* and N88 *P. mirabilis*) harbored *qacEdelta1* genes responsible for mediating the major facilitator superfamily (MFS) antibiotic efflux pump.

All, except 2 (K120 *K. pneumoniae* and K14 *K. pneumoniae*) isolates, harbored *rsmA* genes that conferred resistance-nodulation-cell division (RND) antibiotic efflux pump. One N40 *E. cloacae* isolate carried an efflux pump with reduced permeability *marA* gene in addition with 2 AmpC β-lactamase genes, *bla*$_{AmpH}$ and *bla*$_{ACT-29}$. Carbapenemase genes of the *bla*$_{OXA-232}$ and *bla*$_{NDM-5}$ varieties were detected in one isolate (K14 *K. pneumoniae*) as well as AmpC β-lactamase enzyme, *bla*$_{AmpH}$ and other 3 β-lactamase genes (*bla*$_{CTX-M-15}$, *bla*$_{SHV-1}$ and *bla*$_{TEM-1)}$.

MLST results of the 12 CRE isolates:

Of the 12 CRE isolates, only 2 were evaluable for MLST on the Plasmid Finder. These were *K. pneumoniae* strains K120 and K14, both of which belonged to sequence type ST231. The remaining isolates consisting of 5 *M. morganii*, 4 *P. mirabilis* and 1 *E. cloacae* were not evaluated as *M. morganii* and *Proteus* spp. had no allelic profile developed at the time of our investigation for current DNA-based typing assay.

Analysis of mobile genetic elements (MGEs):

Plasmid Finder showed that K120 *K. pneumoniae* isolate belonged to sequence type ST231 and harbored 6 plasmids; Col(pHAD28), Col440I, IncFIA, IncFIB(pQil), IncFII(K) and IncFII(pAMA1167-NDM-5). In addition, the 115300 bp DNA IncFIB (pQil) plasmid carried *bla*$_{ampH,}$ *bla*$_{SHV-1}$ and *bla*$_{TEM-1}$ genes that mediated β-lactam resistance. Insertion sequences IS26, ISKpn7, ISKpn6 and Tn3 transposase, detected through IS Finder, were also present.

The other sequence type ST231 K14 *K. pneumoniae* isolate carried 7 replicon plasmid types; Col(pHAD28), Col440I, ColKP3, IncFIB (pKPHS1), IncFIB(pQil), IncFII and IncFII(K). ColKP3 plasmid encoded for carbapenemase genes, *bla*$_{OXA-232}$ and *bla*$_{NDM-5}$. Plasmid IncFII harbored *bla*$_{ampH,}$ *bla*$_{CTX-M-15,}$ *bla*$_{SHV-1,}$ and *bla*$_{TEM-1}$ genes. These isolates also carried Insertion sequences IS*Ecp1*IS26, ISKpn7, ISKpn6 and Tn3 transposase.

Table 5: β-lactamase and ESBL resistant genes harbored by CRE isolates in Kuwait and Nigeria

Detected genes	**Number of CRE isolates harboring specific genes**							
	Kuwait (n=12)			**Nigeria (n=12)**				
	K. pneumoniae	***E. coli***	***P. mirabilis***	***K. pneumoniae***	***E. coli***	***P. mirabilis***	***M. morganii***	***E. cloacae***
bla$_{CTX-M-15}$	1	0	0	0	0	0	1	0
bla$_{CTX-M-14}$	0	1	0	0	0	0	0	0
bla$_{CTX-M-65}$	1	0	1	0	0	0	0	0
bla$_{CTX-M-3}$	0	0	0	0	0	0	2	0
bla$_{SHV-1}$	2	0	0	0	0	0	0	0
bla$_{TEM-1}$	0	0	0	0	2	3	0	0
bla$_{CTX-M-15}$ + *bla*$_{SHV-1}$ + *bla*$_{TEM-1}$	2	0	0	0	0	0	0	0
bla$_{CTX-M-15}$ + *bla*$_{CMY-42}$	0	1	0	0	0	0	0	0
bla$_{CTX-M-15}$ + *bla*$_{CMY-166}$	0	1	0	0	0	0	0	0
bla$_{CTX-M-15}$ + *bla*$_{TEM-1}$	1	1	0	1	0	0	0	1
bla$_{CTX-M-3}$ + *bla*$_{TEM-1}$	0	0	0	1	0	0	0	0
bla$_{CTX-M-15}$ + *bla*$_{SHV-37}$ + *bla*$_{TEM-1}$	0	0	0	1	0	0	0	0
Total	**7**	**4**	**1**	**3**	**2**	**3**	**3**	**1**

CRE = Carbapenem-resistant Enterobacteriaceae; ESBL = extended-spectrum β-lactamase

Table 6: AmpC cephalosporinase genes among cefoxitin-resistant and cefepime-susceptible CRE in Kuwait

Detected genes	**Number of CRE isolates harboring specific genes in Kuwait (n=14)**						**Total**
	E. cloacae	***E. coli***	***K. pneumoniae***	***K. aerogenes***	***C. freundii***	***M. morganii***	
bla$_{MOX}$	0	0	0	0	0	0	0
bla$_{CIT}$	0	2	0	0	1	0	3
bla$_{DHA}$	0	0	2	0	0	1	3
bla$_{ACC}$	0	0	0	0	0	0	0
bla$_{EBC}$	4	0	1	3	0	0	8
bla$_{FOX}$	0	0	0	0	0	0	0
Total	**4**	**2**	**3**	**3**	**1**	**1**	**14**

Table 7: Resistance mechanisms of the carbapenemase-negative Enterobacteriaceae isolates to different antimicrobial agents detected by whole genome sequencing

Designated ID number	**Bacterial isolate**	**β-lactam-encoding resistance genes**	**Non-β-lactam-encoding resistance genes**	**Other resistance genes**	**Antibiotic target**	**Gene location**
K120	*K. pneumoniae*	*bla*$_{ampH}$, *bla*$_{SHV-1}$, *bla*$_{TEM-1}$	*AAC(6')-Ib, aadA2, mphA, catI, arr-2, PBP3,* gyrA, *EF-Tu, parC, folP, UhpT, FosA6, QnrS1, dfrA12, sul1, ErmB*	Efflux pump: *marR, oqxA, baeR, H-NS, CRP, adeF, KpnE, KpnF, KpnG, KpnH, emrR, msbA, marA*	Aminoglycoside, macrolide, phenicol, rifamycin, β-lactam, fluoroquinolone, elfamycin, fluoroquinolone, sulfonamides, fosfomycin, quinolone, trimethoprim resistant dihydrofolate reductase dfr, sulfonamide, Erm 23S ribosomal RNA methyltransferase.	Plasmid IncFIB(pQil)
K14	*K. pneumoniae*	*bla*$_{ampH}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-232}$, *bla*$_{NDM-5}$, *bla*$_{CTX-M-15}$, *bla*$_{SHV-1}$, *bla*$_{TEM-1}$	*AAC(6')-Ib, aadA2, mphA, catI, arr-2, PBP3,* gyrB, *EF-Tu, parC, folP, UhpT,*	Efflux pump: *marR, oqxA, baeR, H-NS, CRP, FosA6, adeF, KpnE, KpnF, KpnG, KpnH, emrR, msbA, marA*	Aminoglycoside, macrolide, phenicol, rifamycin, β-lactam, fluoroquinolone, elfamycin, fluoroquinolone, sulfonamides, fosfomycin.	Plasmid IncFIB(pQil), ColKP3, IncFII

K137	*M. morganii*	*bla*$_{DHA-17,}$	*CatIII, PBP3,* gyrB, *EF-Tu*	Efflux pump: *rsmA, CRP, KpnH*	CAT, β-lactam, fluoroquinolone, elfamycin.	Chromosome
K142	*P. mirabilis*		*catA4, catII, sul1, sul2, ANT(3'')-IIa, aadA2, SAT-2, dfrA1, PBP3,* gyrB, *EF-Tu*	Efflux pump: *KpnF*, *adeF*, *CRP, tet(D), qacEdelta1, rsmA, KpnH*	CAT, sulfonamide, aminoglycoside, streptothricin acetyltransferase (SAT), trimethoprim resistant dihydrofolate reductase dfr, β-lactam, fluoroquinolone, elfamycin.	Chromosome
K150	*M. morganii*	*bla*$_{DHA-17}$	*CatIII, mphA, sul1, dfrA24, cmlA6, aadA, ANT(2'')-Ia, PBP3,* gyrB, *EF-Tu, tetR*	Efflux pump: *CRP, KpnH, rsmA, qacEdelta1, tet(B)*	CAT, macrolide phosphotransferase (MPH), Sulfonamide, trimethoprim resistant dihydrofolate reductase dfr, phenicol, aminoglycoside, β-lactam, fluoroquinolone, elfamycin, tetracycline	Chromosome
K171	*M. morganii*	*bla*$_{DHA-13}$	*PBP3,* gyrB, *EF-Tu,*	Efflux pump: *rsmA, tet(D), CRP, KpnH*	β-lactam, fluoroquinolone, elfamycin.	Chromosome
N18	*P. mirabilis*	*bla*$_{TEM-1}$	*APH(6)-Id, catA4, sul1, sul2, QnrD2, PBP3,* gyrB, *EF-Tu,*	Efflux pump: *KpnF*, *adeF*, *CRP, tet(D), qacEdelta1, rsmA, KpnH*	Aminoglycoside, CAT, sulfonamide, quinolone resistance protein (qnr), β-lactam, fluoroquinolone, elfamycin	Chromosome
N40	*E. cloacae*	*bla*$_{ACT-29,}$ *bla*$_{ampH,}$	*FosA2, PBP3, EF-Tu*	Efflux pump: *KpnF*, *adeF*, *CRP, rsmA, KpnE, marA*, ramA, H-NS, *adeF*, *oqx*A, msbA, baeR, emrB, emrR, marR	Fosfomycin, β-lactam, elfamycin,	Chromosome
N62	*M. morganii*	*bla*$_{DHA-1,}$ *bla*$_{CTX-M-15,}$ *bla*$_{OXA-1}$	*CatII, AAC(3)-IIe, APH(6)-Id, sul1, sul2, dfrA1, AAC(6')-Ib-cr, dfrA14, PBP3,* gyrB, EF-Tu	Efflux pump: qacEdelta1, KpnH, CRP, rsmA, *tet(D),*	Phenicol, aminoglycoside, sulfonamide, diaminopyrimidine, fluoroquinolone, aminoglycoside, tetracycline, trimethoprim, β-lactam, fluoroquinolone, elfamycin.	Chromosome
N74	*P. mirabilis*		*CatIII, FosA2, catA4, gyrB, PBP3, EF-Tu, catA4, AAC(2')-Ia*	Efflux pump: KpnH, KpnF, CRP, rsmA, *tet(B), adeF, CRP, tetR, tet(D)*	Chloramphenicol, fosfomycin, chloramphenicol, fluoroquinolone, β-lactam, elfamycin, CAT, aminoglycoside.	Chromosome
N85	*M. morganii*	*bla*$_{DHA-1,}$ *bla*$_{CTX-M-15,}$ *bla*$_{OXA-1}$	*CatII, AAC(3)-IIe, APH(6)-Id, sul1, sul2, dfrA1, AAC(6')-Ib-cr, dfrA14, PBP3,* gyrB, EF-Tu	Efflux pump: qacEdelta1, KpnH, CRP, rsmA, *tet(D)*	Phenicol, aminoglycoside, sulfonamide, diaminopyrimidine, fluoroquinolone, aminoglycoside, tetracycline, trimethoprim, β-lactam, fluoroquinolone, elfamycin.	Chromosome
N88	*P. mirabilis*	*bla*$_{TEM-1}$	*APH(6)-Id, catA4, sul1, sul2, QnrD2, PBP3,* gyrB, *EF-Tu,*	Efflux pump: *KpnF, KpnH adeF, CRP, tet(D), qacEdelta1, rsmA*	Aminoglycoside, CAT, sulfonamide, quinolone resistance protein (qnr), β-lactam, fluoroquinolone, elfamycin.	Chromosome

CAT = chloramphenicol acetyltransferase

Discussion:

Infected patients with CRE have higher mortality and morbidity rates than those infected by susceptible Enterobacteriaceae strains and therefore, require proper and effective antimicrobial therapy. During a one-month point survey, only 14% and 8% of the Enterobacteriaceae isolates were CRE in Kuwait and Nigeria, respectively. The production of carbapenemases mediated by $bla_{\text{OXA-181}}$ gene was a major mechanism of resistance among the CRE isolates in Kuwait. However, carbapenemase production was not a major mechanism of resistance in the CRE from the Nigerian center.

Our data demonstrated that the type of resistance genes harbored by clinical isolates of CRE reported previously from Kuwait differed from our present finding of predominance of OXA-181 carbapenemase (9,10). It is possible that the other genes are being replaced systematically by $bla_{\text{OXA-181}}$, perhaps via horizontal transfer by plasmid encoded resistance factors, in our hospitals. Our present finding was also discordant with some recent surveillance reports emanating from China (23) and several European countries, including Turkey and Israel (24). In the European survey involving 455 sentinel hospitals in 36 countries, 37% of *K. pneumoniae* and 19% of *E. coli* were carbapenemase-producers including KPC, NDM, OXA-48-like, or VIM (24), while in China, NDM and KPC-2 were responsible for 90% of CRE (23). Although the number of CRE reported among the Nigerian isolates were half of those from Kuwait, the carbapenemases produced by CRE from both centers were completely different. In Nigeria, only 2 CRE isolates produced NDM-7 carbapenemase which had never been encountered before among clinical isolates in Kuwait.

In our current survey, 67.8% and 87.5% of CRE isolates were negative for the carbapenemases investigated by conventional PCR assays among the Kuwait and Nigerian isolates, respectively. This large number of NCP CRE in Nigeria is unprecedented in the literature. For instance, in the European survey conducted by Grundmann et al., (24) in 2017, 29% of *K. pneumoniae* and 60% of *E. coli* isolates resistant to carbapenem were negative for KPC, NDM, OXA-48-like, or VIM carbapenemase production. The reason for this observation in both our study and the European study may be due to the production of other non-tested carbapenemase or reduced permeability. For this reason, we randomly selected 12 of such isolates (with MIC ≥ 32µg/ml) for WGS.

The WGS analysis revealed that the mechanism of resistance to the carbapenems in the 12 isolates was mainly due to the presence of multidrug efflux pump (MDEP) genes of the MFS antibiotic efflux pump and resistance-nodulation-cell division (RND) antibiotic efflux pump groups. In addition, one isolate from Kuwait harbored 2 carbapenemase genes, $bla_{\text{OXA-232}}$ and $bla_{\text{NDM-5}}$, that were undetectable by PCR. It is conceivable that these genes were missed because of lack of inclusion of adequate specific primers in the PCR assays. The fact that many of our CRE isolates from both countries did not produce carbapenemases is not new as previous similar studies have demonstrated low- and high-level carbapenem resistance in *Klebsiella* species and *Enterobacter* species without carbapenemase production. In these isolates, resistance was mainly due to combination of production of β-lactamase and impermeability caused by loss of outer membrane proteins (OMPs) (25,26) similar to our findings, ably demonstrated by WGS.

In addition, efflux pump and AmpC overexpression or loss of porins have been described as features of some NCP CRE isolates (26,27) as were the case with our isolates. In a recent report from Nigeria by Akinpelu et al., (28) in 2020, 18 NCP CRE *Klebsiella* spp. isolated from clinical samples were positive for the presence of efflux pump activity tested via ethidium bromide cartwheel method and biofilm forming ability via tissue culture, thereby corroborating our findings on the Nigerian isolates. All the 4 *P. mirabilis* and 5 *M. morganii* isolates also harbored chromosomally encoded β-lactam-encoding resistance gene. However, there were 2 replicon plasmid types (Col3M) detected in N74 *P. mirabilis* with 2 different identities (97.78 and 96.3) and 2 different positions in the contig but with hardly any significant resistance by itself.

Alarmingly, many of the CRE isolates from Kuwait and Nigeria were resistant to the last line antibiotics for the treatment of CRE or MDR infections. Colistin and tigecycline demonstrated relatively unacceptable poor levels of activities against these isolates. This observation may reflect the massive abuse of these antimicrobial agents in our hospitals, particularly in the intensive care unit patients, thereby creating immense selection pressure. However, the resistance level encountered against colistin in our study was lower than the 28.3% resistance rate reported in the European surveillance study (24), but higher than the 1.1–6.2% reported in China (29). The resistance to tigecycline in our study was much higher than

the 5.2% resistance rate reported in the European surveillance study (24).

Analysis of the ESBLs showed that *bla*$_{CTX-M-15}$ and *bla*$_{CTX-M-14}$ were the most commonly identified genes in our isolates. These ESBL gene types have been widely reported worldwide as the genes that encode CTX-M enzymes (*bla*$_{CTX-M}$) which can be horizontally mobilized by different genetic elements (30). Detection of AmpC genes was investigated only in the CRE isolates that were cefoxitin-resistant and cefepime-susceptible, and found only in 14 CRE isolates from Kuwait and none in the Nigerian CRE. The most common genes were *bla*$_{FBC}$, *bla*$_{CIT}$, and *bla*$_{DHA}$. AmpC overexpression phenotype had been demonstrated in a previous report from Switzerland to be a feature of carbapenem-resistant *E. cloacae* (26). This is in keeping with recent reports on acquisition of plasmid-mediated cephalosporinase producing Enterobacteriaceae after a travel to the tropics like Asia, Latin America and Africa (31).

Our study is limited by the fact that only one center per country were included, other mechanisms of carbapenem resistance in all isolates such as permeability problem or porin loss were not investigated, the number of isolates investigated by WGS were relatively small and susceptibility against the new antibiotic combinations such as ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam and ceftolozane-tazobactam were not done; the latter being due to unavailability of these antibiotics in our countries at the time of the study.

In conclusion, the study has revealed that the prevalence of CRE in Kuwait was much higher than in Nigeria, that the resistant isolates were mediated by diverse mechanisms including production of OXA-181, NDM, presence of efflux pumps, combination of ESBLs and AmpC enzymes. It is advocated that preventing CRE infections and containing the spread of carbapenem resistance should be front runners in the infection control guidelines for each hospital.

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Contributions of authors:

All authors made substantial contributions to all of the following; (i) the conception and design of the study, or acquisition of data, or analysis and interpretation of data; (ii) drafting the article or revising it critically for important intellectual content; and (iii) final approval of the version the be submitted. All authors have approved the final manuscript.

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Declaration of interest:

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Original article **Open Access**

Profile of multidrug-resistant clinical bacterial isolates at the National Hospital of Zinder (NHZ), Niger Republic in 2021

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Abstract:

Background: Today, bacterial resistance is a public health challenge throughout the world, and infections caused by resistant bacteria are associated with increased morbidity, mortality and health care costs. The objective of this descriptive study is to determine the prevalence and distribution of multi-drug resistant (MDR) clinical bacteria isolates at the National Hospital of Zinder, Niger Republic in 2021.
Methodology: We conducted a descriptive cross-sectional study of in- and out-patients from whose clinical samples' bacteria were isolated at the bacteriology unit of the laboratory. Bacteria were isolated from the clinical samples following standard aerobic cultures and identified using conventional biochemical test schemes. Antibiotic susceptibility testing (AST) was performed by the agar disk diffusion technique, and categorization of the isolates into sensitive, intermediate or resistant was done according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM) 2020 version 1.2. MDR was defined as resistance to at least one antibiotic in three or more categories, while selected MDR bacteria such as ESBL was identified using double disk synergy test, and MRSA by cefoxitin disk diffusion test.
Results: Seventy-seven (6.7%) bacterial species were isolated from 1153 clinical samples processed at the bacteriology unit of the hospital laboratory between June and December 2021, of which 65.0% (50/77) were members of the order *Enterobacteriales. Escherichia coli* represented 40.3% (40/77) of the isolated bacteria, *Staphylococcus aureus* 13.0% (10/77) and *Pseudomonas aeruginosa* 11.7% (9/77). The overall prevalence of MDR was 44.2% (34/77), including 61.8% (21/34) ESBL-producing *Enterobacteriales* (ESBL-E), 26.5% (9/34) multi-resistant *P. aeruginosa* and 11.7% (4/34) MRSA, with 67.6% (23/34) of the MDR isolates from out-patients. Resistance rates of the *Enterobacteriales* to ciprofloxacin, gentamicin, amikacin and imipenem were 62.0%, 52.0%, 38.0% and 8.0% respectively. Resistance rates of *P. aeruginosa* were 100.0%, 88.9%, 77.8%, 33.3%, 22.2%, and 22.2% respectively to ceftazidime, ticarcillin, imipenem, ciprofloxacin, levofloxacin, and amikacin. Resistance rates of *S. aureus* were 100.0%, 50.0%, 40.0%, 10.0%, 0% and 0% to penicillin G, erythromycin, cefoxitin, tetracycline, fusidic acid, and chloramphenicol respectively. ESBL-E were 47.6%, 85.7% and 0% resistant to amikacin, ciprofloxacin and imipenem, and MRSA resistance rates were 75.0%, 75.0%, 50.0% and 0% to erythromycin, tetracycline, gentamicin, and chloramphenicol respectively.
Conclusion: This study reports high prevalence of MDR bacteria, mainly ESBL-E, with concerning high resistance to carbapenem. Rational use of antibiotics and implementation of surveillance system for MDR bacteria must be implemented in order to limit the emergence and spread of MDR bacteria in Niger Republic.

Keywords: MDR bacteria; inpatient; outpatient; Zinder; Niger Republic

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Profil des souches bactériennes multirésistantes isolées à l'Hôpital National de Zinder (HNZ), République du Niger en 2021

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Résumé:

Contexte: Aujourd'hui, la résistance bactérienne est un défi de santé publique dans le monde entier, et les infections causées par des bactéries résistantes sont associées à une augmentation de la morbidité, de la mortalité et des coûts des soins de santé. L'objectif de cette étude descriptive est de déterminer la prévalence et la distribution des isolats cliniques de bactéries multirésistantes (MDR) à l'Hôpital National de Zinder, République du Niger en 2021.
Méthodologie: Nous avons mené une étude transversale descriptive des patients hospitalisés et ambulatoires dont les bactéries des échantillons cliniques ont été isolées à l'unité de bactériologie du laboratoire. Les bactéries ont été isolées des échantillons cliniques à la suite de cultures aérobies standard et identifiées à l'aide de schémas de tests biochimiques conventionnels. Les antibiogrammes (AST) ont été réalisés par la technique de diffusion sur disque de gélose, et la catégorisation des isolats en sensibles, intermédiaires ou résistants a été faite selon les recommanda- tions du Comité Antibiogramme de la Société Française de Microbiologie (CA-SFM) 2020 version 1.2. La MDR a été définie comme la résistance à au moins un antibiotique dans trois catégories ou plus, tandis que certaines bactéries MDR telles que les BLSE ont été identifiées à l'aide d'un test de synergie à double disque et le SARM par le test de diffusion sur disque de céfoxitine.
Résultats: Soixante-dix-sept (6,7%) espèces bactériennes ont été isolées à partir de 1153 échantillons cliniques traités à l'unité de bactériologie du laboratoire hospitalier entre juin et décembre 2021, dont 65,0% (50/77) appartenaient à l'ordre des *Enterobacteriales*. *Escherichia coli* représentait 40,3% (40/77) des bactéries isolées, *Staphylococcus aureus* 13,0% (10/77) et *Pseudomonas aeruginosa* 11,7% (9/77). La prévalence globale des MDR était de 44,2% (34/77), dont 61,8% (21/34) d'*Enterobacteriales* productrices de BLSE (BLSE-E), 26,5% (9/34) de *P. aeruginosa* multirésistantes et 11,7% (4/34) SARM, avec 67,6% (23/34) des isolats de MDR provenant de patients externes. Les taux de résistance des *Enterobacteriales* à la ciprofloxacine, à la gentamicine, à l'amikacine et à l'imipénème étaient respectivement de 62,0%, 52,0%, 38,0% et 8,0%. Les taux de résistance de *P. aeruginosa* étaient respectivement de 100,0%, 88,9%, 77,8%, 33,3%, 22,2% et 22,2% à la ceftazidime, à la ticarcilline, à l'imipénème, à la ciprofloxacine, à la lévofloxacine et à l'amikacine. Les taux de résistance de *S. aureus* étaient respectivement de 100,0%, 50,0%, 40,0%, 10,0%, 0% et 0% à la pénicilline G, à l'érythromycine, à la céfoxitine, à la tétracycline, à l'acide fusidique et au chloramphénicol. Les BLSE-E étaient de 47,6%, 85,7% et 0% de résistance à l'amikacine, à la ciprofloxacine et à l'imipénème, et les taux de résistance au SARM étaient respectivement de 75,0%, 75,0%, 50,0% et 0% à l'érythromycine, la tétracycline, la gentamicine et le chloramphénicol.
Conclusion: Cette étude rapporte une prévalence élevée de bactéries MDR, principalement des BLSE-E, avec une résistance élevée aux carbapénèmes. L'utilisation rationnelle des antibiotiques et la mise en place d'un système de surveillance des bactéries MDR doivent être mises en œuvre afin de limiter l'émergence et la propagation des bactéries MDR en République du Niger.

Mots-clés: Bactérie MDR; patient hospitalisé; ambulatoire; Zinder; République du Niger

Introduction:

The discovery of antibiotic and its development for therapy from 1940s have considerably reduced the mortality associated with infectious diseases. However, the widespread use and misuse have resulted in the adaptation of bacteria, with emergence and dissemination of resistant bacteria that continue to be major cause of mortality and increased health care costs from resistant infections for over 50 years (1,2).

The World Health Organization (WHO) has estimated that infectious disease account for 45% of deaths in Africa and South-East Asia and bacterial infections account for a significant proportion of these in Africa (3). More recent estimates indicate 4.95 million associated deaths and 1.27 million attributable deaths due to AMR infections (4). Also, the WHO describes gaps in surveillance despite the threat presented by AMR pathogens. This lack of quality data is problematic and leads to treatment guidelines that are not adapted to the local context (5). In order to better understand the problems and to effectively tackle these, it is necessary to conduct epidemiological surveillance of bacteria.

In Niger Republic, few studies have been conducted on the epidemiology and antimicrobial susceptibility of bacteria to antibiotics. It is for this reason that we conducted this study to determine the epidemiology and susceptibility profiles of clinical bacteria isolates at the National Hospital of Zinder (NHZ) in Niger Republic.

Materials and method:

Study setting and population

We conducted a descriptive cross-sectional study at the National Hospital of Zinder, Niger Republic, from June to December 2021. National Hospital of Zinder is 740 bed capacity hospital with several clinical service departments and units. The study population was composed of all patients (in-patients and outpatients) from whom clinical samples (urine, pus, stool and others) were collected and submitted to the bacteriology unit of the laboratory for analysis.

Only patients from whom bacteria were isolated from their samples during the period of the study were included. Clinical and laboratory variables of the patients such as age, sex, bacterial species isolated, antibiotic sensitivity profile, and multidrug-resistant (MDR) patterns were collected with a designed data collection form.

Ethical approval

The study was approved by the National Hospital of Zinder ethical committee. Anonymity and confidentiality of the data were guaranteed.

Isolation and identification of bacteria

After macroscopic and microscopic examinations of clinical samples, pus was plated on Chapman (Mannitol salt agar) and fresh blood agar; urine was plated on CLED agar; stool samples were plated on Hektoen enteric medium for *Salmonella* and *Shigella*, and on Sorbitol MacConkey agar for *Escherichia coli*. The inoculated media were incubated aerobically at 37°C for 18-24 hours. The identification of bacterial isolates on culture plates was performed on the basis of their cultural characteristics and conventional biochemical test schemes (6)

Antibiotic susceptibility testing

Antibiotic susceptibility testing (AST) was performed using the agar disk diffusion technique (7) against selected antibiotics including penicillin (penicillin, amoxicillin, amoxicillin-clavulanate, piperacillin, piperacillin-tazobactam, ticarcillin, ticarcillin-clavulanic acid), cephalosporin (cefalotin, cefoxitin, ceftriaxone, cefixime, cefotaxime, ceftazidime), aztreonam, carbapenem (imipenem) aminoglycoside (gentamicin, kanamycin, amikacin, tobramycin), fluoroquinolone (ciprofloxacin. levofloxacin), sulfonamide (cotrimoxazole), macrolide (erythromycin), tetracycline, chloramphenicol and fusidic acid. The categorization of the isolates into sensitive, intermediate or resistant was done according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM) 2020 version 1.2. Multi-drug resistance was defined as resistance to at least one antibiotic in three or more antibiotic categories (8).

Detection of selected multi-resistant bacteria

ESBL phenotype was detected on standard antibiogram according to the double disk synergy technique described by Jarlier et al, (9), which entails demonstrating synergy between clavulanic acid disc and a third-generation cephalosporin (cefotaxime, ceftazidime or ceftriaxone) separated by 30 mm on the agar plate. The presence of ESBL was determined after 18 to 24 hours of incubation by the appearance of 'champagne cork' synergistic action between the two antibiotics. The detection of methicillin resistance in *S. aureus* was performed by measuring the zone of inhibition around a 30µg cefoxitin disk (as surrogate for methicillin resistance) of less than 22 mm on Mueller Hinton agar plate after 24 hours of incubation (7).

Statistical analysis

The data were entered into Microsoft Excel 2013 and statistical analysis was performed using EPI INFO software v 3.5.4.

Results:

Sociodemographic characteristics of patients

A total of 77 patients from whose clinical samples' bacteria were isolated formed the study participants, with a male to female ratio of 1.5. The mean age of the patients was 34.2±22.85 years, with a range of 5 months to 78 years. The socio-demographic data of the patients are summarized in Table 1.

The bacteria isolates were predominantly recovered from clinical samples of outpatients (62.3%, 48/77), urine was the most common specimens (56.7%, 40/77), and *Enterobacteriales* constituted majority of the isolates (65%, 50/77).

Distribution of isolated bacteria by species

Of the 77 bacteria isolates, *E. coli* represents 40.3% (n=31) and thus constitutes the majority of the species isolated, followed by *S. aureus* and *P. aeruginosa* representing respectively 13% and 11% of the isolated bacteria (Table 2).

Table 1: Distribution of the study population by age, gender, patient type and sample

Characteristics	Frequency	Percentage
Gender		
Male	47	61.0
Female	30	39.0
Mean age (years)	34.2±22.85	
Age range	5 months-78 years	
Type of patients		
Inpatient	29	37.7
Outpatient	48	62.3
Types of samples		
Urine	40	56.7
Pus	28	36.4
Others	9	6.9

Table 2: Distribution of isolated bacteria by Taxonomical groups and species

Isolated bacteria	Numbers	Percentage
Order Enterobacteriales (n=50)		
Escherichia coli	31	40.3
Klebsiella pneumoniae	6	7.8
Klebsiella oxytoca	2	2.6
Klebsiella spp	1	1.3
Citrobacter koseri	1	1.3
Morganella morganii	1	1.3
Proteus mirabilis	3	39
Providencia stuartii	1	1.3
Serratia odorifera	2	2. 6
Salmonella spp	2	2. 6
Order Pseudomonadales (n=10)		
Pseudomonas aeruginosa	9	11.7
Acinetobacter baumannii	1	1.3
Gram positive bacteria (n=17)		
Staphylococcus aureus	10	13.0
Coagulase negative staphylococci	6	7.8
Enterococcus faecalis	1	1.3
Total	**77**	**100. 0**

Antibiotic susceptibility of bacterial isolates

All *Enterobacteriales* isolates (n=50) were resistant to amoxicillin (Table 3). However, the activity of amoxicillin on them was recovered by the combination with clavulanic acid. High level resistance was expressed by the *Enterobacteriales* to 3rd generation cephalosporins and other antibiotics, with resistance rates to ceftriaxone, cefixime, ciprofloxacin and gentamicin of 60%, 64%, 62% and 52% respectively. Amikacin was the most active antibiotic on the *Enterobacteriales* with 62% sensitivity of the isolates.

All the *S. aureus* (n=10) isolates were resistant to penicillin G, and 40% were resistant to cefoxitin, which was used as surrogate for MRSA detection. Aminoglycosides, fusidic acid and cotrimoxazole had good anti-bacterial activity with sensitivity rates of 75% to gentamicin, 87.5% to fusidic acid and 62.5% to cotrimoxazole. The best activity was obtained with chloramphenicol and clindamycin for which no isolate expressed resistance (100% sensitivity). In contrast, the lowest activity was obtained with levofloxacin with a sensitivity rate of only 28.6%.

Pseudomonas aeruginosa (n=9) isolates exhibited high resistance to carboxy- and ureido-penicillins, aminoglycosides and the carbapenems, with resistance rates of 85.7% to ticarcillin, 100% to piperacillin, 71.4% to tobramycin, and 100% to imipenem and ceftazidime. Nevertheless, levofloxacin and amikacin had high activity, with sensitivity rates of 71.4% for levofloxacin and 85.7% for amikacin (Table 3).

Prevalence of multidrug-resistant bacteria

A total of 34 of the 77 isolates were multi-drug-resistant (MDR) representing a prevalence of 44.2% (Fig 1). They were isolated most often in the male patients (46.8%, 22/47) but this was not significantly different from isolation rate from the female patients (40.0%, 12/30) (OR=1.320, 95%CI= 0.5216 -3.341, p=0.7253) as shown in Table 4. The ESBL-E constituted the majority of the isolated MDR bacteria (61.8%, 21/34), followed by multi-resistant *P. aeruginosa* (resistant to ceftazidime and/or imipenem) (26.5%, 9/34) and MRSA 11.7% (4/34) (Fig 2).

The MDR bacteria were isolated more from samples of outpatients (47.9%, 23/48) than from samples of inpatients (37.9%, 11/29) but the isolation rate was not significantly different (OR=0.6643, 95%CI=0.2595-1.701, p=0.5365). Urine (47.5%, 19/40) was the most frequent specimens from which MDR bacteria were isolated, followed by pus (39.3%, 11/28) and others (22.2%, 2/9), but this isolation rate was also not significantly different (x^2=2.026, p=0.3630) (Table 4).

Table 3: Antibiotic resistance rates of some selected bacteria isolates to antimicrobial agents

Antibiotics	*Enterobacteriales* (%) (n=50)	*E. coli (%)* (n=31)	*Klebsiella* spp (%) (n=9)	*P. mirabilis* (%) (n =3)	*P. aeruginosa* (%) (n=9)	*S. aureus* (%) n=10
PG	NT	NT	NT	NT	NT	10 (100.0)
AMX	50 (100.0)	31 (100.0)	9 (100.0)	3 (100.0)	NT	NT
AMC	15 (30.0)	8 (25.8)	-	2 (66.6)	NT	NT
PIP	46 (92.0)	30 (96.8)	2 (22. 2)	3 (100.0)	9 (100.0)	NT
TIC	46 (92.0)	29 (93.5)	9 (100.0)	3 (100.0)	9 (100.0)	NT
PIT	46 (92.0)	30 (96.8)	9 (100.0)	2 (66.7)	9 (100.0)	NT
TCC	45 (90.0)	29 (93.5)	9 (100.0)	1 (33.3)	8 (88.9)	NT
KF	48 (94.0)	30 (96.8)	9 (100.0)	2 (66,7)	NT	NT
CX	19 (38.0)	12 (38.7)	3 (33.3)	1 (33.3)	NT	4 (40.0)
CTR	30 (60.0)	20 (64.5)	6 (66.7)	1 (33.3)	NT	NT
CFM	32 (64.0)	21 (67.7)	6 (67.7)	1 (33.3)	NT	NT
CAZ	33 (66.0)	22 (71.0)	6 (66.7)	1 (33.3)	9 (100.0)	NT
CTX	32 (64.0)	21 (67.7)	6 (67.7)	1 (33.3)	NT	NT
IMP	4 (8.0)	3 (9.7)	0	0	7 (77.8)	NT
ATM	28 (56.0)	20 (64.5)	5 (55.6)	0	NT	NT
K	NT	NT	NT	NT	NT	3 (30.0)
AK	19 (38.0)	14 (45.2)	1 (11.1)	1 (33.3)	2 (22.2)	NT
CN	26 (52.0)	16 (51.6)	5 (55.6)	1 (33.3)	NT	2 (20.0)
TOB	26 (52.0)	17 (54.8)	4 (44.4)	1 (33.3)	6 (66.7)	2 (20.0)
CIP	31 (62.0)	21 (67.7)	5 (55.6)	1 (33.3)	3 (33.3)	NT
LEV	NT	NT	NT	NT	2 (22.2)	7 (70.0)
TET	NT	NT	NT	NT	NT	10 (100.0)
AF	NT	NT	NT	NT	NT	1 (10.0)
CHL	NT	NT	NT	NT	NT	0
E	NT	NT	NT	NT	NT	4 (40.0)
COT	NT	NT	NT	NT	NT	2 (20.0)

PG=Penicillin G, AMX=Amoxicillin, AMC=Amoxicillin-clavulanate, PIP=Piperacillin, TIC=Ticarcillin, PIT=Piperacillin-Tazobactam, TCC=Ticarcillin-clavulanic acid, KF=Cefalotin, CX=Cefoxitin, CTR=Ceftriaxone, CFM=Cefixime, CAZ=Ceftazidime, CTX=Cefotaxime, IMP=Imipenem, ATM=Aztreonam, K=Kanamycin, AK=Amikacin, CN=Gentamicin, TOB=Tobramycin, CIP=Ciprofloxacin, Lev=Levofloxacin, TET=Tetracycline, AF=Fusidic acid, CHL=Chloramphenicol, E=Erythromycin, COT=Cotrimoxazole, NT=Not tested ; n= number of resistant isolates

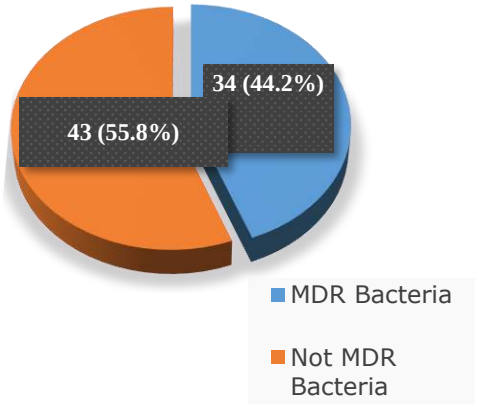

Fig 1: Prevalence of MDR bacteria in the study

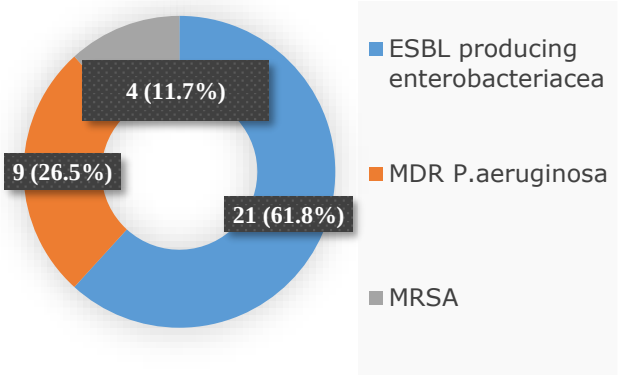

Fig 2: Distribution of MDR bacteri*a* species

Table 4: Statistical analysis of some variables with prevalence of MDR bacteria

Characteristic variables	Number of patients with MDR	x^2	OR (95% CI)	*p* value
Gender				
Male (n=47)	22 (46.8)	0.1235	1.320 (0.5216-3.341)	0.7253$^+$
Female (n=30)	12 (40.0)			
Total (n=77)	34 (44.2)			
Type of patients				
Inpatient (n=29)	11 (37.9)	0.3822	0.6643 (0.2595-1.701)	0.5365$^+$
Outpatient (n=48)	23 (47.9)			
Types of samples				
Urine (n=40)	19 (47.5)	2.026	NA	0.3630$^+$
Pus (n=28)	11 (39.3)			
Others (n=9)	2 (22.2)			

MDR=multi-drug resistance; x^2=Chi square; OR=Odds ratio; CI=Confidence interval; NA=Not applicable; +=not statistically significant

Table 5: Resistance rates of MRSA and ESBL-E to selected antibiotics

Antibiotics	ESBL-E (%) (n=21)	MRSA (%) (n=4)
Amikacin	10 (47. 6)	NT
Kanamycin	NT	3 (75)
Gentamicin	14 (66.7)	2 (50)
Tobramycin	13 (61.9)	2 (50)
Imipenem	0	NT
Ciprofloxacin	18 (85.7)	NT
Cefoxitin	11 (52.4)	2 (50)
Cotrimoxazole	NT	3 (75)
Erythromycin	NT	3 (75)
Chloramphenicol	NT	0
Fusidic acid	NT	1 (25)
Clindamycin	NT	0
Levofloxacin	NT	4 (100)

MRSA=methicillin-resistant *Staphylococcus aureus*; ESBL-E=Extended spectrum β-lactamase-*Enterobacteriales*; NT=No tested

Antibiotic profile of selected MDR bacteria

ESBL-producing *Enterobacteriales* isolates exhibited high resistance to the antibiotics tested in this study. The resistance rates to ciprofloxacin, gentamicin and tobramycin were 85.7%, 66.7% and 61.9% respectively. Imipenem was the most active among the antibiotics tested against ESBL-E with sensitivity rate of 100%, followed by amikacin with sensitivity rate of 52.4% (Table 5).

Resistance rates of MRSA to kanamycin, gentamicin and cotrimoxazole were 75%, 50% and 75% respectively. The highest antibiotic activities against MRSA isolates were obtained with clindamycin (100% sensitivity), chloramphenicol (100% sensitivity) and fusidic acid (75% sensitivity) (Table 5).

Discussion:

In this study, the majority of bacterial species isolated were *E. coli* (40.3%), which is identical to the results obtained by Nadembega et al., (10) in Burkina Faso with 40.2%. Also, Salou et al., (11) in Togo and Okalla Ebongue et al., (12) in Cameroon reported predominance of *E. coli* among the species of bacteria isolated in clinical settings.

Members of the order *Enterobacteriales* isolated from this study showed high resistance rates to penicillins with 100% resistance to amoxicillin. Gangoué-Piéboji (13) reported 87% resistance rate of *Enterobacteriaceae* isolates to amoxicillin in Cameroon. Our finding could be explained by the very frequent use of amoxicillin, especially for self-medication. However, the combination with clavulanic acid restored the activity of amoxicillin on the *Enterobacteriales* of up to 70% in our study. Imipenem and amikacin were the most active antimicrobial molecules on the *Enterobacteriales* with *in vitro* inhibition of 92% and 62% respectively. These results are in line with those from Okalla Ebongue et al., (12) in Cameroon and Affolabi et al., (14) in Benin Republic.

The resistance rate of *P. aeruginosa* to ceftazidime was 100% and 85.7% to imipenem in this study. A low resistance rate to imipenem was reported by Ettu et al., (15) in Nigeria, but Osundiya et al., (16) in Lagos reported high ceftazidime resistance rate of 79.4%, while ceftazidime resistance rate of 23.5% was reported by Kpoda et al., (17) in Burkina Faso. Our study report resistance rate of *P. aeruginosa* to ciprofloxacin of 33.3% but a higher rate (73.8%) was reported by Manga et al., (18) in Nigeria. Levofloxacin and amikacin had the best activity against *P. aeruginosa* isolates with sensitivity rate of 77.2% for each antibiotic in our study. These results are in agreement with those reported by Sakr et al., (19).

All the *S. aureus* isolates in our study were resistant to penicillin G, which is similar to the results reported by Andrianarivelo et al., (20) in Madagascar and by Salem et al., (21) in Mauritania. Resistance rates to cefoxitin used as surrogate for MRSA was 40% in our study. A cefoxitin resistance rate of 54% was reported by Olabi et al., (22) and Adegoke et al., (23) reported a similar result in Nigeria. The sensitivity rate of *S. aureus* to cotrimoxazole was 80% in our study, which is similar to the rate reported by Salem et al., (21) in Nouakchott Mauritania, but higher rate of 91.7% was reported by Elhamzaoui et al., (24) in Morocco. Resistance rate of *S. aureus* to gentamicin was 20% in our study but lower rates were reported in different studies conducted in Madagascar with 5.67% (18), Burkina Faso with 0% (25), Uganda with 0% (26) and Morocco with 2.2% (24). The best anti-staphylococcal activities were exhibited by chloramphenicol (100% sensitivity), clindamycin (100% sensitivity) and fusidic acid (90% sensitivity), which was in concordance with results from studies conducted in Burkina Faso (25) and Madagascar (20).

The overall MDR prevalence rate of 44% (34/77) was obtained for the isolates in our study. Lower prevalence rate was reported in Tunisia by Kooli et al., (27) but a higher rate of 68% was reported by Metwally et al., (28). ESBL-producing *Enterobacteriales* constituted the majority of the MDR bacteria (61.8%. 21/34), followed by MDR *P. aeruginosa* (26.5%, 9/34) and MRSA (11.7%, 4/34). Saidani et al., (29) had previously reported a predominance of ESBL-producing *Enterobacteriaceae* in their study. In our study, ESBL-E expressed high resistance to aminoglycosides with 66.7% to gentamicin, 61.9% to tobramycin and 47.6% to amikacin. A study carried out in Madagascar reported resistance rates of 78.3% to gentamicin, 26.1% to tobramycin, but absolute sensitivity to amikacin (30). The sensitivity to amikacin seems to be more preserved compared to the other aminoglycosides. Imipenem remained active on all the ESBL-E isolates in our study. These results are consistent with those of Rakotovao in Madagascar (30), and in Burkina Faso by Zongo et al., (31). Ciprofloxacin showed low activity on ESBL-E with 85.7% resistance rate. This high level of resistance among *Enterobacteriales* to fluoroquinolones is alarming, and is thought to be the result of the selection pressure created by over- and mis-use of fluoroquinolones, especially in the treatment of urinary and digestive infections.

In our study, the resistance rates of MRSA isolates to macrolides and aminoglycosides were 75% for erythromycin and 50% for gentamicin. The study by Ojulong et al., (32) in Uganda reported 58.8% resistance to gentamicin and 88.2% to erythromycin but absolute resistance of MRSA isolates to gentamicin and erythromycin was reported by Onwubiko et al., (33) in a study conducted in Kano, Nigeria. However, due to the low number of MRSA isolates in our study, interpretation of our findings have to be done with caution.

Conclusion:

The phenomenon of antibiotic resistance is a reality in Zinder, Niger Republic. Our study reports high antibiotic resistance expressed by clinical bacteria isolates, with high prevalence of MDR bacteria circulating both in the community and the hospital. Appropriate control measures must be desi-

gned and implemented to reduce the prevalence of MDR bacteria in Republic of Niger.

Contributions of authors:

All authors contributed equally to the study design, methodology, manuscript preparation and validation of the original version submitted for publication.

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No conflict of interest is declared

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Original Article **Open Access**

In vitro antibiotic susceptibility of bacterial pathogens and risk factors associated with culture positive neonatal sepsis in two hospitals, Katsina metropolis, Nigeria

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Abstract:

Background: Neonatal sepsis is one of the most important causes of morbidity and mortality among neonates, particularly in developing countries. This study aimed to determine the risk factors and *in vitro* antibiotic susceptibility patterns of bacterial pathogens associated with neonatal sepsis in Federal Medical Centre (FMC) and Turai Umaru Yar'adua Maternal and Children Hospital (TUYMCH), Katsina, Nigeria.
Methodology: A total of 60 hospitalized neonates evaluated for neonatal sepsis at the special care baby units (SCBU) of the two healthcare facilities whose parents gave informed consent were enrolled for the study between July and December 2020. Blood samples were aseptically collected from the neonates and cultured on BacT/Alert automated platform (BioMérieux, Mercy-Etoile, France) machine. Bacteria were identified from all positive cultures and *in vitro* susceptibility test was performed on the isolates to determine their minimum inhibitory concentrations (MICs) to eight selected antibiotics using the Vitek-2 compact system. Data were analyzed by SPSS version 22.0.
Results: A total of 60 neonates with clinical features suggestive of sepsis were enrolled. The mean age of the neonates is 1.35±0.48 days while the mean weight is 2.13±0.89 kg. Neonates with early onset sepsis (<3 days) constituted 65% while those with late-onset sepsis (>3 days) constituted 35%. Thirty-one (51.7%) neonates were culture positive while 29 (48.3%) were culture negative for bacterial pathogens. Gram-positive bacteria predominated, constituting 80.6% while Gram-negative bacteria constituted 19.4%. The most frequent Gram-positive bacteria were coagulase-negative staphylococci (51.6%, 16/31), with *Staphylococcus haemolyticus* 5 (16.1%) predominating, while the most frequent Gram-negative bacteria isolate was *Escherichia coli* 2 (6.5%). A high degree of antibiotic resistance (>50% rate) was exhibited by the isolates against most of the tested antibiotics including third generation cephalosporins and fluoroquinolones. Gentamicin was the only antibiotic effective, with 65.5% of all isolates sensitive to it; 68.0% Gram-positives and 50.0% Gram-negatives. Vancomycin was also effective against Gram-positive bacteria, with 68.0% of the isolates sensitive to it. Previous premature delivery (64.5%, 20/31) and baby delivery at home were respectively the only maternal and neonatal factors significantly associated with culture-positive neonatal sepsis (OR=2.975, 95% CI=1.040-8.510). There was no significant difference between culture positive and negative neonatal sepsis with respect to clinical manifestations such as refusal of feeds, fever, jaundice, fast breathing, convulsion and body temperature (p>0.05).
Conclusion: Neonatal sepsis is a substantial cause of mortality and morbidity among neonates admitted at the FMC and TUYMCH, Katsina, Nigeria. There is a need for regular surveillance of the risk factors, causative organisms, and antibiotic susceptibility patterns of isolated pathogens, to inform the choice of empirical antibiotic treatment pending the results of blood cultures.

Keywords: neonates, sepsis, risk factor, antibiotic, bacteria.

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Sensibilité aux antibiotiques in vitro des pathogènes bactériens et facteurs de risque associés à une septicémie néonatale à culture positive dans deux hôpitaux, métropole de Katsina, Nigeria

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Résumé:

Contexte: La septicémie néonatale est l'une des causes les plus importantes de morbidité et de mortalité chez les nouveau-nés, en particulier dans les pays en développement. Cette étude visait à déterminer les facteurs de risque et les schémas de sensibilité aux antibiotiques in vitro des agents pathogènes bactériens associés à la septicémie néonatale dans le Centre Médical Fédéral (FMC) et le Turai Umaru Yar'adua Hôpital de la Mère et de l'Enfant (TUYMCH), Katsina, Nigeria.
Méthodologie: Un total de 60 nouveau-nés hospitalisés évalués pour une septicémie néonatale dans les unités de soins spéciaux pour bébés (SCBU) des deux établissements de santé dont les parents ont donné leur consentement éclairé ont été inclus dans l'étude entre juillet et décembre 2020. Des échantillons de sang ont été prélevés de manière aseptique sur les nouveau-nés et cultivés sur la plate-forme automatisée BacT/Alert (BioMérieux, Mercy-Etoile, France). Les bactéries ont été identifiées à partir de toutes les cultures positives et un test de sensibilité in vitro a été effectué sur les isolats pour déterminer leurs concentrations minimales inhibitrices (CMI) à huit antibiotiques sélectionnés à l'aide du système compact Vitek-2. Les données ont été analysées par SPSS version 22.0.
Résultats: Au total, 60 nouveau-nés présentant des caractéristiques cliniques évoquant une septicémie ont été recrutés. L'âge moyen des nouveau-nés est de 1,35±0,48 jours alors que le poids moyen est de 2,13±0,89 kg. Les nouveau-nés atteints d'un sepsis précoce (<3 jours) représentaient 65% tandis que ceux atteints d'un sepsis tardif (>3 jours) représentaient 35%. Trente et un (51,7%) nouveau-nés étaient positifs à la culture tandis que 29 (48,3%) étaient négatifs à la culture pour les pathogènes bactériens. Les bactéries Gram-positives prédominaient, constituant 80,6% tandis que les bactéries Gram-négatives constituaient 19,4%. Les bactéries à Gram positif les plus fréquentes étaient les staphylocoques à coagulase négative (51,6%, 16/31), *Staphylococcus haemolyticus* 5 (16,1%) prédominant, tandis que l'isolat de bactéries à Gram négatif le plus fréquent était *Escherichia coli* 2 (6,5%). Un degré élevé de résistance aux antibiotiques (> 50% de taux) a été présenté par les isolats contre la plupart des antibiotiques testés, y compris les céphalosporines de troisième génération et les fluoroquinolones. La gentamicine était le seul antibiotique efficace, avec 65,5% de tous les isolats qui y étaient sensibles; 68,0% de Gram positifs et 50,0% de Gram négatifs. La vancomycine était également efficace contre les bactéries Gram-positives, 68,0% des isolats y étant sensibles. Un accouchement prématuré antérieur (64,5%, 20/31) et un accouchement à domicile étaient respectivement les seuls facteurs maternels et néonatals significativement associés à une septicémie néonatale à culture positive (OR=2,975, IC 95%=1,040-8,510). Il n'y avait pas de différence significative entre la septicémie néonatale positive et négative à la culture en ce qui concerne les manifestations cliniques telles que le refus de s'alimenter, la fièvre, la jaunisse, la respiration rapide, les convulsions et la température corporelle (p>0,05).
Conclusion: La septicémie néonatale est une cause importante de mortalité et de morbidité chez les nouveau-nés admis au FMC et TUYMCH, Katsina, Nigeria. Il est nécessaire de surveiller régulièrement les facteurs de risque, les organismes responsables et les schémas de sensibilité aux antibiotiques des agents pathogènes isolés, afin d'éclairer le choix d'un traitement antibiotique empirique en attendant les résultats des hémocultures.

Mots-clés: nouveau-nés, septicémie, facteur de risque, antibiotique, bactérie.

Introduction:

Sepsis is a significant cause of neonatal morbidity and mortality, especially in developing countries. The microbial organisms of sepsis and their antimicrobial susceptibility patterns are dynamic (1). Despite several attempts to lessen its effect, neonatal sepsis has remained a significant cause of morbidity and mortality in neonates. It remains one of the leading causes of death in neonates, both in the developed and developing countries (2). The neonatal period is the most endangered period of life due to vulnerability to infectious agents. Neonates are deficient in both cell-mediated and humoral immunity due to relative immaturity of their immune systems and lack of exposure to infectious pathogens, and the fact that they produce immunoglobulins at lower proportion compared to adults (2).

Neonatal sepsis is a clinical condition comprising of non-specific signs and symptoms of infection, accompanied by bacteremia in the first 28 days of life. It is characterized by systemic signs of circulatory compromise such as decrease peripheral perfusion, pallor, hypothermia, and poor responsiveness (3). Neonatal sepsis and mortality risk increase with decreasing birth weight and gestational age (4). Neonatal sepsis may also refer to neonatal systemic infection, including septicemia, pneumonia, meningitis, urinary tract infection, arthritis, and osteomyelitis (5). Neonatal sepsis could be early-onset in the first 72 hours of life and known to be acquired through prenatal and intrapartum maternal transmission, while late-onset sepsis starts from the fourth day to the fourth week of life (5).

Globally, about 40% of deaths in under-five children occur in the neonatal period resulting in about 2.9 million neonatal deaths each year (6). The highest mortality rates for neonates occur in the developing and poorest countries and a third of these deaths are ascribed to

infections acquired by the neonate during labor and delivery or immediately after birth (6).

Nigeria is said to account for the highest number of neonatal deaths in Africa and third in the world, after India and China, with sepsis responsible for about 30% to 50% of deaths (7). Prevalence of sepsis in neonates reported from earlier hospital-based studies ranges between 7.04 and 22.9 per 1000 live births (8). Sepsis-related case fatality rates are mostly preventable with appropriate antimicrobial use and aggressive supportive care. Nevertheless, neonatal sepsis has no pathognomonic features and the clinical presentations also varies (5). Poor or delayed laboratory services also make laboratory diagnosis problematic in resource-limited settings. As a result, neonatal healthcare providers in resource-poor settings make an uncertain diagnosis and empirical treatment of neonatal sepsis, using the new neonatal international management guidelines of the World Health Organization (3).

Certain pathogens implicated in neonatal sepsis have progressively developed increased resistance to frequently used antibiotics due to the selection burden, which is an unavoidable phenomenon in antimicrobial use and thus makes treatment of neonatal sepsis very difficult (2). However, the variety of organisms causing neonatal sepsis differs significantly across different regions and countries, and changes over time, even in the same place. This variation may affect the achievement of empirical management (3). In the developed countries, the most common organisms of neonatal sepsis are Group B streptococci (GBS), *Escherichia coli,* and *Listeria monocytogenes* while Gram-negative bacteria and coagulase-negative staphylococci are the commonest in the developing countries (9).

It is notable that the rising incidence of drug-resistant bacteria has also made treatment more problematic and expensive (10). It is important, therefore, that the epidemiology of neonatal sepsis should be regularly updated to provide information required for regular review of the choice of antimicrobials most appropriate for the treatment of sepsis in neonates, in different places and at different times (11).

The choice of antibiotic therapy for the treatment of neonatal sepsis is still challenging due to the emergent antibiotic resistance to the common antibiotics used in the treatment of infection. The aim of this study therefore is to determine the risk factors and *in vitro* antibiotic susceptibility of bacterial pathogens associated with neonatal sepsis in Katsina metropolis of Nigeria.

Materials and method:

Study area

The study was carried out at the special care baby units (SCBU) of the Federal Medical Centre, Katsina, a tertiary health facility, and Turai Umar Yar'adua Maternity and Children Hospital, Katsina, northwest Nigeria. Katsina is situated about 260 kilometres east of the city of Sokoto and 135 kilometres northwest of Kano, and shares international border with the Niger Republic, and has a total area of 24,192 km^2 and geographical coordinates of $12^0$15'N and $7^0$30'E. As at 2016, the estimated population of Katsina metropolis was 505,000.

Katsina is predominantly an agrarian society with majority of the inhabitants being of the Hausa-Fulani tribe. The two hospitals were preferred as a study area due to availability of the study population and laboratory facilities required for the research. The hospitals provide newborn healthcare services to the population of Katsina metropolis and the surrounding local government areas.

Study design and duration

This was a hospital-based descriptive observational study of neonates with clinical features of sepsis, and involved collection and microbiological analysis of blood specimens as well as administration of structured questionnaire on each enrolee to collect relevant socio-demographic and clinical information. The study was carried out between July and December 2020.

Ethical consideration

Ethical approvals were obtained from the Ethic and Research Committees of the Federal Medical Centre, Katsina (FMCNHREC.REG. N003/082012) and the Katsina State Ministry of Health (MOH/ADM/SUB/1152/1/350). The study was carried out in line with the WHO guidelines for research conduct on human subjects. Participant information sheet was issued and consent form was signed by the parents of selected neonates.

Sample size, selection criteria, and sampling method

A total of 60 neonates admitted into the special care baby units (SCBU) of the two hospitals being evaluated for sepsis during the period of the study were included**.** Neonates whose parents or guardians declined consent, and those already commenced on antibiotics were excluded from the study.

Data and sample collection

The socio-demographic and clinical information of each enrolee were collected using a structured and interviewer-administered questionnaire, consisting of variables such as gender, age (in days), gestational age at birth, birth weight, place of delivery, presenting symptoms, onset of symptom, and clinical signs as well as maternal variables such as history of maternal fever during pregnancy, age, weight, ethnicity, and antenatal care during pregnancy. The questions were interpreted verbally into Hausa language where necessary.

About 2 millilitres of blood samples were collected from each neonate enrolee before administration of antibiotic therapy, from a peripheral vein using aseptic technique. The blood was inoculated immediately into a properly labeled Bact/Alert sample bottle, which was then transferred to a closed container and immediately transported to the laboratory.

Bacterial detection using the BacT/Alert blood culture detection system

The BacT/Alert microbial detection system and culture bottle offer both microbial detection system and culture media with appropriate nutritional and environmental conditions for microorganisms that may be present in the test samples. Inoculated bottles are placed into the device where they are incubated and continuously observed for the presence of microorganisms that will grow in the Bact/Alert bottles (12). The system uses a colorimetric sensor and reflected light to monitor the existence and production of carbon dioxide (CO_2) which is dissolved in the culture medium. With the presence of microorganisms in the test sample, carbon dioxide is produced as the organisms metabolize the substrates in the culture medium, which cause the color of the gas-permeable sensor at the bottom of the culture bottle to change to yellow (12).

The BacT/Alert disposable culture bottle contains 40 ml pancreatic digest of casein (1.7% w/v), papain digest of soybean meal (0.3% w/v), sodium polyanethol sulfonate (SPS) (0.035% w/v), pyridoxine HCl (0.001% w/v), supplementary complex amino acid and carbohydrate substrates in purified water. The inoculated culture bottles were loaded into the BacT/Alert machine for aerobic incubation for 5 days or until culture positive bottles, recognized by color change to yellow, are acknowledged and removed for bacterial identification through Gram stain smears and sub-cultures on blood and MacConkey agar plates. Negative cultures were checked by smear and subculture at interval prior to discarding them as negative if there were no growth on sub-cultures. The procedures for loading and unloading the culture bottles into the BacT/Alert machine were done in line with the manufacturers' user-instruction manual.

Bacterial identification and antibiotic susceptibility test

The Vitek-2 compact system (30 card capacity), a computerized microbiology device that utilizes growth-based technology (13,14) was used for bacterial identification and susceptibility testing of the isolates. The machine uses a fluorogenic procedure for microbial identification and a turbidimetric technique for susceptibility testing to generate minimum inhibitory concentration (MIC) data, using a 64 well card which is barcoded with data on card type, expiration date, lot number, and unique card identification number. The test kits used were ID-GN (for Gram-negative bacilli identification), ID-GP (for Gram-positive cocci identification), AST-GN (for Gram-negative susceptibility) and AST-GP (for Gram-positive susceptibility). The Vitek-2 ID-GN card recognizes 154 species of *Enterobacteriaceae* and a select group of glucose non-fermenting Gram-negative bacteria within about 10 hours. The Vitek-2 ID-GP card recognizes 124 species of staphylococci, streptococci, enterococci, and a group of Gram-positive bacteria within about 8 hours. The AST results were available for bacteria in less than 18 hours.

The identification cards were inoculated with bacterial suspensions by means of an integrated vacuum device. A test tube holding the suspension was placed in a distinct rack (called cassette) and the identification card was placed in the adjacent slot while implanting the transfer tube into the corresponding suspension tube. The filled cassette was then placed manually into a vacuum chamber station. The Vitek-2 card and sample were connected via barcode. Once the cassette was loaded, the device handles all successive steps for inoculation and interpretation with regards to identification and susceptibility test. Eight pre-installed antibiotics (gentamicin, ciprofloxacin, cefuroxime, ceftriaxone, ceftazidime, augmentin, meropenem, and vancomycin) were tested for each isolate, except vancomycin for only Gram-positive isolates. The manufacturer's instructions were strictly followed during bacterial identification and antibiotic susceptibility test.

Data processing and statistical analysis:

All demographic and clinical data were analyzed using SPSS version 22.0. The Chi-square (x^2) test and Odds ratio (with 95% confidence interval) were used to determine associa-

tion of risk factors with culture-positive and culture-negative sepsis, and *p* value less than 0.05 was considered statistically significant.

Results:

A total of 60 hospitalized neonates evaluated for suspected neonatal sepsis at the special care baby units (SCBU) of the Federal Medical Centre (FMC) and Turai Umaru Yar'adua Maternal and Children Hospital (TUYMCH), Katsina, Nigeria, were enrolled into study between July and December 2020. The clinical parameters and laboratory culture results of neonates are presented in Table 1. The mean age of the neonates is 1.35±0.48 days, while the mean weight is 2.13±0.89 kg. Neonates with early onset sepsis (<3 days) constituted 65.0% while those with late-onset sepsis (>3 days) were 35.0%. Of the 60 blood samples collected from the neonates, 31 (51.7%) were culture positive while 29 (48.3%) were culture negative.

Table 2 shows the distribution of bacteria associated with neonatal sepsis among the neonates. Gram-positive bacteria predominated, constituting 80.6% (25/31) while Gram-negative bacteria constituted just 19.4% (6 of 31). The most frequent Gram-positive bacteria were coagulase-negative staphylococci (51.6%, 16/31), with *Staphylococcus haemolyticus* 5 (16.1%), *Staphylococcus epidermidis* 4 (12.9%) and *Staphylococcus hominis* 2 (6.5%). *Staphylococcus aureus* constituted 12.9% (4/31) while *Enterococcus* spp constituted 9.7% (3/31). The most frequent Gram-negative bacteria isolates was *Escherichia coli* 2 (6.5%).

Table 3 shows the susceptibility profile of the isolated bacteria pathogens. A high degree of antibiotic resistance was exhibited by the isolates against the eight commonly used antibiotics tested. Gentamicin and vancomycin were the only antibiotics that exhibited some *in vitro* inhibitory actions against Gram-positive bacteria, with 68% (17/25) of the isolates sensitive to them, while ciprofloxacin and meropenem exhibited inhibitory actions against the Gram-negative bacteria tested, with 100% (4/4) and 75% (3/4) susceptibility rates respectively.

Table 1: Clinical parameters and laboratory culture results of neonates with suspected sepsis at FMC and TUYMCH, Katsina

Clinical parameters and culture result	Frequency (%)
Mean age (days)	1.35±0.48
Mean weight (kg)	2.13±0.89
Onset of infection	
Early onset (< 3 days)	39 (65.0)
Late onset (> 3 days)	21 (35.0)
Culture result	
Culture positive	31 (51.7)
Culture negative	29 (48.3)

Table 2: Distribution of bacteria associated with neonatal sepsis among neonates at FMC and TUYMCH Katsina, Nigeria

Bacteria isolates	Frequency (%)
Gram positive	**25 (80.6)**
Staphylococcus aureus	4 (12.9)
Staphylococcus haemolyticus	5 (16.1)
Staphylococcus epidermidis	4 (12.9)
Staphylococcus hominis	2 (6.5)
Staphylococcus lentus	1 (3.2)
Staphylococcus gallinarum	1 (3.2)
Staphylococcus saprophyticus	1 (3.2)
Staphylococcus xylosus	1 (3.2)
Staphylococcus pseudointermedius	1 (3.2)
Enterococcus faecalis	2 (6.5)
Enterococcus faecium	1 (3.2)
Streptococcus agalactiae	1 (3.2)
Globicatella sanguinis	1 (3.2)
Gram negative	**6 (19.4)**
Escherichia coli	2 (6.5)
Klebsiella pneumoniae	1 (3.2)
Acinetobacter baumannii	1 (3.2)
Sphingomonas paucimobilis	1 (3.2)
Ralstonia mannitolilytica	1 (3.2)
Total	**31 (100.0)**

Table 3: Percentage susceptibility of bacteria associated with neonatal sepsis among neonates at FMC and TUYMCH, Katsina, Nigeria

Isolates/antibiotics		**GEN (%)**	**CIP (%)**	**CEF (%)**	**CTR (%)**	**CFZ (%)**	**AUG (%)**	**MER (%)**	**VAN (%)**
Gram positive (n=25)	S	17 (68)	8 (32)	4 (16)	6 (24)	4 (16)	3 (12)	1 (4)	17 (68)
	R	8 (32)	17 (68)	21 (84)	19 (76)	21 (84)	22 (88)	24 (96)	8 (32)
S. aureus (n=4)	S	3 (75)	0	1 (25)	2 (50)	2 (50)	0	1 (25)	1 (25)
	R	1 (25)	4 (100)	3 (75)	2 (50)	2 (50)	4 (100)	3 (75)	3 (75)
S. haemolyticus (n=5)	S	3 (60)	1 (20)	0	0	0	0	0	5 (100)
	R	2 (40)	4 (80)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	0
S. epidermidis (n=4)	S	3 (75)	2 (50)	1 (25)	1 (25)	1 (25)	1 (25)	0	2 (50)
	R	1 (25)	2 (50)	3 (75)	3 (75)	3 (75)	3 (75)	4 (100)	2 (50)
S. hominis (n=2)	S	2 (100)	1 (50)	0	0	0	0	0	2 (100)
	R	0	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	0
S. lentus (n=1)	S	1 (100)	1 (100)	0	0	0	0	0	1 (100)
	R	0	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0
S. gallinarum (n=1)	S	1 (100)	0	1 (100)	1 (100)	0	1 (100)	0	0
	R	0	1 (100)	0	0	1 (100)	0	1 (100)	1 (100)
S. saprophyticus (n=1)	S	1 (100)	1 (100)	0	0	0	0	0	1 (100)
	R	0	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0
S. xylosus (n=1)	S	1 (100)	0	0	0	0	0	0	1 (100)
	R	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0
S. pseudointermedius (n=1)	S	1 (100)	0	0	0	0	0	0	1 (100)
	R	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0
Streptococcus agalactiae (n=1)	S	0	0	0	0	0	1 (100)	0	0
	R	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)
Enterococcus (n=2) (*E. faecalis* and *E. faecium*)	S	0	2 (67)	0	1 (33)	0	0	0	3 (100)
	R	3 (100)	1 (33)	3 (100)	2 (67)	3 (100)	3 (100)	3 (100)	0
Globicatella sanguinis (n=1)	S	1 (100)	0	1 (100)	1 (100)	1 (100)	0	0	0
	R	0	1 (100)	0	0	0	1 (100)	1 (100)	1 (100)
Gram negative (n=4)	S	2 (50)	4 (100)	1 (25)	1 (25)	1 (25)	2 (50)	3 (75)	NA
	R	2 (50)	0	3 (75)	3 (75)	3 (75)	2 (50)	1 (25)	NA
Escherichia coli (n=2)	S	2 (100)	2 (100)	0	0	1 (50)	2 (100)	2 (100)	NA
	R	0	0	2 (100)	2 (100)	1 (50)	0	0	NA
Klebsiella pneumoniae (n=1)	S	0	1 (100)	0	0	0	0	1 (100)	NA
	R	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)	0	NA
Ralstonia mannitolilytica (n=1)	S	0	1 (100)	1 (100)	1 (100)	0	0	0	NA
	R	1 (100)	0	0	0	1 (100)	1 (100)	1 (100)	NA
Total bacterial (n=29)*	S	19 (65.5)	12 (41.4)	5 (17.2)	7 (24.1)	5 (17.2)	5 (17.2)	4 (13.8)	NA
	R	10 (34.5)	17 (58.6)	24 (82.8)	22 (75.8)	24 (82.8)	24 (82.8)	25 (86.2)	NA

S-Sensitivity, R-Resistance, GEN-Gentamicin, CIP-Ciprofloxacin, CEF-Cefuroxime, CTR-Ceftriaxone, CFZ-Ceftazidime, AUG-Augmentin, MER-Meropenem, VAN-Vancomycin; NA=Not applicable; *=*Acinetobacter baumannii* (n=1) and *Sphingomonas paucimobilis* (n=1) were not included

Table 4 shows the association between sociodemographic characteristics of the neonates in relation to culture positive and negative sepsis. There was no statistically significant association between culture positive and negative neonatal sepsis with respect to gender (OR= 1.014, 95% CI=0.3662-2.805, *p*=1.000), age of sepsis onset (OR=0.527, 95% CI=0.1786-1.558, *p*=0.3715), gestational age at birth (OR=0.5102, 95% CI=0.1789-1.455, p=0.3164), birth weight (OR=0.5464, 95% CI=0.1897-1.574, *p*=0.3903) and mode of delivery (OR = 07788, 95% CI= 0.1586 - 3.824, *p*=0.7577). However, with respect to place of delivery, neonates delivered at home were almost 3 times more likely to have culture positive sepsis than children delivered in the hospital (OR=2.975, 95% CI=1.040-8.510, *p*=0.0717).

Table 4: Neonatal risk factors associated with culture positive neonatal sepsis

Risk factors		Neonatal sepsis		x^2	OR (95% CI)	*p* value
		No positive (%)	No negative (%)			
Gender	Male	14 (51.9)	13 (48.1)	0.000	1.014 (0.3662 - 2.805)	1.000
	Female	17 (51.5)	16 (48.5)			
Age (days)	≤ 3 (EOS)	18 (46.2)	21 (53.8)	0.7987	0.527 (0.1786 - 1.558)	0.3715
	>3 (LOS)	13 (61.9)	8 (38.1)			
Gestational age at birth (weeks)	<37 (Preterm)	10 (41.7)	14 (58.3)	1.004	0.5102 (0.1789 - 1.455)	0.3164
	>37 (Term)	21 (58.3)	15 (41.7)			
Birth weight (kg)	< 2.5 (LBW)	17 (45.9)	20 (54.1)	0.738	0.5464 (0.1897 - 1.574)	0.3903
	>2.5 (NBW)	14 (60.9)	9 (39.1)			
Mode of delivery	Vaginal	27 (50.9)	26 (49.1)	0.09516	0.7788 (0.1586 - 3.824)	0.7577
	Caesarean section	4 (57.1)	3 (42.9)			
Place of delivery	Home	20 (64.5)	11 (35.5)	3.243	2.975[+] (1.040 - 8.510)	0.0717[+]
	Hospital	11 (37.9)	18 (62.1)			

x^2 = Chi square; OR = Odds ratio; CI = Confidence interval; EOS = early-onset sepsis; LOS = late-onset sepsis; LBW = low birth weight; NBW = normal birth weight; [+] = Although the *p* value here is >0.05, the 95% CI of the OR indicated that culture positive neonatal sepsis occurred significantly more (by a factor of 2.975) following home delivery (64.5%) than following hospital delivery (37.9%);

Table 5: Maternal risk factors associated with culture positive neonatal sepsis

Risk factors		Neonatal sepsis		x^2	OR (95% CI)	*p* value
		No positive (%)	No negative (%)			
Application of traditional substances to the umbilicus	Yes	16 (61.5)	10 (38.5)	1.161	2.027 (0.7158 - 5.738)	0.2813
	No	15 (44.1)	19 (55.9)			
Premature rupture of membrane	Yes	10 (43.5)	13 (56.5)	0.540	0.5861 (0.2050 - 1.675)	0.4623
	No	21 (56.8)	16 (43.2)			
Mothers' level of education	None	20 (66.7)	10 (33.3)	7.167	NA	0.0668
	Primary	3 (25.0)	9 (75.0)			
	Secondary	5 (38.5)	8 (61.5)			
	Tertiary	3 (60.0)	2 (40.0)			
Mothers' residence	Rural	8 (57.1)	6 (42.9)	0.585	NA	0.7465
	Semi-urban	15 (53.6)	13 (46.4)			
	Urban	8 (44.4)	10 (55.6)			
Mothers' occupation	Housewife	28 (50.9)	27 (49.1)	1.517	0.6914 (0.1070 - 4.468)	0.6969
	Civil servant	3 (60.0)	2 (40.0)			
Previous premature delivery	Yes	20 (64.5)	11 (35.5)	3.243	2.975[+] (1.040 - 8.510)	0.0717[+]
	No	11 (37.9)	18 (62.1)			

x^2 = Chi square; OR = Odds ratio; CI = Confidence interval; [+] = Although the *p* value here is >0.05, the 95% CI of the OR indicated that culture positive neonatal sepsis occurred significantly more (by a factor of 2.975) following previous premature delivery (64.5%) than when there was no previous premature delivery (37.9%); NA = Not applicable

Table 5 shows association between maternal risk factors in relation to culture positive and negative neonatal sepsis. There was no statistically significant association between culture positive and culture-negative neonatal sepsis with respect to maternal factors such as application of traditional substance for umbilical care (OR = 2.027, 95% CI = 0.7158 - 5.738, p=0.2813), premature rupture of membranes (OR=0.5861, 95% CI = 0.2050 - 1.675, p=0.4623), mother's educational level (x^2=7.167, p=0.0668), mother's place of residence (x^2=0.585, p=0.7465) and mother's occupation (OR = 0.6914, 95% CI = 0.1070 - 4.468, p=0.6969). However, mothers with previous history of premature delivery are about 3 times more likely to have culture positive neonatal sepsis compared to mothers who had no previous history of premature delivery (OR=2.975, 95% CI=1.040-8.510, p=0.0717).

Table 6 shows the clinical manifestations of culture-positive and culture-negative neonatal sepsis. The commonest presentations of neonatal sepsis were fever (76.7%, 46/60), pyrexia with temperature > 36.2°C (50%, 30/60), refusal to suck (35.0%, 21/60), fast breathing (26.7%, 16/60) and hypothermia (23.3%, 14/60). However, there was no significant difference in any of the clinical manifestations between culture-positive and culture-negative neonatal sepsis (p>0.05).

Discussion:

In this study, 63.3% of the neonates studied had early onset sepsis while 36.7% had late-onset sepsis, indicating that early onset sepsis was more common than late-onset sepsis in agreement with the findings of Dedeke et al., (1) in Abeokuta, Nigeria and from similar studies conducted in Cameroon (18) and Bangladesh (19). However, this contradicts the findings of Mokuolu et al., (15) in Ilorin, Nigeria (15), and of some studies from other developing countries including Pakistan (16) and Libya (17), where late-onset sepsis was reported to be commoner than early onset sepsis.

The prevalence of culture-positive neonatal sepsis (51.7%) in our study is remarkably higher compared to 30.8% reported by Mokuolu et al., (15) in Ilorin and 34.0% by Shobowale et al., (2) in Lagos, Nigeria. The high prevalence in our study may be due to the seasonality of bacteria aetiology of neonatal sepsis but the small sample size in our study requires us to cautiously interpretate our findings. Gram-positive bacterial pathogens predominated in our study, constituting 80.6% while Gram-negative bacteria constituted just 19.4%. The most frequent Gram-positive bacteria were coagulase-negative

Table 6: Clinical presentations of the study subjects in relation to neonatal sepsis

Clinical presentation		**Neonatal sepsis**		**x^2**	**OR (95% CI)**	***p* value**
		No positive (%)	**No negative (%)**			
Fever	Yes	23 (52.3)	21 (47.7)	0.02427	1.095 (0.3485-3.442)	0.8762
	No	8 (50.0)	8 (50.0)			
Jaundice	Yes	5 (45.5)	6 (54.5)	0.01498	0.7372 (0.1983-2.741)	0.9026
	No	26 (53.1)	23 (46.9)			
Fast breathing	Yes	8 (50.0)	8 (50.0)	0.02427	0.9130 (0.2906-2.869)	0.8762
	No	23 (52.3)	21 (47.7)			
Refusal to suck	Yes	13 (61.9)	8 (38.1)	0.7987	1.896 (0.6420-5.599)	0.3715
	No	18 (46.2)	21 (53.8)			
Convulsion	Yes	5 (71.4)	2 (28.6)	0.5053	2.596 (0.4619-14.592)	0.4772
	No	26 (49.1)	27 (50.9)			
Temperature	< 36.6°C (hypothermia)	9 (64.3)	5 (35.7)	3.864	NA	0.1449
	36.6 - 37.2°C (normal)	5 (31.3)	11 (68.8)			
	> 37.2°C (pyrexia)	17 (56.7)	13 (43.3)			

x^2 = Chi square; OR = Odds ratio; CI = Confidence interval; NA = Not applicable

staphylococci (51.6%) consisting of mainly *S. haemolyticus* (16.1%), *S. epidermidis* (12.9%) and *S. hominis* (6.5%). *Staphylococcus aureus* constituted 12.9% of the isolates while *Enterococcus* spp constituted 9.7%, with the most frequent Gram-negative bacteria isolate being *E. coli* (6.5%). These findings are in consonance with those of Mokuolu et al., (15) where Gram-positive bacteria particularly *Staphylococcus* spp were the commonest pathogens associated with neonatal sepsis. In a similar study by Labi et al., (21), *Staphylococcus* spp were also the most common bacteria causing neonatal sepsis. Our study however contradicted the findings of Dedeke et al., (1) and Shobowale et al., (2), where *K. pneumoniae* was the predominant bacterium causing neonatal sepsis in their studies. Even though some of the isolated bacteria in our study were normal commensals of the skin and gastrointestinal tract, they are pathogenic in neonates due to their weak and immature immune system.

Gentamicin and vancomycin were the only antibiotics that exhibited above average *in vitro* inhibitory actions against Gram-positive bacteria in our study, with 68% of the isolates sensitive to them. This is similar to the findings of Yadav et al., (23), who reported that the antibiotic with *in vitro* inhibitory activity against mostly Gram-positive bacterial isolates in their study was gentamicin (93% sensitivity). Vinodkumar et al., (24) also reported low resistance of their isolates to gentamicin. Our finding however contrasted the study conducted by Mustafa and Ahmed (22), who reported low sensitivity of their isolates to gentamicin. The sensitivity rate of 68% for vancomycin against the Gram-positive isolates in our study is similar to the findings of Singh et al., *(*32). Gentamicin and vancomycin still remains the drug of choice for empirical treatment of neonatal sepsis, but recently resistance to these drugs has been reported especially in the developing countries due to decreesing costs of these drugs and increased availability, which may have led to their overuse in empirical therapy of sepsis and other life-threatening infections.

The high degree of resistance exhibited by the isolates to commonly used antibiotics including broad-spectrum cephalosporins, reported in our study is similar to reports of other studies (18). Rizwan et al., (27) and Kayange et al., (28) reported only moderate sensitivity to ciprofloxacin by the isolates in their study while the sensitivity of the isolates in our study to ceftriaxone (a third-generation cephalosporin) is below 50%. However, the study by Abdelsalam et al., (29) reported overall high sensitivity of the bacterial isolates in their study to ciprofloxacin, which contrasted the findings of our current study. The studies by Dedeke et al., (1) and Aku et al., (25) reported low sensitivity of the bacterial isolates to cefuroxime, which agrees with our current study of 17.2% sensitivity to cefuroxime. Reduced susceptibility of bacterial isolates to cephalosporins and fluoroquinolones is a big challenge in the empirical treatment of neonatal sepsis (30,31).

Reports of multi-drug resistant bacteria causing sepsis in neonates, especially in developing countries are on the increase (20). Downie et al., (26) reported that over 40% of cases of neonatal sepsis were due to bacteria that were resistant to the antibiotic combination of ampicillin and penicillin as well as gentamicin, or the commonly used alternative third-generation cephalosporins. These reports support the need for continuous review of empirical antibiotics used in the treatment of sepsis in neonates, to ensure optimal antimicrobial use. The high resistance rate against many of the antibiotics was not surprising because inappropriate and overuse of antibiotics in both humans and animals in Nigeria might have accounted for the high resistance rates detected in our study.

In this study, 51.7% (31/60) of the neonates were delivered at home while 48.3% (29/60) were delivered in hospital, but neonates delivered at home (64.5%) were about 3 times more likely to have culture-positive neonatal sepsis compared to those delivered in hospital (35.5%) (OR=2.975, 95% CI=1.040-8.510). This is in agreement with the findings of Shobowale et al., (2), who reported that babies who were not delivered in hospital were more likely to develop sepsis when compared to babies delivered in hospital. Previous history of premature delivery was significantly associated with culture-positive neonatal sepsis in this study (OR= 2.975, 95% CI = 1.040 - 8.510). Studies have shown that previous premature delivery is a risk factor for another premature delivery, while prematurity is a risk factor for neonatal sepsis. This is because underdeveloped immune system, common in premature babies, is associated with higher risk of infection (33). Darmstadt et al., (34) reported that premature neonates have 3 to10 fold higher occurrence of infections than term and normal birth weight infants.

The most common clinical manifestations of neonatal sepsis in our study were fever (76.7%), refusal to suck (35.0%), fast breathing (26.7%) and hypothermia (23.3%), which agree with some of the findings of Dedeke et al., (1), who reported the common clinical presentations among babies with sepsis to be fever, respiratory distress, and refusal to suck. However, a similar study by Karthikeyan and Prem-

kumar (20) in India reported respiratory distress to be the major presenting feature of neonatal sepsis among their patients. With respect to culture positivity, there was no significant difference in the clinical manifestations of sepsis between culture-positive and culture-negative neonates in our study, which implies that clinical diagnosis of neonatal sepsis is very vital in the initial critical period in the management of neonatal sepsis.

Conclusion:

Neonatal sepsis is common among newborns in Federal Medical Centre (FMC) and Turai Umaru Yar'adua Maternal and Children Hospital (TUYMCH), Katsina, Nigeria, with early onset more common than late-onset sepsis. Gram-positive bacteria are the major pathogens with coagulase-negative staphylococci dominating, while the most frequent Gram-negative bacteria are *E. coli*. The isolates exhibited high resistance (>50% rate) to most of the tested antibiotics including third generation cephalosporins and fluoroquinolones. Gentamicin and vancomycin were the only antibiotics effective, with 65.5% of all isolates sensitive to gentamicin, and 68% of Gram-positive isolates sensitive to vancomycin.

Previous premature delivery and delivery at home were factors significantly associated with culture-positive neonatal sepsis, but there is no significant difference between culture-positive and culture-negative neonatal sepsis with respect to clinical manifestations. There is need for regular surveillance of causative organisms of neonatal sepsis and monitoring of their susceptibility patterns, to inform the choice of empirical antibiotic treatment pending results of blood culture tests.

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Contributions of authors:

OHK, AB and AS conceived idea and developed the study concept. OHK performed the laboratory analysis, while AB and AS supervised the study. All authors discussed the results and contributed to the final manuscript draft for submission.

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Widal antibody titre test versus blood culture; which is a better diagnostic for typhoid fever?

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Abstract:

Background: The importance of accurate diagnosis of infectious diseases is central and crucial to the effectiveness of treatment and prevention of the associated long-term complications of such infections. The objective of this study was therefore to determine the accuracy of the Widal antibody titre test in the diagnosis of typhoid fever relative to the gold standard blood culture technique.
Methodology: A total of 40 students attending the Olabisi Onabanjo University Health Services, Ago-Iwoye, Ogun State, Nigeria on account of suspected typhoid fever by positive Widal test (≥ 1/80) and not on antibiotic therapy, were recruited for the study. Stool and blood samples were collected from each participant and analysed at the medical laboratory of the health center using conventional culture techniques and confirmation of isolates by simplex and multiplex polymerase chain reaction (PCR) amplification assays of *hilA* (*Salmonella enterica*), *ipaH* (*Shigella* spp), *rfc* (*Shigella flexneri*) and *wbgZ* (*Shigella sonnei*) genes. Antibiotic susceptibility testing (AST) of isolated bacteria to 10 panel of antibiotics was done using the Kirby Bauer disk diffusion test and interpreted according to the Clinical and Laboratory Standards Institute (CSLI) guideline.
Results: Of the 40 patients with suspected typhoid fever by the Widal test, 9 yielded *Salmonella enterica* giving a 22.5% isolation rate, with *Salmonella enterica* serovar Typhi (*Salmonella* Typhi) confirmed as sole bacterium from blood cultures in 5 (12.5%) patients and co-infection of *Salmonella* and *Shigella* from stool samples in 4 (10.0%) patients. A total of 52 enteric bacteria isolates were recovered from blood and stool samples of the 40 patients made of *Salmonella enterica* 9 (17.3%), *Shigella* spp 20 (38.5%), *S. flexneri* 9 (17.3%) and *S. sonnei* 14 (26.9%). All the enteric isolates were multi-drug resistant (MDR), with resistance rates to the antibiotic panel ranging from 33.3%-100%, and all the isolates were resistant to ceftriaxone and pefloxacin. *Salmonella* isolates were also 100% resistant to nitrofurantoin, ofloxacin and ciprofloxacin; *S. flexneri* were 100% resistant to nitrofurantoin, amoxicillin, cotrimoxazole, ofloxacin and ciprofloxacin; and *S. sonnei* were 100% resistant to nitrofurantoin and cotrimoxazole.
Conclusion: These results showed that only 12.5% of typhoid fever diagnosis by Widal test had *Salmonella* Typhi isolated from their blood cultures while *Salmonella enterica* and *Shigella* spp were isolated from stool samples of other cases. There is need to adopt culture techniques for laboratory diagnosis of febrile illnesses in order to improve treatment regimen. The fact that AST can also be performed with culture technique could further guide antibiotic prescription and reduce the risk of emergence of resistant bacteria.

Keywords: Blood culture; Typhoid fever; Widal test; *Salmonella enterica*; *Shigella* spp.

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Test de titre d'anticorps Widal versus hémoculture; quel est le meilleur diagnostic pour la fièvre typhoïde?

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Résumé:

Contexte: L'importance d'un diagnostic précis des maladies infectieuses est centrale et cruciale pour l'efficacité du traitement et la prévention des complications à long terme associées à ces infections. L'objectif de cette étude était donc de déterminer la précision du test de titre d'anticorps de Widal dans le diagnostic de la fièvre typhoïde par rapport à la technique d'hémoculture de référence.
Méthodologie : Un total de 40 étudiants fréquentant les services de santé de l'Université Olabisi Onabanjo, Ago-Iwoye, État d'Ogun, Nigéria en raison d'une fièvre typhoïde suspectée par un test Widal positif (≥ 1/80) et non sous antibiothérapie, ont été recrutés pour l'étude. Des échantillons de selles et de sang ont été prélevés sur chaque participant et analysés au laboratoire médical du centre de santé à l'aide de techniques de culture conventionnelles et de confirmation des isolats par des tests d'amplification par réaction en chaîne par polymérase (PCR) simplex et multiplex de *hilA* (*Salmonella enterica*), *ipaH* (*Shigella* spp), les gènes *rfc* (*Shigella flexneri*) et *wbgZ* (*Shigella sonnei*). Les tests de sensibilité aux antibiotiques (AST) des bactéries isolées à 10 groupes d'antibiotiques ont été effectués à l'aide du test de diffusion sur disque de Kirby Bauer et interprétés conformément aux directives du Clinical and Laboratory Standards Institute (CSLI).
Résultats: Sur les 40 patients suspects de fièvre typhoïde par le test de Widal, 9 ont révélé Salmonella enterica donnant un taux d'isolement de 22,5%, avec *Salmonella enterica* sérovar Typhi (*Salmonella* Typhi) confirmée comme bactérie unique à partir d'hémocultures chez 5 (12,5%) patients et co-infection de *Salmonella* et *Shigella* à partir d'échantillons de selles chez 4 (10,0%) patients. Un total de 52 isolats de bactéries entériques ont été récupérés à partir d'échantillons de sang et de selles des 40 patients constitués de *Salmonella enterica* 9 (17,3%), *Shigella* spp 20 (38,5%), *S. flexneri* 9 (17,3%) et *S. sonnei* 14 (26,9%). Tous les isolats entériques étaient multirésistants (MDR), avec des taux de résistance au panel d'antibiotiques allant de 33,3% à 100%, et tous les isolats étaient résistants à la ceftriaxone et à la péfloxacine. Les isolats de *Salmonella* étaient également résistants à 100% à la nitrofurantoïne, à l'ofloxacine et à la ciprofloxacine; *S. flexneri* était résistant à 100% à la nitrofurantoïne, à l'amoxicilline, au cotrimoxazole, à l'ofloxacine et à la ciprofloxacine; et *S. sonnei* étaient 100% résistants à la nitrofurantoïne et au cotrimoxazole.
Conclusion: Ces résultats ont montré que seuls 12,5% des diagnostics de fièvre typhoïde par le test de Widal avaient *Salmonella* Typhi isolée à partir de leurs hémocultures, tandis que *Salmonella enterica* et *Shigella* spp ont été isolées à partir d'échantillons de selles d'autres cas. Il est nécessaire d'adopter des techniques de culture pour le diagnostic en laboratoire des maladies fébriles afin d'améliorer le schéma thérapeutique. Le fait que l'AST puisse également être réalisée avec une technique de culture pourrait guider davantage la prescription d'antibiotiques et réduire le risque d'émergence de bactéries résistantes.

Mots clés: Hémoculture; La fièvre typhoïde; test Widal; *Salmonella enterica*; *Shigella* spp

Introduction:

The need to properly identify the aetiological agents of infection is a serious concern considering the elevated trends of false positive test especially in the diagnosis of typhoid fever in Africa (1). This is necessary for appropriate treatment regimen (2), as the quality of microbiology laboratory diagnosis is central and crucial to proper diagnosis of infectious diseases (3). Failure to appropriately provide true positive tests in disease diagnosis represents low sensitivity and specificity of diagnostic tests (4, 5).

In Nigeria where majority of the populace live below the estimated poverty level and are mostly of low socio-economic class that is known to be frequently associated with poor sanitation, and toilet systems among others (6), it is expected that the level of enteric infection transmission could be high (7), and this could easily be misdiagnosed due to the similarities in the manifestations of these infections (4). Infections may also occur concurrently or superimpose on the other (3,8).

Generally, enteric infections continue to be global health challenge, with an estimated 21.6 million people suffering from febrile illnesses and resulting in estimated 200,000 deaths every year (9). In a study conducted by Folorunso et al., (10), isolation of *Salmonella* Typhi was reported in 17.77% of aetiological agents of pyrexia of undetermined origin. It is thus imperative to evaluate the sensitivity and specificity of Widal antibody titre test relative to blood culture by evaluating patients with

significant typhoid antibody titre and comparing with blood culture technique, in order to elucidate the effect of cross-reactions from infections caused by other enteric pathogens such as *Salmonella* Paratyphi and *Shigella*. This study was therefore aimed at evaluating diagnostic accuracy of the Widal test for diagnosis of typhoid fever relative to the blood culture technique.

Materials and method:

Study setting, participants and ethical approval

A total of 40 students attending Olabisi Onabanjo University Health Services, Ago-Iwoye who have been diagnosed with typhoid fever by the Widal test significant antibody titre (≥1/80), were recruited into this study after obtaining their informed consents. Students already on antibiotic treatment were excluded from the study. The study was approved by the Ethics Committee of the Directorate of Health Services, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

Sample collection and bacterial isolation

Approximately 2ml of venous blood and 2g of faecal samples were aseptically collected from each consenting participant at the medical laboratory of the Olabisi Onabanjo University Health Services. About 1ml of the blood and a loopful of the faecal samples were aseptically introduced into separate MacCartney bottles containing 10ml each of pre-sterilized Rappaport Vasiliadis and nutrient broth. The mixtures were gently shaken to enhance homogeneity and then incubated at 37^{o}C for an initial period of 48 hours which served as inoculum for subsequent analyses. The inoculum (blood and faecal samples introduced into the Rappaport Vassiliadis broth) was then inoculated onto *Salmonella-Shigella* agar (SSA) and MacConkey agar plates by streaking method after which the plates were incubated aerobically at 37^{o}C for 24 hours. The colonies from the culture plates were sub-cultured on nutrient agar for purity plating prior to identification with molecular technique.

Molecular identification of isolates

The molecular identification of bacterial isolates from suspected typhoid fever patients was carried out in a polyphasic manner. First, each of the isolated bacterium was first pre-incubated at 99°C for 20 mins and DNA extracted using QIAamp DNA mini kit (Qiagen) following the manufacturer's instructions. The extracted DNA was then amplified in simplex and multiplex polymerase chain reaction (PCR) assays using specific primers for *Salmonella enterica* (*hilA*) and *Shigella* species (*ipaH*), and subsequently, additional primer pairs specific for *Shigella sonnei* (*wbgZ*) and *Shigella flexneri* (*rfc*) were used (11).

The condition for PCR amplification was performed in a reaction mixture with total volume of 25µL, containing 2.5µL 10xTaq polymerase buffer, 0.3µL dNTPs (10mmol/L), 1U Taq DNA polymerase, 0.6µL $MgCl_2$ (50mmol/L) and 0.3 mol/L of each primer pair. The PCR procedure was as follows; initial denaturation step at 94°C for 5 minutes followed by 30 cycles, consisting of denaturation (94°C for 1 min), annealing (58°C *ipaH*, 65°C *hilA*, 60°C *wbgZ* and *rfc*) for 1 min, was set separately for each primer pair, and extension at 72°C for 1 min, followed by final extension step at 72°C for 5 min (11). Electrophoresis of the amplified products along with a molecular DNA marker was carried out on 1.5% agarose gel, visualization of the ethidium-stained gel was done in a UV transilluminator, and was photographed with a camera.

Widal antibody agglutination test

Only patients with positive Widal test (≥1:80) were recruited for the study. The Widal agglutination test was conducted to detect *Salmonella* agglutinin (antibody) from the serum of the patients. Briefly, the Widal test was performed with Cromatest kit as follows; serum samples were screened using the slide agglutination test which measures agglutinating antibodies against the lipopolysaccharide 'O' and protein flagellar 'H' antigens of *Salmonella* Typhi and *Salmonella* Paratyphi A and B. Serial dilution of sera starting at a dilution of 1:40 were made with 0.9% saline and examined for visible agglutination. Widal test was considered positive when a titre of ≥ 1:80 was observed according to routine laboratory procedures (12).

Table 1: Oligonucleotide primers used in this study (11)

Primer target		Primer sequence (5′ – 3′)	Amplicon size (bp)
ipaH	*ipaH-F*	GTTCCTTGACCGCCTTTCCGATACCGTC	619
	ipaH-R	GCCGGTCAGCCACCCTCTGAGAGTAC	
hilA	*hilA-F*	CGGAACGTTATTTGCGCCATGCTGAGGTAG	784
	hilA-R	GCATGGATCCCCGCCGGCGAGATTGTG	
Sflex-rfc	*Sflex-rfc-F*	TTTATGGCTTCTTTGTCGGC	537
	Sflex-rfc-R	CTGCGTGATCCGACCATG	
Sson-wbgZ	*Sson-wbgZ-F*	TCTGAATATGCCCTCTACGCT	430
	Sson-wbgZ-R	GACAGAGCCCGAAGA	

Antimicrobial susceptibility testing

Antimicrobial susceptibility test (AST) was performed in duplicate on each isolated bacterium using the disk diffusion method of Bauer et al., (13) against 10 selected antibiotics and the average diameter of inhibition zone for each isolate was taken, and interpreted as susceptible or resistant following the recommended guideline of the Clinical and Laboratory Standards Institute (13).

Statistical analysis

Data were presented using frequency distribution tables and descriptive statistics. The prevalence of each isolate was determined by dividing the number of isolates by the total number of all the isolates, expressed in percentages. The prevalence of infection caused by each isolate was also determined by dividing the number of each isolate recovered from the patients by the total number of patients (expressed in percentages).

Results:

Of the 40 patients with suspected typhoid fever by the Widal test, 9 yielded distinct growth of *Salmonella enterica* confirmed by PCR, giving a 22.5% rate, with *Salmonella enterica* serovar Typhi (*Salmonella* Typhi) as sole bacterium in 5 (12.5%) patients and co-infection with *Shigella* spp in 4 (10.0%) patients (Tables 2 and 3). All the 40 patients cultured positive for enteric bacteria, with a total of 52 PCR-confirmed isolates; *S. enterica* 9 (17.3%), *Shigella* spp 20 (38.5%), *Shigella flexneri* 9 (17.3%) and *Shigella sonnei* 14 (26.9%) (Table 3). The gel electrophoresis of the PCR amplicons of representative *Salmonella* and *Shigella* isolates on simplex and multiplex PCR amplifications are depicted in plates 1 and 2.

The antibiotic susceptibility test (AST) in Table 4 showed that all the enteric bacterial isolates were multi-drug resistant (resistant to ≥ 3 antibiotic classes), and all were resistant to ceftriaxone and pefloxacin. *Salmonella enterica* isolates were 33.3% resistant to amoxicillin, 44.4% to augmentin and gentamicin, 55.5% to tetracycline, and 100% to ceftriaxone, nitrofurantoin, ofloxacin, ciprofloxacin and pefloxacin. *Shigella* spp were 70% resistant to gentamicin and tetracycline, 80% to augmentin, 85% to amoxicillin, 90% to cotrimoxazole, 95% to nitrofurantoin, ofloxacin and ciprofloxacin, and 100% to ceftriaxone and pefloxacin. *Shigella flexneri* isolates were 55.5% resistant to gentamicin and tetracycline, 66.6% to augmentin, and 100% to amoxicillin, cotrimoxazole, nitrofurantoin, ceftriaxone, ofloxacin, ciprofloxacin and pefloxacin. *Shigella sonnei* isolates were 64.3% resistant to gentamicin and amoxicillin, 78.6% to tetracycline, 85.7% to augmentin, 92.9% to ofloxacin and ciprofloxacin, and 100% to cotrimoxazole, nitrofurantoin, ceftriaxone and pefloxacin.

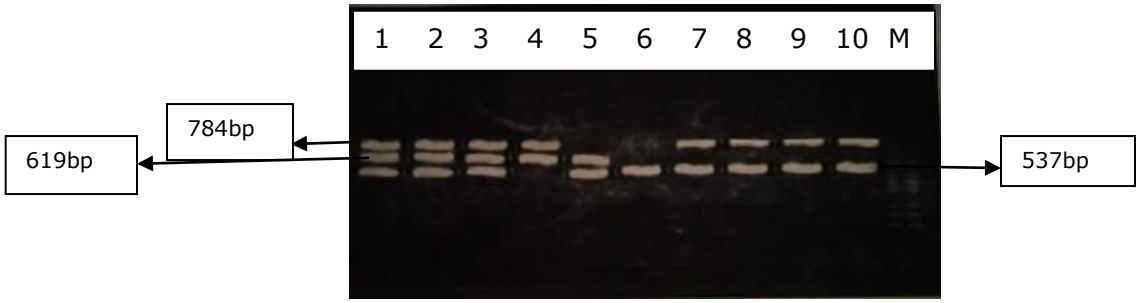
Table 2: Distribution of PCR-confirmed bacterial isolates from typhoid fever patients with positive Widal test

Laboratory code	Bacterial isolates	Frequency
1459	*Salmonella enterica*, *Shigella* spp	2
1460	*Salmonella enterica* serovar Typhi (*Salmonella* Typhi)	1
1461	*Salmonella enterica*, *Shigella* spp	2
1462	*Shigella* spp, *Shigella sonnei*	2
1463	*Shigella* spp	1
1464	*Shigella* spp, *Shigella flexneri*	2
1471	*Salmonella enterica* serovar Typhi (*Salmonella* Typhi)	1
1473	*Salmonella enterica*, *Shigella* spp	2
1474	*Shigella* spp, *Shigella sonnei*	2
1481	*Shigella flexneri*	1
1482	*Salmonella enterica* serovar Typhi (*Salmonella* Typhi)	1
1485	*Salmonella enterica* serovar Typhi (*Salmonella* Typhi)	1
1487	*Shigella flexneri*	1
1488	*Shigella* spp, *Shigella sonnei*	2
1489	*Shigella sonnei*	1
1566	*Shigella* spp	1
1569	*Shigella flexneri*	1
1570	*Shigella* spp	1
1571	*Shigella* spp, *Shigella sonnei*	2
1572	*Shigella* spp, *Shigella sonnei*	2
1574	*Shigella* spp	1
1578	*Shigella sonnei*	1
1579	*Shigella sonnei*	1
1580	*Salmonella enterica* serovar Typhi (*Salmonella* Typhi)	1
1581	*Shigella* spp, *Shigella sonnei*	2
1582	*Shigella* spp, *Shigella sonnei*	2
1637	*Shigella* spp	1
1639	*Shigella* spp	1
1641	*Shigella* spp	1
1642	*Shigella flexneri*	1
1643	*Shigella sonnei*	1
1644	*Shigella sonnei*	1
1645	*Shigella flexneri*	1
1646	*Shigella sonnei*	1
1647	*Shigella* spp	1
1667	*Shigella flexneri*	1
1668	*Salmonella enterica*, *Shigella sonnei*	2
1669	*Shigella flexneri*	1
1678	*Shigella* spp	1
1684	*Shigella flexneri*	1

Table 3: Distribution of enteric bacterial isolates by species

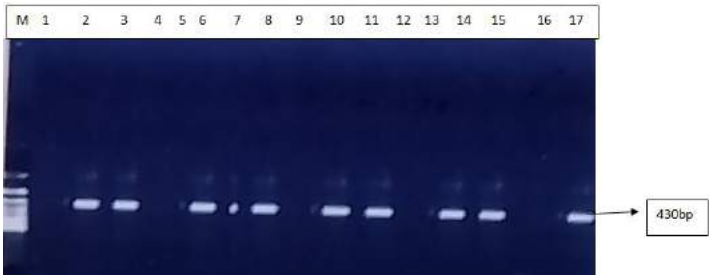
Bacterial isolate	Number of isolates (%) (n=52)	***Number of patients (%) (n=40)
Salmonella enterica	9 (17.3)*	9 (22.5)**
Shigella spp	20 (38.5)	20 (50.0)
Shigella flexneri	9 (17.3)	9 (22.5)
Shigella sonnei	14 (26.9)	14 (35.0)

* = 5 (9.6%) of 52 isolates (from blood) are *Salmonella enterica* serovar Typhi; ** = 5 (12.5%) of 40 patients had *Salmonella enterica* serovar Typhi isolated from their blood; *** = 12 patients had had two bacterial species while 28 patients had one bacterial specie isolated from their samples



Shigella species *ipaH* amplicon (molecular weight = 619bp), *Salmonella* species *hilA* amplicon (molecular weight = 784bp), *Shigella flexneri rfc* amplicon (molecular weight = 537bp)

Plate 1: Gel electrophoresis of multiplex PCR amplicon of representative *Salmonella* and *Shigella* isolates



Shigella sonnei wbgZ amplicon (molecular weight = 430bp)

Plate 2: Gel electrophoresis of PCR amplicon of representative *Shigella sonnei* isolates

Table 4: Antibiotic resistance profile in enteric bacterial isolates

Antibiotics	***Salmonella enterica* (n=9)**	***Shigella* species (n=20)**	***Shigella flexneri* (n=9)**	***Shigella sonnei* (n=14)**
Amoxicillin	3 (33.3)	17 (85.0)	9 (100.0)	9 (64.3)
Augmentin	4 (44.4)	16 (80.0)	6 (66.6)	12 (85.7)
Ceftriaxone	9 (100.0)	20 (100.0)	9 (100.0)	14 (100.0)
Nitrofurantoin	9 (100.0)	19 (95.0)	9 (100.0)	14 (100.0)
Gentamicin	4 (44.4)	14 (70.0)	5 (55.5)	9 (64.3)
Cotrimoxazole	8 (88.9)	18 (90.0)	9 (100.0)	14 (100.0)
Tetracycline	5 (55.5)	14 (70.0)	5 (55.5)	11 (78.6)
Ofloxacin	9 (100.0)	19 (95.0)	9 (100.0)	13 (92.9)
Ciprofloxacin	9 (100.0)	19 (95.0)	9 (100.0)	13 (92.9)
Pefloxacin	9 (100.0)	20 (100.0)	9 (100.0)	14 (100.0)

Discussion:

The importance of precise and timely diagnosis of typhoid fever in early stages of infection is crucial to identifying the etiological agent and carriers that might serve as source of transmission during outbreak (3), and this may subsequently avert inaccurate estimation of the global burden of this disease (2,14). In this study, a total of 52 PCR-confirmed bacterial isolates were recovered from blood and stool samples of 40 patients clinically suspected to have typhoid fever from positive Widal test; *Salmonella enterica* (17.3%, 9/52), *Shigella* spp (38.5%, 20/52), *Shigella flexneri* (17.3%, 9/52) and *Shigella sonnei* (26.9%, 14/52). Five of the *S. enterica* were recovered as sole isolates from blood cultures of only 5 (12.5%) of the 40 patients, and because only serovar Typhi frequently cause blood stream infection, these five *S. enterica* isolates are assumed to be *Salmonella* Typhi. This low *S.* Typhi isolation rate from patients considered to have typhoid fever by positive Widal test (≥1:80) and the high rate of isolation of other enteric pathogens (*Shigella* spp) aside *S.* Typhi emphasizes the low sensitivity and specificity (from possible cross-reactivity from other enteric pathogens) of the Widal test in the diagnosis of typhoid fever (5,15). Although, several studies have documented inherent variabilities of the Widal test, difficulty in establishing a steady state baseline titre, and lack of reproducibility of the test result (16,17,18), evidence from our study might suggest that a positive Widal test correlates more with infections caused by other enteric bacteria aside typhoid fever.

Shigella spp (especially *S. dysenteriae*) which was the most predominant bacterium in this study is known to cause inflammation of the large intestines, resulting in diarrhea stool containing blood or mucus. On the other hand, *S. sonnei* and *S. flexneri* are known for enterotoxin production (19). *Salmonella* Typhi, which had a low prevalence in this study, is known to be responsible annually for over 3.4 million typhoid fever cases with about 2 million deaths globally (9), and 681,316 deaths in Africa (20). Our study however showed that the true prevalence of the aetiological agents of enteric fever can only be known through appropriate laboratory diagnosis. Consequently, the true prevalence of typhoid fever in our study area was found to be approximately 12.5%, indicating that for every 100 patients who tested positive for Widal test, only 12 actually have the disease. Previous studies have reported comparable prevalence of 11.3%, 14.1% and 18.7% of culture-confirmed typhoid fever (21,22,23). In contrast to our findings, some previous studies reported higher typhoid fever prevalence of 22.1% to 55% among febrile patients in various settings in Africa and Asia from blood cultures (5,24,25,). Another factor to be considered with cultural method is the opportunity of determining the susceptibility of the isolated aetiological agents of enteric fever, which is not possible with the Widal test. This is particularly imperative now that the world is fighting the serious challenge of antimicrobial resistance (AMR) with attendant prolonged hospitalization and healthcare cost, treatment failure and increased mortality and morbidity (23,26-29). To avert such negative trend, it is thus necessary to know the resistance pattern of aetiological agents of enteric fever.

In our study, high antimicrobial resistance (AMR) rates were observed to selected antibiotics, ranging from 33.3% to 100%, with *Salmonella enterica* being the only enteric isolates showing > 50% sensitivity to augmentin (55.6%), gentamicin (55.6%) and amoxicillin (66.7%), while all other isolates exhibited resistant rates of >50% to all the antibiotics. Only gentamicin among all the selected antibiotics showed some *in vitro* inhibitory activity on *S. enterica* (55.6% sensitivity), *S. flexneri* (44.5% sensitivity), *S. sonnei* (35.7% sensitivity) and *Shigella* spp (30% sensitivity). Ceftriaxone and pefloxacin were the two antibiotics with 100% of the isolates resistant to them. Our findings are in agreement with previous studies from other sub-Saharan Africa which reported MDR rates of 50% and 52% among typhoid isolates in Ethiopia and Ghana respectively (30-32). Also, Ohanu et al., (23) reported high resistant rates (22.8%-100%) for typhoid isolates to several older and relatively newer antibiotics. These high antibiotic rates may have, among other possible causes, resulted from antibiotic selection pressure created by the overuse and misuse of antibiotics for treatment of typhoid fever that have been misdiagnosed from the wrong use of the Widal test.

Conclusion:

The results of our study showed that only 12.5% of typhoid fever cases diagnosed by the Widal test had *Salmonella* Typhi isolated from their blood cultures while *Salmonella enterica* and *Shigella* spp were isolated from stool samples of other cases, with all the bacterial isolates being multi-drug resistant. There is need to adopt culture techniques for

laboratory diagnosis of febrile illnesses in order to improve treatment regimen. The fact that antimicrobial susceptibility test can also be performed with culture technique could further guide antibiotic prescription and reduce the risk of emergence of resistant bacteria.

Contributions of authors:

POD, TBT and FJB conceptualized the study; POD drafted the first manuscript. TBT and FJB contributed to the design of the study and revised the final draft of the manuscript. BHT and AHA supervised sample collections by OQO and CMO. POD, TBT, BHT, AHA and FJB supervised laboratory analysis by OQO and CMO. Data analyses and interpretations were done by TBT. All the authors approved the final version of the manuscript

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Conflict of interest:

Authors declare no conflict of interest

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Original article **Open Access**

Epidemiology of Dengue in patients with febrile syndrome at Saint Camille Hospital, Ouagadougou, Burkina Faso from 2020 to 2021

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Abstract:

Background: Dengue is still a public health problem in tropical countries. This disease, which had almost disappeared in some areas of the world, has become re-emergent in certain parts of the world including Africa. The aim of this study is to determine the seroprevalence and evolution of Dengue virus (DENV) infection from 2020 to 2021 at the Hospital Saint Camille de Ouagadougou (HOSCO), Burkina Faso.
Methodology: This was a descriptive analytical study of patients seen in general practice with febrile syndrome referred for serological diagnosis of Dengue at the HOSCO laboratory over a period of 2 years (January 1, 2020 – December 31, 2021). The "Dengue Duo (AgNS1/IgM/IgG)" kit from SD Bioline was used for the rapid diagnosis through the detection of NS1 antigen and IgM/IgG antibodies in plasma. Data were analysed with SPSS version 20.0 software. Association between demographic data and prevalence of DENV infection was determined by Chi square test and odds ratio (with 95% confidence interval). P value less than 0.05 was considered statistical significance.
Results: A total of 2957 patients aged 0-94 years were referred for serological diagnosis of DENV infection at the HOSCO laboratory over the period 2020-2021, comprising 56.3% females and 43.7% males. The overall prevalence of acute DENV infection (NS1Ag positive) was 5.4% (159/2957), with 2.4% (41/1700) in 2020 and 9.4% (118/1257) in 2021 (OR=4.192, 95% CI=2.915-6.028, p<0.0001). The prevalence of acute DENV infection of 7.0% (91/1292) in the males was significantly higher than 4.1% (68/1665) in the females (OR=1.779, 95% CI=1.288-2.458, p=0.0005), and also significantly higher in age groups 20-29 years (7.6%), 10-19 years (6.9%) and 40-49 years (5.8%) than other age groups (X^2=14.928, p=0.0107). The overall prevalence of DENV IgM and IgG antibodies was 3.2% and 37.3% respectively. The prevalence of DENV IgG antibodies was significantly higher in males (44.0%) than females (32.1%) (OR=1.667, 95%CI=1.434-1.938, p<0.0001) and in age groups 30-39 (43.4%), 40-49 (44.0%) and >50 years (49.3%) than other age groups (X^2=121.0, p<0.0001), indicating that past exposure to DENV infection is higher among males and older age groups. The peak of DENV infection was between October and November with 84.3% (134/159) of NS1Ag positivity occurring during this period.
Conclusion: The present study reports a high prevalence of acute Dengue virus infection in patients from October to November. To eradicate Dengue which has become a tropical silent epidemic, interventions such as vector control, availability of and accessibility to diagnostic tests, and good therapeutic management are of great importance.

Keywords: Epidemiology; Dengue; NS1Ag; Burkina Faso

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Epidémiologie de la Dengue chez les patients atteints du syndrome fébrile à l'Hôpital Saint Camille, Ouagadougou,

Burkina Faso de 2020 à 2021

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Résumé:

Contexte: La dengue reste un problème de santé publique dans les pays tropicaux. Cette maladie, qui avait quasiment disparu dans certaines régions du monde, est devenue ré-émergente dans certaines parties du monde dont l'Afrique. Le but de cette étude est de déterminer la séroprévalence et l'évolution de l'infection par le virus de la Dengue (DENV) de 2020 à 2021 à l'Hôpital Saint Camille de Ouagadougou (HOSCO), Burkina Faso.
Méthodologie: Il s'agissait d'une étude analytique descriptive de patients vus en médecine générale avec syndrome fébrile adressés pour diagnostic sérologique de la Dengue au laboratoire HOSCO sur une période de 2 ans (1er Janvier 2020 – 31 Décembre 2021). Le kit "Dengue Duo (AgNS1/IgM/IgG)" de SD Bioline a été utilisé pour le diagnostic rapide grâce à la détection de l'antigène NS1 et des anticorps IgM/IgG dans le plasma. Les données ont été analysées avec le logiciel SPSS version 20.0. L'association entre les données démographiques et la prévalence de l'infection par le DENV a été déterminée par le test du chi carré et l'Odds ratio (avec un intervalle de confiance à 95%). Une valeur P inférieure à 0,05 a été considérée comme une signification statistique.
Résultats: Au total, 2957 patients âgés de 0 à 94 ans ont été référés pour un diagnostic sérologique de l'infection par le DENV au laboratoire HOSCO sur la période 2020-2021, comprenant 56,3% de femmes et 43,7% d'hommes. La prévalence globale de l'infection aiguë par le DENV (NS1Ag positif) était de 5,4% (159/2957), avec 2,4% (41/1700) en 2020 et 9,4% (118/1257) en 2021 (OR=4,192, IC à 95%=2,915-6,028, p<0,0001). La prévalence de l'infection aiguë par le DENV de 7,0% (91/1292) chez les hommes était significativement supérieure à 4,1% (68/1665) chez les femmes (OR=1,779, IC à 95%=1,288-2,458, p=0,0005), et également significativement plus élevé dans les tranches d'âge 20-29 ans (7,6%), 10-19 ans (6,9%) et 40-49 ans (5,8%) que les autres tranches d'âge (X^2=14,928, p=0,0107). La prévalence globale des anticorps IgM et IgG contre le DENV était de 3,2% et 37,3% respectivement. La prévalence des anticorps IgG DENV était significativement plus élevée chez les hommes (44,0%) que chez les femmes (32,1%) (OR=1,667, IC à 95%=1,434-1,938, p <0,0001) et dans les tranches d'âge 30-39 (43,4%), 40-49 (44,0%) et > 50 ans (49,3%) que les autres groupes d'âge (X^2=121,0, p<0,0001), ce qui indique que l'exposition antérieure à l'infection par le DENV est plus élevée chez les hommes et les groupes d'âge plus âgés. Le pic d'infection au DENV a eu lieu entre octobre et novembre avec 84,3% (134/159) de positivité NS1Ag survenant au cours de cette période.
Conclusion: La présente étude rapporte une prévalence élevée d'infection aiguë par le virus de la dengue chez les patients d'octobre à novembre. Pour éradiquer la Dengue qui est devenue une épidémie tropicale silencieuse, des interventions telles que la lutte antivectorielle, la disponibilité et l'accessibilité des tests de diagnostic, et une bonne prise en charge thérapeutique sont d'une grande importance.

Mots clés: Epidémiologie; Dengue; NS1Ag; Burkina Faso

Introduction:

Dengue is an arbovirosis transmitted to humans by diurnal mosquitoes of the genus Aedes subgenus Stegomyia, particularly the species Aedes aegypti and Aedes albopictus. Dengue is the most widespread arbovirosis in tropical and subtropical countries of the world (1). Dengue virus (DENV), the etiological agent of dengue fever, belongs to the genus Flavivirus, and to the family Flaviviridae. DENVs are grouped into four genetically related but antigenically distinct serotypes; DEN-1, DEN-2, DEN-3 and DEN-4 (2), which are single-stranded RNA viruses with positive polarity. They are extremely limited in their natural vertebrate host range, which includes only primates (3). Recently a fifth serotype, DENV-5 was isolated in 2013 in Sarawak in the Malaysian part of the island of Borneo without having caused any human cases to date, because it follows a purely sylvatic cycle unlike the other four serotypes which follow the human cycle (4).

This disease, which had almost disappeared in some areas of the world, is appearing again with high incidence, that qualifies it as a re-emerging infection (5). Thus, given the increase in its incidence over the last fifteen years, it is one of the infectious diseases of public health today (5). According to the World Health Organization (WHO), 3.9 billion people

(40 to 50% of the world's population) are exposed to the risk of DENV infection, with 500,000 cases of severe forms of the disease per year, and approximately 30,000 deaths (6). Dengue is a major public health threat in low-and-middle-income countries where the disease is endemic. It has been reported in 34 countries in Africa over the past decades (7). West Africa has been identified as a potential dengue hotspot due to a rapid growth of urban areas without proper sanitation that creates large areas in which the *Aedes aegypti* vector proliferates (8,9).

In Burkina Faso, the first Dengue epidemic occurred in 1925. Subsequently, a significant number of cases were identified in the 1980s. Burkina Faso is one of 34 countries in Africa where Dengue cases have been reported since 2000 (7), and is considered by the WHO to be an endemic country. Due to under-reporting of the disease and under-utilization of health services, particularly in resource-limited countries, the true health and economic burden of DENV infections is difficult to estimate (10). There is a real problem of differential diagnosis with the risk of misdiagnosis of Dengue as malaria. More than 70% of febrile illnesses are treated as suspected malaria without laboratory diagnosis in areas where malaria is prevalent (11).

Forecasts in Africa are notoriously underestimated. However, the most severe forms of the disease are Dengue hemorrhagic fever (DHF) and the Dengue shock syndrome (DSS) characterized by thrombocytopenia and vascular leakage leading to hypervolemia, with high mortality without prompt management. Although severe Dengue disease has historically been associated with pediatric populations in areas of hyper-endemia (12,13), recent trends indicate that adults may also be at risk (14,15).

There is currently no specific viral drug treatment for Dengue, therefore, treatment is symptomatic. Clinical signs are not specific to a serotype and infection by a serotype confers prolonged immunity to that same serotype but there is no cross-protection between serotypes. Therefore, a person may present several episodes of infection with more severe symptoms with each new infection by a different serotype than the one of the presenting infections. Vaccination could be one of the ideal methods to fight dengue, and it is interesting to note the progress made in developing vaccines against dengue viruses (16). The first licensed Dengue vaccine CYD-TDV (Dengvaxia®), has been registered in several countries but is not yet widely used and other vaccine candidates are being tested.

Biological diagnosis of Dengue involves detection of the virus, its genome or viral antigens, which is the direct diagnosis reserved for the early stage of the disease. Antibody detection, or indirect diagnosis, is preferred from day 5 of the disease (17). The detection of IgM antibodies uses capture ELISA technique, while the detection of IgG antibodies uses indirect ELISA technique (18). Molecular methods based on reverse transcriptase-polymerase chain reaction (RT-PCR) have helped to improve the diagnosis of Dengue in the symptomatic phase and to detect the serotypes involved (19). Viral isolation combined with sequencing allows for molecular epidemiological studies useful for health authorities and for understanding the circulation of dengue virus strains.

In 2013, three serotypes were isolated after another Dengue epidemic in Burkina Faso (18). Dengue remains a public health problem in Burkina Faso, therefore, regular data on the circulation of the virus and cases of infection are necessary for proper surveillance of a possible epidemic. The objective of this study was to determine the seroprevalence and evolution of Dengue infection from 2020 to 2021 at the Hospital Saint Camille de Ouagadougou (HOSCO), Burkina Faso.

Materials and method:

Study setting and design

This is a descriptive analytical study conducted over a period of 2 years (January 1, 2020 to December 31, 2021) at the Hospital Saint Camille de Ouagadougou (HOSCO) in Ouagadougou, the capital of Burkina Faso. HOSCO is one of the major health centers of the Burkinabe capital and receives patients from all over the city and from surrounding towns.

Study population and sampling

The study population consisted of 2957 patients (children and adults) of both sexes seen consecutively at the HOSCO general medical clinic with a febrile syndrome and at the laboratory of Hospital Saint Camille in Ouagadougou for the serological diagnosis of Dengue over the period of study. Demographic informations of each subject were collected from registers and patients' files. Blood samples from each patient were collected into ethylene diamine tetra-acetic acid (EDTA) bottles. After centrifugation at 4000g for 5min, the plasma was separated and used for the serological diagnosis of Dengue fever.

Ethical considerations

All participants in the study gave their free and informed consent. The confidentiality and anonymity of the information obtained from the various registers and patient files are kept strictly confidential.

Serodiagnosis of Dengue fever

The detection of Dengue virus from

the plasma sample was performed using the "Dengue Duo (NS1Ag+IgM/IgG)" from SD Bioline (Standard Diagnostic Inc., Korea) according to the protocols provided by the manufacturer. The test is based on detection of NS1 antigen of Dengue virus in serum as well as IgG and IgM antibodies produced by the body against the virus. The principle is immunochromatography on cassette. The anti IgG and IgM antibodies of the sample react with the recombinant envelope proteins present in the colloid conjugate to form an Ag-Ab complex. This complex migrates by capillary action and captured by human anti IgG and/or anti IgM antibodies immobilized on the two lines, generating a colored line.

For AgNS1 detection using a micropipette, 100 µl of plasma was deposited in the sample deposit area of the cassette dedicated to AgNS1 detection. Still on the same cassette but in the compartment dedicated to IgG and IgM detection, 5 µl of plasma was also deposited in the square S sample well followed by 90 µl of diluent in the diluent deposit area. After 15 to 20 minutes the result was interpreted according to the manufacturer's prescription. The presence of a single-colored line in the control window indicated a negative result and when the control line did not appear the result was invalid. The presence of a colored line in the test window for any of the markers AgNS1, IgG, IgM indicated a positive result for that marker.

Data analysis

Data were entered into Microsoft Excel 2016 software, and SPSS version 20.0 software was used for data analysis. The results were considered statistically significant at $p < 0.05$. The Odds ratio (OR) and 95% confidence intervals (CI) were calculated to estimate the effect of patient sex and age on Dengue virus infection using EPI INFO 7.0.

Results:

Socio-demographic characteristics of the study population

The study included 2,957 patients who were referred for serological diagnosis of Dengue at the HOSCO laboratory, with age range of 0 to 94 years. The study population was composed of 56.3% females and 43.7% males; 56.1% (953/1,700) females and 43.9% (747/1,700) males in 2020, and 56.6% (712/1,257) females and 43.4% (545/1,257) males in 2021. The age group 20-39 years was the most represented with 43.8% (746/1700) and 51.9% (652/1,257) in 2020 and 2021 respectively. Adolescents and children under 20 years of age represented 30.9% (525/1700) in 2020 versus 23.3% (293/1257) in 2021 (Table 1).

Prevalence of Dengue virus infection by NS1 antigen and IgG/IgM antibody tests

Three Dengue virus markers (NS1 Ag, IgM, IgG) were tested in symptomatic patients with febrile syndrome. The overall prevalence of acute Dengue infection (NS1 Ag+) is 5.4% (159/2957), with 7.0% (91/1292) in males and 4.1% (68/1665) in females (X^2=11.946, OR=1.779, 95% CI=1.288-2.458, p=0.0005) (Table 2). The prevalence of acute DENV infection was significantly higher in age groups 20-29 years (7.6%), 10-19 years (6.9%) and 40-49 years (5.8%) than other age groups (X^2=14.928, p=0.0107). Univariate analysis shows that the prevalence was significantly higher in age group 20-29 years (p=0.01) (Table 3).

Table 1: Demographic characteristics of the study population

Demographics/ Year	2020		2021		Combined 2020 and 2021	
	Number	Percentage	Number	Percentage	Number	Percentage
Gender						
Male	747	43.9	545	43.4	1,292	43.7
Female	953	56.1	712	56.6	1,665	56.3
Total	**1700**	**100.0**	**1257**	**100.0**	**2957**	**100.0**
Age group (years)						
<10	328	19.3	144	11.5	472	16.0
10-19	197	11.6	149	11.8	346	11.7
20-29	389	22.9	323	25.6	712	24.1
30-39	357	21.0	329	26.2	686	23.2
40-49	171	10.0	138	11.0	309	10.4
≥50	258	15.2	174	13.9	432	14.6
Total	**1700**	**100.0**	**1257**	**100.0**	**2957**	**100.0**

Table 2: Prevalence of Dengue infection by the NS1 antigen, IgG and IgM antibodies in the study population in 2020 and 2021

DENV serology	NS1 Ag+ (%)	IGM+ (%)	IGG+ (%)
Gender			
Male (n=1292)	91 (7.0)	50 (3.9)	569 (44.0)
Female (n=1665)	68 (4.1)	45 (2.7)	534 (32.1)
Total (n=2957)	159 (5.4)	95 (3.2)	1103 (37.3)
x^2	11.946	2.823	44.045
OR (95% CI)	1.779 (1.288 – 2.458)	1.449 (0.9622-2.183)	1.667 (1.434-1.938)
p value	0.0005*	0.0929	< 0.0001*
Age group (years)			
< 10 (n=472)	19 (4.0)	6 (1.3)	85 (18.0)
10-19 (n=346)	24 (6.9)	14 (4.0)	119 (34.4)
20-29 (n=712)	54 (7.6)	27 (3.8)	252 (35.4)
30-39 (n=686)	28 (4.1)	25 (3.6)	298 (43.4)
40-49 (n=309)	18 (5.8)	11 (3.6)	136 (44.0)
≥ 50 (n=432)	16 (3.7)	12 (2.8)	213 (49.3)
Total (n=2957)	159 (5.4)	95 (3.2)	1103 (37.3)
x^2	14.928	8.057	121.10
p value	0.0107*	0.1531	< 0.0001*

OR = Odd Ratio; X^2= Chi square; CI = Confidence interval; * = Statistically significant; DENV=Dengue virus

Table 3: Univariate analysis of gender and age group with prevalence of acute Dengue infection (Ag NS1+)

DENV serology	NS1 Ag+	NS1 Ag-	OR	95% CI	*p* value
Gender					
Male	91	1128	Réf		
Female	68	1501	1.78	1.28 - 2.46	0.0005*
Age group (years)					
< 10	19	453	Réf		
10-19	24	322	0.56	0.30 - 1.04	0.08
20-29	54	658	0.51	0.29 - 0.87	0.01*
30-39	28	658	0.98	0.54 - 1.78	1.00
40-49	18	291	0.67	0.35 - 1.31	0.30
-*≥ 50	16	416	1.09	0.55 - 2.14	0.86

$NS1^+$= NS1 positive antigen; NS1-=NS1 negative antigen; OR=Odds Ratio; CI=Confidence interval; *=statistically significant; DENV=Dengue virus

The overall prevalence of DENV IgM and IgG antibodies was 3.2% and 37.3% respectively. The prevalence of DENV IgG antibodies was significantly higher in the males (44.0%) than in females (32.1%) (OR=1.667, 95%CI=1.434-1.938, p<0.0001) and in age groups 30-39 (43.4%), 40-49 (44.0%) and >50 years (49.3%) than the other age groups (X^2=121.0, p<0.0001), indicating that past exposure to DENV infection is higher among males and older age groups (Table 2).

Of 1,700 samples analyzed in 2020, 2.4% (41/1,700) had recent or acute DENV infection (NS1 Ag+), 35.5% (603/1700) had secondary infection or past exposure to DENV (IgG+), 1.5% (25/1,700) had late primary or recent secondary DENV infection (IgM+/IgG+), 0.1% (2/1700) had primary acute DENV infection (NS1 Ag+/IgM+) and 0.1% (2/1700) of patients were positive for all the three markers (NS1+/IgM+/IgG+) (Table 4).

Of 1257 samples analyzed in 2021, 9.4% (118/1,257) had recent or acute DENV infection (NS1 Ag+), 39.8% (500/1,700) had secondary infection or past exposure to DENV (IgG+), 5.0% (63/1,700) had late primary or recent secondary DENV infection (IgM+/IgG+), 2.3% (29/1257) had primary acute DENV infection (NS1 Ag+/IgM+), and 2.1% (27/1257) of the patients were positive for the three DENV serological markers (NS1+/IgM+/IgG+) (Table 4).

Table 4: Comparative prevalence of Dengue infection by the NS1 antigen, IgG and IgM antibodies in the study population in 2020 and 2021

Year/ DENV serology	2020			2021			
	NS1 Ag+ (%)	IgM+ (%)	IgG+ (%)		NS1 Ag+ (%)	IgM+ (%)	IgG+ (%)
All subjects (n=1700)				**All subjects (n=1257)**			
	41 (2.4)	27 (1.5)	603 (35.5)		118 (9.4)	68 (5.4)	500 (39.8)
Gender				**Gender**			
Male (n=747)	23 (3.1)	14 (1.9)	366 (49.0)	Male (n=545)	68 (12.5)	36 (6.6)	203 (37.2)
Female (n=953)	18 (1.9)	13 (1.4)	237 (24.9)	Female (n=712)	50 (7.0)	32 (4.5)	297 (41.7)
X^2	2.040	0.4089	105.45	X^2	10.116	2.292	2.387
OR (95% CI)	1.65 (0.9-3.1)	1.38 (0.6-2.9)	2.90 (2.3-3.6)	OR (95% CI)	1.89 (1.3-2.8)	1.50 (0.9-2.5)	0.83 (0.7-1.0)
p value	0.1532	0.5225	< 0.0001*		0.0014*	0.1300	0.1223
Age group (years)				**Age group (years)**			
< 10 (n=328)	8 (2.4)	2 (0.6)	60 (18.3)	< 10 (n=144)	11 (7.6)	4 (2.8)	25 (17.4)
10-19 (n=197)	9 (4.6)	4 (2.0)	67 (34.0)	10-19 (n=149)	15 (10.1)	10 (6.7)	52 (34.9)
20-29 (n=389)	9 (2.3)	5 (1.3)	130 (33.4)	20-29 (n=323)	45 (13.9)	22 (6.8)	122 (37.8)
30-39 (n=357)	5 (1.4)	7 (2.0)	154 (43.1)	30-39 (n=329)	23 (7.0)	18 (5.5)	144 (43.8)
40-49 (n=171)	4 (2.3)	5 (2.9)	69 (40.4)	40-49 (n=138)	14 (10.1)	6 (4.3)	67 (48.6)
≥ 50 (n=258)	6 (2.3)	4 (1.6)	123 (47.7)	≥ 50 (n=174)	10 (5.7)	8 (4.6)	90 (51.7)
X^2	5.473	4.755	70.918	X^2	13.466	4.213	49.218
p value	0.3609	0.4465	< 0.0001*	*p* value	0.0194*	0.5191	< 0.0001*

OR = Odd Ratio; X^2= Chi square; CI = Confidence interval; * = Statistically significant

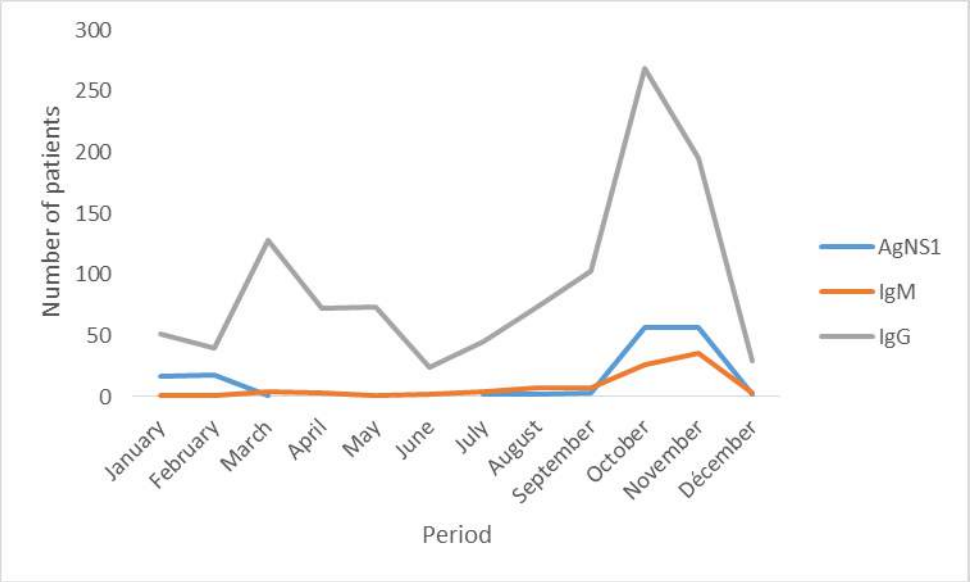

Fig 1: Evolution of dengue infection markers from 2020 to 2021 according to months

Majority of suspected DENV infections were reported during the last months (September-November) of the year. Patients positive for NS1 Ag during this period accounted for 84.3% (134/159) of all the positive patients in the entire study (Fig 1).

Discussion:

In this study, female represented the majority of the study population with 56.3% (56.1% in 2020 and 56.6% in 2021). Our results are similar to those of a previous study conducted at HOSCO where Ouattara et al., (21) reported 56.2% and 57.3% of females in 2016 and 2017 respectively, but slightly lower than the 69.2% reported in another study conducted in Ouagadougou (22). The predominance of females could be explained by the high representation of women in the general population of Burkina Faso (51.7%) (23) as well as the fact that females are more likely than males to attend health facilities for care.

The age of the study population ranged from 0 to 94 years with a means age of 29±14.46 years. Adolescents and children under 20 years of age represented 30.9% (525/1700) in 2020 and 23.3% (293/1257) in 2021. These results agree with 24.1% in 2016 and 17.8% in 2017 reported by Ouattara et al., (21). The prevalence of acute Dengue (NS1 Ag+) infection in our study population was 5.4%; 2.4% (41/1,700) in 2020 and 9.4% (118/1,257) in 2021, showing significantly higher rate in 2021 than 2020 (OR= 1.779, 95% CI = 1.288-2.458, p = 0.0005). A previous study conducted at HOSCO reported a prevalence of 17.3% over the period 2016 to 2017 (21), another study in Togo reported a prevalence of 17% in 2017 (24) and 15.1% in Ivory Coast (25). The decrease in the prevalence of Dengue in Burkina Faso could be explained by various reasons, including the COVID-19 pandemic, which has kept some patients away from health centers, forcing them to take care of asymptomatic or mild cases of Dengue themselves, and also the effect of the sensitization of the population and healthcare personnel on Dengue and the prevention strategies (urban sanitation, anti-vectorial fight) put in place by the Burkina Faso health authorities since the Dengue peaks of 2013 (21,26).

Our study shows that males were significantly more infected with DENV 7.0% (91/1,292) compared to 4.1% (68/1,665) in females (OR = 1.779, 95% CI = 1.288 - 2.458, p=0.0005). Previous studies have reported higher prevalence of DENV infection in men than in women (21,27,28). On the other hand, a study conducted in Vietnam found that males were underrepresented among Dengue cases, but with higher risk of developing the severe form of the disease than females (29), while in Nigeria, Ayolabi et al., (30) reported that women were significantly more infected than men in their study population.

Adolescent and children < 20 years of age (n=818) in our study also appeared to be affected by acute DENV infection (NS1 Ag+), with prevalence of 5.3% (43/818); 3.2% (17/525) in 2020 and 8.9% (26/293) in 2021 (OR = 2.90, 95%CI = 1.551 - 5.459, p= 0.001). According to some studies, children are the most affected by severe forms of DENV infection associated with mortality (31,32). However, in our study, the prevalence of DENV infection was high in age group 20-29 years with 7.6% (54/712), which was statistically significant on univariable analysis (OR=0.51, 95%CI=0.29-0.87, p=0.01). These results are similar to those of a study conducted in Malaysia which showed that adults are not spared from the threat of Dengue (33). Also in Burkina Faso, a study reported that adults are at risk of Dengue virus infections (34).

In the semi-urban areas of Kaya and Zorgho, DENV prevalence was 9.9% and 2.7% respectively (35). The level of urbanization between localities could explain these differences, especially since it is known that the population density is a factor conducive to the expansion of the DENV due to the insalubrity that favors the development of *Aedes,* which are the vectors (36,37). However, the difference between the results of 2014 Ridde et al., (35) study and the current study could be explained by the fact that the former only considered children aged 0 to 10 years.

Approximately 35% of the patients in 2020 and 39.8% in 2021 had secondary or prior DENV infection with positive IgG while IgM was positive in 1.5% in 2020 versus 5.4% in 2021, suggesting recent DENV infection. The prevalence of DENV IgG was significantly higher in males (44.0%) than females (32.1%) (OR=1.667, 95% CI = 1.434-1.938, p<0.0001) and in age groups 30-39 (43.4%), 40-49 (44.0%) and >50 years (49.3%) than other age groups (X^2=121.0, p<0.0001). This indicates that past exposure to DENV infection is higher among males and older age groups. The results of the three Dengue virus markers also showed that 42.9% of the patients were positive for at least one of the three markers. Ouattara et al., (21) reported 40.1% in the same facility.

It was noted in our study that acute DENV infections were rare from January to August but became increasingly common from September onwards. The majority of NS1 Ag positive patients were reported during the months of September to December, representting 84.3% (134/159) of total DENV infections, with peak infections in October and November. Our findings are similar to those of Ouattara et al., (21) who reported the peak of Dengue evolution between mid-October and mid-November. High prevalence of acute DENV infection was reported between September and December in Burkina Faso (38,39). The peak of the infection at this period is justified by the fact that after the rainy season, the climate is favorable to the multiplication of breeding sites and the proliferation of *Aedes*.

Conclusion:

The results of this study showed that the Dengue viral infection in Burkina Faso is a re-emerging disease and still constitutes a public health challenge. Constant surveillance to control the spread of DENV infection is necessary. The high prevalence of acute DENV infection reported during the last three months of the year, especially from October to November suggests an overlap between Dengue virus and malaria parasite transmissions at the end of the rainy season. In a

context of endemicity of both diseases, it is recommended to perform adequate tests to diagnose them in febrile patients. In addition, the proliferation of the vector and the transmission of the virus must be controlled. But with the resistance of *Aedes aegypti* and *Aedes albopictus* to insecticides, would it not be appropriate to explore new avenues of research such as "*genes drive*" technology (genetic forcing), nano-biotechnology or trials of larvicidal plant extracts for better prevention strategy of Dengue?

Contributions of authors:

SOTB wrote the first draft of the manuscript and revised the different versions of the manuscript AAZ, LT, AKO, and TMZ performed statistical analysis of all the data and revised different versions of the manuscript. AH, SZ, AA, and ST collected the data and revised the different versions of the manuscript. AD and JS designed the work and revised the different versions of the manuscript.

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Conflict of interest:

No conflict of interest is declared.

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Original Article **Open Access**

Mobile phones of hospital workers: a potential reservoir for the transmission of pathogenic bacteria

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Abstract:

Background: Mobile phones are increasingly associated with the transmission of pathogenic microbial agents. In the clinical setting where there is usually high exposure to pathogens, these devices may serve as vehicles for the transmission/spread of pathogens. This study determined the prevalence of bacterial contamination of mobile phones of health workers and the predisposing factors, in order to ascertain the risk of transmission of pathogenic bacteria through mobile phones.
Methodology: This study was carried out in a private medical center at Mbouda, Cameroon, involving 78 health workers including health professionals (nurses, physicians, laboratory scientists) and hospital support workers (cleaners, cashiers and security guards), recruited by convenient sampling. Sterile swab sticks moistened with physiological saline were used to swab about three quarter of the surface of each phone. The swabs were cultured on MacConkey and Mannitol Salt agar plates which were incubated aerobically at 37°C for 24 hours, while Chocolate agar plate was incubated in a candle extinction jar for microaerophilic condition. The isolates were identified using standard biochemical tests including catalase, coagulase, and the analytical profile index (API) system. Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0.
Results: Mobile phones of 75 of the 78 (96.2%) health workers were contaminated, with highest contamination rates for the phones of laboratory scientists (100%, 12/12), followed by support staff (98.9%, 13/14), nurses (97.7%, 43/44) and physicians (87.3%, 7/8), but the difference in contamination rates was not statistically significant (p=0.349). A total of 112 bacteria belonging to 12 genera were isolated, with predominance of *Staphylococcus aureus* (31.3%, n=35), *Micrococcus* spp (30.4%, n=34), coagulase negative staphylococci (10.7%, n=12) and *Pseudomonas* spp (5.4%, n=6). The laboratory (18.8%, 21/112) and medical wards (16.1%, 18/112) had the highest bacterial contamination of mobile phones (p=0.041), and more bacterial species were isolated from smartphones (68.8%, n=77/112) than keypad phones (31.2%, n=35/112) (p=0.032). There was no significant difference between phone contamination rates and the practice of hand hygiene or decontamination of work surfaces (p>0.05).
Conclusion: The presence of potentially pathogenic bacteria on cell phones of health-care workers emphasizes the role of fomites in the transmission of infectious diseases. Consequently, good hand hygiene and decontamination practices are encouraged among health workers in order to limit the spread of hospital-acquired infections.

Keywords: mobile phones, bacterial contamination, hospital workers, risk factors, nosocomial infections

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Les téléphones portables des personnels hospitaliers: un réservoir potentiel de transmission de bactéries pathogènes

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Résumé:

Contexte: Les téléphones portables sont de plus en plus associés à la transmission d'agents microbiens pathogènes. Dans le cadre clinique où il y a généralement une forte exposition aux agents pathogènes, ces dispositifs peuvent servir de véhicules pour la propagation de la transmission des agents pathogènes. Cette étude a déterminé la prévalence de la contamination bactérienne des téléphones portables des agents de santé et les

facteurs prédisposants, afin de déterminer le risque de transmission de bactéries pathogènes par les téléphones portables.
Méthodologie: Cette étude a été réalisée dans un centre médical privé à Mbouda, au Cameroun, impliquant 78 agents de santé, y compris des professionnels de la santé (infirmiers, médecins, scientifiques de laboratoire) et des agents de soutien hospitalier (agents de nettoyage, caissiers et agents de sécurité), recrutés par échantillonnage pratique. Des écouvillons stériles humidifiés avec du sérum physiologique ont été utilisés pour écouvillonner environ les trois quarts de la surface de chaque téléphone. Les écouvillons ont été cultivés sur des plaques de gélose MacConkey et Mannitol Salt qui ont été incubées en aérobiose à 37°C pendant 24 heures, tandis que la plaque de gélose au chocolat a été incubée dans un pot d'extinction de bougie pour une condition microaérophile. Les isolats ont été identifiés à l'aide de tests biochimiques standard, notamment la catalase, la coagulase et le système d'indice de profil analytique (API). Les données ont été analysées à l'aide du package statistique pour les sciences sociales (SPSS) version 20.0.
Résultats: Les téléphones portables de 75 des 78 agents de santé (96,2 %) étaient contaminés, avec les taux de contamination les plus élevés pour les téléphones des scientifiques de laboratoire (100%, 12/12), suivis par le personnel de soutien (98,9%, 13/14), infirmières (97,7%, 43/44) et médecins (87,3%, 7/8), mais la différence de taux de contamination n'était pas statistiquement significative (p=0,349). Au total, 112 bactéries appartenant à 12 genres ont été isolées, avec une prédominance de *Staphylococcus aureus* (31,3%, n=35), *Micrococcus* spp (30,4%, n=34), staphylocoques à coagulase négative (10,7%, n=12) et *Pseudomonas* spp (5,4%, n=6). Le laboratoire (18,8%, 21/112) et les services médicaux (16,1%, 18/112) avaient la contamination bactérienne la plus élevée des téléphones portables (p=0,041), et plus d'espèces bactériennes ont été isolées des smartphones (68,8%, n=77/112) que les téléphones à clavier (31,2%, n=35/112) (p=0,032). Il n'y avait pas de différence significative entre les taux de contamination du téléphone et la pratique de l'hygiène des mains ou de la décontamination des surfaces de travail (p>0,05).
Conclusion: La présence de bactéries potentiellement pathogènes sur les téléphones portables des travailleurs de la santé souligne le rôle des fomites dans la transmission des maladies infectieuses. Par conséquent, de bonnes pratiques d'hygiène des mains et de décontamination sont encouragées chez les agents de santé afin de limiter la propagation des infections nosocomiales.

Mots-clés: téléphones portables, contamination bactérienne, personnels hospitaliers, facteurs de risque, infections nosocomiales

Introduction:

Mobile phones are wireless hand-held electronic devices that are used primarily for communication (1). There are three main categories of mobile phones. The basic phones used for basic communication purposes such as making voice calls, sending and receiving short message service (SMS) messages and making use of unstructured supplementary services data (USSD) (1). The feature phones offer features additional to a basic phone, including cameras and increased storage, as well as the ability to access the Internet. On the other hand, smartphones offer advanced capabilities and features over feature and basic phones. Most smartphones run a full-featured operating system, allow users add applications to their phones and have wireless-fidelity (Wi-Fi) capabilities. These additional features make smartphones more widely used than feature and basic phones. It is estimated that more than 50% of the world's population make use of mobile phones and this number is expected to increase especially with the advent of the internet (2).

Despite the importance of the mobile phones as effective communication tools, these devices are increasingly being associated with the transmission of disease agents from person to person (3-5). In the hospital milieu, mobile phones, though not direct medical tool per se, are constantly being used in nearly all the sectors. It is a fact that the hospital environment usually harbors a variety of pathogenic microbes which can be transmitted from hospital staff to patients and vice versa (5-8).

It has been observed that health care professionals constantly handle mobile phones without disinfection in their bags and pockets or on their hands in the clinical setting (6); these practices may enhance the spread of pathogens. Thus, mobile phones of hospital workers may serve as important vehicles for nosocomial transmission of infections. Various research studies have shown that some microbes have the ability to persist on inanimate surfaces such as plastics, wooden material and mobile phones (9-13). High rates of contamination of mobile phones of health workers with potentially pathogenic microorganisms have been reported by many studies (13-16).

Amongst these microorganisms are bacterial species such as coagulase negative staphylococci, *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella* spp, viridians streptococci and *Bacillus* spp (13-16). There are reports that poor hand washing, absence of regular phone disinfection practices by health professionals predispose their mobile phones and those of others to colonization by bacteria (14-16). In this light, the usage of mobile phones by health workers within the hospital milieu, coupled with poor hygienic practices may be associated with high infection risks.

Although there are several reports on microbial contamination of mobile phones of health workers, there are limited data in our setting. This study was aimed at investigating the bacterial species that contaminate mobile phones of health workers and the factors ass-

ociated with these phones' contamination.

Materials and method:

Study area:

The study site was Adlucem Medical Foundation (AMF) hospital located at Mbouda, the chief town of Bambotus division in the western region of Cameroon. This hospital is a private health institution with about 80 staff, including physicians, nurses, laboratory technicians and other paramedical staff. The hospital consists of various units; notably, emergency, laboratory, imaging, antenatal & infant welfare clinic, pharmacy, theatre, dentistry, otorhinolaryngology unit, preventive medicine, mortuary, almoner and administrative unit. It also has several wards which include pediatric, male and female medical and surgical wards, gynecological ward, post-natal ward, intensive care unit/reanimation and nursery.

Research design, participant recruitment and ethical approval:

The study is a cross sectional hospital-based design involving health professionals (nurses, physicians, laboratory scientists) and hospital support workers (cleaners, cashiers and security guards). The study participants were recruited by convenient sampling and included all consenting hospital workers present at AMF hospital during the study period.

A structured questionnaire was administered on each participant to obtain information on demographics and risk factors for mobile phone contamination. The research protocol was reviewed and approved by the Faculty of Health Sciences Ethics Review Board of University of Bamenda, Cameroon.

Sample collection

A mobile phone was sampled for each participant and for those who have more than one phone, only the most commonly used phone was sampled. The mobile phones were categorized into two groups; the smartphones and the keypad phones (comprising all the basic type phones).

Samples were collected aseptically from each participant's mobile phone as previously reported (17). Briefly, the external surface of each phone was swabbed using sterile swab slightly moistened with sterile physiological saline. About three quarter of the surface of each phone was swabbed with key areas being the mouth piece, keys buttons and outlets. Swabbing of each phone was done through the entire front and sides to the back of the phone for about 3 minutes under aseptic conditions. The samples were transported immediately to the laboratory and kept at 4^{o}C prior to analyses.

Isolation and identification of bacteria:

The swabs were inoculated onto Chocolate agar (CHA), Blood agar (BLA), MacConkey agar (MAC) and Mannitol Salt agar (MSA) plates. The BLA, MAC and MSA plates were incubated aerobically at 37^{o}C for 24hrs while CHA plates were incubated at 37^{o}C in a candle extinction jar for the isolation of fastidious bacteria.

Presumptive identification of bacterial isolates from culture plates was based on colony morphology and Gram stain reaction, as previously described (18). Further confirmation of Gram-positive bacteria was done with catalase and coagulase tests while Gram-negative bacterial isolates were identified using Analytical Profile Index for Enterobacteriaceace (API-20E) according to the manufacturer's instruction (BioMerieux, Marcy-l'Etoile, France).

Data analysis

Data from the questionnaires and laboratory results were entered in Microsoft Excel office version 24 and analysis was done using the Statistical Package for Social Sciences (SPSS) version 20.0. The Chi-square test was used to compare proportions and to establish a statistical significance between the investigated variables. Statistical significance was established when the p value was < 0.05.

Results:

Sociodemographic characteristics of study participants

A total of 78 health workers were recruited from 19 different units of the AMF and a cell phone of each participant was sampled. The socio-demographic information of participants is presented in Table 1. There were more females (52.6%, n=41) than males (47.4%, n=37), however, this difference was not statistically significant. All the participants were above 20 years of age, with the majority in the age group 20-30 years (35.9%, n=28) and age group 30-40 years (34.6%, n=27). In addition, 40 out of the 78 (51.3%) participants were married while 33 (48.7%) were single.

The study participants were categorized into four groups based on profession, with physicians (10.3%, n=8), nurses (56.4%. n=44), laboratory scientists (15.4%, n=12) and support staff (17.9%, n=14).

Table 1: Socio-demographic characteristics of participating health worker at Adlucem Medical Foundation Hospital, Mbouda, Cameroon

Demographic parameters		Number	Percentage
Gender	Male	37	47.4
	Female	41	52.6
Age group (years)	< 20	0	0.0
	20 -30	28	35.9
	30-40	27	34.6
	>40	23	29.5
Marital status	Married	40	51.3
	Single	38	48.7
Category of hospital worker	Physicians	8	10.3
	Nurses	44	56.4
	Laboratory scientist	12	15.4
	Support staff	14	17.9
Type of phone	Smartphone	52	66.7
	Keypad phone	26	33.3

Table 2: Contamination rates of mobile phones of participating health workers at Adlucem Medical Foundation Hospital, Mbouda, Cameroon

Category of hospital worker	No of phones analysed	Number of phones contaminated	Percentage of phones contaminated	*p*-value
Physician	8	7	87.3	
Nurse	44	43	97.7	0.349
Laboratory Scientist	12	12	100.0	
Support staff	14	13	98.9	
Total	**78**	**75**	**96.2**	

Prevalence of bacterial contamination of the mobile phones of health workers

Out of the 78 phones examined, bacterial contamination occurred with 75, giving an overall bacterial contamination rate of 96.2% (Table 2). Mobile phones of laboratory scientists had the highest contamination rate (100%, 12/12) while those of physicians had the lowest contamination rate (87.3%, 7/8). However, the difference in the contamination rates of mobile phones of the various categories of health workers was not statistically significant (p=0.349).

Based on the type of phones, all the keypad phones (100%, 26/26) were contaminated with at least one bacterial genus while 49 of 52 (94.2%) smart phones were contaminated. On the other hand, mobile phones from the laboratory and medical wards had the highest bacterial contamination rate (18.6%, 14/75) each, followed by those from re-animation (10.6%, 8/75), surgery (9.3%, 7/75) and the PMI (8.0%, 6/75) units.

The level of contamination was also analysed with respect to the number of bacterial species isolated from each mobile and the categories are as follows; single-contamination (single bacterial species); double-contamination (two bacterial species) and multi-contamination (three or more bacterial species). It was observed that single-contamination of mobile phones was more frequent (52%) while multi-contami nation was least frequent (5.3%) (Fig. 1)

Bacterial species isolated from phones of health workers

A total of 112 bacterial pathogens belonging to 12 bacterial genera were isolated from phones of participating health workers (Table 3). Gram-positive bacteria (72.3%, n= 81) were commonly isolated than Gram-negative bacteria (27.7%, n=31), however, 10 species of Gram-negative bacteria were isolated compared to 2 species of Gram-positive bacteria. The predominant bacterial species were *Staphylococcus aureus* (31.3%, n= 35), *Micrococcus* spp (30.4%, n=34), coagul-

ase negative staphylococci (10.7%, n=12), *Pseudomonas* spp (5.4%, n=6) and *Haemophilus* spp (5.4%, n=6).

Generally, more bacterial species were isolated from smartphones (68.8%, 77/112) than keypad phones (31.2%, 35/112) (Table 3). Regarding the individual bacterial species, *S. aureus* was isolated about 7 times more frequently from smartphones (91.4%, n=32) than keypad phones (8.6%, n=3), and this difference was statistically significant (OR= 7.585; 95% CI=2.135-26.944, *p*=0.0004).

The distribution of bacterial isolates with respect to the various hospital units was statistically significant (*p*=0.041), with laboratory (18.8%, 21/112) having the highest proportion, followed by medical wards (16.1%, 18/112), PMI (8.9%, 10/112), reanimation (8.0%, 9/112), surgery (7.1%, 8/112) and reception (7.1%, 8/112) (Fig 2). Although Gram-positive bacteria were predominantly isolated, Gram-negative bacteria were isolated more frequently than Gram-positive bacteria from mobile phones in PMI (16.1% and 6.2%, respectively) and mortuary (9.7% and 0.0% respectively).

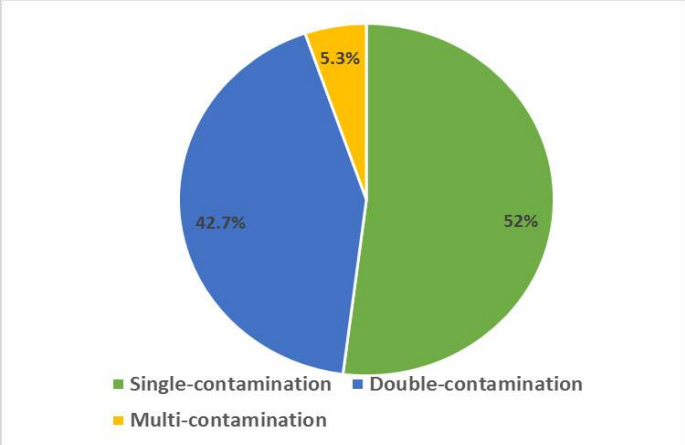

Fig 1: Percentage contamination of mobile phones based on the number of bacterial species
(Single-contamination: one bacterial species; double-contamination: two bacterial species; multi-contamination: three or more bacterial species)

Table 3: Bacterial pathogens isolated from mobile phones of participating health workers at Adlucem Medical Foundation Hospital, Mbouda, Cameroon

Bacterial species	**Number of bacteria isolated from phones (%)**				
	Smartphone	**Keypad phone**	**Total**	**OR (95% CI)**	***p* value**
Staphylococcus aureus	32 (91.4)	3 (8.6)	35 (31.3)	7.585 (2.135-26.944)	0.0004*
CoNS	7 (58.3)	5 (41.7)	12 (10.7)	0.600 (0.1762-2.043)	0.5115
Micrococcus spp	19 (55.9)	15 (44.1)	34 (30.4)	0.4524 (0.1937-1.056)	0.0782
Klebsiella spp	1 (33.3)	2 (66.7)	3 (2.7)	0.2171 (0.019-2.480)	0.2297
Citrobacter spp	2 (66.7)	1 (33.3)	3 (2.7)	0.9067 (0.079-10.351)	1.000
Pseudomonas spp	3 (50.0)	3 (50.0)	6 (5.4)	0.4324 (0.0828-2.260)	0.3744
Haemophilus spp	3 (50.0)	3 (50.0)	6 (5.4)	0.4324 (0.0828-2.260)	0.3744
Escherichia coli	1 (100)	0	1 (0.9)	1.392 (0.055-35.055)	1.000
Serratia spp	4 (100)	0	4 (3.6)	4.347 (0.228-83.036)	0.3075
Enterobacter spp	0	1 (100)	1 (0.9)	0.1484 (0.0059-3.738)	0.3125
Acinetobacter spp	1 (50.0)	1 (50.0)	2 (1.8)	0.4474 (0.0272-7.370)	0.5293
Cedecea spp	1 (50.0)	1 (50.0)	2 (1.8)	0.4474 (0.0272-7.370)	0.5293
Burkholderia spp	3 (100)	0	3 (2.7)	3.336 (0.168-66.381)	0.5507
Total	**77 (68.8)**	**35 (31.2)**	**112 (100)**		

CoNS = coagulase negative staphylococci; OR=Odds ratio; CI = Confidence Interval; * = statistically significant

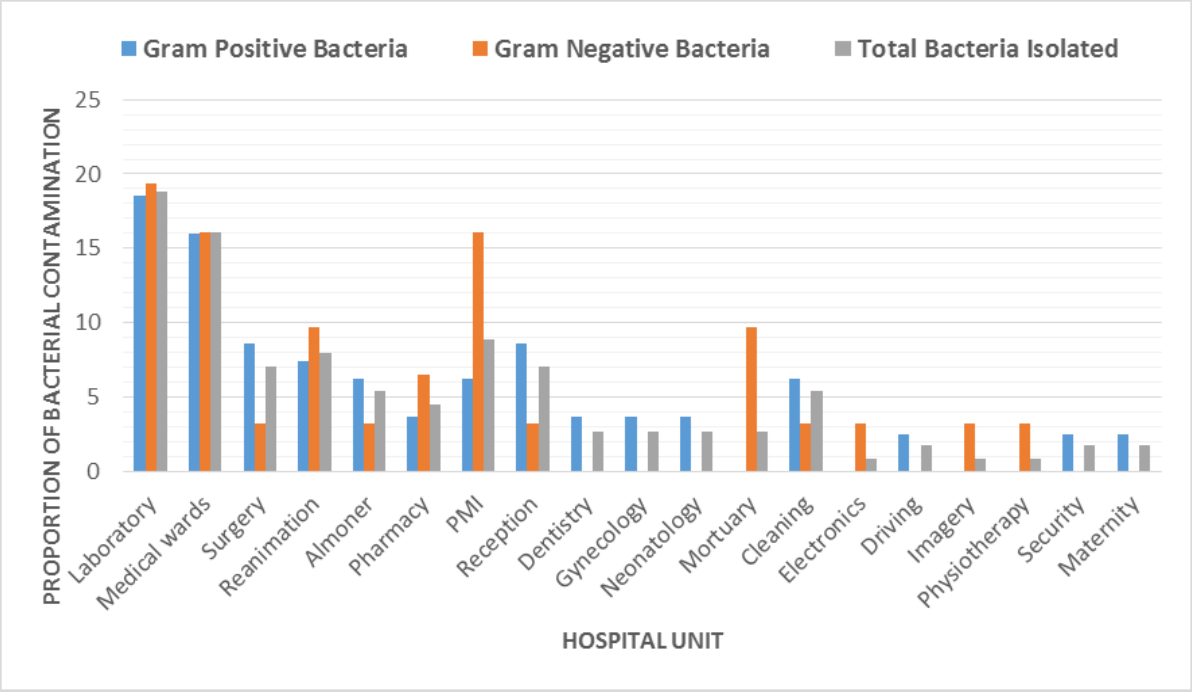

Fig 2: Distribution of bacterial isolates from mobile phones from different hospital units

Table 4: Prevalence of bacterial contamination of mobile phones of participating health workers at Adlucem Medical Foundation Hospital, Mbouda, Cameroon with respect to gender and age

Parameter	No contaminated (%)	No not contaminated (%)	*p* value
Gender			
Female	39 (95.1)	2 (4.9)	0.6179
Male	36 (97.3)	1 (2.7)	
Total	75 (96.2)	3 (3.8)	
Age group (years)			
20-30	27 (96.4)	1 (3.6)	0.9886
30-40	26 (96.3)	1 (3.7)	
>40	22 (95.7)	1 (4.3)	
Total	75 (96.2)	3 (3.8)	

No = Number

Predisposing factors for bacterial contamination of mobile phones:

The rate of contamination of phones was analysed with respect to the age group and gender of the study participants and the results presented in Table 4. There was no statistical significance difference in the rate of contamination based on gender (p=0.6179) and age group (p=0.9886).

Table 5 shows the rate of contamination of mobile phones with respect to hand hygiene practice, disinfection of work surface, and frequency of phone usage while working. More than half of the participants (52.0%, n=40) admitted they wash their hands with water and soap while 19 (24.6%) wash their hands with water/soap or detergent and also sanitize their hands with alcohol solution.

In addition, majority of the participants (52.1%, n=37) always disinfect their work surfaces with 10% hypochlorite before and after work. Meanwhile, 40 (51.3%) rarely use their mobile phones (<5 times) and 38 (48.7%) often use phones while working. However, there was no significant difference between the practice of hand hygiene, decontamination of work surfaces, and frequency of phone usage with the contamination rates of the mobile phones (p>0.05).

Table 5: Prevalence of bacterial contamination with respect to hand hygiene and work surface decontamination

Factors	Response	Contaminated (%)	Not contaminated (%)	*p* value
Hand hygiene (n=77)	With water only	4 (100.0)	0	0.2102
	With water and soap	39 (97.5)	1 (2.5)	
	With water and detergent	4 (80.0)	1 (20.0)	
	With alcohol solution only	8 (88.9)	1 (11.1)	
	A mixture of all	19 (100.0)	0	
Total		**74 (96.1)**	**3 (3.9)**	
Work surface decontamination (n=71)	Before work only	18 (100.0)	0	0.4108
	After work only	9 (100.0)	0	
	Before and after work	34 (91.9)	3 (8.1)	
	Before or after work	7 (100.0)	0	
Total		**68 (95.8)**	**3 (4.2)**	
Frequency of phone usage (n=78)	Rarely	39 (97.5)	1 (2.5)	0.9639
	Often	36 (94.7)	2 (5.3)	
Total		**75 (96.2)**	**3 (3.8)**	

Discussion:

Although mobile phones play a significant role in communication in the healthcare sector, they may act as vehicles in the spread of nosocomial infections. Studies have shown that mobile phones of health workers harbor significant amounts of potentially pathogenic microbes most of which are bacterial species (19-26). The present study investigated the mobile phones of 78 hospital workers including health professionals and support staff to ascertain the level of contamination and the factors associated with risk of contamination.

The overall contamination of mobile phones of hospital workers in our study was 96.2%, and mobile phones of laboratory scientists showed the highest contamination rate (100%) while those of physicians had the least contamination rate (87.3%). These results are similar to previous findings in Saudi Arabia (13), Cameroon (14), Ethiopia (15,16) and Iran (27) in which the prevalence of contamination of mobile phones of healthcare professionals ranged from 94-100%. Although relatively lower contamination rates have been reported by others (28), these are still high (>50%). Also, based on the type of phones, all (100%) keypad phones were contaminated with at least one bacterial genus while 94.2% of the smartphones were contaminated. Although this difference was not statistically significant, keypad phones are more likely to have debris on them over a longer period due to the roughness of their surfaces (29). As a result, it might be more difficult to clean or disinfect the surfaces of key pad phones than smartphones and this may encourage the growth of microbes.

On the other hand, mobile phones from laboratory (18.7%) and medical wards (18.7%) had the highest contamination rates. Previous studies have reported varied contamination levels of mobile phones from different units of the hospital. In one of such studies, Asfaw and Genetu (15) reported higher rates of bacterial contamination of the mobile phones in the intensive care unit (22.6%), the surgical ward (17.8%) and the laboratory (17.8%) compared with other hospital units. The relatively higher contamination rate in the laboratory may due to the numerous specimens handled in this unit (30). Also, the difference in the contamination rates in the various hospital units may be accounted for by the poor adherence to infection control practices.

With regard to the types of bacterial species isolated, Gram-positive bacteria (72.3 %) were more frequently isolated than Gram negative bacteria (27.7%), and the predominant species were skin commensals such as *S. aureus*, *Micrococcus* spp and coagulase negative staphylococci (CoNS), *Pseudomonas* spp and *Haemophilus* spp. Similar findings have been reported in Cameroon and elsewhere (13,14,28). In Zambia, Mushabati et al., (28) reported common bacterial isolates from phones to be CoNS, *S. aureus* and *Bacillus* spp while the study by Bodena et al., (16) in Ethiopia had CoNS, *S. aureus* and *Klebsiella* spp as predominant bacteria from mobile phones. The study conducted by Sedighi and colleagues (27) in Iran isolated more of CoNS, *S. aureus* and *P. aeruginosa*.

It is common to find Gram-positive bacteria contaminating surfaces especially the skin. *Staphylococcus* aureus is commonly found in the anterior nares of healthcare workers and can easily be transmitted on surfaces via contaminated hands (24). The predominance of CoNS reflects the fact that normal commensal of the skin can easily be transferred to any object that comes in contact with body surfaces. The combination of constant handling and heat generated during receiving phone calls may facilitate the survival and growth of microorganisms on the cell phone surface (21,31). Though CoNS is a nor-

mal skin flora, in hospital setting, this bacterium could emerge as a pathogenic microorganism causing nosocomial infections (32).

Important Gram-negative nosocomial pathogens such as *P. aeruginosa* and *E. coli* were isolated in our study. According to previous report, *Pseudomonas* is metabolically versatile, ubiquitous in both terrestrial and aquatic environments and infection caused by the organism is very difficult to manage (32). The presence of this bacterium on mobile phones of medical personnel should be very concerning. *Escherichia coli* was the most prevalent aerobic bacteria in human/animal faeces and its presence may suggests faecal contamination (21). Poor personal hygiene or contamination from already contaminated sites may account for the presence of this organism (33). Contrary to our study, Asfaw and Genetu (15) isolated more of Gram-negative (53.6%) than Gram-positive bacteria (46.4%). These differences may reflect variations in the source of contamination, hand hygiene and other infection control practices, especially as the Gram-positive bacteria isolated were mostly skin commensals while the majority of the Gram-negative bacteria were enteric bacilli.

Although more keypad phones were contaminated compared with smartphones, it was observed that more bacterial species were significantly isolated from smartphones (68.8%) than keypad phones (31.2%). It is a fact that smartphones have more features than keypad phones and coupled with their advantage of internet accessibility, smartphones are frequently being used than keypad phones (34). The high frequency of touch of these phones may explain the higher bacterial isolation rate compared with keypad phones. In the present study, there was no significant difference in the rate of contamination of the phones with respect to gender and age. Our findings are similar to those of Mushabati et al., (28) who reported no association between bacterial contamination of mobile phones and age, gender and profession. Notwithstanding, some previous studies significantly associated bacterial contamination with male gender (15, 16) and age group 20 to 35 years (15)

According to the World Health Organization (WHO), proper hand hygiene and sanitation practices may help reduce the spread of nosocomial infections (33). However, our study did not find any association between contamination rates of mobile phones and practice of hand hygiene, disinfection of work surfaces and frequency of phone usage while working. This discrepancy may be as a result of the small number of samples (n=78) used in our study as well as variations in the type of detergents used especially as some domestic soap may not be antiseptic, as previously reported (31). Bodena and colleagues (16) reported that the absence of regular hand washing was significantly associated with mobile phone contamination, and another study in Ethiopia reported a significantly higher mobile phone contamination rate among healthcare workers in intensive care unit (ICU) who neither disinfected their phones nor wash their hands, compared to their counterparts who performed these tasks (15). A similar study carried out in Cameroon reported that using mobile phones during working hours, moving around patients with phones, and lack of hand hygiene practices of health professionals were risk factors for the dissemination of multi-drug resistant (MDR) pathogens (14).

Conclusion:

The bacterial species isolated from mobile phones of hospital workers in our study were mostly skin commensals and enteric bacteria. More bacterial species were isolated from smartphones than keypad phones and the laboratory and medical units had more contamination than other units. The presence of potentially pathogenic bacteria on mobile phones of healthcare workers emphasizes the role of fomites in the transmission of infectious diseases. Consequently, good hand hygiene and decontamination practices are encouraged among healthcare workers in order to limit the spread of hospital-acquired infections.

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Conflict of interest:

Authors declare no conflict interest

Authors contributions:

MEAB was responsible for conception and design of the study, laboratory analyses, data validation and analyses, revision, general supervision and final approval of the manuscript. MM was involved in sample collection, laboratory analyses, data validation and analyses, and writing of the manuscript.

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Original article **Open Access**

Serological study of leptospirosis in cats from Algeria

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Abstract:

Background: By the nature of their environment and behavior, stray cats are at risk of exposure to leptospirosis. Leptospirosis is an emerging zoonotic disease with worldwide distribution. The prevalence of leptospirosis in the feline species in Algeria is unknown. The main objectives of this study are to determine the seroprevalence and identify the most common *Leptospira* serovars in stray cats in the Algiers region.
Methodology: Serum samples from 144 randomly selected healthy stray cats from 57 municipalities of the Algiers region were analyzed by the microscopic agglutination test (MAT). The MAT was performed to determine the antibody titers against nine *Leptospira* serovars (Canicola, Copenhageni, Icterohaemorrhagiae, Autumnalis, Grippotyphosa, Bratislava, Pomona, Pyrogenes, Patoc). The age of each cat was estimated based on dentition and physical appearance, and information on cat sex, breed and clinical status were collected. Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 17.0
Results: *Leptospira* antibodies were detected in 8 of 144 healthy stray cats, giving a seroprevalence rate of 5.6% [95% confidence interval (CI)=1.814-9.297]. The antibody titers ranged from 1:100 to 1:3200. Serovars Pyrogenes (1:100) and Patoc (1:100) were the most prevalent serovars detected in 2.8% (4/144) of the cats, followed by serovars Icterohaemorrhagiae (1:100) and Bratislava (1:100) detected in 2.1% (3/144) of the cats. The seroprevalence of 7.8% (7/90) in the male cats was higher than 1.9% (1/54) in the female cats but this did not reach a significant difference (OR=4.47, 95% CI=0.5344-37.387, p=0.2586). All the positive cats were over one year of age.
Conclusion: This study showed that stray cats in Algiers are exposed to leptospirosis. In addition, the serovars detected are very common serovars in dogs and humans. The control of leptospirosis is largely dependent on general hygiene measures and the control of animal reservoirs. Additional investigations are necessary to clarify the epidemiology of the disease in the different regions of Algeria.

Keywords: *Leptospira*, cats, serology, MAT, Algiers

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Etude sérologique de la leptospirose chez les chats d'Algérie

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Résumé:

Contexte: De par la nature de leur environnement et de leur comportement, les chats errants sont à un risque d'exposition à la leptospirose. La leptospirose est une maladie zoonotique émergente de distribution mondiale. La prévalence de la leptospirose chez l'espèce féline en Algérie est inconnue. Les principaux objectifs de cette étude sont de déterminer la séroprévalence et d'identifier les sérovars de *Leptospira* les plus fréquents chez les chats errants de la région d'Alger.
Méthodologie: Des échantillons de sérum de 144 chats errants sanitaires sélectionnés au hasard dans 57 communes de la région d'Alger ont été analysés par le test d'agglutination microscopique (MAT). Le MAT a été réalisé pour déterminer les titres d'anticorps contre neuf sérotypes de *Leptospira* (Canicola, Copenhageni, Icterohaemorrhagiae, Autumnalis, Grippotyphosa, Bratislava, Pomona, Pyrogenes, Patoc). L'âge de chaque chat a été estimé sur la base de la dentition et de l'apparence physique, et des informations sur le sexe, la race et l'état clinique du chat ont été collectées. Les données ont été analysées à l'aide du package statistique pour les sciences sociales (SPSS) version 17.0
Résultats: Des anticorps contre *Leptospira* ont été détectés chez 8 des 144 chats errants sanitaires, donnant un taux de séroprévalence de 5,6% [intervalle de confiance (IC) à 95%=1,814-9,297]. Les titres d'anticorps variaient de 1:100 à 1:3200. Les sérovars Pyrogenes (1:100) et Patoc (1:100) étaient les sérovars les plus prévalents détectés chez 2,8 % (4/144) des chats, suivis des sérovars Icterohaemorrhagiae (1:100) et Bratislava (1:100) détectés chez 2,1% (3/144) des chats. La séroprévalence de 7,8 % (7/90) chez les chats mâles était supérieure à 1,9 % (1/54) chez les chattes, mais cela n'a pas atteint une différence significative (OR=4,47, IC 95%=0,5344-37,387, p=0,2586). Tous les chats positifs avaient plus d'un an.
Conclusion: Cette étude a montré que les chats errants d'Alger sont exposés à la leptospirose. De plus, les sérovars détectés sont des sérovars très répandus chez le chien ou chez l'homme. Le contrôle de la leptospirose est largement tributaire des mesures d'hygiène générales et de la lutte contre les réservoirs animaux. Des investigations complémentaires sont nécessaires pour préciser l'épidémiologie de la maladie dans les différentes régions de l'Algérie.

Mots clés: *Leptospira*, chats, sérologie, MAT, Alger

Introduction:

Leptospirosis is a global disease affecting many domestic and wild animal species, and is considered a zoonotic disease. It causes serious problems in tropical and temperate climates whether in urban or rural environments (1). This zoonosis is caused by pathogenic spirochetes of the genus *Leptospira* which colonize the renal tubules where they reproduce before being excreted in the urine (2). Infected urine or contaminated water are sources of leptospirosis and *Leptospira* can enter the body of mammalian hosts through lacerations of skin, mucous membranes, conjunctiva and inhalation of aerosols (3,4).

Previously, domestic cats were thought to be resistant to leptospirosis and many practitioners do not consider feline leptospirosis in the differential diagnosis with other diseases (5). However, recently published reports on feline leptospirosis concluded that cats are exposed to *Leptospira* and may play a role in the epidemiology of this disease (6,7). The presence of viable pathogenic Leptospires in the urine of cats has been proven (8,9). Therefore, the species can play a role in the transmission of the zoonosis.

Our previous study in Algiers was molecular and did not demonstrate carriage and urinary excretion of pathogenic Leptospires in cats (10). According to Hartmann et al., (11), renal carriage and leptospiruria in naturally infected cats may have been underestimated. Rodents are known to serve as the main reservoir of pathogenic *Leptospira*. Thus, the number of infected cats could also be high, as rodent hunting is believed to be the main source of infection in cats (11). Infection from water or urine of cohabiting dogs seems to play a minor role in cats (11). In Algeria, leptospirosis poses a real public health problem and every year there are human cases that occur however, animal studies are limited in the region (10,12-15). Cats are becoming increasingly popular as pets in Algiers and therefore it is important to have data to assess at what rate cats pose a risk for human leptospiral infection.

The aims of the present study were to determine the seroprevalence of *Leptospira* and the most prevalent serovars in stray cats in the region of Algiers, using the most common serological test for the diagnosis of leptospirosis, micro-agglutination test (MAT).

Materials and method:

Study design and sampling

In order to carry out a serological survey and detect the incriminated serovars, 144 stray cats were randomly selected from the 57 municipalities of Algier region. About 5 to 10 ml of venous blood were collected in dry tubes. The sera were obtained by centrifugation for 5 to 10 minutes at 3000 rpm and then stored at −20°C until serological tests were performed. The age of each cat was

estimated based on dentition and physical appearance. Information regarding the sex, breed and clinical status was recorded.

Ethical statement

The study was approved by the ethics committee and decision board (number 416/2017) of Entreprise Publique à Caractère Industriel et Commercial - Hygiene Urbaine et Protection de l'Environnement (EPIC-H.U.P.E) of Wilaya of Algiers (Ex: HURBAL).

Microscopic agglutination test

The microscopic agglutination test (MAT) with 92% sensitivity and 60-100% specificity is regarded as the 'gold standard' method for the diagnosis of leptospirosis, and detects different serovars (16). The MAT was performed according to the Office International des Epizooties (OIE) standards 2008. The MAT was carried out at *Leptospira* unit, Pasteur Institute of Algiers, Algeria, and antibody titres were determined against nine *Leptospira* serovars (Canicola, Copenhageni, Icterohaemorrhagiae, Autumnalis, Grippotyphosa, Bratislava, Pomona, Pyrogenes, and Patoc) (Table 1). The MAT titre equal to or higher than 1:100 was considered positive.

Table 1: Panel of *Leptospira* strains used for MAT

Serogroups	Serovars	Strains
Canicola	Canicola	Hond Utrecht
Icterohaemorrhagiae	Copenhageni	Willinberg
Icterohaemorrhagiae	Icterohaemorrhagiae	Verdun
Autumnalis	Autumnalis	Akiyami A
Grippotyphosa	Grippotyphosa	Moskva V
Australis	Bratislava	Jez Bratislava
Pomona	Pomona	Pomona
Pyrogenes	Pyrogenes	Salinem
Semaranga	Patoc	Patoc1

Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL). Association of variables (sex, age, breed, clinical signs) with the seroprevalence of leptospirosis was done using Chi-square ($\chi 2$) test (Yates corrected). P values lower than 0.05 were considered as indicative of significance.

Results:

Microscopic agglutination test result

Antibodies were detected samples of 8 of the 144 cats, representing a sero-prevalence of 5.6% [95% confidence interval (CI) =1.814-9.297]. All the 8 cats tested positive to at least one *Leptospira interrogans* serovar at a dilution of ≥ 1:100. Serovars Pyrogenes (1:100) and Patoc (1:100) were the most prevalent, detected in 2.8% (4/144) of the cats, followed by serovars Icterohaemorrhagiae (1:100) and Bratislava (1:100), detected in 2.1% (3/144) of the cats. One serovar each was detected in 3 cats, two serovars each in 4 cats and three serovars in 1 cat (Table 2).

Characteristics and risk factors associated with seropositivity to *Leptospira*

All the stray cats sampled were cats of common breed, hence all the 8 cats seropositive for *Leptospira* were common breed. There were 90 (62.5%) male and 54 (37.5%) female cats; 112 (77.8%) cats were over one year while 32 (22.2%) cats were under one year old. The seroprevalence of 7.8% (7/90) in the male cats was higher than 1.9% (1/54) in the female cats but this did not reach a significant difference (OR = 4.47, 95% CI = 0.5344-37.387, p=0.2586). All the stray cats seropositive for *Leptospira* were over 1 year old. Also, all the stray cats sampled were healthy and did not show any clinical signs of disease (Table 3).

Table 4 shows published articles on leptospirosis from other countries over the last five years (17-24), with seroprevalence rates reported in cats varying between 0% and 42%. The table provides infirmation on study area, period of study, way of life, clinical status, number of serum samples collected, positive results, predominant serovars or serogroups, and cut-off values used in each study.

Discussion:

In the present study, we evaluated the prevalence of *Leptospira* infection among cats in Algiers regions of Algeria. Serological method by MAT was used in order to determine the infectious status of the 144 stray cats. Looking at studies carried out in other countries published over the last five years, the seroprevalence observed in cats varies between 0% and 42% (17-24). Our study records a low seroprevalence of 5.6% in stray cats (95%CI=1.814-9.297). As there is no systematic vaccination program of the feline population against leptospirosis in Algiers, all the more reason that these animals live outside, these positive sera can be considered as active or previous infections. The lack of vaccine against leptospirosis for cats is justified by the low morbidity of the disease. Therefore, seropositivity is a true indicator of exposure and cannot be confused with post-vaccination seropositivity (23).

Our prevalence rate is similar to that reported in several studies (18,20,25) except for some studies where the prevalence was higher due to the cut-off value of 1:50 used (22-24). In cats, antibody levels are commonly low and often lower than those in other animals (26-29). Environmental factors such as outdoor habits, presence of farm

animals that may shed Leptospires in the neighborhood, hunting habits, or even the season of the year, can explain the broad ranges of antibody prevalence reported in the literature (20). Reports have shown that leptospirosis prevalence can differ not only according to country, but to particular region also (30), even as different cut off values (≥1:100) and serovar panels used in laboratories may affect the prevalence. There is no consensus on the most appropriate cut-off value to choose in cats, and cats are thought to respond to infection with low antibody titers, ranging from 1:30 to 1:400, as has been demonstrated in experimental and natural infections (22). The results of our study suggest that stray cats in Algiers are in contact with pathogenic Leptospires, probably through other maintenance host species such as rodents.

Table 2: Antibody titers to various *Leptospira* serovars in the serum of stray cats from Algiers, as assessed by MAT

Serovars / **Cases**	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**	**Case 7**	**Case 8**
Canicola	-	-	-	-	-	-	-	-
Copenhageni	-	-	-	-	-	-	-	-
Icterohaemorrhagiae	-	-	1600	-	-	100	1600	-
Autumnalis	-	-	-	-	-	-	-	-
Grippotyphosa	-	-	-	-	-	-	-	-
Bratislava	-	-	-	100	200	-	-	100
Pomona	-	-	-	-	-	-	-	-
Pyrogenes	100	200	3200	-	100	-	-	-
Patoc	100	100	-	100	200	-	-	-

MAT = Microscopic agglutination test

Table 3: Seroprevalence and univariable analysis of risk factors associated with *Leptospira interrogans* among sampled cats

Independent Variables	**Categories**	**Number of cats sampled**	**Total number of positive results (%)**	**OR (95% CI)**	***p* value**
Breed	Common breed	144	8 (5.6)	-	-
Sex	Female	54	1 (1.9)	4.47 (0.5344-37.387)	0.2586[+]
	Male	90	7 (7.8)		
Age (years)	< 1 year	32	0	0.1891 (0.01062-3.369)	0.1996[+]
	> 1year	112	8 (7.1)		
Clinical sign		144	8 (5.6%)	-	-
Total			144		

OR=Odds ratio; CI=Confidence interval; + = not statistically significant

In the present study, cross-reactions between two or more serogroups were present in all cases. Serovars Pyrogenes and Patoc were the most detected servars, followed by Icterhemorrhagiae and Bratislava. It is possible that cats were infected with serovars not included in the panel or unknown serovars. The simultaneous seropositivity exhibited by some cats, not only in our study but also in several other studies, could be explained either by true cross-reactivity in the cats, or by simultaneous exposure of animals to different serovars (20). The high titers observed for the serovar Icterohemorrhagiae (1:1600) in this study, would suggest that rats are the source of infection for some cats. Serovar Canicola was not detected in this study, knowing that it is the major serovar, as far as canine leptospirosis is concerned. It has been already reported that serovars Icterohemorrhagiae, Canicola, Grippotyphosa, Pomona and Bratislava are the most common *Leptospira* serovars isolated from cats (Table 4). However, the range of serovars should not be limited to local strains as the infection may be caused by a rare serovar or a strain not previously described.

We included the saprophytic strains (Patoc) in our diagnostic panel, which can cross-react with antibodies produced by certain pathogenic serovars (20). Four of the 8 cats with antibodies against pathogenic *Leptospira* did not have antibodies against saprophytic serovars. This can be explained by the fact that infections are old and saprophytic serovars, in particular serovar Patoc, have limited ability to detect cross-reactions with antibodies from past infections (31). According to the guidelines of the International Leptospirosis Society, the range of serovars should not be limited to local strains and thus, serovar Patoc should be included, because it cross-reacts with human or animal antibodies generated by a number of pathogenic serovars (18).

None of the seropositive cats presented with clinical signs compatible with leptospirosis such as fever, weight loss, jaundice, lethargy, ascites, renal failure or hepatitis (19). In addition to low serological response, cats are reported to rarely develop clinical leptospirosis (5,32,33). Nevertheless, clinical cases of leptospirosis have been reported in cats infected with higher titers (1:800) of serovar Pomona (19). Conclusively, cats do not seem clinically sensitive to *Leptospira* serotypes circulating in Algiers.

In our study, male cats were more seropositive than female cats, which is similar to what was reported in the study by Weis et al., (34), while the study by Bourassi et al., (22) noted that seropositive cats were more females than males. In many studies however, gender was not significantly associated with *Leptospira* seropositivity in cats (6,7,18,28,35-37). The seropositive cats in our study were all over one year old. Bourassi et al., (22) reported the same result that positive cats were more adults than juveniles. On the other hand, in the study by Milan et al., (27), seropositivity was more frequent in juvenile than in adult cats. Older age was already reported in other studies as a risk factor for *Leptospira* infection in cats (35-37).

Conclusion:

To the best of our knowledge, this is the first report of seroprevalence of pathogenic Leptospires in cats from Algiers by the MAT serological technique. In addition, the serovars detected are very frequent serovars in dogs and humans. Control of leptospirosis largely depends on general hygiene measures and control of animal reservoirs. Additional investigations are necessary to clarify the epidemiology of the disease in other animal species and in other regions of Algeria.

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Authors contributions:

ZS conceptualize the study, and was involved in methodology, visualization, and writing of the manuscript; AKA was involved in conceptualization, methodology, resources provision, supervision and visualization; BeA was involved in the methodology and manuscript writing; BoA was involved in methodology and visualization; HNK was involved in the methodology; HD.j was involved in the methodology and manuscript writing; and BI was involved in resources provision, supervision and visualization

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Conflict of interest:

Authors declare no conflict of interest

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Table 4: Leptospirosis infection rates and commonly reported serovars in cats from studies conducted worldwide

Region (country)	Year of the study	Way of life	Clinical status	Number of Sera collected	Positive results	Prevalence (%)	Dominated Serovars/‖Serogroups	Cut-off value	References
Quebec (Canada)	1974-1976	NI	Suspected of having leptospirosis	19	0	0	Nd	1: 100	38
Trinidad and Tobago	NI	NI	NI	40	5	12.5	‖Canicola, Icterohaemorrhagiae, Hebdomadis	-	39
New Zealand	NI	House	NI	225	20	8.8	Hardjo, Pomona, Ballum, Copenhageni, Balanica, Canicola	1:24	29
São Paulo (Brazil)	NI	NI	NI	172	22	12.8	Pomona	1:100	35
South East Australia	1988-1990	NI	NI	59	10	16.9	Pomona, Copenhageni, Grippotyphosa, Tarassovi	1:50	40
Glasgow (Scotland)	NI	NI	Ill, leptospirosis was not suspected	87	8	9.2	Hardjo, Icterohaemorrhagiae Autumnalis	1:30	26
South India	2000	Living on the premises of rice mill	NI	9	6	66.6	‖Autumnalis, Canicola, Icterohaemorrhagiae	1:80	41
Thessaloniki (Greece)	1997-1998	Owned	Total	99	33	33.3	Rachmati, Bratislava, Ballum, Bataviae, Canicola, Panama, Pyrogenes	1:50	28
			Ill	51	18	35.3			
			Healthy	48	15	31.3			
France	NI	NI	Ill	98	47	48.0	‖Canicola, Sejroë, Australis, Icterohaemorrhagiae	-	42
Southern Kyushu (Japan)	NI	NI	NI	117	9	7.7	Autumnalis, Hebdomadis, Australis, Icterohaemorrhagiae, Pyrogenes	1:50	43
Tehran (Iran)	2003	Total	NI	111	30	27.0	Canicola, Hardjo, Icterohaemorrhagiae	1:100	44
		Stray		89	19	21.3			
		Household		22	11	50.0			

Andalucia (Spain)	2004-2007	Feral	NI	53	7	14.0	Icterohaemorrhagiae, Ballum	1:100	27
Goiânia (Brazil)	2008	NI	Healthy	330	23	6.9	Cynopteri, Djasiman, Butembo, Castellonis, Patoc	1:100	45
Mahalla (Egypt)	2006-2007	Feral	NI	2	1	100.0	Grippotyphosa	1:50	46
Ahvaz (Iran)	2007-2008	Stray	NI	102	5	4.9	Ballum, Australis	1:100	36
United States	2010	Feral	NI	63	3	4.8	Autumnalis, Pomona, Icterohaemorrhagiae, Bratislava	1:100	47
Reunion Island	2009	Stray	NI	30	8	26.6	‖Panama	1:100	48
Quebec (Canada)	2007	NI	Presented for different clinical signs	40	10	25.0	Bratislava, Autumnalis	1:100	49
Southern Chile	2011-2012	Urbanand rural cats	NI	124	10	8.1	Autumnalis, Canicola, Bataviae	1:100	6
Paraíba (Brazil)	2011	Total	Healthy	129	7	5.43	Pomona	1:100	37
		Owned		61	4	6.56			
		Stray		68	3	4.41			
Southern Thailand	2010-2011	Total	NI	225	21	9.3	Shermani, Javanica, Icterohaemorrhagiae, Australie, Pyogenes	1:100	50
		Stray cats		155	17	11.0			
		Household cats		70	4	5.7			
Quebec (Canada)	2010-2012	NI	Healthy cats	125	9	7.2	Pomona, Bratislava, Grippotyphosa Icterohaemorrhagiae	1:100	7
			Cats with kidney disease	114	17	14.9			
Belgrade (Serbia)	2012-2013	Healthy	Stray	161	45	26.7	Grippotyphosa, Icterohaemorrhagiae, Pomona, Canicola, Batavie, Australis	1:100	30
Mashhad (Iran)	2008-2010	Total	Healthy	147	10	6.8	Hardjo, Pomona, Icterohaemorrhagiae	1:100	51
		Households		42	0	0			
		Stray		52	1	0.52			
		Rural		53	9	4.77			

Northeastern Brazil	2013-2015	Rural	NI	43	2	4.7	Andamana, Patoc	1:100	52
Merida (Mexico)	2005	Owned free roaming cats	NI	13	3	23.2	Canicola, Australis	1:100	53
Saint-Christophe Island	2014-2015	NI	NI	50	2	4.0	Cynopteri, Pomona	1:100	25
Munich (Germany)	2013-2015	Outdoor	Various clinical signs	195	35	17.9	Australis, Bratislava, Grippotyphosa, Copenhageni	1:100	34
Iowa (USA)	2015-2016	Stray and shelter	NI	139	12	8.6	Pomona, Icterohaemorrhagiae, Bratislava, Hardjo, Grippotyphosa	1:100	17
Thailand	2016-2017	NI	Healthy except three	260	14	5.4	Anhoa, Autumnalis, Celledoni, Copenhageni, Djasiman, Icterohaemorrhagiae, Patoc	1:20	18
Malaysia	2017-2018	Shelter	Healthy	82	21	25.6	‖Bataviae, Javanica, Ballum	1:100	19
Spain	2017-2018	Stray and shelter	NI	244	10	4.1	Cynopteri, Ballum, Bratislava, Grippotyphosa, Proechimys	1:20	20
Appalachia (USA)	2017-2018	Shelter	NI	43	0	0	--	1:100	21
Prince Edward Island, Canada	2017-2018	Feral cats	Healthy (presented for sterilization)	20	200	10.0	Icterohaemorrhagiae, Canicola, Grippotyphosa, Bratislava, Pomona	1:50	22
Northern and western Reunion Island	2013	Domestic cats	Healthy	50	21	42.0	Icterohaemorrhagiae, Ballum (Castellonis)	1:40	23
		Stray cats (Urban)		42	13	31.0			
Okinawa Island, Japan	2016-2018	Stray	-	241	40	16.6	‖Javanica, Hebdomadis	1:80	24

NI= not identified, not cited‖= Serogroup

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Case Series Communication **Open Access**

Emergence of nosocomial-acquired extensively drug-resistant and pandrug-resistant Enterobacterales in a teaching hospital in Kuwait

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Abstract:

Background: The emergence and high ascendancy of infections caused by extensively-drug-resistant (XDR) and pandrug-resistant (PDR) Enterobacterales isolates is a serious clinical and public health challenge. Isolation of PDR Gram-negative bacteria (GNB) in clinical setting is very rare and rarer is the infection caused by XDR GNB. Apart from restricted therapeutic options, these infections are associated with increased mortality and morbidity. Urgent studies to re-evaluate existing therapeutic options and research into new antibiotic molecules are desperately needed. The objectives of this study are to report the emergence of rarely encountered multidrug-resistant (MDR), difficult-to-threat, CRE infections in our hospital and investigate their molecular epidemiology.
Methodology: This was a retrospective observational analysis of six patients with severe infections caused by XDR and PDR Enterobacterales isolates at Mubarak AL Kabeer Teaching Hospital, Jabriya, Kuwait, over a period of one and half years. The mechanisms of resistance in these isolates were then prospectively investigated by molecular characterization and genomic studies.
Results: The majority of infections were caused by *Klebsiella pneumoniae* (83.3%, 5/6) and one (16.6%) was caused by *Escherichia coli*. Three patients had bloodstream infection (BSI), one had both BSI and urinary tract infection (UTI), one had respiratory tract infection, and the last one had UTI. Two patients were infected with OXA-48 producers, one patient was infected with NDM-1 producer, one patient was infected with NDM-5 producer, one patient was infected with both NDM-1 and OXA-48 producer and the last patient was infected with both NDM-5 and OXA-181 producer. For definite treatment, all patients received combination therapy. The mortality rate was high (50.0%).
Conclusion: The high mortality rate associated with XDR and PDR Enterobacterales infections and the limited antimicrobial options for treatment highlight the need for improved detection of these infections, identification of effective preventive measures, and development of novel agents with reliable clinical efficacy against them.

Keywords: Extensively-resistant; pandrug-resistant; Enterobacterales; nosocomial; infections; treatment; resistance genes; Kuwait

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Émergence d'Entérobactéries nosocomiales acquises ultrarésistantes aux médicaments et pandrug-résistantes dans un hôpital universitaire au Koweït

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Résumé:

Contexte: L'émergence et la montée en puissance des infections causées par des isolats d'entérobactéries ultrarésistantes (XDR) et pandrug-résistantes (PDR) constituent un sérieux défi clinique et de santé publique. L'isolement de bactéries Gram-négatives PDR (GNB) en milieu clinique est très rare et plus rare est l'infection causée par XDR GNB. En dehors des options thérapeutiques restreintes, ces infections sont associées à une augmentation de la mortalité et de la morbidité. Des études urgentes pour réévaluer les options thérapeutiques existantes et la recherche de nouvelles molécules antibiotiques sont désespérément nécessaires. Les objectifs de cette étude étaient de signaler l'émergence d'infections à CRE multirésistantes (MDR), difficiles à menacer, rarement rencontrées dans notre hôpital et d'enquêter sur leur épidémiologie moléculaire.
Méthodologie: Il s'agissait d'une analyse observationnelle rétrospective de six patients atteints d'infections graves causées par des isolats d'entérobactéries XDR et PDR à l'hôpital universitaire Mubarak AL Kabeer, Jabriya, Koweït, sur une période d'un an et demi. Les mécanismes de résistance de ces isolats ont ensuite été étudiés de manière prospective par caractérisation moléculaire et études génomiques.
Résultats: La majorité des infections ont été causées par *Klebsiella pneumoniae* (83,3%, 5/6) et une (16,6%) a été causée par *Escherichia coli*. Trois patients avaient une infection du sang (BSI), un avait à la fois une BSI et une infection des voies urinaires (UTI), un avait une infection des voies respiratoires et le dernier avait une UTI. Deux patients ont été infectés par des producteurs d'OXA-48, un patient a été infecté par un producteur de NDM-1, un patient a été infecté par un producteur de NDM-5, un patient a été infecté par un producteur de NDM-1 et d'OXA-48 et le dernier patient a été infecté avec le producteur NDM-5 et OXA-181. Pour un traitement définitif, tous les patients ont reçu une thérapie combinée. Le taux de mortalité était élevé (50.0%).
Conclusion: Le taux de mortalité élevé associé aux infections XDR et PDR Enterobacterales et les options antimicrobiennes limitées pour le traitement soulignent la nécessité d'améliorer la détection de ces infections, l'identification de mesures préventives efficaces et le développement de nouveaux agents avec une efficacité clinique fiable contre elles.

Mots-clés: extrêmement résistant; résistant aux pandrogues; les Entérobactéries; nosocomiale; infections; traitement; gènes de résistance; Koweit

Introduction:

Global emergence of multidrug-resistant (MDR) Gram-negative bacteria is of major public health concern because of limited treatment options, increased morbidity and mortality, and lack of uniform infection prevention and control guidelines (1,2). Mortality rates reported with carbapenemase-producing *Klebsiella pneumoniae* infections vary from 22-72% (2-5). This wide range in mortality rates is dependent on the types of population studied and analyzed such as age, underlying disease and comorbidity. The rate may also be affected by inclusion of patients colonized by multi-drug resistant organisms (MDROs) rather than true infections with MDROs, which is what can affect the patient outcome and success of therapy.

Carbapenems are a class of antibiotics considered the drugs of choice for treatment of life-threatening infections (6). The emergence of carbapenem-resistant Enterobacterales (CRE) has added a new dimension to the burden of limited therapeutic options available to clinicians. In addition, these MDROs have the propensity to spread rapidly as attested to by their worldwide spread in India, Pakistan, United Kingdom, the Gulf region and in some low-income resource countries like Nigeria (7-9). The Centers for Disease Control and Prevention (CDC) has noticed an increase in CRE-related infections in the US and consequently advised on possible procedures to avoid their spread (10). As previously seen with ESBLs, patients colonized with CRE are at higher risk of infections due to these CREs (11).

In recent years, cases of extensively drug-resistant (XDR) and pandrug-resistant (PDR) Enterobacterales infections with unfavorable consequences have been reported around the globe (2,12-14). The choice of appropriate antimicrobial agents is a big challenge due to their XDR or PDR phenotype and co-resistance with other β-lactams, aminoglycosides, quinolones, and fosfomycin. Pandrug-resistant Enterobacterales have been reported sporadically in many countries (14-17). However, PDR Enterobacterales associated with clinical infections in Kuwait are still rare and have only been reported in a few studies (17).

In this communication, we report the characteristic features of patients with infections caused by XDR and PDR Enterobacterales isolates, the molecular analysis of the genes mediating their resistance phenotypes and the associated relatively high mortality rates seen in Kuwait.

Patients and methods:

Study setting:

This study was conducted on infected patients seen and managed at the Mubarak AL Kabeer Teaching Hospital, Jabriya, Kuwait.

Study design:

The first part of the study was a retrospective observational analysis of six patients with severe infections caused by XDR and PDR Enterobacterales isolates. The isolates were then secondarily investigated prospectively by molecular characterization and genomic analyses.

Ethical approval:

Institutional ethical approval was obtained from the Health Sciences Centre Ethical Committee, Health Sciences Centre, Kuwait University (permit number VDR/EC/4025). No waiver was obtained for the study. Collection of the specimens was conducted according to the Declaration of Helsinki and with particular institutional ethical and professional standards. No additional specimens were collected from the patients for this study. Informed consent was obtained from all participants of the study or the legally authorized representative for unconscious patient. The patient identities were kept anonymous.

Data collection:

The data concerning the patients were collected retrospectively from laboratory and medical in-patient records during a period of one and half year (January 2018 – June 2019). Important bio-data and useful information collected were age, gender, ethnic origin, past medical history prior to admission, diagnosis on admission, date infected, length of hospital stays, history of previous hospital admission, previous antibiotics used, co-morbidities, travel history outside the country within 12 months preceding admission and countries visited.

Case presentations:

Case 1:

This was a 39-year-old lady admitted with a slipped right-sided Double-J (DJ) stent and symptoms of urinary tract infection (UTI) on April 11, 2019. Her past medical history included prior admission for right kidney obstruction, hydronephrosis and acute respiratory distress syndrome (ARDS). During the current admission, she underwent ureteroscopy and replacement of DJ stent. Urine sample obtained via right-sided percutaneous nephrostomy (PCN) was sent to the microbiology laboratory for culture and susceptibility testing. The urine culture yielded $>10^5$ CFU/ml of XDR *Klebsiella pneumoniae* (designated KP1a) susceptible to only colistin and tigecycline. In a repeat septic workup, the blood culture and PCN culture yielded PDR *K. pneumoniae* (KP1b) resistant to all antibiotics including colistin and tigecycline.

The patient was started on empirical intravenous (IV) meropenem 1g 8 hourly. Despite this therapy, she went into septic shock requiring inotropic support and necessity for intensive care unit (ICU) admission where IV amikacin 700 mg once daily was empirically added. Upon isolation of XDR *K. pneumoniae*, she was immediately transferred into an isolation room and strict contact precaution protocol was initiated. Intravenous colistin, 9 million IU loading dose, followed by 2 million IU 12 hourly for 21 days was added. The PCN was replaced with a new DJ stent. The patient remained in the ICU isolation room, responded very well to the therapy and was discharged home after 3 weeks. There was no recurrence of her infections at outpatient (OP) follow-up 4 weeks after discharge.

Case 2:

A 73-year-old diabetic man with considerable comorbidities; nephropathy, hypertension, dyslipidemia, osteoarthritis and immune thrombocytopenic purpura (on oral prednisolone), peripheral vascular disease, and bilateral chronic lower limb ischemia, underwent coronary artery bypass grafting for ischemic heart disease (multi-vessel disease) in February of 2017. He was on intermittent hemodialysis via right subclavian long-term hemodialysis catheter. At the admission on May 17, 2019, he presented with signs and symptoms of pulmonary edema and acute-on-chronic kidney disease. Shortly after admission, he developed severe shortness of breath requiring intubation with ventilator support and was shifted to the ICU. On the 3rd day post-intubation, he developed fever (Temp of 39^{o}C) and leukocytosis (WBC count of 15,000/µL). Samples of blood, urine and respiratory secretion were sent to the laboratory for investigations. Culture of endotracheal secretion yielded XDR *K. pneumoniae* (KP3) resistant to all antibiotics including colistin, aminoglycosides and the fluoroquinolones, except tigecycline (MIC=1.5 µg/ml). His rectal swabs were positive for XDR *K. pneumoniae* with the same susceptibility pattern as KP3.

He was put on contact isolation and empirically started on IV meropenem 1g 12-hourly. The antibiotic regimen was changed to IV colistin, 9 million IU loading dose, followed

by 2 million IU 12 hourly, nebulized colistin, 3 million IU daily, and IV tigecycline, 100 mg loading dose, followed by 50mg 12-hourly. There was remarkable improvement after 7 days on this therapy and was subsequently extubated and discharged to the ward on the same antibiotics which continued for a period of 14 days. He was discharged home hale and hearty. His out-patient follow-up was unremarkable.

Case 3:

This was an 85-year-old male patient, a known case of diabetes mellitus (DM), hypertension (HBP), old cerebrovascular accident (CVA) sustained in 2008, bedridden on naso-gastric tube feeding, urethral stricture on permanent Foley's catheter, benign prostatic hypertrophy (BPH) for which he underwent transurethral prostate resection in 2012, and normal pressure hydrocephalous. In addition, he also had a history of retinal detachment back in 2014. Six months before presentation, he was diagnosed as a case of myasthenia gravis in Jordan and was given IV immunoglobulin with slight improvement. He had recently developed bilateral pressure sores on the heels necessitating admission to our hospital on January 3, 2019.

The intervention given included debridement of his wound and was administered IV meropenem, 1g 8 hourly for 7 days. Subsequently, he was well enough to be discharged home. However, on February 10 2019, he was brought back to the emergency room (ER) desaturated and semi-comatose. He was intubated and shifted directly to the ICU. IV norepinephrine bitartrate (levophed) 2 µg/kg/min and IV dopamine 10 µ/kg/min were started to maintain his blood pressure. After collecting appropriate specimens for sepsis work-up, he was started empirically on IV piperacillin-tazobactam, 4.5g 6 hourly and IV levofloxacin, 500 mg daily. On day 2 of admission to the ICU, the result of his blood culture revealed the growth of XDR *K. pneumoniae* (KP4) susceptible only to amikacin and tigecycline. The antibiotic regimen was changed to IV colistin 9 million, IU loading dose, followed by 4 million IU 12 hourly and IV tigecycline 100 mg loading dose followed by 50 mg 12 hourly.

He was isolated in a single room under contact precaution. On day 7 post-ICU admission, he showed some improvement and was transferred to the ward. However, while on the ward, his condition deteriorated, he developed disseminated intravascular coagulopathy (DIC), and went into cardiac arrest. All efforts to resuscitate him failed and he was pronounced dead a few hours after.

Case 4:

This was a 68-year-old patient, known hypertensive patient with uncontrolled DM. Past medical history revealed previous hemicolectomy and ileostomy done in 2014 for colon carcinoma for which he was also treated with chemotherapy. He was admitted to our hospital on April 25, 2018 with complaints of swollen and tender right thigh accompanied with limitation of movement and fever (Temp: 39^{o}C). Ultrasonography (US) of the affected limb showed ilio-psoas abscess extending to the right thigh.

The initial intervention was prompt incision and drainage of the abscess with the pus specimen sent to the microbiology laboratory for culture and susceptibility testing. He was started on empirical IV piperacillin-tazobactam, 4.5g 8 hourly. The pus yielded methicillin-sensitive *Staphylococcus aureus* (MSSA). As a result, IV clindamycin, 900 mg 8 hourly was added. There was an initial improvement followed by sudden drop in the blood pressure with accompanying abdominal pain but abdominal x-ray did not reveal any underlining cause. Repeat blood culture was negative. His blood pressure continued to drop requiring administration of vasopressors and transfer to the ICU. The next blood culture, taken on June 22 2018, yielded mixed infections with susceptible *K. aerogenes* and XDR *Escherichia coli* (EC1) susceptible only to amikacin and tigecycline. Strict contact precautions and isolation were promptly instituted. The treatment regimen was replaced by IV amikacin 15mg/kg 8 hourly plus IV tigecycline 100 mg loading dose, followed by 50mg 12 hourly. There was no clinical improvement and the patient passed away 48 hours after.

Case 5:

A 63-year-old diabetic patient with high BP, congestive cardiac failure (CHF), left ventricular hypertrophy and multiple myeloma was admitted to our hospital on January 12, 2018 with a 10-day history of shortness of breath that worsened on exertion, associated with orthopnea and productive cough. She had a past medical history of previous admission at an Indian hospital for chest infections caused by H1N1 Influenza A virus two months prior to presentation. On examination, she was afebrile with stable vital signs. Her chest examination revealed bilateral basal crepitations. Chest radiograph (CXR) ordered at this time showed bi-

lateral pleural effusion.

She was promptly started on diuretics and oral levofloxacin 750mg once daily. On day 2 of admission, she developed tachypnea and was put on bi-level positive airway pressure (BiPAP) therapy. Repeat CXR showed worsening of pleural effusion necessitating pleural tap. On day 3, the pleural fluid was reported as sterile by the microbiology laboratory. She was then seen by a consultant haematology/oncologist who prescribed interventional chemotherapy which was stoutly rejected by the patient family. On day 6, she developed high grade fever (Temp: 40^{o}C). Hematological investigations revealed leukocytosis with a shift to the right (22,000 WBC/µL). IV meropenem, 1g 8 hourly was added to the levofloxacin. Her level of consciousness deteriorated but her family once again refused ICU admission. On day 7, she developed evidence of UTI and acute renal shut down requiring hemodialysis. Urine culture yielded significant growth of PDR *K. pneumoniae* (KP5) resistant to all antibiotics tested. She was isolated with strict contact precautions and put on inotropes and vasopressors for BP maintenance. Despite all the efforts, she succumbed to the ongoing sepsis due to PDR organism and passed away on day 8 of admission.

Case 6:

This 36-year-old patient was a known case of Down's syndrome, hiatus hernia, peptic ulcer disease, bronchial asthma (on nebulizer) and, in addition, had laparoscopic cholecystectomy done 3 years prior to presentation. There was history of multiple admissions to our hospital because of exacerbation of his asthma and community-acquired pneumonia (CAP) during the preceding 6 months. On this hospital visit on June 23 2019, he was admitted to the surgical ward for elective repair of hiatus hernia. Post-operatively, he was moved to the ICU for observation. Without appropriate microbiology/ infectious diseases consultation, he was started on empirical IV piperacillin-tazobactam 4.5g 8-hourly. On day 2 post-op, he vomited coffee-ground contents and a diagnosis of bleeding peptic ulcers was confirmed by upper GI endoscopy. A naso-jejunostomy tube was inserted.

On day 5 post-op, he developed septic shock requiring intubation and inotropic support via peripherally inserted central catheter (PICC) line. Full septic work-up was ordered and his antibiotic therapy changed to IV meropenem 1g 8 hourly. His blood culture taken from peripheral line yielded XDR *K. pneumoniae* (KP6) resistant to all antibiotics except tigecycline, trimethoprim-sulfamethoxazole and aminoglycosides. Meropenem was stopped and IV trimethoprim-sulfamethoxazole 960 mg 12 hourly combined with IV gentamicin 5mg/kg/ day, in divided doses, 8-hourly, was commenced. He was isolated under strict contact precautions. There was remarkable improvement on this combination therapy and he was discharged to another hospital in USA for further non-infectious management.

Microbiology investigations:

Bacterial isolates:

All specimens were transported to the routine clinical diagnostic microbiology laboratory and processed according to the standard operational protocols (SOPs). Briefly, 10ml of blood was drawn from each patient as indicated and dispensed into 2 bottles of BD BACTEC 9240 (Microbiology System, Becton Dickinson, Massachusetts, USA) and incubated in BACTEC Blood Culture System (BD) for 24 h to 5 days at preset temperature of 37^{o}C. Other specimens were inoculated onto appropriate selective and non-selective culture media according to instructions in the SOPs and incubated in air at 37^{o}C for 24-48 hours or in the Anoxomat Anaerobic System (bioMérieux, Marcy, L'Étoile, France) that generated CO_2 (10%), H_2 (10%) and N_2 (80%), for 24 –hours to 7 days.

Representative isolates from these patients' specimens were stored at -80^{o}C in Cryo-Bank beads (Mast Group Limited, Merseyside, UK) in the Anaerobe/Hospital Infection Reference Laboratory in the Department of Microbiology, Faculty of Medicine, Kuwait University. They were later retrieved, thawed and investigated for the genes mediating their resistance phenotypes by molecular characterization.

Re-isolation and identification of isolates:

The thawed bacterial suspensions were sub-cultured onto MacConkey agar (Oxoid, Basingstoke, UK) and blood agar (Oxoid). The inoculated plates were incubated in air at 37^{o}C for 24 h. Representative colonies on the agar were then re-identified to species level by VITEK-2 ID System (bioMérieux, Marcy, L'Etoile, France).

Antibiotic susceptibility testing (AST):

AST was performed by determining the minimum inhibitory concentrations (MICs) of clinically relevant antibiotics using the E-test (bioMérieux) in accordance with the manufacturer's protocol, and by the agar dilution method where applicable (e. g. colistin susceptibility by CLSI) (18). In each test run, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *E. coli* ATCC 35218 were included as potency and media controls. Results were

interpreted according to the interpretative criteria of the Clinical and Laboratory Standards Institute (18), and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Guidelines for tigecycline and colistin (19).

Phenotypic and genotypic tests for detection of carbapenemase

The Modified-Hodge test and metallo-beta-lactamase (MBL) E-test were performed according to standard methods (20) to phenotypically detect metallo-beta-lactamases. Bacterial strains phenotypically MBL-positive were screened for the presence of genes mediating carbapenemase production, specifically *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{KPC}$, *bla*$_{OXA-23}$, *bla*$_{OXA-48}$, *bla*$_{OXA-181}$, *bla*$_{NDM-1}$, and *bla*$_{NDM-5}$, using multiplex PCR assay method with previously published primers (21-23), as well as by gene sequencing. Colistin resistance gene was investigated by multiplex PCR assay (24).

Definitions:

According to the European Centre of Disease Prevention and Control (ECDC) and Centre of Disease Control and Prevention (CDC), MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories; XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories while PDR is defined as non-susceptibility to all commercially available antimicrobial categories (25). Carbapenem-resistant Enterobacterales (CRE) was defined as isolate that showed decrease susceptibility to the carbapenems (ertapenem MIC > 0.5 µg/ml; imipenem, MIC > 1 µg/ml and/or meropenem, MIC > 1 µg/ml) regardless of carbapenem resistance (18).

Results:

The demographic profiles, underlying morbidities and empirical antibiotic therapy of the cases are summarized in Table 1. Three (50%) of the 6 patients had bloodstream infection (BSI) and 2 (33.3%) had UTI. There were 4 males and 2 females with male-to-female ratio of 2:1. Their ages ranged from 36 to 85 years (mean age of 60.7 years). Four (67%) of the 6 patients were diabetic. A total of 4 (67%) patients were treated with colistin, 3 (50%) with tigecycline, 2 (33.3%) with meropenem and 1 (16.7%) with trimethoprim-sulfamethoxa zole.

As shown in Table 2, 3 (50%) of the 6 patients infected by XDR and PDR isolates died, and 3 (50%) survived and discharged home in good health. All but one of them were infected by *K. pneumoniae*. A total of 8 *K. pneumoniae* and 1 *E. coli* were isolated from the patients. The first patient was infected by the same phenotypically identical *K. pneumoniae* from urine, PCN and blood cultures.

Table 3 shows that all the clinical isolates were highly resistant to all the cephalosporins, fluoroquinolones, carbapenems, colistin and most of the aminoglycosides. The 3 blood isolates, KP4, KP6 and ECI, were susceptible to tigecycline with MICs of 2µg/ml, 2µg/ml and 0.38µg/ml, respectively. The results of multiplex PCR and sequencing showed that the isolates harbored multiple genes that mediated resistance, namely; *bla*$_{NDM-1}$, *bla*$_{NDM-5}$, *bla*$_{OXA-48}$, and *bla*$_{OXA-181}$ (Table 4), but *bla*$_{VIM}$, *bla*$_{IMP}$, and *bla*$_{KPC}$ were not detected. All the colistin resistant isolates were negative for the presence of *mcr*-1 plasmid genes.

Discussion:

In this communication, we presented a series of six cases encountered in our hospital over one and half-year period with high mortality rate of 50%. Approximately, 83.3% of the patients were infected by MDR *K. pneumoniae* resistant to almost all the available antibiotics in our hospital formulary. These isolates were encountered in different clinical specimens including blood, urine and respiratory secretions. An interesting commonality was the history of previous multiple hospitalizations by all patients either in the same clinical center or different centers or even in different countries altogether.

Table 1: Characteristics of patients infected by multidrug-resistant microorganisms during hospital admission in Kuwait

S/N	Age (years)	Past medical history and previous hospital admission	Duration of current hospitalization before infection	Category of current admission	Current admitting diagnosis	Comorbidities	Empirical intervention
1	39	Insertion of Double-J stent; Kidney obstruction and hydronephrosis in the Surgical ward	3 days	ICU	Septic shock	Acute respiratory distress syndrome (ARDS)	Ureteroscopy, Inotropic support, IV meropenem 1g 12 hly and amikacin 500 mg 12 hly
2	73	Multiple hospital admissions, known case of DM, previous IHD with coronary bypass surgery. on hemodialysis	3 days	ICU	Pulmonary edema, acute-on-chronic renal failure, severe shortness of breath	Nephropathy, hypertension, dyslipidemia, osteoarthritis, immune thrombocytopenia purpura, bilateral chronic lower limb ischemia	Intubation with ventilator support, IV meropenem 1g 12 hly
3	85	Known case of DM, CVA, BPH, TUPR in 2012, normal pressure hydrocephalous, Myasthenia gravis. Previous hospital admission for bilateral pressure sores of the heels and debridement	2 days	ICU	Desaturation and loss of consciousness at home.	Low blood pressure, disorientation, urethral stricture, BPH	Intubation, IV levophed 2 mcg/kg/min, IV dopamine 10 mcg/kg/min, IV piperacillin-tazobactam 4.5 mg 6 hly, IV levofloxacin 500 mg daily
4	68	Known HBP, DM, previous hemicolectomy and ileostomy for colon cancer, chemotherapy.	53 days	Surgical ward → ICU	Ilieo-psoas abscess, fever, intra-abdominal pain.	Uncontrolled DM, HBP	Ultrasonography, incision and drainage, IV piperacillin-tazobactam 4.5g 8 hly, IV clindamycin 900mg 8 hly. Later, IV amikacin 15mg/kg 8 hly, IV tigecycline 100 mg start and 50 mg x 12 hly.
5	63	DM, HBP, CCF, multiple myeloma, previous hospital admission in India, H1N1 flu chest infection	6 days	ICU	Bilateral pleural effusion, UTI, acute renal failure	DM, HBP, high fever (T=40^{o}C), leukocytosis	Per oral levofloxacin 750 mg od, bi-level positive airway pressure, IV meropenem 1g 8 hly
6	36	Known case of Down's syndrome, hiatus hernia, PU, asthma, CAP, cholecystectomy at previous hospital admission 3 years back, history of multiple hospital admissions	6 days	Surgical ward → ICU	Hiatus hernia	PU, bronchial asthma	Naso-jejunostomy tube, IV piperacillin-tazobactam 4.5 g 8 hly, later IV meropenem 1g 8 hly

DM=diabetes mellitus; CCF=congestive cardiac failure; HBP=high blood pressure; IHD=ischemic heart disease; CVA=cerebrovascular accident; TUPR=transurethral prostatic resection; BPH=benign prostatic hypertrophy; PU=peptic ulcer; CAP=community-acquired pneumonia; IV=intravenous; ICU=intensive care unit; od=once daily; S/N=serial number

Table 2: Characteristics of patients' pan-resistant and extensively-resistant isolates, site of infection, antimicrobial therapy and outcome of infections during hospital admission

S/N of patient (isolate designation)	Isolate	Site of infection	Total duration of hospital admission	Prior antibiotic therapy	Antimicrobial treatment/duration	Outcome
1 (KP1a)	*Klebsiella pneumoniae*	Urine culture	30 days	IV meropenem 1g 8 hly	IV meropenem 1g 8hly + IV amikacin 700 mg OD. Later, IV colistin 9 million IU loading dose followed by 2 million IU 12 hly + IV meropenem 1g 8 hly x 21 days.	Survived
(KP1b)	*Klebsiella pneumoniae*	PCN culture				
(KP1c)	*Klebsiella pneumoniae*	Blood culture				
2 (KP2a)	*Klebsiella pneumoniae*	Respiratory secretion	21 days	IV meropenem 1g 8 hly	IV colistin 9 million IU loading dose, followed by 2 million IU 12 hly + nebulized colistin 2 million IU od + IV tigecycline 100 mg start followed by 50 mg 12 hly x 21 days	Survived
(KP2b)	*Klebsiella pneumoniae*	Rectal swab				
3 (KP3)	*Klebsiella pneumoniae*	Blood culture	14 days	IV piperacillin-tazobactam 4.5 g 6 hly	IV colistin 9 million IU start, followed by 2 million IU 12 hly + IV tigecycline 100 mg loading dose followed by 50 mg 12 hly	Expired
4 (EC1)	*Escherichia coli*	Blood culture	58 days	IV piperacillin-tazobactam 4.5g 8 hly, IV clindamycin 900 mg tid	IV amikacin 15/kg 8 hly + IV tigecycline 100 mg loading dose, followed by 50 mg 12 hly	Expired
5 (KP4)	*Klebsiella pneumoniae*	Urine culture	10 days	Oral levofloxacin 750 mg OD	IV meropenem 1g 8 hly + IV levofloxacin 750 mg/150 ml	Expired
6 (KP5)	*Klebsiella pneumoniae*	Blood culture	14 days	IV piperacillin-tazobactam 4.5 g 8 hly, IV meropenem 1g 8 hly	IV trimethoprim-sulfamethoxazole 960 mg 12 hly + IV gentamicin 5 mg/kg/day in divided dose 8 hly	Survived

Kp1a=*Klebsiella pneumoniae* isolated from urine culture of case no 1; Kp1b=*K. pneumoniae* isolated from percutaneous nephrostomy tube culture of case no 1; Kp1c=*K. pneumoniae* isolated from blood culture of case no1; Kp2a=*K. pneumoniae* isolated from respiratory secretion of case no 2; Kp2b=*K. pneumoniae* isolated from rectal swab of case no 2; Kp3=*K. pneumoniae* isolated from blood culture of case no 3; EC1=*E. coli* isolated from blood culture of case no 4; Kp4=*K. pneumoniae* isolated from blood culture of case no 5; Kp5=*K. pneumoniae* isolated from blood culture of patient no 6; S/N=serial number; tid=three times a day

The *K. pneumoniae* isolates harbored multiple genes that encoded carbapenemases production such as NDM and OXA classes which sequence analysis revealed to be NDM-1, NDM-5, OXA-48 and OXA-181. Selection of appropriate antimicrobial agents for the treatment of infections caused by these CRE strains was a clinical challenge. Despite concerns about being nephrotoxic and neurotoxic, the use of old antibiotics, such as colistin, as a 'last resort' treatment have come to the forefront (26). Unfortunately, colistin is being overused, or used with suboptimal dosing regimen in some settings, including our hospital, and this has inadvertently led to growing resistance of Gram-negative bacteria to this life-saving agent (26,27).

All but one of the clinical isolates in our study were phenotypically resistant to colistin. It is conceivable that this was, in part, due to the overuse of this agent in our hospital. PCR for *mcr* gene mediating resistance to colistin was negative in all our isolates, a finding that is not surprising as most cases of plasmid-mediated *mcr*-1 genes have been found mainly in *E. coli* isolates.

Table 3: Minimum inhibitory concentrations of the antimicrobial agents tested against the clinical isolates

Antibiotic (breakpoint in µg/ml)	KP1a/KP1b/KP1c (MIC µg/ml)	KP2a/KP2b (MIC µg/ml)	KP3 (MIC µg/ml)	EC1 (MIC µg/ml)	KP4 (MIC µg/ml)	KP5 (MIC µg/ml)
Cefotaxime (4)	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)
Ceftazidime (4)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)
Cefepime (16)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)
Piperacillin/tazobactam (128/4)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)
Ertapenem (2)	R (>32)	R (>32)	R (>32)	R (>256)	R (>32)	R (>256)
Imipenem (4)	R (>32)	R (>32)	R (8)	R (>256)	R (>32)	R (>32)
Meropenem (4)	R (>32)	R (>32)	R (>32)	R (>256)	R (>32)	R (32)
Amikacin (64)	R (>256)	R (>256)	S (4)	R (>256)	S (6)	S (16)
Gentamicin (16)	R (768)	R (>1024)	R (768)	R (512)	R (512)	S (1)
Ciprofloxacin (1)	R (>32)	R (24)	R (>32)	R (>32)	R (>32)	R (32)
Levofloxacin (2)	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)	R (32)
Tigecycline	R (6)	S (1.5)	S (2)	R (3)	S (0.38)	S (2)
Colistin	R (24)	R (12)	R (12)	R (24)	R (4)	R (12)
Trimethoprim/sulfamethoxazole (4)	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)	S (<20)
Rifampicin	R	R	R	R	-	-
Chloramphenicol (32)	R (32)	R (32)	R (>256)	R (48)	R (32)	-

Kp1a = *Klebsiella pneumoniae* isolated from urine culture of case no 1; Kp1b = *K. pneumoniae* isolated from percutaneous nephrostomy tube culture of case no 1; Kp1c = *K. pneumoniae* isolated from blood culture of case no1; Kp2a = *K. pneumoniae* isolated from respiratory secretion of case no 2; Kp2b= *K. pneumoniae* isolated from rectal swab of case no 2; Kp3 = *K. pneumoniae* isolated from blood culture of case no 3; EC1 = *E. coli* isolated from blood culture of case no 4; Kp4 = *K. pneumoniae* isolated from blood culture of case no 5; Kp5 = *K. pneumoniae* isolated from blood culture of patient no 6; MIC = Minimum inhibitory concentration µg/ml; S = sensitive; R = resistant. Rifampicin disc diffusion method was used as there was no E-test available.

Table 4: Molecular characterization of the genes mediating carbapenemase production in the XDR/PDR isolates.

Serial number	Organism	*bla*$_{NDM}$	*bla*$_{KPC}$	*bla*$_{OXA}$	*bla*$_{VIM}$	*bla*$_{IMP}$	*bla*$_{mcr-1}$
1	KP1a/KP1b/KP1c	*bla*$_{NDM-5}$	-	-	-	-	-
2	KP2a/KP2b	*bla*$_{NDM-1}$	-	*bla*$_{OXA-48}$	-	-	-
3	KP3	-	-	*bla*$_{OXA-48}$	-	-	-
4	EC1	-	-	*bla*$_{OXA-181}$	-	-	-
5	KP4	*bla*$_{NDM-1}$	-	-	-	-	-
6	KP5	*bla*$_{NDM-5}$	-	*bla*$_{OXA-181}$	-	-	-

Kp1a = *Klebsiella pneumoniae* isolated from urine culture of case no 1; Kp1b = *K. pneumoniae* isolated from percutaneous nephrostomy tube culture of case no 1; Kp1c = *K. pneumoniae* isolated from blood culture of case no1; Kp2a = *K. pneumoniae* isolated from respiratory secretion of case no 2; Kp2b= *K. pneumoniae* isolated from rectal swab of case no 2; Kp3 = *K. pneumoniae* isolated from blood culture of case no 3; EC1 = *E. coli* isolated from blood culture of case no 4; Kp4 = *K. pneumoniae* isolated from blood culture of case no 5; Kp5 = *K. pneumoniae* isolated from blood culture of patient no 6.

Another class of new antibiotic is tigecycline, a minocycline derivative known as glycylcycline, which is being exploited and overused in many hospitals globally. It has a broad-spectrum activity against many Gram-positive, Gram-negative as well as anaerobic pathogens (28,29). It is usually prescribed as part of a combination therapy against CRE because of its good *in vitro* activity against many MDR and XDR Enterobacterales (30,31). In our series, two isolates were resistant to tigecycline thus reducing the treatment option. Even in isolates that were susceptible to tigecycline, the therapeutic use of this drug has a serious limitation because of its poor achievable serum and urine concentrations according to many studies (28, 32).

The first 3 patients in our series were

treated with combination therapies involving colistin and tigecycline (2 cases) or colistin and meropenem (1 case). Two of these patients survived. Colistin and tigecycline have been used as first line agents for the treatment of infections caused by MDR Enterobacterales. However, there are uncertainties with respect to their efficacy as the mortality rates from these septic episodes still remain high. On the other hand, 2 patients (case nos. 4 and 5) not treated with combinations involving colistin died. The third case (case no. 6) was on trimethoprim-sulfamethoxazole and his condition improved remarkably by the time his family requested for his discharge to seek treatment abroad. Although recently, some new and combination agents with activity against MDR organisms and CRE, such as ceftazidime-avibactam, ceftolozane-tazobactam, meropenem-vaborbactam, imipenem-cilastin-relebactam, plazomicin, eravacycline and cefiderocol, have been approved for clinical use or are at the final stages of development, none of these agents is available in our country at this time.

It is of interest to note that the first case who had UTI and bloodstream infections with different XDR and PDR *K. pneumoniae* strains survived on prolonged colistin plus meropenem combination therapy. For all the cases, bundles interventions including enhanced environmental cleaning, contact precautions as well as antimicrobial stewardship were instituted promptly. As Enterobacterales are among the leading causes of healthcare associated infections, early identification of resistant bacteria is of paramount importance to the success of infection control efforts. In our hospital, active surveillance of patients helped to improve infection control by detecting colonization and preventing horizontal spread by these dangerous MDR pathogens. The CDC guidelines for surveillance, recommends the use of active surveillance in outbreaks, and that even non-endemic acute care facilities should review all clinical cultures within the last 6-12 months for previously unrecognized carbapenemase-producing Enterobacterales (CPE) (33). We believe that the aggressive infection control efforts in our hospital, including the bundle interventional guidelines introduced at the appropriate time, have been effective in decreasing rates of infections with CPE.

Currently, the therapeutic options for highly resistant carbapenemase-producing organisms in our hospital are limited. Prudent antimicrobials use becomes increasingly important as we are faced with little or no bright future for procurement of effective alternate drugs. Information on how to treat infections with CPE is still surprisingly limited, in spite of rapidly increasing prevalence of these organisms. Comprehensive clinical studies of the main therapeutic options, broken down by pathogens, enzymes and clinical syndrome, are definitely lacking. Clinicians need accurate susceptibility data to provide effective therapy.

Conclusion:

Rapid routine molecular detection is essential to optimize therapy, improve outcomes and limit the spread of CRE through aggressive infection control measures, including screening of potentially colonized high-risk patients (31). Even when we eventually introduce new antimicrobial agents into the hospital armamentarium, antimicrobial stewardship will play crucial important role, as these drugs will come with their own strengths and caveats.

Contributions of authors:

All authors made substantial contributions to all the following; (i) the conception and design, or acquisition of data, or analysis and interpretation of data; (ii) drafting the article, or revising it critically for important intellectual content; and final approval of the revision to be submitted. All authors have approved the final manuscript.

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No conflict of interest is declared

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Short Communication **Open Access**

Prevalence and distribution of cervical high-risk human papillomavirus infection in a rural community of Edo State, Nigeria

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Abstract:

Background: Human papillomaviruses (HPVs) are non-enveloped, double-stranded DNA viruses and most women in the world are probably infected with at least one type of the virus during their sexual life. Oncogenic HPVs are predominantly sexually-transmitted pathogens and several high-risk types are associated with nearly all cases of cervical cancer worldwide. In view of paucity of data on the prevalence and distribution of various high risk HPV subtypes, this study was carried out to provide evidence based local data for cervical cancer preventive programs within this region.
Methodology: This was a descriptive cross-sectional study involving 145 consenting women living in Ugbegun rural community of Edo central senatorial district, Edo State, Nigeria. Informed consent of each participant was obtained and socio-demographic information collected through interviewer-administered collection tool. Cervical swab sample was collected using the female cervical cell collection kit for HPV DNA testing. HPV DNA was detected by the Hybribio 21 HPV Geno array test kit which uses polymerase chain reaction (PCR) amplification and flow through hybridization assay. Summary statistics were presented as mean, standard deviation, median, frequency and proportions as appropriate using the Statistical Package for the Social Sciences (SPSS) version 22.0. Association of socio-demographic characteristics of the women with HPV prevalence was done using the '*t*' test, with *p* value less than 0.5 considered statistical significance.
Results: Twenty four of the 145 women tested positive, giving HPV prevalence of 16.6%. Six HPV serotypes were detected; types 16, 18, 35, 45, 52 and 58. HPV types 16 and 18 were most frequent, contributing 54.2%, and co-infection occurred in 29.2%. HPV-positive women had significantly higher mean number of life time sexual partners (p=0.046) and mean parity (p=0.0001) compared to HPV-negative women. The mean age of the women (p=0.710), mean age at menarche (p=0.570) and mean age at coitarche (p=0.940) were not significantly associated with prevalence of HPV
Conclusion: This study showed predominance of oncogenic cervical HPV types 16 and 18 within this sub region of rural Nigeria. Strengthening reproductive and sexual education in both males and females with focus on HPV vaccination, delaying sexual activities and reduction in number of child birth are strategies which could prevent high risk HPV infection and cervical cancer in rural communities.

Keywords: high-risk; cervical; HPV; rural community; prevalence

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Prévalence et répartition de l'infection cervicale à haut risque par le virus du papillome humain dans une communauté rurale de l'État d'Edo, au Nigéria

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Résumé:

Contexte: Les papillomavirus humains (VPH) sont des virus à ADN double brin sans enveloppe et la plupart des femmes dans le monde sont probablement infectées par au moins un type de virus au cours de leur vie sexuelle. Les VPH oncogènes sont principalement des agents pathogènes sexuellement transmissibles et plusieurs types à haut risque sont associés à presque tous les cas de cancer du col de l'utérus dans le monde. Compte tenu du manque de données sur la prévalence et la distribution de divers sous-types de VPH à haut risque, cette étude a été réalisée pour fournir des données locales fondées sur des preuves pour les programmes de prévention du cancer du col de l'utérus dans cette région.

Méthodologie: Il s'agissait d'une étude transversale descriptive impliquant 145 femmes consentantes vivant dans la communauté rurale d'Ugbegun du district sénatorial central d'Edo, dans l'État d'Edo, au Nigeria. Le consentement éclairé de chaque participant a été obtenu et les informations socio-démographiques ont été collectées via un outil de collecte administré par l'intervieweur. Un échantillon d'écouvillon cervical a été prélevé à l'aide du kit de collecte de cellules cervicales féminines pour le test ADN du VPH. L'ADN du VPH a été détecté par le kit de test Hybribio 21 HPV Geno array qui utilise une amplification par réaction en chaîne par polymérase (PCR) et un test d'hybridation en flux continu. Les statistiques sommaires ont été présentées sous forme de moyenne, d'écart-type, de médiane, de fréquence et de proportions, selon le cas, à l'aide de la version 22.0 du package statistique pour les sciences sociales (SPSS). L'association des caractéristiques sociodémographiques des femmes avec la prévalence du VPH a été réalisée à l'aide du test «*t*», avec une valeur de p inférieure à 0,5 considérée comme une signification statistique.

Résultats: Vingt-quatre des 145 femmes ont été testées positives, ce qui donne une prévalence du VPH de 16,6 %. Six sérotypes de VPH ont été détectés ; types 16, 18, 35, 45, 52 et 58. Les types de VPH 16 et 18 étaient les plus fréquents, contribuant à 54,2%, et une co-infection s'est produite dans 29,2%. Les femmes séropositives pour le VPH avaient un nombre moyen de partenaires sexuels (p=0,046) et une parité moyenne (p=0,0001) significativement plus élevés que les femmes séronégatives pour le VPH. L'âge moyen des femmes (p=0,710), l'âge moyen à la ménarche (p=0,570) et l'âge moyen à la coïtarche (p=0,940) n'étaient pas significativement associés à la prévalence du VPH

Conclusion: Cette étude a montré la prédominance des types de VPH cervicaux oncogènes 16 et 18 dans cette sous-région du Nigéria rural. Le renforcement de l'éducation reproductive et sexuelle chez les hommes et les femmes en mettant l'accent sur la vaccination contre le VPH, le report des activités sexuelles et la réduction du nombre de naissances sont des stratégies qui pourraient prévenir les infections à VPH à haut risque et le cancer du col de l'utérus dans les communautés rurales.

Mots-clés: à haut risque; cervical; VPH; communauté rurale; prévalence

Introduction:

Human papillomaviruses (HPVs) are non-enveloped, double-stranded DNA viruses. These viruses are ubiquitous and most women in the world are probably infected with at least one type of the virus during their sexual life, with a point prevalence rate of 10.1% (1,2). Genital HPV types are categorized, according to epidemiologic association with cervical cancer and oncogenic potentials, as low risk types (6, 11, 41, 44), and high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52 ,56, 58, 59, and 68) (3).

Oncogenic HPVs are predominantly sexually-transmitted pathogens and several high-risk types are associated with nearly all cases of cervical cancer worldwide. Cervical cancer is the second most common cancers in women constituting about 12% of all cancers. Globally, 80% of new cases of cervical cancer occur in the developing countries, but conversely only 5% of women in these countries have ever had preventive screening test such as HPV typing. The impact and threat of cervical cancer to the lives of women worldwide is indisputable (4,5).

The need for prevention of HPV-associated cancers in developing countries cannot be over-emphasized. On a nationwide basis, HPV vaccination still remains a veritable option in prevention of cervical cancer. In the absence of HPV vaccination or improvements in screening and treatment framework, deaths due to cervical cancer is projected to rise significantly.

There are few studies in Nigeria that have evaluated the prevalence and distribution of various high risk HPV subtypes (6,7). In view of the paucity of information on this, it was necessary to conduct this study to provide evidence based local data for decision making which would be useful in devising the optimum strategy for HPV vaccination and other cervical cancer preventive programs within this region of Edo State, Nigeria.

Materials and method:

Study setting, design and participants

This was a descriptive cross-sectional study of 145 women from Ugbegun community of Esan central senatorial district in Edo State, Nigeria conducted in April 2021. The study participants included non-menstruating women who were 18 years and above, living within the selected rural community.

Sample size determination:

The sample size was calculated using the formula for cross sectional study (8); $n=Z^2pq/d^2$, where 'n' is the sample size, 'Z' is standard normal deviation (1.96), 'p' is the proportion of subjects positive for high-risk HPV which is taken as 21.6% (0.216) based on a previous study (9), 'q' is 1-p (1-0.216=0.784) and 'd' is the assumed observed difference at 5% (0.5) level of significance. This gave the calculated sample size of 71, which was increased to 145 to increase the power of the study.

Sampling technique

The sampling method employed was multistage sampling technique. Esan central is made up of four clans and villages which include Irrua (as the administrative headquarter), Ewu, Opoji and Ugbegun kingdoms. Irrua was excluded because it has been designated as an urban community (10). Simple random sampling by ballot without replacement was used to select one of the remaining three communities, and Ugbegun community was selected as the rural community for the study.

Community engagement and participant recruitment

By local custom, community meetings were held prior to recruitment. This was done after a proper community entry through the opinion leaders and the women leaders with proper explanation of the purpose of the research. The study days and times were advertised throughout the communities through 'word-of-mouth'. On the study days, potential participants arrived at a community healthcare centre and were voluntarily recruited into the study.

Informed consent and data collection:

Women who agreed to participate in the study had their informed consent obtained after due explanation. Interviewer obtained informed consent was done in the privacy of an interview room. A printed consent form was translated verbally by a trained investigator into the local language and read to the study participant in some cases. The interview was then administered in a private area. Socio-demographic information of each participant was obtained using a design data collection form.

Specimen collection and laboratory analysis:

Each consenting participant entered a private examination room, where pelvic examination and cervical specimen collection were performed by the attending medical doctor trained for the study. Cervical swab sample was collected using the female cervical cell collection kit for HPV DNA testing.

The residual cell suspensions from the female cervical cell collection kit were frozen at -20°C and transported to molecular diagnostic laboratory, Nigeria Limited, Lagos State, Nigeria, in iced cold packs for analysis. HPV DNA was detected using the Hybribio 21 HPV Geno array test kit which uses polymerase chain reaction (PCR) amplification and flow through hybridization (9).

Data analysis

Statistical analysis of data was done using the Statistical Package for the Social Sciences version 22.0 software. The mean and standard deviations were calculated for quantitative variables, while charts, graphs and tables were used to depict qualitative variables. The Chi-square test was used to compare the differences between proportions while Students' '*t*' test was used to compare the mean differences between continuous variables. P value less than 0.5 was considered statistical significance.

Results:

The mean age of the 145 women studied was 37.0±13.0 years. Majority (66.2%) of the women did not have tertiary level of education, and 54% were married in monogamous setting (Table 1).

Table 1: Socio-demographic characteristics of participants

Socio-demographic variables	Frequency (%) (n=145)
Age group (years)	
18 – 24	23 (15.9)
25 – 34	54 (37.2)
35 - 44	29 (20.0)
45 – 54	15 (10.3)
≥ 55	24 (16.6)
Mean age (± SD) in years	37.193 ± 13.01
Education status	
No Education	11 (7.6)
Primary	33 (22.7)
Secondary	52 (35.9)
Tertiary	49 (33.8)
Marital status	
Single	39 (26.9)
Married	80 (55.1)
Divorced	0
Separated	12 (8.3)
Widowed	14 (9.7)
Marital setting	
Single	35 (24.1)
Monogamy	78 (53.8)
Polygamy	32 (22.1)

A total of 24 women tested positive, giving HPV prevalence rate of 16.6% in the

study. Six HPV serotypes; 16, 18, 35, 45, 52 and 58 were detected (Table 2). HPV subtypes 16 and 18 were most frequent types, contributing 54.2% (14/25). Co-infection occurred in 29.2% (7/24) of these women who tested positive for high-risk HPV infection. Serotype 45 did not occur in isolation, but coexisted with other high-risk HPV subtypes as shown in the Table 2.

Table 2: Distribution of high-risk HPV subtypes

HPV serotypes		**Number (%) of HPV positive women**
Viral serotype	16	9 (37.5)
	18	4 (16.7)
	16,18	1 (4.2)
	35	4 (16.7)
	16, 45	3 (12.5)
	45, 52	2 (8.3)
	45, 58	1 (4.2)
Total		**24 (100)**

HPV = human papillomavirus

HPV-infected women had significantly higher mean number of life time sexual partners (p=0.046) and mean parity (p=0.0001) compared to those who were HPV negative (Table 3). The age of the women (p=0.710), age at menarche (p=0.570) and age at coitarche (p= 0.940) were not significantly associated with prevalence of HPV

Table 3: Statistical analysis of socio-demographic characteristics of high-risk HPV-positive and HPV-negative women

Socio-demographic variables	**Mean ± SD values of women**		**p-value**
	Positive	**Negative**	
Age in years	31.0 ± 12.6	38.6 ± 14.1	0.710
Age at menarche	13.3 ± 1.0	15.4 ± 1.0	0.570
Age at coitarche	18.3 ± 1.7	21.1 ± 2.8	0.940
Sexual partners	3.7 ± 1.1	2.7 ± 1.5	0.046*
Parity	2.4 ± 2.0	1.2 ± 2.7	0.0001*

SD=Standard deviation; * = statistically significant; HPV=human papillomavirus

Discussion:

The prevalence of high-risk HPV infection in this study was 16.6%, which is similar to the study from southwest Nigeria with 21.6% reported in Ile-Ife (9), but at variance with the study from Kano, northcentral Nigeria which reported 76% (11). A similar study conducted in Mali reported a prevalence of 12% (12). The International Agency for Research on Cancer (IARC) surveys of HPV in various countries described inconsistent trends in HPV infections in Africa (13), consistent with the finding in our study and those of others in Nigeria, hence the need for more studies in various geographical regions of the world.

The viral serotypes identified in this study were types 16, 18, 35, 45, 52 and 58. HPV subtypes 16 and 18 were the most frequent subtypes with 54.2%. This is worrying, considering the oncogenic potential of these two subtypes, as over 70% of cervical cancer cases have been attributed to infections with these two subtypes. Type 45 did not occur in isolation but coexisted with other HPV types. In Mozambique, HPV serotype 35 was found to be slightly higher than HPV 16 among women without cervical cancer (14).

It has been established that transmission of HPV is predominantly by sexual contact. Early sexual debut and multiple sexual partners are known risk factors, while other routes have been shown to be of lesser significance (15). In our study, sexual activities irrespective of the number of partners and higher parities were significantly associated with positive high-risk HPV infection.

Conclusion:

Our study showed predominance of oncogenic HPV types 16 and 18 within this subregion of rural Nigeria. Strengthening reproductive and sexual education in both males and females with focus on HPV vaccination, delaying sexual activities and reduction in number of child birth are strategies which could prevent high-risk HPV infections and cervical cancer in these communities.

Contributions of authors:

OJ and SSO conceptualized the study; OJ and ASC performed literature search; OJ and NA collected the cervical specimens; NA and OJ collected the participants data; SSO and OJ performed the laboratory works; OJ, ASC, OGBO and SSO were involved in the write-up and review of the manuscript. All authors agreed to the final manuscript draft submitted.

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Conflict of interest:

No conflict of interest is declared

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Case Report **Open Access**

Cerebrospinal fluid xanthochromia in acute bacterial meningitis as a red herring for subarachnoid haemorrhage: A case report

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Abstract:

This article presents a case that highlights the importance of excluding underlying intracranial pathology in a patient presenting with severe headache and positive xanthochromia. This case report demonstrated that false-positive xanthochromia without subarachnoid haemorrhage (SAH) is possible in acute bacterial meningitis when there is a combination of traumatic lumbar puncture and either hyperbilirubinaemia or raised cerebrospinal fluids (CSF) protein.

Keywords: cerebrospinal fluid; acute bacterial meningitis; subarachnoid haemorrhage; xanthochromia

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Xanthochromie du liquide céphalo-rachidien dans la méningite bactérienne aiguë comme hareng rouge pour une hémorragie sous-arachnoïdienne: à propos d'un cas

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Résumé:

Cet article présente un cas qui met en évidence l'importance d'exclure une pathologie intracrânienne sous-jacente chez un patient présentant une céphalée sévère et une xanthochromie positive. Ce rapport de cas a démontré qu'une xanthochromie faussement positive sans hémorragie sous-arachnoïdienne (HSA) est possible dans la méningite bactérienne aiguë lorsqu'il existe une combinaison de ponction lombaire traumatique et d'hyperbilirubinémie ou d'augmentation de la protéine du liquide céphalo-rachidien (LCR).

Mots clés: liquide céphalo-rachidien; méningite bactérienne aiguë; hémorragie sous-arachnoïdienne; xanthochromie

Introduction:

Otitis media, and less commonly otitis externa, is a well-known cause of bacterial meningitis (1) . Common clinical presentations of acute bacterial meningitis following otitis infection are fever, otalgia, neck stiffness, headache, and confusion (1). One important differential is subarachnoid haemorrhage (SAH), which typically occurs between ages 40 and 60, with the peak frequency between 55 and 60 years (2). The Cooperative study found that intracranial aneurysms were the causative factors in 54% of the initial SAH, while arteriovenous malformations (AVMs) accounted for 6%, and other aetiologies for the remaining 40% (3). After negative computed tomography (CT) of brain for SAH, CT angiography (CTA) is the next line of investigation to identify and characterise berry aneurysms or AVMs (Fig 1). Therefore, there is a clinical justification for patients to have an outpatient magnetic

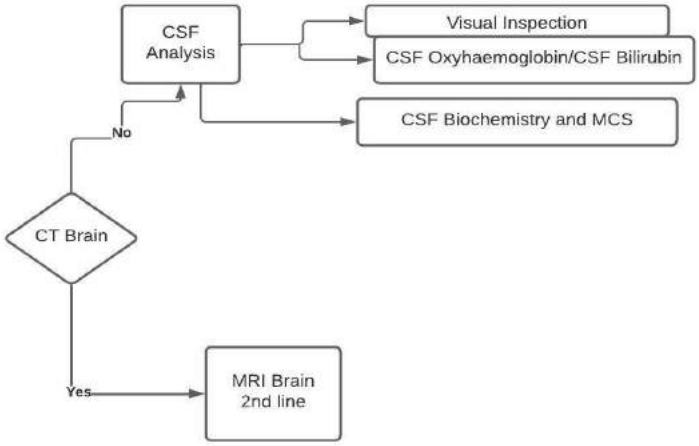

Fig 1: Diagnostic flowchart for suspected subarachnoid haemorrhage and central nervous system infections

Table 1: Causes of false-positive and false-negative cerebrospinal fluid xanthochromia for subarachnoid haemorrhage

False positive result	False negative result
1. Increased oxyhaemoglobin and bilirubin typical of SAH	
i. Repeat LP	i. LP performed < 12 hours after onset of symptoms
ii. Traumatic LP and increased CSF protein	ii. CSF sample exposed to light
iii. Traumatic LP and hyperbilirubinaemia	iii. Prolonged delay in CSF sample analysis
2. Increased bilirubin (late presenting SAH)	
i. Raised CSF protein	i. CSF sample exposed to light
ii. Hyperbilirubinaemia	ii. Prolonged delay in CSF analysis

LP=Lumbar puncture; CSF=cerebrospinal fluid; SAH=subarachnoid haemorrhage

resonance imaging (MRI) of head with contrast carried out to exclude any underlying cause.

It is possible that a combination of traumatic lumbar puncture (LP) and either hyperbilirubinemia or raised CSF protein may lead to false-positive tests for xanthochromia (4,5). Notable causes of false-positive and negative CSF results for SAH are shown in Table 1. In this report, we discuss a case which highlights the importance of excluding underlying intracranial pathology in a patient presenting with severe headache and positive xanthochromia.

Case presentation:

A 61-year-old man with a past medical history of systemic hypertension and bilateral hip replacements presented to the Emergency department (ED) after a few weeks of experiencing tinnitus, ear ache and giddiness. He was managed as otitis externa a week before his admission. A day before admission, he had an MRI brain in a private hospital to rule out acoustic neuroma. He presented to the ED following a collapse episode, which according to his wife, had prior complaints of severe headache and acute confusion but no previous fever. At ED triage, he was noted to have slurred speech, slight facial droop on the right with temperature of 39.4^{o}C. On examination, he had a left-sided hemiparesis with power of 4/5, Glasgow Coma Score of 13/15, and was deaf in the right ear. The report of the standard MR brain and both internal auditory meatus (IAM) were normal for age, except for a minor paranasal sinus mucopolyposis.

On admission to the ED, the CT head (axial view) revealed no acute abnormality. The patient's blood sample analysis showed raised inflammatory markers (Table 2). The CSF collected in aliquots of 4 bottles following lumbar puncture, was cloudy macroscopically (but not blood-stained) with subsequent analysis revealing white cell count of 1530 cells/ml; 95% polymorphs and 5% lymphocytes. The detailed results of the CSF analysis are shown in Table 3. The CSF and blood cultures

Table 2: Blood sample analysis results of the patient

Test (Normal values)	Day 1	Day 12
Hydrogen ions (35-45 nmol/l)	29.3	
Glucose (3.2-6.1 mmol/l)	10.7	
Lactate (0.7-2 mmol/l)	2.4	
White cell count (3.7-9.5 x10^{9}/l)	17.2	9.6
Neutrophils (1.5-6.5 x10^{9}/l)	15.2	6.5
Lymphocytes (1.1-5 x10^{9}g/l)	0.5	0.8
C-reactive protein (2-10 mg/l)	59	39
Urea (2.5-7.5 mmol/l)	6.1	4.4
Creatinine (50-125 mmol/l)	96	79
Ferritin (20-100 U/l)	1305	1102
Platelet (150-400 ng/ml)	124	347

grew *Streptococcus pneumoniae* and the CSF pneumococcal antigen was also positive. The 4th sample bottle which was used for CSF xanthochromia spectrophotometry analysis revealed the presence of oxyhaemoglobin and bilirubin, indicative of xanthochromia. Blood borne viruses (BBV) screening was negative for HIV and hepatitis, and ECG shows only sinus tachycardia.

Table 3: Cerebrospinal fluid sample analysis results of the patient

Parameter	Test sample	Bottle 2	Bottle 3	Bottle 4
CSF RBC count	-	423	403	209
CSF protein	5.4			
CSF glucose	1.4			
Blood glucose	10.3			

Based on the history and examination findings at the ED, the patient was treated for possible CSF infection and started on treatment to cover for both bacterial meningitis and encephalitis, after the initial CT head excluded SAH, intracranial bleed or space-occupying lesion (SOL). The patient received intravenous (IV) crystalloids infusion, empirical IV ceftriaxone 2g and acyclovir 800mg as well as antipyretics, until his fever subsided, and was thereafter admitted into the medical ward. The positive finding of CSF xanthocromia necessitated request for an outpatient MRI brain (or the 'goal standard' CTA or MRA brain) to exclude AVM or berry's aneurysm underlying probable SAH, judging by the fact that CT head diagnosis of SAH diminishes with time (5).

On day 3 of admission, he was commenced on IV dexamethasone to mitigate the possibility of cerebral edema developing. On day 10 of admission, the patient became positive for coronavirus disease-19 (COVID-19), which was likely from cross-infection, and was transferred to the COVID ward where he remained asymptomatic. The patient was discharged home on day 12 with a request for outpatient MRA brain to exclude AVM/berry's aneurysm.

The MRI reported a subtle subcentimetre focus of T2 hyperintensity within the subcortical white matter of the right frontal lobe on the FLAIR sequence only. This was considered non-specific and of doubtful significance with no apparent underlying mass lesion. There were no evidences of haemorrhage, infarct, expansible mass lesion, gross atrophy or small vessel ischaemia, diffusion restriction, arterial or venous sinus lesion, and no AVM or berry aneurysm was identified. Only mucosal retention cysts were noted in the right maxillary sinus.

Discussion:

The 'gold standard' investigation for the diagnosis of SAH in 98% of patients presenting within 12 hours is CT of the brain, but the sensitivity drops to 50% if patients present one week after onset of SAH (6). CSF xanthochromia may remain positive up to 2 weeks post-SAH event (6). The diagnostic test for both meningitis and SAH is LP for CSF analysis. LP should be performed less than 12 hours after the SAH event ideally and this typically reveals xanthochromia to confirm the diagnosis, which is usually detected by CSF spectrophotometry (7).

Researchers in Aberdeen have previously reported a review of 316 CSF samples analysed by spectrophotometry between 1 February 2011 and 31 January 2012, with 26 patients positive for CSF xanthochromia, 6 of whom had aneurysms on CTA (all 6 aneurysms were subsequently clipped or coiled), one patient had encephalitis and another one had malignant meningitis (8). Therefore, it is ideal to determine in CT brain-negative patients in whom clinical suspicion of SAH remains high, the need for further imaging in form of angiography.

The guideline for diagnosis of SAH is CSF with increased oxyhaemoglobin and bilirubin spectrophotometry (6). Isolated elevation of CSF bilirubin spectrophotometry finding however occur when either CSF proteins or serum bilirubin are raised (6). Traumatic LP which occurs commonly can cause raised CSF oxyhaemoglobin, however, this does not cause CSF bilirubin rise, as CSF bilirubin needs to be synthesized *in vivo* (9). The basis of CSF analysis using UV-Vis spectrophotometer is to determine oxyhaemoglobin at the maximum absorbance between 410 and 418 nm while CSF bilirubin is determined at broad absorption maximum in the range of 450 to 460 nm or as a shoulder adjacent to the oxyhaemoglobin peak (Fig 2).

As second line to CT brain, MRI brain is the preferred option. In the case presented, subsequent outpatient MRI brain turned out normal, excluding the possibility of underlying AVM or berry's aneurysm causing an intracranial bleed. This case report described the basis of CSF analysis in form of spectrophotometry and highlighted the need to use MRI/MRA brain to exclude relevant intracranial pathology. It also emphasized the need to confirm the CSF xanthochromia seen in this patient as a red herring, not indicative of SAH, and is in fact due to raised CSF protein from bacterial meningitis, inspite of clinical features suggestive of intracranial bleed.

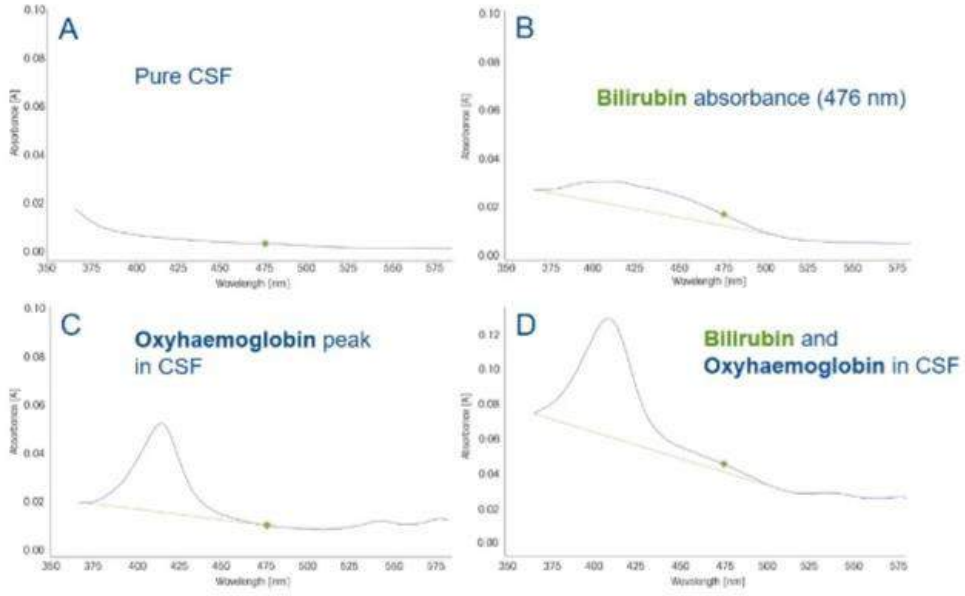

Fig 2: Specifics of CSF analysis in SAH/CNS infections using UV-Vis spectrophotometer

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AMA is responsible for study design, conceptualization, interpretation and writing of the manuscript. ARA is responsible for critical review and writing of the manuscript. The authors actively contributed to the writing and reviewing of the manuscript and approve the final submitted version.

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Letter to the Editor **Open Access**

Harness innovation to reduce the malaria disease burden and save lives

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Exploiter l'innovation pour réduire le fardeau du paludisme et sauver des vies

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Dear Editor,

The World Health Organization (WHO) theme for the year 2022 on the World Malaria Day is "Harness innovation to reduce the malaria disease burden and save lives". This theme raises a few questions relating to the issue of controlling malaria. These are; (1) Can malaria be eliminated without a vaccine? (2) As the theme suggests, what are the innovations needed to eliminate malaria? (3) What are the current figures in terms of annual malaria mortality, incidence and economic costs in Nigeria? (4) What is the Nigerian situation with respect to resistance of malaria vector to insecticides and malaria parasites to the antimalarial drugs of choice (artemisinin combination therapy ACT), which has been reported in most parts of the world? (5) With China's *Artemisia annua* (Chinese salad plant) for ACTs, showing that plants are the future of medicine, what should Nigeria do about this considering the fact that there are effective herbal drugs available for malaria therapy in the country? and (6) How has Nigeria fared with the issue of proliferation of fake and adulterated antimalarial drugs?

We have provided below brief answers to these questions for the benefit of the scientific, research and development community.

1. Can malaria be eliminated without vaccine?

It is unlikely that malaria will be eliminated without vaccine because in recent years, there has not been a significant reduction in morbidity and mortality especially in areas where malaria is endemic despite the preventive and treatment measures that have been in place for years. The availability of vaccine may also not be sufficient to eliminate malaria because the efficacy of the current vaccine (RTS,S/AS01) is 36.3% at first dose against clinical malaria in children, 25.9% in infants while efficacy against severe malaria

stands at 32.2% and 17.3% in children and infants respectively (1). Although people in endemic regions develop immunity to malaria in adulthood, they are not immune to new infection and therefore the spread of the parasite is inevitable.

The early candidate vaccines were based on sporozoite antigens however, it became evident that they did not completely block infection (2). On the other hand, those vaccine candidates based on synthetic peptides (3) and recombinant proteins that combined sporozoite antigens with blood stages (4), had produced mixed efficacy results. Unless vaccines that are able to prevent infection are developed and backed up with 100% uptake, it is most unlikely that the current vaccines would be able to eliminate malaria. Bearing in mind that malaria vaccines are developed to reduce disease and deaths, but not to completely eliminate disease, it will take a combination of strategies, including the use of existing control and preventive measures as well as development of new tools to control and ultimately eliminate malaria.

2. As the theme suggests, what are the innovations needed to eliminate malaria?

There is need to scale up available preventive and control measures currently in use which include; use of long-lasting insecticide treated bed nets (LLINs), indoor residual spraying (IRS), larviciding, seasonal chemoprevention, intermittent preventive therapy in pregnancy, prompt diagnosis with RDT/microscopy and treatment with artemisinin combination therapy (ACT). In addition, other innovative measures that are needed include; research and development of new generations of highly effective vaccines, newer drugs to prevent resistance, new generation of chemicals required for IRS and larviciding, and research into sterilization of mosquitoes, amongst others.

3. What are the current figures in terms of annual malaria mortality, incidence and economic costs in Nigeria?

The Nigeria Demographic and Health Survey (DHS) data (5) shows that over 110 million malaria cases occur yearly, and this is responsible for 11% maternal mortality, 25% infant mortality, and 30% of under-five mortality, with associated social and economic burdens including retardation of the country gross domestic products (GDP) by 40% annually and estimated 480 billion Naira loss due to malaria related out-of-pocket (OOP) treatment, prevention costs, absenteeism and productivity losses (5).

The 2015 National Malaria Indicator Survey (NMIS), which was the second malaria indicator survey in Nigeria, however showed that prevalence of malaria reduced to 45% by RDT and 27% by microscopy, from 52% by RDT and 42% by microscopy in the 2010 NMIS data (6). The 2018 DHS data also showed a reduction in malaria prevalence in children from 42% in 2010 to 27% in 2015 and 23% in 2018 (5). However, global estimates from the 2021 World Malaria Report (7) showed that Nigeria recorded an increase in the proportion of cases and deaths attributable to malaria from 23% in 2020 to 27% in 2021, which made Nigeria the country with the highest number of malaria cases and deaths worldwide, put at approximately 200,000 deaths (7). Malaria therefore remains a foremost public health challenge for Nigeria and a major cause of morbidity and mortality, which government should be determined to change.

Nigeria is tackling this challenge through the National Malaria Policy, launched in February 2015, which expresses the desire and commitment of the Government at all levels to ensure the elimination of malaria. The National Malaria Strategic Plan (NMSP) 2021-2025 target is to reduce malaria parasite prevalence to less than 10% and mortality attributable to malaria to less than 50 deaths per 1,000 live births by 2025 (8). However, there are concerns that Nigeria may miss the 2025 malaria elimination target as Nigeria did not make the list of countries that met the 2020 milestone of NMSP 2014-2020, and hence not projected by the WHO with the potential to stamp out malaria by 2025 (9).

4. What is the Nigerian situation with respect to resistance of malaria vector to insecticides and malaria parasites to the antimalarial drugs of choice (artemisinin combination therapy ACT)?

There is evidence to show that there is widespread resistance of *Anopheles* mosquitoes to a number of the insecticides used for IRS, such as deltamethrin, and DDT. Extreme resistance of malarial vector to DDT and possible resistance to bendiocarb have been reported (10,11). In a report (11), *Anopheles coluzzii*, and *Anopheles gambiae* showed resistance to three classes of insecticides (DDT, permethrin and bendiocarb) approved by the WHO. These underscore the need for constant analysis and improvements in research and development to ensure that newer, more effective insecticides are developed.

Unlike in Asia and some other countries around the world where there is high resistance to ACTs, there has not been significant resistance of malaria parasites to ACTs in Nigeria (12,13). This is good news but also a reason to be cautious to ensure that ACT use is not abused so as to prevent resistance.

5. With China's *Artemisia annua* (Chinese salad plant) for ACTs, showing that plants are the future of medicine, what should Nigeria do about this considering the fact that there are

effective herbal drugs available for malaria therapy in the country?

Focus group discussions and interviews (14) were held about plants often found useful for malaria therapy in the community. Fifty species (local names) of plants including *Morinda lucida* (Oruwo), *Enantia chlorantia* (Awopa), *Alstonia boonei* (Ahun), *Azadirachta indica* (Dongoyaro) and *Khaya grandifolia* (Oganwo) were found to be in use for malaria therapy at Okeigbo, Southwest, Nigeria (14). Many other drugs have been developed from plant extracts but the main issues with the direct use of these plants are possible harm to people from certain toxic components in them and lack of information on appropriate dosages.

Nigeria should henceforth begin to conduct her own research and analysis of the natural resources within the country to extract the components in the plants which are beneficial for therapy of malaria and other disease conditions.

6. How has Nigeria fared with the issue of proliferation of fake and adulterated anti-malarial drugs?

Research has shown that 1 in 5 anti-malarial products in circulation is fake (15). Fake and adulterated drugs are a major challenge especially in low-and-middle income countries (LMICs) where there is lack of advanced technology and inadequate regulatory bodies necessary to combat this challenge. Tackling this issue will require; improving the economy, strengthening technical capacity, providing regulatory oversight, ensuring punitive action, improving consumer and health worker knowledge about product authenticity, and regulating the private sectors purchase of drugs.

7. When is Nigeria going to deploy the new malaria vaccine?

The Federal Government of Nigeria has begun setting up mechanisms to facilitate implementation of the malaria vaccine by putting together a committee to implement national response, while collaborating with various stakeholders, including discussions with Program for Appropriate Technology in Health (PATH) to develop a roadmap for vaccine deployment in Nigeria and to ensure smooth roll out (15).

Financing malaria vaccine purchase is being supported by GAVI, the vaccine alliance, the Global Fund to fight AIDS, TB and malaria, and UNITAD, while the Federal Government is expected to provide counterpart funding. The Nigerian Government and Prince Ned Nwoko malaria eradication foundation have applied for purchase of the vaccine which will be rolled out in phases starting with States with the highest malaria burden (16). Deploying the vaccine will require adequate planning (17) to ensure successful implementation.

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AO and AB designed the topic; MBTO, BM, SO, NM and ASA reviewed literature on different aspects of the work; AB and MBTO merged the various write-up and edited the final manuscript.

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doi: 10.4314/ajtcam.v4i2.31207

3. Bakare, R. A., Oni, A. A., Okesola, A. A., et al. Efficacy of pefloxacin on acute uncomplicated gonococcal urethritis. Nig Qt J Hosp Med. 1996; 6: 335

For books:

1. Arya, O. P., Osoba, A. O., and Bennett, P. Tropical Venereology, Churchill Livingstone, Edinburgh, 1980

When referring to a chapter in a book and where the names of authors are also given, the reference should be;

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