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ORIGINAL ARTICLE

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NOSOCOMIAL URINARY INFECTIONS AT THE UROLOGY UNIT OF THE NATIONAL UNIVERSITY HOSPITAL (YALGADO OUÉDRAOGO), OUAGADOUGOU: FEB.-SEPT. 2012

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ABSTRACT

Objective: The aim of this study was to identify the risk factors and the microorganisms susceptibilities of nosocomial urinary infections at the urology unit of the national university hospital of Ouagadougou in Burkina Faso.

Method: From February to September 2012, two bacteriological analyzes have been performed for any of the 75 in-patients in the urology unit of the national university hospital of Ouagadougou in Burkina Faso.

Results: During the study period, 43 cases of nosocomial urinary infection were identified (57.3%) and we found no statistically significant associated risk factors with age groups, sex, arterial blood pressure, kidney illness and urinary obstructive pathologies.

The most frequently isolated bacteria were *Escherichia coli* (30.9%), *Klebsiella spp* (26.9%) and *Staphylococcus spp* (15.4%). The yeasts strains were very sensitive to antifungal but the bacteria susceptibility rate to antibiotics was very variable. Thus, the cocci were rather sensitive to association clavulanic acid + amoxicilline and ceftriaxone and enough sensitive to gentamicine ; the bacilli were enough sensitive to gentamicin and very sensitive to imipenem.

Conclusion: From the antibiogram results, we recommend gentamicin in combination with penicillin or metronidazole as the first antibiotics to be used in the treatment of nosocomial urinary tract infections.

Keywords: urinary infection, nosocomial infection, bacteria, antibiotics

INFECTIONS URINAIRES NOSOCOMIALES AU SERVICE D'UROLOGIE DU CENTRE HOSPITALIER UNIVERSITAIRE (YALGADO OUÉDRAOGO), OUAGADOUGOU (de février à septembre 2012)

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RÉSUMÉ

Titre : Infections urinaires nosocomiales au service d'urologie du centre hospitalier universitaire (Yalgado Ouédraogo), Ouagadougou (de février à septembre 2012).

Objectif : Identifier les facteurs de risque et la sensibilité aux antimicrobiens des germes responsables d'infection urinaire nosocomiale dans le service d'urologie du CHU-YO.

Matériel et méthode : Entre février et septembre 2012, des facteurs de risque d'infection urinaire ont été recherchés chez des patients hospitalisés dans le service d'urologie du CHU-YO. Pour chaque patient, un examen cytot bactériologique des urines a été réalisé et la sensibilité aux antimicrobiens des germes éventuellement responsables a été déterminée.

Résultats : Sur 75 patients hospitalisés, 43 ont présenté au moins un épisode d'infection urinaire (57,3%). Bien que présentant des risques d'infection plus importants, des variables comme l'âge avancé, le sexe, l'hypertension artérielle, l'insuffisance rénale et les uropathies obstructives n'étaient pas statistiquement plus associés aux infections urinaires nosocomiales.

Les germes isolés étaient dominés par *Escherichia coli* (30,9%), les Klebsielles (26,9%) et les staphylocoques (15,4%). Les levures étaient très sensibles aux antifongiques mais les bactéries ont montré des taux de sensibilité très variables. Ainsi, les cocci étaient assez sensibles à l'association amoxicilline-acide clavulanique, à la ceftriaxone et assez sensibles à la gentamicine ; les bacilles étaient assez sensibles à la gentamicine et très sensibles à l'imipénème.

Conclusion : A partir de ces résultats, nous recommandons la gentamicine en association avec une pénicilline ou le métronidazole dans le traitement en première intention des infections urinaires nosocomiales.

Mots clés : infection urinaire, infection nosocomiale, bactéries, antibiotiques

INTRODUCTION

Nosocomial infections are the most undesirable among the hospital care events with more than 50% of the cases [1, 2]. Their incidence rates are related to the medical development level of the country; and to the hygienic level at the hospital unit. A study carried out in France hospitals showed the national prevalence of nosocomial infection at 1% approximately [3]. This phenomenon is largely studied in the developed countries through the national committees against nosocomial infections, is not well documented in developing countries.

Among the nosocomial infections, those of urines are the most frequent with 34 to 40% of the cases [4, 5, 6]. They are supported by the urinary catheterization during long periods, the use of large spectrum antibiotics and the hygienic deficit during the nursing [2, 7, 8].

In Burkina Faso, a study conducted at the national university hospital "Yalgado Ouédraogo" indicated a nosocomial urinary infection rate of 14.8% and 67.3% respectively in the medicine ward and in the urology unit [9, 10]. The contamination occurs by ascension along urethra of endogenous or exogenous microorganisms and the more incriminated bacteria are *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [6, 11, 12]. Usually, these bacteria are characterized by their antibiotics resistance due to the selection pressure existing in hospital [9, 11, 12].

The purposes of this study is to identify the risk factors related to nosocomial urinary infections among patients hospitalized in the urology unit of the national university hospital "Yalgado Ouédraogo" and to determine the antimicrobials susceptibilities of implicated agents.

MATERIAL AND METHODS

This study conducted from February to September 2012 in the urology unit of the national university hospital "Yalgado Ouédraogo" included all in-patients who stayed at least one week in the urology unit, who performed at least two bacteriological analyses of urines during their staying and who gave their consent to be enrolled in the study.

For each patient, the first morning urines collected in sterile bottles have been subjected to:

- A bacterial count using immersed blade method (Uriline*, France)
- Leucocytes count with a Malassez calibrated cell
- Microscopic examination after centrifugation at 3,000 rounds/min x 5 minutes to look for red blood cells,

parasites, yeasts, crystals and over elements

- Gram stain to appreciate bacteria morphologies
- Culture on usual agar plates (Bromocresol pourpre, Eosin methylen Blue [Biomérieux, France]) and another culture medium chosen according to the microscopic examination results. For example Chapman agar for selecting Gram positive cocci evoking *Staphylococcus* genus; blood agar plate for cocci evoking *Streptococcus* genus; Eosine methylen blue agar for Gram negative bacilli; and Sabouraud agar for yeasts.

After 18 to 24 hours of incubation in 37°C the isolated microorganisms have been identified on the basis of their morphological, cultural, and metabolic nature. For each identified germ, the antimicrobial profile has been determined by the diffusion method in Müller-Hinton plate agar as recommended by the "European Committee on Antimicrobial Susceptibility Testing Version (2.0, valid from 2012-01-01). The obtained results have been confirmed with the automat "MicroscanWalkAway 40 IF" (Siemens, Germany) and the quality control has been done with bacteria of the American Type Culture Collection (ATCC) *E. coli* 25922, *Pseudomonas aeruginosa* 27853 and *Staphylococcus aureus* 25923. The statistical analysis was done with "Epi-Info 7".

The operational definitions used for this study were:

- urinary infection : when bacteria count $\geq 10^4$ bacteria/mL of urine.
- nosocomial urinary infection : when the first bacteriological analysis of urine is negative and the second positive or when both (first and second) are positive with different germs.

RESULTS

Characteristics of the studied population

During the study period, 243 in-patients have been recorded in the urology unit of the national university hospital of Ouagadougou of whom 75 (30.9%) have been enrolled in the study. Among these 75 patients:

- 61 (81.3%) were male,
- 32 (42.7%) were 60 years old and more,
- 17 (22.7%) have an antecedent of urinary infection,
- 58 (77.3%) presented an obstructive urologic pathologies,
- 43 (57.3%) presented a nosocomial urinary infection among whom six did two episodes of urinary infection.

Nosocomial urinary infection and studied risk factors

The sex, the age-groups, the renal insufficiency, high arterial blood pressure, obstructive

urologic pathologies and bladder washing are not risk factors of nosocomial urinary infection (Table I).

TABLE I: NOSOCOMIAL URINARY INFECTIONS ACQUISITION AND RISK FACTORS STUDIED

Risk factors		Number	Infected patients n %		Chi-square	P-value
Sex	Male	61	35	57.4	0.00	0.987
	Female	14	8	57.1		
Age groups	<40 years	26	13	50.0	5.152	0.076
	[40-60[	17	7	41.2		
	≥60 years	32	23	71.9		
Kidney deficiency	Yes	11	5	45.5	0.744	0.389
	No	64	38	59.4		
High blood pressure	Yes	19	14	73,7	2,781	0,095
	No	56	29	51,8		
Origin of patient	Health-care facil	19	13	68,4	1,279	0,258
	Home	56	30	53,6		
Urologic pathologies	Obstructive	58	36	62,1	2,346	0,126
	no obstructive	17	7	41,2		
Bladder cleaning	Yes	20	14	70,0	1,789	0,181
	No	55	29	52,7		
Surgery act	Yes	63	37	58,7	0,314	0,575
	No	12	6	50,0		
Urinary catheterization	Yes	66	40	60,6	2,408	0,120
	No	9	3	33,3		

Bladder cleaning= Bladder washing ; Origin of patient= where patient comes from ; Health-care facil =Health care facilities

Identified micro-organisms

A total of 52 microbial strains were identified, generally in mono-microbial culture. Microbial association was found in three samples: *Klebsiella pneumoniae* + *Candida not albicans*;

Acinetobacter baumanii + *Candida albicans*; and *Escherichia coli* + *Staphylococcus aureus*. The most frequently isolated germs were *Enterobacteria* (76.7%) and *Staphylococcus* (15.4%) (Table II).

TABLE II: THE 52 MICRO-ORGANISMS ISOLATED FROM THE NOSOCOMIAL URINARY INFECTIONS

Groups	Genus	Species	Number	(%)
Enterobacteria (35)	<i>Escherichia</i>	<i>E. coli</i>	16	30,8
	<i>Klebsiella</i>	<i>K. pneumonia</i>	12	23,1
		<i>K. ozenae</i>	2	3,85
	<i>Enterobacter</i>	<i>E. cloacae</i>	1	1,9
		<i>E. sakazakii</i>	1	1,9
	<i>Proteus</i>	<i>P. mirabilis</i>	2	3,85
	<i>Providencia</i>	<i>P. stuartii</i>	1	1,9
Non-fermentative Gram negative bacilli (5)	<i>Pseudomonas</i>	<i>P. aeruginosa</i>	4	7,7
	<i>Acinetobacter</i>	<i>A. baumanii</i>	1	1,9
Gram positif cocci (8)	<i>Staphylococcus</i>	<i>S. aureus</i>	5	9,6
		<i>S. epidermidis</i>	3	5,8
Yeasts (4)	<i>Candida</i>	<i>C. not albicans</i>	3	5,8
		<i>C. albicans</i>	1	1,9
Total			52	100

Antimicrobials activities

The most efficient antibiotics were imipenem for bacilli and gentamicine for cocci (Table III).

TABLE III: SUSCEPTIBILITY RATES TO ANTIBIOTICS OF ISOLATED BACTERIA FROM NOSOCOMIAL URINARY INFECTION

NFGNB= No-Fermentative Gram Negative Bacilli ; Amoxi + clav ac = amoxicilline + clavulanic acid

Antibiotics	Enterobacteria : n=35 (%)	NFGNB n=5 (%)	Staphylococcus : n=8 (%)
Amoxicillin	0		50
Amoxi + clav ac	8,6		87,5
Ceftriaxone	8,6		87,5
Ciprofloxacin	28,6		87,5
Cotrimoxazole	20		25
Gentamicine	60	80	100
Imipenem	100	100	
Cefixime		60	
Cefpodoxime		60	
Pipemidique acide		60	
Netilmicine		40	
Nitrofurantoïne		40	
Pristinamycine		40	

Specifically, the sensitivity rates were for *Eschechia coli* (n=16) 100% with nitrofurantoïne and 87.5% with netilmicine; for *Klebsiella spp* (n=14) 71.4% with gentamicine and 100% with nystatine, econazole and miconazole for *Candida spp* (n=4).

Evolution after treatment

After treatment, the evolution was favourable for all the 43 patients with nosocomial urinary infection (100%). The first therapeutic profile using gentamicine or ciprofloxacin or pipemidic acid has been corrected for 28 patients (65.1%) according to the antibiogram or antifongigram results. The average duration of treatment was 12 days but it lasted one to two weeks for 39 patients (90.7%) and two to three weeks for four patients (9.3%). Any case of death related to the nosocomial urinary infection was noted.

DISCUSSION

During this study conducted in the urology unit of the national university hospital (Yalgado Ouédraogo) of Ouagadougou in Burkina Faso, the nosocomial urinary infection prevalence rate was 57.3% within the studied population. This result indicates that the nosocomial urinary infection is an important public health problem in our region. Our studied population was dominated by male sex (81.33%) but the infection rate was approximately 57% in men and women contrarily in developed countries where males seem to be more infected [13, 14].

Generally, infection occurs among patients with urinary catheter (69.7%) especially for long period. Effectively, it has been demonstrated that the contamination probability increases 10 to 30% per day of catheterization and reaches 100% after two months [15]. The nosocomial urinary infection is more frequent in patients 60 years old and after (71.9%) however, age groups are not

statistically associated to the infection as previously observed [16].

The infection incidence was higher in high blood pressure patients (73.7%) compared to patients with normal blood pressures (51.8%), although the difference was not statistically significant. In our studied population no case of severe high blood pressure has been noted (diastolic ≥ 14 ; systolic ≥ 9).

The origin of patient was not a significant factor of urinary infection occurring : 53.6% for patients coming directly from their residence versus 68.4% for patients coming from another health-care facilities (P=0.258). However, it's known that in these secondary medical centres hygiene is often defective and medical treatments are often unsuited because of lack of specialists [17, 18].

During this study, the incidence of the nosocomial urinary infection was more important in case of obstructive pathologies (62.1%), bladder cleaning (70%), bladder catheterization (60.6%) and urologic surgery (58.7%). But, these events are not statistically risk factors (p=0.126; p=0.181; p=0.120; p=0.575 respectively). However, many publications recognized that in urology, obstructive pathologies and surgical acts increase the risks of urinary infection [19, 20, 21, 22].

A total of 48 bacteria and 4 yeasts have been identified from the urinary samples among whom three were bi-microbial with following associations: *Klebsiella pneumoniae* + *Candida* not albicans, *Acinetobacter baumannii* + *Candida albicans* and *Escherichia coli* + *Staphylococcus aureus*. The most frequent identified genus

were Enterobacteria, (76.7%) and Staphylococcus (15.4%) which are current aetiologies of urinary infections [5, 10]. The most frequently identified species were *Escherichia coli* (30.8%), *Klebsiella spp* (26.9%) and *Staphylococcus spp* (15.4%) as demonstrated by many authors [9, 21, 22, 23, 24, 25, 26, 27, 28, 29].

In our study, the resistance rates of the isolated Enterobacteria were more than 71% with amoxicillin, amoxicillin + clavulanic acid, ceftriaxone, ciprofloxacin and cotrimoxazole. The high resistance level of nosocomial urinary infection bacilli is well documented and is supported by antibiotic selection pressure leading to resistant samples in hospital [9, 25, 26, 28]. Despite their susceptibility to imipenem, the five strains of non-fermentative bacilli showed resistance rates from 40 to 60% with piperimic acid, cefixim, cefpodoxim, Netilmicin, Nitrofurantoin and pristinamycin. The eight strains of *Staphylococcus spp* were susceptible to amoxicillin + clavulanic acid, ceftriaxone, ciprofloxacin and very sensitive to gentamicin as earlier demonstrated [25, 27, 28]. All the four samples of *Candida spp* were very sensitive to nystatine and econazole.

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- None of the antibiotics tested were sufficiently active on the bacilli and the cocci. The best therapy seems to be imipenem in association with gentamicin ; unfortunately imipenem is very expensive in our context.
- The antibiogram permitted to adjust antibiotherapy for 28 patients (57.1%) and evolution was favourable for all the patients after an average of 12 days of antibiotic treatment. This treatment duration is not very different from that observed in the hospitals of developed countries where hygiene around the patients is recommended to limit the infection risks [7, 8]

CONCLUSION

During this study, nosocomial urinary infections were very frequent in the urology unit of the national university hospital (Yalgado Ouédraogo), but we found no statistically significant risk factors. To reduce this infection rate, hygiene must be reinforced in our healthcare facilities and "Committees Against Nosocomial Infections" must be created in every hospital in Africa. In case of infection, we propose gentamicin in combination with penicillin or metronidazole as first line of chemotherapy.

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URINARY TRACT INFECTIONS AMONGST PREGNANT WOMEN ATTENDING A MEDICAL CENTRE IN KADUNA, NIGERIA

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ABSTRACT

Urinary tract infection (UTI) constitutes a major health problem in pregnant women due to their relatively short urethra, which promotes the ascending of the pathogens to the bladder, urethra and the kidneys. It is also more common in pregnant women due to the anatomical and physiological changes that occur during pregnancy.

Aim: To determine the incidence of Urinary Tract Infections and the antimicrobial susceptibility of the microbial isolates from the urine samples of pregnant women prior to treatment.

Methods: Fifty (50) mid stream urine (MSU) samples were collected and analyzed using standard Microbiological Techniques, and the antimicrobial sensitivity tests determined using Kirby Bauer disc diffusion techniques.

Results: Of the 50 urine samples obtained from pregnant women, 3 different microbes were isolated indicating 28%. *Staphylococcus aureus* 18%, *Escherichia coli* 8%, *Candida albicans* 4%; and a 2% co-infection of *Candida albicans* and *Staphylococcus aureus*.

Staphylococcus aureus and *Escherichia coli* were highly sensitive to Ciprofloxacin, Ofloxacin (Cilox), Paflacin and Cephalosporine.

Conclusion: Undetected and untreated urinary tract infection in pregnancy leads to discomfort associated with abdominal pains, itching, vaginal discharge and dysuria which may lead to more serious medical complications.

Keywords: Microbial isolates; pregnant women; anti-microbial susceptibility; microbiological techniques; Disc diffusion.

INFECTIONS DE VOIES URINAIRES PARMI LES FEMMES ENCEINTES EN CONSULTATION AU CENTRE MEDICAL DE KADUNA, NIGERIA

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RÉSUMÉ

L'infection de voies urinaires (ITU) constitue un problème majeur de santé chez les femmes enceintes due à leur court urètre, ce qui favorise l'ascendance des agents pathogènes dans la vessie, l'urètre et les reins. Il est également plus fréquent chez les femmes enceintes en raison des modifications anatomiques et physiologiques qui surviennent pendant la grossesse.

But : déterminer l'incidence des infections de voies urinaires et la sensibilité antimicrobienne des souches microbiennes isolées des échantillons d'urine chez les femmes enceintes avant les traitements.

Matériel et méthodes : Cinquante (50) échantillons d'urine du milieu de jet ont été collectés et analysés selon les techniques microbiologiques standards et les tests de sensibilité aux antibiotiques selon les techniques de diffusion de disque de Kirby Bauer.

Résultats : sur 50 échantillons d'urine obtenus chez les femmes enceintes, 3 agents microbiens différents ont été isolés représentant 28%. *Staphylococcus aureus* 18%, *Escherichia coli* 8%, *Candida albicans* 4%, et 2% de co-infection de *Candida albicans* et *Staphylococcus aureus*. *Staphylococcus aureus* et *Escherichia coli* sont très sensibles à la ciprofloxacine, à l'ofloxacine (Cilox), au Paflacine et à la céphalosporine.

Conclusion : l'infection de voies urinaires non détectée et non traitée pendant la grossesse entraîne de l'inconfort associée à des douleurs abdominales, des démangeaisons, un écoulement vaginal et une dysurie qui peuvent conduire à des complications médicales graves.

Mots-clés: isolats microbiens; les femmes enceintes; sensibilité aux agents antimicrobiens; techniques microbiologiques; Diffusion du disque.

INTRODUCTION

Urinary tract infection (UTI) is a common bacterial infection during pregnancy and a significant cause of perinatal and maternal morbidity and mortality (1). It may be symptomatic, in form of urethritis, cystitis, pyelonephritis; or it may remain asymptomatic (2).

Urinary Tract Infection is more common in women than in men due to their short urethra, promoting ascending infection to the bladder (cystitis) and occasionally the kidney (2).

When it affects the lower urinary tracts, it is known as cystitis and when it affects the upper urinary tracts, it is known as pyelonephritis. Acute cystitis refers to infection of the bladder (lower urinary tract); it can occur alone or in conjunction with pyelonephritis (infection of the kidney-the upper urinary tract) (3). There has been an increasing resistance by the bacterial agents to the commonly available antibiotics (1,2)

The prevalence of UTI is increased by several risk factors. Poor socio-economic status is reported to be a major risk factor with indigent patients having a five-fold increased risk (4). Other risk factors include age, high parity, poor perinatal hygiene, history of recurrent UTI, diabetes mellitus, neurogenic bladder retention and anatomic bladder retention, anatomic or functional urinary tract abnormality and increased frequency of sexual activity (1, 5 ,6).

More than 90% of urinary tract infections are caused by bacteria species that are part of the normal body flora; and consequently can readily contaminate the genital area and invade the urinary tract(7)

Staphylococcus aureus has been reported to colonize the vagina in 4%-22% of pregnant women (8). *Escherichia coli* is responsible for between 72% and 55% of cystitis cases in younger women, and more than 50% in women over 50 years (9).

Of 500 asymptomatic pregnant women screened, 433 clinical specimens showed significant bacteriuria, representing an incidence of 86.6% (10). Of this number, 38 (7.4%) were mixed bacteria colonies, while 395 (91%) were of single bacteria colonies. *Staphylococcus aureus* (29.8%), *Escherichia coli* (29.1%) and *Klebsiella pneumoniae* (21.5%) were most frequently isolated pathogens. On the average, the pathogens were sensitive to Ciprofloxacin (99.7%),

Ceftazidime (81.6%), Cotrimoxazole (79.4%), Augmentin (71.4%), Nalidixic Acid (61.7%), Nitrofurantoin (61%), Gentamicin (56.9%) and Ampicillin (25.4%) (10).

The positive culture rate seen in previous studies carried out in Nigeria are: 46.5% in Ebonyi, Eastern Nigeria; 35.5% in Ilorin (North Central Nigeria); 31.6% in Kano (Northern Nigeria); 32.7% in Benin (Southern Nigeria) (1,12,13).

There have been reported cases of resistance to antibiotics by the UTI-causing organisms (1,11,14).

Following frequent use of broad spectrum antibiotics, the prevalence of these resistant bacteria is mainly due to widespread use of antibiotics in people and animal feeds (1,11, 15).

The aim of this retrospective study is to determine the incidence of Urinary Tract Infections and the antimicrobial susceptibility of the microbial isolates from the urine samples of pregnant women attending Shehu Muhammed Kangiwa Clinic of the Kaduna Polytechnic, prior to treatment.

MATERIALS AND METHODS

COLLECTION OF URINE SAMPLES

Fifty (50) sterile universal containers were distributed to pregnant women registered with the ante natal clinic of the Shehu Muhammed Kangiwa medical centre, Kaduna Polytechnic. The study period was March to May 2012.

They were advised on how to collect clean-catch mid stream urine sample which includes the cleansing of the urethral opening with sufficient amount of clean water, dry the area, and collect the urine with the labia held apart (16). The first portion of the urine voided and about 10-15 ML of the mid stream urine samples was collected into the sterile universal bottles containing 0.15g of Boric acid crystals (1% w/v) (16). The bottles were labeled correctly and distributed to the patients. The staff within the antenatal clinic helped to supervise these patients; and the specimens were brought to the laboratory for analysis. Using standard microbiological techniques, microscopy, culture and sensitivity tests were carried out on the urine samples (16).

MICROSCOPY

5-10ML of The urine samples were centrifuged at 2500 Rpm to obtain deposit. The supernatant were discarded into a disinfectant jar. The bolt (base) of the test tube was tapped with finger to agitate the deposit. A drop of the deposit was placed onto a clean microscope slide after being cultured, covered with cover slip and examined microscopically for presence of pus cells, epithelial cells, casts, and RBC.

Candida yeasts are seen microscopically as small, oval with their characteristic budding. On Sabouraud agar, Candida albicans appears as cream colored pasty colonies after 24 hours incubation at 37°C. (7).

CULTURE

A well mixed urine sample was inoculated on CLED and MacConkey agar plates using aseptic procedures. Standard wire loop was used in streaking the specimen on the solidified, dried culture media in order to obtain discrete microbial colonies. It was incubated at 37°C overnight and the plates read after 24 hours.

The presence of 10^5 single bacterial colony counts per ML of urine and a microscopy of 3-5 pus cells per high field were used as baseline for determining significant bacteriuria.

Suspected pathogens were identified using morphological features of the colonies and standard biochemical and sugar utilization tests (16).

Following isolation and identification, the microbial isolates were subjected to antibiotic sensitivity testing using the disc diffusion techniques. The measurement of zone of inhibition taken in accordance with the chart used in Kirby- Bauer method (16).

Information on socio-demographic factors were obtained from patients. these includes age, parity, religion, gestational age, marital status and socio-economic status as determined by (17).

All information about a client was entered into a standard register specially designed for this study and are treated with utmost confidentiality

RESULTS

Of the 50 samples analyzed, there was a monomicrobial growth in 13, representing 26% of the samples. Polymicrobial growth in 1 patient represented 2%; and no microbial isolate in 36, representing 72% (TABLE 1).

9 (18%) patients were infected with *Staphylococcus aureus*; 4 (8%) patients were infected with *Escherichia coli*; and 1 (2%) patient was co-infected with *Staphylococcus aureus* and *Candida albicans*, (TABLE 2).

TABLE 1: INCIDENCE OF MONOMICROBIAL /POLYMICROBIAL GROWTH

NUMBER OF ORGANISM	NUMBER OF CASES	PERCENTAGE
No growth	36	72
One	13	26
Two	1	2
TOTAL	50	100

TABLE 2: NUMBER OF ORGANISMS

ORGANISM	NUMBER	PERCENTAGE
<i>Staphylococcus aureus</i>	9	18
<i>Escherichia Coli</i>	4	8
<i>Candida albicans</i>	1	2
TOTAL	14	28

Majority of the patients were between 25 and 35 years. Higher infection rates were associated with this age group and 40 years and above, although the association was not statistically significant (TABLE 3).

TABLE 3: MICROBIAL ANALYSIS ACCORDING TO AGE GROUP OF URINE SAMPLES OBTAINED FROM 50 PREGNANT WOMEN

AGE GROUP	NUMBER OF SAMPLES SCREENED	NUMBER OF INFECTED SAMPLES	NUMBER OF UN-INFECTED SAMPLES	PERCENTAGE OF INFECTED SAMPLES
15-19	1	-	1	-
20-24	7	2	5	14.29
25-29	15	4	11	28.57
30-34	13	3	10	21.43
35-39	7	1	6	7.14
40 and above	7	4	3	28.57
TOTAL	50	14	36	100

On the average, the pathogens were highly sensitive to quinolones (Ciprofloxacin (99.8%), Cilox (98.7%),

TABLE 4: ORGANISMS WITH ANTIBIOTIC SENSITIVITY (%)

Antibiotic	Staphylococcus aureus	Escherichia coli	% sensitive
Amoxycillin	10	80	45
Ceftazidime	97	98	97.5
Ofloxacin(Cilox)	100	97	98.7
Peflacin	99.2	98.0	98.6
Ciprofloxacin	100	97.6	98.8
Erythromycin	45.1	40	42.6
Genticin	75	60	67.5
Septtrin	35	15	25
Tetracycline	30	10	20
Penicillin	26	-	26

DISCUSSION

The positive culture rate seen in previous studies carried out in Nigeria are: 46.5% in Ebonyi, Eastern Nigeria, 35.5% in Ilorin (North Central Nigeria), 31.6% in Kano (Northern Nigeria), and 32.7% in Benin(Southern Nigeria)(1,12,13). Non pregnant women have lower positive culture rates (10).

The usefulness of Quinolones for the treatment of UTI found in this study is similar to other studies elsewhere (18),(19). Quinolones are however associated with teratogenicity in the first trimester of pregnancy; and are secreted in the breast milk. They

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pefloxacin (98.6%)), and Cephalosporin (ceftazidime) (97.5%) (TABLE 4).

are therefore not recommended for the treatment of UTI in pregnancy.

Cephalosporines, though expensive, are known to be safe in pregnancy.

RECOMMENDATIONS

The above results indicated that the microbes found to be responsible for the urinary tract infection in pregnant women studied are: *Staphylococcus aureus*, *Escherichia coli* and *Candida albican*. It is therefore recommended that UTI in pregnancy be treated with a cephalosporin; and with antifungal agent where *Candida* is found.

Couples should be educated on ways of preventing urinary tract infections. It is highly recommended that pregnant women be properly treated to avert the negative effects of urinary tract infections on both the mother and the foetus.

Government should further encourage the funding for the diagnosis and treatment of urinary tract infections in pregnancy in the healthcare service plans.

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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF URINARY PATHOGENS ISOLATED FROM TWO TERTIARY HOSPITALS IN SOUTHWESTERN NIGERIA

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ABSTRACT

Background: Urinary tract infection (UTI) is among the most common reasons for patients to seek health assistance that is commonly encountered in office practices. This is also a leading cause of Gram negative sepsis in hospitalized patients.

Objectives: This study was carried out in order to isolate, characterize and identify the pathogens associated with UTI in two teaching hospitals at Osun state, Nigeria and to determine their antimicrobial susceptibility patterns.

Methods: This was a prospective observational study involving standard microbiological procedures for analysing urine samples of inpatient and outpatient cases of UTI. Identification of these pathogens was performed using Microbact/API identification system.

Results: Out of the 300 urine samples cultured, 88 (29.3%) yielded significant growth of urinary pathogens while 212 (70.7%) yielded either insignificant growth or no growth of any urinary pathogen. *Escherichia coli* 19 (21.6%) were the commonest pathogen isolated followed by *Klebsiella pneumoniae* 14 (15.9%), *Staphylococcus aureus* 12(13.6%), *Candida albicans* 12(13.6%), *Pseudomonas aeruginosa* 9 (10.2%), *Klebsiella oxytoca* 8 (9.1%), *Staphylococcus saprophyticus* 6 (6.8%), *Serratia rubidaea* 3 (3.4%), *Enterobacter agglomerans* 2 (2.3%), *Acinetobacter iwoffii* 1 (1.1%), *Acinetobacter baumannii* 1 (1.1%), *Providencia rettgeri* 1 (1.1%). The susceptibility of Gram negative bacteria (GNB) were mainly toward parenteral antibiotic rather than oral one, while most of the common antibiotic showed a resistant pattern. UTI was more prevalent among patients within hospital setting 71(80.7%) than out-patients 17(19.3%).

Conclusion: This study justifies the necessity to treat patients with UTI based on antimicrobial susceptibility test result in order to prevent evolution of resistant pathogens. Since UTI has large impact on the socio-economy and emergence of bacterial resistance, periodic surveillance of antibiotic susceptibility is strongly recommended.

Keywords: Microbact; antimicrobial resistance; UTI and Osogbo.

SENSIBILITE ANTIMICROBIENNE DE PATHOGENES URINAIRES ISOLEES AU NIVEAU DE DEUX HOPITAUX TERTIAIRES AU SUD-OUEST DU NIGERIA

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RÉSUMÉ

Contexte: L'infection des voies urinaires (IVU) est parmi les raisons les plus courantes pour les patients à demander de l'assistance médicale qui est couramment rencontré dans les clientèles privés. C'est aussi la principale cause de septicémie de bactéries à Gram négatif chez les patients hospitalisés.

Objectifs: Cette étude a été réalisée afin d'isoler, caractériser et identifier les agents pathogènes associés aux infections urinaires dans les deux centres hospitaliers universitaires de l'Etat d'Osun au Nigéria et pour déterminer leurs profils de sensibilité aux antimicrobiens.

Méthodes: C'est une étude prospective observationnelle impliquant des procédures microbiologiques standard pour analyser des échantillons d'urine de patients hospitalisés et ambulatoires de cas d'infection des voies urinaires. L'identification de ces agents pathogènes a été réalisée en utilisant le système d'identification Microbact /API.

Résultats: Sur les 300 échantillons de culture urinaire, 88 (29,3%) ont eu une croissance importante de pathogènes urinaires tandis que 212 (70,7%) ont eu une croissance soit insignifiante ou pas de croissance d'aucun agent pathogène urinaire. *Escherichia coli* 19 (21,6%) était le germe pathogène le plus fréquent isolé suivi de *Klebsiella pneumoniae* 14 (15,9%), *Staphylococcus aureus* 12 (13,6%), *Candida albicans* 12 (13,6%), *Pseudomonas aeruginosa* 9 (10,2%), *Klebsiella oxytoca* 8 (9,1%), *Staphylococcus saprophyticus* 6 (6,8%), *Serratia rubidaea* 3 (3,4%), *Enterobacter agglomerans* 2 (2,3%), *Acinetobacter iwoffii* 1 (1,1%), *Acinetobacter baumannii* 1 (1,1%), *Providencia retgeri* 1 (1,1%). La sensibilité de bactéries à Gram négatif (BGN) était essentiellement à un antibiotique parentéral plutôt que par voie orale, alors que la plupart d'antibiotiques fréquents a montré une résistance. L'infection de voies urinaires était plus fréquente chez les patients hospitalisés 71 (80,7%) par rapport aux patients externes 17 (19,3%).

Conclusion: Cette étude justifie la nécessité de traiter les patients souffrant d'infection urinaire en se basant sur le résultat du test de sensibilité aux antimicrobiens afin de prévenir l'évolution de pathogènes résistants. Depuis, l'infection de voies urinaires a un impact important sur le développement socio-économique et l'émergence de la résistance bactérienne; la surveillance périodique de la sensibilité aux antibiotiques est fortement recommandée.

Mots-clés: Microbact; la résistance aux antimicrobiens; Infection des voies urinaires et Osobgo.

INTRODUCTION

Urinary tract infections (UTI) are among the most common conditions encountered in office practices. This is also leading cause of Gram negative sepsis in hospitalized patients [1]. UTI is the most common reason for patients to seek health assistance, accounting for about seven million patients visit every year. Majority are otherwise healthy women who typically present with dysuria each year. Approximately 20% of women develop UTI sometime during their life time. Above Age 50, the incidence of UTI is similar in men and women [2].

UTI encompasses both asymptomatic microbial colonization of the urine and symptomatic infection with microbial invasion and inflammation of urinary tract structures. Microbiologically, growth of more than 10^5 colony forming unit (cfu) /mL from a properly collected midstream urine samples indicates infection. However, a smaller number of bacteria (10^2 - 10^4 /mL) may signify infection in specimen from suprapubic aspiration, catheter samples and immunocompromized patients. The etiology of UTI and the antimicrobial susceptibility of urinary pathogens in both the community and hospitals have been changing, and in recent years antibiotic resistance has become a major problem worldwide due to several factors related to the genetic nature of the organisms and selective antimicrobial pressure in humans and animals.

Drug resistance is a large and growing problem in infection that accounts for most of Africa's disease burden e.g. malaria, TB, HIV, respiratory and diarrheal diseases. Currently, the prevalent pathogens of UTI have been resistant to most chemotherapeutic

agents making antimicrobial susceptibility highly unpredictable without laboratory support. These would have profound impact on future management of infection with these drugs [3,4].

Furthermore, prevalence of the urinary pathogens and their susceptibility reactions to antibiotics differ from places to places with time. It is essential to know the current trends of UTI in these two places to ease diagnosis and thus establish the suitable antimicrobial agents for such infections in order to facilitate quick recovery, prevent/minimise complications of antimicrobial resistance.

Variability of Pathogens in UTI and Antimicrobial Susceptibility in Nigeria and Neighbouring countries
The prevalence of antimicrobial resistance in urinary pathogens is increasing worldwide. along with temporal and local variables affecting the pattern of pathogens. An accurate bacteriological records of UTI from studies locally and regionally, may provide guidance for empiric therapy with antimicrobial agents in UTI before the culture and sensitivity patterns are available [5,6,7].

In our regional context, variable results on the prevalence of pathogens and their antimicrobial susceptibility pattern in UTI were obtained in recent and past studies: at Abeokuta, Nigeria, Ojo *et al.*, 2007 reported that the overall prevalence of UTI was 52% with *E.coli* (28%), *Klebsiella pneumoniae* (16%) and *Pseudomonas aeruginosa* (8%). The organisms were susceptible to ofloxacin, nitrofurantoin, ciprofloxacin and gentamicin ($P = 0.0001$) but resistant to ampicillin ($P = 0.153$) [7].

Similarly, Okesola and Oni, 2005 (6) at the University College Hospital (UCH), Ibadan, Nigeria, showed that the most prevalent bacterial pathogen in urine were *Staphylococcus aureus* (47.5%), *Pseudomonas aeruginosa* (24.6%), *Klebsiella species* (23%), *Proteus spp* (3.3%), *E. coli* (1.6%). Older generation antibiotics like streptomycin and chloramphenicol were more resistant than the newer gentamycin and ciprofloxacin [8].

Jumbo *et al.*, 2011 from Calabar, Nigeria said the incidence of UTI was 7.7% (Male 46.7% and Female 53.3%). 69.2% were of community acquired while 30.8% were nosocomial in origin [9].

Obiogbolu *et al.*, 2009 from Akwa metropolis, South-eastern Nigeria also reported that *E.coli* was the commonest urinary tract pathogen and the study indicated a high incidence of UTIs (54%) in pregnant women [10].

At Imo State University Teaching Hospital, Orlu, Nigeria, it was also showed that *E. coli* accounted for 52.5% infection; *S. aureus* (33.9%), *P. mirabilis* (8.5%), *Enterococcus species* (5.0%) and *N. gonorrhea* (1.7%). These organisms were most sensitive to the quinolones compared to the penicillins and aminoglycosides [11].

Similar results were obtained at Port Harcourt, where most of the pathogens were resistant to tetracycline, ampicillin and cotrimazole but exhibited sensitivity to nitrofurantoin, gentamicin and nalidixic acid [11].

Kenenchukwu *et al.*, 2006 at Enugu, Nigeria, reported the commonest isolates to be *E.coli*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Proteus spp*. The Gram positive organisms were very sensitive to Augmentin and the fluoroquinolones. *Escherichia coli* showed the highest sensitivity to nitrofurantoin (76%) and the fluoroquinolones (74%). The study clearly showed that nitrofurantoin is a very effective first line drug for UTIs [11].

From Makurdi, North central Nigeria, Umeh *et al.*, 2007 reported that bacteriuria was approximately 5 times high in women as in men. The attributed risk was 30.15%. Gram positive bacteria predominated in the males and were responsible for 60% of bacteriuria in males. In females, the Gram negatives accounted for 66.7% of the bacteria isolated. Antibiotic susceptibility testing of *Staphylococcus aureus* and the coliform bacteria to commonly used antimicrobial drugs showed a higher resistance pattern [12].

In similar studies carried out in Kano Nigeria, Adeleke and Asani, 2009 reported that UTI was caused predominantly by *Staphylococcus aureus*

(67.9%), *Klebsiella species* (17.9%), and *Pseudomonas* (14.2%). There was high in-vitro resistance of these organisms to nalidixic acid and ampicillin but sensitive to cefotaxime, ceftriazone and ciprofloxacin [13].

At Yola, Adamawa, Nigeria, it was reported that 37.1% of uropathogens were obtained from male suspects, while the remaining 62.9% were from female subjects. Gram negative bacteria had the highest frequency of occurrence with 63.6% than Gram positive with 36.4% with *E.coli* as the commonest [14].

Zaria *et al.* also reported from Maiduguri, Nigeria, a high incidence of UTI both in pregnant and non-pregnant women, *E.coli* being the prominent organism, and highly sensitive to quinolone group while resistant to Co-amoxicillin, Cotrimoxazole and Nalidixic acid [15]. Similar results were observed in Kaduna, Nigeria [16].

Apart from Nigeria, in Koforidua, Ghana, the most common urinary pathogens isolated were *E.coli* (58.6%) and *Klebsiella spp.* (20.6%). The majority of isolates (78%) were from females. The highest percentage of isolates (7.9%) was in age group 21-30 yrs. All pathogens were resistant to Ampicillin (95.8%) and cotrimoxazole (97.7%) but were sensitive to Nitrofurantoin (71.2%) and Gentamicin (51.75%) [17].

In Karachi, Pakistan, prevalence of asymptomatic bacteriuria (ASB) was 6.2%. *E.coli* and *Staphylococcus saprophyticus* showed 66.67% resistance to ampicillins and sulphonamides, while *Enterobacters* showed 100% resistance to ampicillins, cephalosporins and nitrofurantoin [18]. In Tehran, Iran, the most prevalent urinary pathogen was *E.coli* (56.6%) [19]. At Al-Khobar, Saudi Arabia, Bukharie and Saeed, 2001 reported that *E.coli* and *Klebsiella spp.* were also the most common pathogens of urine, accounting for 79% of the Nalidixic acid and Nitrofurantoin were least resistant (13%) while cotrimoxazole was most (39%) with Augmantin 19%. The best susceptibility pattern was seen with quinolones [20].

MATERIALS AND METHODS

This prospective observational study was carried out at Ladoke Akintola University Teaching Hospital (LTH), Osogbo and Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) Ile Ife. The study subjects were from both out-patients clinics and in-patient general wards of these two hospitals.

Study Urine Collection

Each subject was given a sterile plastic bottle to collect mid-stream urine (MSU) after they were taught how

to collect it aseptically. 300 sterile urine samples were thus collected for subsequent bacteriological analysis [21].

Urinalysis

5mls each of the urine samples was centrifuged at 2,500g for 5 minutes. The supernatant was discarded and the deposit re-suspended with the small amount of urine left in the tube by tapping the base of the centrifuge tube. A drop was placed on cleaned grease free slide, covered with cover slip and examined under microscope using 10x and 40x objective lenses for the presence of white blood cells, red blood cells, epithelia cells, casts, crystals, bacteria, yeast cells and Trichomonas [21].

Culture and Sensitivity

A calibrated standard wire loop was used for inoculating culture plate. A loopful (0.002mL) of well mixed un-centrifuged urine was plated on a dried CLED and MacConkey agar media. These plates were incubated aerobically at 37°C for 24hours. Colony counts were determined at the end of incubation period. Each urine sample with over 10⁵ CFU per milliliter was followed up as significant bacteria growth and isolated colonies were sub-cultured.

Subculture of isolated colony

To obtain pure isolates, discrete colonies of pathogens isolated were inoculated in a well dried MacConkey agar media. The plates were incubated aerobically at 37°C for 24hours. After overnight incubation, colonies were subjected to biochemical tests for identification [21].

Characterization of Isolates

The various isolates obtained were subjected to morphological, physiological, and biochemical tests.

Morphological characterization

The isolated colonies were examined and recorded based on the type of growth, elevation, size, colour, margin, edge, consistency, opacity, and change in medium [21].

Gram staining

A thin smear of each isolate was made on clean grease free glass slide, air dried and heat fixed by passing it gently over flame and then Gram stained. Gram positive cells stained purple while the Gram negative cells stained pink [21].

Biochemical tests

The *Candida albicans* were identified by performing Germ Tube Test (GTT) on any isolate whose Gram result shows yeasts. Catalase test was done on the Gram positive cocci to differentiate *Staphylococcus spp*

from *Streptococcus spp*. Coagulase test was done to identify *S. aureus* which produces the enzyme coagulase.

Oxidase test was done on the Gram negative bacilli (GNB) to identify *Psuedomonas spp* from other Gram Negative bacilli. MICROBACT (API) identification system was used to identify the species of the oxidase negative GNB.

Microbat identification

MICROBACT™ GRAM-NEGATIVE 12A was used. It is a range of simple, standardised systems for the rapid identification of Gram-negative bacteria.

Principle

Each kit contains 12 miniature biochemical tests. Organism identification is based on pH change and substrate utilisation. Microbact™ Gram-negative 12A (microplate format) was used alone for the identification of oxidase-negative, nitrate-positive glucose fermenters (comprising 15 genera) and is useful for screening pathogenic Enterobacteriaceae from enteric and urine specimens.

Antibiotic Susceptibility Test

Antibiotic susceptibility of pure culture of confirmed isolate was performed on diagnostic sensitivity test agar (Mueller Hinton agar) by the Kirby Bauer disc diffusion method, using the appropriate Gram positive and Gram negative discs. Isolates were considered sensitive after incubation for 24 hours at 37°C by measuring zone of inhibition with meter rule which was then compared with zone diameter interpretative to National committee for clinical Laboratory standard (CLSI chart) for different organisms and different antibiotics [22].

To guarantee precision and reliability of antibiogram data, quality control strains of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 supplied by department of Pharmaceutics, Obafem Awolowo University, Ile-Ife, Nigeria were used.

Statistical Analysis

Data were analyzed using SPSS version 18.0 window based program. Discrete variables were expressed as percentages and proportions were compared using the Chi-square test. Statistical significant difference was considered at value of $P \leq 0.05$.

RESULTS

Table 1 shows the prevalence of UTI organisms in the two major tertiary institutions in Osun State. Of the 300 subjects, 100 (33.3%) were from LTH and 200 (66.7%) from OAUTHC. 88 (29.3%) of the 300 samples yielded significant growth of organisms; giving UTI a prevalence of 29.3% in Osun State.

The male and female ratio were comparable, however, there was a propensity of higher incidence of UTI in female (Table 2 and Table 3).

From the 88 samples that yielded significant growth, 19 (21.6%) were *Escherichia coli*, 14 (15.9%) *Klebsiella pneumoniae*, 12 (13.6%) *Staphylococcus aureus*, 12 (13.6%) *Candida albicans*, 9 (10.2%) *Pseudomonas aeruginosa*, 8 (9.1%) *Klebsiella oxytoca*, 6 (6.8%) *Staphylococcus saprophyticus*, 3 (3.4%) *Serratia rubidaea*, 2 (2.3%)

Enterobacter agglomerans, 1 (1.1%) *Acinetobacter iwoffii*, 1 (1.1%) *Acinetobacter baumannii* and 1 (1.1%) *Providencia rettgeri*. (Fig. 1 and Fig. 2). The infection was found to be more common among the in patients than the out patients (Fig. 3).

Contrary to the expectation, UTI was more common in non-pregnant women than the pregnant patients (66.7% vs 33.3%, Table 4).

Table 3 shows organisms in female infections with *K. pneumoniae* 10 (21%) out of the 48 isolates from female patients. Making *K. pneumoniae* highest UTI organisms in females followed by *C. albicans* 9 (19%), *E. coli* 8 (17%), *K. oxytoca* 6 (13%), *S. aureus* 6 (13%), *S. saprophyticus* 5 (10%), *P. aeruginosa* 2 (4%), *S. rubidaea* 1 (2%) and *E. agglomerans* 1 (2%).

TABLE 1: PREVALENCE OF UTI IN OSUN STATE AT LARGE

Hospital	Growth (H%) (O%)	No Growth (H%) (O%)	Total (H%) (O%)
LTH	40 (40) (13.3)	60 (60) (20)	100 (100) (33.3)
OAUTHC	48 (24) (16)	152 (76) (50.7)	200 (100) (66.7)
Total (Osun)	88 (29.3)	212 (70.7)	300 (100)

H = Hospital O = Overall and Total (Osun) = H (LTH) + H (OAUTHC). Ladoke Akintola University Teaching Hospital (LTH),

Osogbo and Obafemi Awolowo University Teaching Hospital Complex (OAUTHC)

TABLE 2: AGE GROUP AND SEX DISTRIBUTION OF SUBJECTS ATTENDING TERTIARY HEALTH INSTITUTIONS IN OSUN STATE

Age Group	Gender		Total (%)
	Male (%)	Female (%)	
0 – 9	16 (5.3)	10 (3.3)	26 (8.7)
10-19	7 (2.3)	8 (2.7)	15 (5.0)
20-29	44 (14.7)	46 (15.3)	90 (30.0)
30-39	31 (10.3)	53 (17.7)	84 (28.0)
40-49	9 (3.0)	4 (1.3)	13 (4.3)
50 & above	53 (17.7)	19 (6.3)	72 (24)
Total	160 (53.3)	140 (46.7)	300 (100)

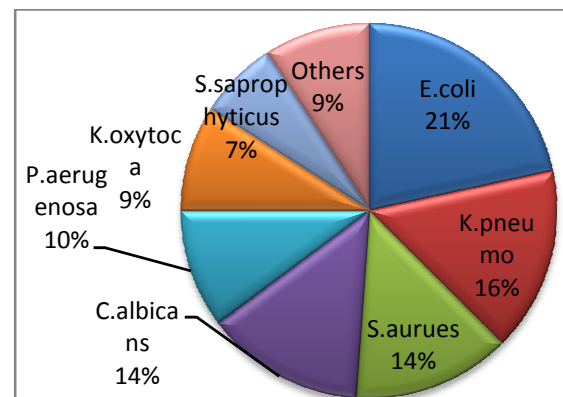


FIG. 1: MICROBIAL ISOLATES FROM SAMPLES

TABLE 3: MICROBIAL ISOLATES FROM URINE OF SUBJECTS ATTENDING BOTH HOSPITALS

Isolate	Male (%)	Female (%)	Total (%)
<i>Escherichia coli</i>	11 (12.5)	8 (9.1)	19 (21.6)
<i>Klebsiella pneumoniae</i>	4 (4.5)	10 (11.4)	14 (15.9)
<i>Staphylococcus aureus</i>	6 (6.8)	6 (6.8)	12 (13.6)
<i>Candida albicans</i>	3 (3.4)	9 (10.2)	12 (13.6)
<i>Pseudomonas aeruginosa</i>	7 (8.0)	2 (2.3)	9 (10.2)
<i>Klebsiella oxytoca</i>	2 (2.3)	6 (6.8)	8 (9.1)
<i>Staph. Saprophyticus</i>	1 (1.1)	5 (5.7)	6 (6.8)
<i>Serratia rubidaea</i>	2 (2.3)	1 (1.1)	3 (3.4)
<i>Enterobacter agglomerans</i>	1 (1.1)	1 (1.1)	2 (2.3)
<i>Acinetobacter iwoffii</i>	1 (1.1)	0 (0.0)	1 (1.1)
<i>Acinetobacter baumannii</i>	1 (1.1)	0 (0.0)	1 (1.1)
<i>Providencia retgerri</i>	1 (1.1)	0 (0.0)	1 (1.1)
Total	40 (45.5)	48 (54.5)	88 (100)

Table 5 shows the pathogens isolated in relation to different age groups. Of the 88 samples that yielded

significant growth, the highest number of isolates 31 (35.2%) was found among the age group 50 and above, followed by the age group 30-39 with 26 (29.5%) isolates, 22 (25%) in age group 20-29, 6 (6.8%) in 0-9 years, 2 (2.3%) in 10-19 and the least 1 (1.1%) seen in age group 40-49.

TABLE 4: ISOLATES FROM FEMALES RELATING PREGNANT TO NON-PREGNANT WOMEN

Isolate	Pregnant	Non-pregnant	Total (%)
<i>E.coli</i>	1	7	8 (16.7)
<i>K.pneumo</i>	2	8	10 (20.8)
<i>S.aureus</i>	3	3	6 (12.5)
<i>C.albicans</i>	3	6	9 (18.8)
<i>Pseudomonas spp</i>	1	1	2 (4.2)
<i>K.oxytoca</i>	3	3	6 (12.5)
<i>S.saprophyticus</i>	2	3	5 (10.4)
<i>S.rubidaea</i>	0	1	1 (2.1)
<i>E.agglomerans</i>	1	0	1 (2.1)
Total (%)	16 (33.3)	32 (66.7)	48 (100)

TABLE 5: PREVALENCE OF ISOLATES AMONG AGE GROUPS

Isolate	Age						Total
	0 - 9	10 - 19	20 - 29	30 - 39	40 - 49	50 >	
<i>E.coli</i>	2	0	6	3	0	8	19
<i>K.pneumo</i>	0	0	2	6	0	6	14
<i>S.aureus</i>	0	0	2	4	0	6	12
<i>C.albicans</i>	1	2	1	6	0	2	12
<i>Pseudomonas spp</i>	1	0	2	1	0	5	9
<i>K.oxytoca</i>	1	0	4	3	0	0	8
<i>S.saprophyticus</i>	0	0	3	1	0	2	6
<i>S.rubidaea</i>	0	0	0	1	0	2	3
<i>E.agglomerans</i>	0	0	1	1	0	0	2
<i>A.iwoffii</i>	0	0	1	0	0	0	1
<i>A.baumannii</i>	0	0	0	0	1	0	1
<i>P.retgerri</i>	1	0	0	0	0	0	1
Total (%)	6 (6.8)	2 (2.3)	22 (25)	26 (29.5)	1 (1.1)	31 (35.2)	88 (100)

Gram Positive Isolates

All the Gram positive bacteria were resistant to tetracycline and cotrimoxazole. *Staphylococcus aureus* showed varying susceptibility patterns to the antibiotics; augmentin and erythromycin (66.7%), chloramphenicol (58.3%), vancomycin (50%),

gentamicin (33.3%) and amoxicillin, cloxacillin and ceftazidime (16.7%). *Staphylococcus saprophyticus* was resistant (100%) to amoxicillin, gentamicin and ceftazidime, mildly sensitive (66.7%) to erythromycin and cloxacillin and moderately sensitive (83.3%) to

augmentin, chloramphenicol and vancomycin (Table 6).

Gram Negative Isolates

All organisms were sensitive to imipenem except *Pseudomonas aeruginosa* which had 22.2% resistance. All the organisms were resistant (100%) to cotrimoxazole. *Escherichia coli* was moderately sensitive to gentamicin (84.2%) and ofloxacin (73.7%), mildly sensitive (36.8%) to nitrofurantoin and tetracycline, (31.6%) to ciprofloxacin, very low sensitivity (21.1%) to ceftazidime, (10.5%) to amoxycillin and (5.3%) to nalidixic acid with 100% resistance to augmentin. *Klebsiella pneumonia* was moderately sensitive to gentamicin (64.3%) and 57.1%) to ofloxacin and ciprofloxacin, mildly sensitive to ceftazidime (50%) and nitrofurantoin (42.9%), very low sensitivity (21.4%) to augmentin, (7.1%) to amoxycillin and nalidixic acid with 100% resistance to tetracycline. *Pseudomonas aeruginosa* was moderately sensitive (88.9%) to gentamicin and ofloxacin, (77.8%) to imipenem, mildly sensitive (55.6%) to nitrofurantoin, ceftazidime and ciprofloxacin, (44.4%) to nalidixic acid and tetracycline with 100% resistance to amoxycillin and augmentin.

Klebsiella oxytoca was moderately sensitive (62.5%) to ofloxacin and ceftazidime, mildly sensitive (50%) to nitrofurantoin and gentamicin, (37.5%) to augmentin and ciprofloxacin, very low sensitivity (25%) to nalidixic acid and tetracycline and (12.5%) to amoxicillin. *Serratia rubidaea* was highly sensitive (100%) to gentamicin, moderately sensitive (66.7%) to ofloxacin and ciprofloxacin, mildly sensitive (33.3%) to amoxicillin, nitrofurantoin, augmentin, tetracycline and ceftazidime with 100% resistance to nalidixic acid.

Enterobacter agglomerans was highly sensitive (100%) to nitrofurantoin, gentamicin and ceftazidime, mildly sensitive (50%) to amoxicillin, nalidixic acid, ofloxacin, augmentin and ciprofloxacin with 100% resistance to tetracycline. *Acinetobacter iwoffii* was highly sensitive (100%) to gentamicin, nalidixic acid, ofloxacin, tetracycline and ceftazidime with 100% resistance to amoxycillin, nitrofurantoin, augmentin and ciprofloxacin. *Acinetobacter baumannii* was highly sensitive (100%) to gentamicin and ofloxacin with 100% resistance to amoxycillin, nitrofurantoin, nalidixic acid, augmentin, tetracycline, ceftazidime and ciprofloxacin. *Providencia rettgeri* was highly sensitive (100%) to ceftazidime and ciprofloxacin with 100% resistance to amoxycillin, nitrofurantoin, gentamicin, nalidixic acid, ofloxacin, augmentin and tetracycline. (Table 7)

FIG. 2: ISOLATES FROM SAMPLES IN RELATION TO SEX

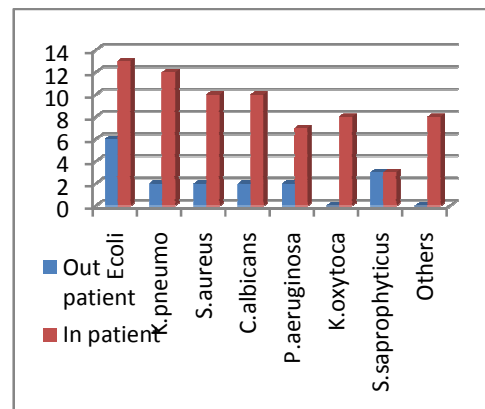
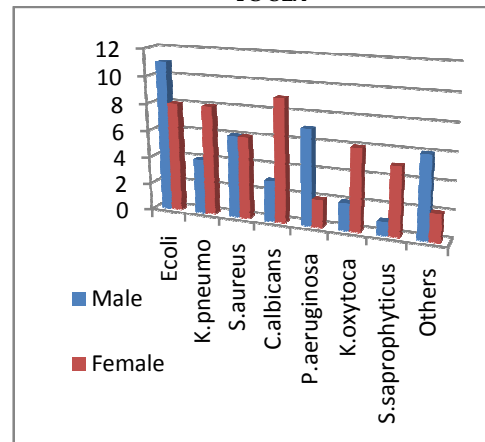


FIG. 3: MICROBIAL ISOLATES RELATING HOSPITAL ACQUIRED TO COMMUNITY ACQUIRED UTI

TABLE 6: ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF THE GRAM POSITIVE ISOLATES

Isolate		AUG	AMX	ERY	TET	CXC	GEN	COT	CHL	VA	CAZ
<i>Staph.aureus</i>	S	8(66.7)	2(16.7)	8(66.7)	0(0.0)	2(16.7)	4(33.3)	0(0.0)	7(58.3)	6(50)	2(16.7)
N=12	R	4(33.3)	10(83.3)	4(33.3)	12(100)	10(83.3)	8(66.7)	12(100)	5(41.7)	6(50)	10(83.3)
<i>S.saprophyticus</i>	S	5(83.3)	0(0.0)	4(66.7)	0(0.0)	4(66.7)	0(0.0)	0(0.0)	5(83.3)	5(83.3)	0(0.0)
N=6	R	1(16.7)	6(100)	2(33.3)	6(100)	2(33.3)	6(100)	6(100)	1(16.7)	1(16.7)	6(100)

AUG = Augmentin, AMX = Amoxycillin, ERY = Erythromycin, TET = Tetracycline, CXC = Coxacillin, GEN = Gentamicin, COT = Cotrimoxazole, CHL = Chloramphenicol, VA = Vancomycin, CAZ = Ceftazidime

TABLE 7: ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF THE GRAM NEGATIVE ISOLATES

Isolate		AMX	COT	NIT	GEN	NAL	OFL	AUG	TET	CAZ	IPM	CIP
<i>E.coli</i>	S	2(10.5)	0(0.0)	7(36.8)	16(84.2)	1(5.3)	14(73.7)	0(0.0)	7(36.8)	4(21.1)	19(100)	6(31.6)
N=19	R	17(89.5)	19(100)	12(63.2)	3(15.8)	18(94.7)	5(26.3)	19(100)	12(63.2)	15(78.9)	0(0.0)	13(68.4)
<i>K.pneumoniae</i>	S	1(7.1)	0(0.0)	6(42.9)	9(64.3)	1(7.1)	8(57.1)	3(21.4)	0(0.0)	7(50)	14(100)	8(57.1)
N=14	R	13(92.9)	14(100)	8(57.1)	5(35.7)	13(92.9)	6(42.9)	11(78.6)	14(100)	7(50)	0(0.0)	6(42.9)
<i>Pseudomonas</i>	S	0(0.0)	0(0.0)	5(55.6)	8(88.9)	4(44.4)	8(88.9)	0(0.0)	4(44.4)	5(55.6)	7(77.8)	5(55.6)
<i>spp</i> N=9	R	9(100)	9(100)	4(44.4)	1(11.1)	5(55.6)	1(11.1)	9(100)	5(55.6)	4(44.4)	2(22.2)	4(44.4)
<i>K.oxytoca</i>	S	1(12.5)	0(0.0)	4(50)	4(50)	2(25)	5(62.5)	3(37.5)	2(25)	5(62.5)	8(100)	3(37.5)
N=8	R	7(87.5)	8(100)	4(50)	4(50)	6(75)	3(37.5)	5(62.5)	6(75)	3(37.5)	0(0.0)	5(62.5)
<i>S.rubidaea</i>	S	1(33.3)	0(0.0)	1(33.3)	3(100)	0(0.0)	2(66.7)	1(33.3)	1(33.3)	1(33.3)	3(100)	2(66.7)
N=3	R	2(66.7)	3(100)	2(66.7)	0(0.0)	3(100)	1(33.3)	2(66.7)	2(66.7)	2(66.7)	0(0.0)	1(33.3)
<i>E.agglomerans</i>	S	1(50)	0(0.0)	2(100)	2(100)	1(50)	1(50)	1(50)	0(0.0)	2(100)	2(100)	1(50)
N=2	R	1(50)	2(100)	0(0.0)	0(0.0)	1(50)	1(50)	1(50)	2(100)	0(0.0)	0(0.0)	1(50)
<i>A.woffii</i>	S	0(0.0)	0(0.0)	0(0.0)	1(100)	1(100)	1(100)	0(0.0)	1(100)	1(100)	1(100)	0(0.0)
N=1	R	1(100)	1(100)	1(100)	0(0.0)	0(0.0)	0(0.0)	1(100)	0(0.0)	0(0.0)	0(0.0)	1(100)
<i>A.baumannii</i>	S	0(0.0)	0(0.0)	0(0.0)	1(100)	0(0.0)	1(100)	0(0.0)	0(0.0)	0(0.0)	1(100)	0(0.0)
N=1	R	1(100)	1(100)	1(100)	0(0.0)	1(100)	0(0.0)	1(100)	1(100)	1(100)	0(0.0)	1(100)
<i>P.retgerri</i>	S	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100)	1(100)	1(100)
N=1	R	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0.0)	0(0.0)	0(0.0)

AMX = Amoxycillin, COT = Cotrimoxazole, NIT = Nitrofurantoin, GEN = Gentamicin, NAL = Nalidixic acid, OFL = Ofloxacin, AUG = Augmentin, TET = Tetracycline, CAZ = Ceftazidime, IPM = Imipenem, CIP = Ciprofloxacin

DISCUSSION

The study showed that, from 300 urine samples cultured 88(29.3%) yielded significant growth of urinary pathogens while 212 (70.7%) yielded either insignificant growth or no growth of any urinary pathogen. This 29.3% UTI prevalence from the study was lower in comparison to the finding of Yengkokpam *et al.* (40.4%) from India. However, this was in agreement with other study conducted by Chhetri *et al.* (21.8%), Rai *et al.* (28.6%), and Kumari *et al.* (25.7%) from Nepal [23,24,25,26]. All the significant culture growths were monomicrobial. There was higher number of males 160 (53.3%) attending the hospitals on UTI cases than females 140 (46.7%). The age group, 20-29, recorded the highest attendance with 90 (30%) while 40-49 age group had the lowest attendance 13 (4.3%).

The isolated organisms in this study showed that *Escherichia coli* were the most common UTI pathogens and this agrees with previous report of Obiogbolu *et al.* and Kumari *et al.* They found that *E.coli* was the commonest urinary tract pathogen which indicated a high incidence of UTIs (54%) and (42%) respectively [10,26]. Incidence value of *E.coli* in this work is lower than in many previous works. This is probably because some organisms were identified in this work, which were not identified in the previous works. This may be due to the Microbact identification system used and emergence of new strains of pathogens.

Also, the result showed that females 48 (54.5%) were more infected than males 40 (45.5%). This is in accordance with the patterns that UTIs follow universally. Close proximity of perineal structures along with the short urethra predisposes female to be at a higher risk than male. Moreover, the use of diaphragm with spermicide and indiscriminate use of antimicrobial agents may kill or reduce the number of the normal floras of the vagina thereby giving room for pathogens to have a field day [27]. In addition, the antibacterial properties of prostatic fluid play a preventive role in male [28]. Some species of organisms were found to be more in males (*E.coli* and *P.aeruginosa*), others are more in females (*K.pneumoniae*, *C.albicans*, *K.oxytoca* and *S.saprophyticus*) while some were equally distributed (*S.aureus*).

Interestingly, UTI was more prevalent in the patients within the hospital settings, 80.4% in-patient versus 19.3% out-patient cases. Patients' comorbidity, immune status and catheter association might account for this [27,28]. All isolated strains were highly prevalent among in-patients than out-patients except for *S.saprophyticus* with equal distribution. From the studies, it was shown that the most common pathogen of female UTI is *K.pneumoniae* (21%), followed by *C.albicans* (19%), *E.coli* (17%), *K.oxytoca* (13%), *S.aureus* (12%), *S.saprophyticus* (10%), *P.aeruginosa* (4%), *S.rubidaea* (2%) and *E.agglomerans* (2%). The study also

revealed that UTI was more prevalent in non-pregnant 32 (66.7%) than in pregnant 16 (33.3%) when female population was considered in general.

The high prevalence of UTI observed in the aged people may be solely due to the inability of their immune systems to fight or resist bacterial infection (Table 5).

Antibiotic sensitivity pattern of organisms is changing rapidly in recent period. It is especially true for developing countries (like Nigeria) where antibiotics are prescribed not only by the medical practitioners but also purchased directly over the counter from pharmacy.

Most of the gram positive cocci (GPC) were sensitive to common antibiotics except tetracycline and cotrimoxazole (Table 6). *Staphylococcus aureus* was moderately sensitive to augmentin, erythromycin, chloramphenicol, vancomycin, gentamicin, amoxicillin, cloxacillin and ceftazidime. *Staphylococcus saprophyticus* was highly resistant to amoxicillin, gentamicin and ceftazidime, mildly sensitive to erythromycin and cloxacillin and moderately sensitive to augmentin, chloramphenicol and vancomycin. This implies that, in UTI with *Staphylococcus spp* within Osun state (or Nigeria as a whole), tetracycline and cotrimoxazole should not be given empirically. But drugs like augmentin, erythromycin and vancomycin could be used instead.

All the gram negative bacteria (GNB) were sensitive to imipenem except *Pseudomonas aeruginosa*, and were largely resistant to cotrimoxazole. This finding is in consonance with the findings of Theodros [29]. It is obvious that cotrimoxazole is no more useful against GNB causing UTI as all the isolates were resistant to it. Previously this antibiotic was used as the drug of choice for empirical treatment of UTI. The broad spectrum activity of fluoroquinolones has made them as one of the best therapeutic options for UTI. In the present study the isolates showed low degree of susceptibility to fluoroquinolones which indicates that they can no more be opted for treating UTI empirically.

Resistance of the isolates to some of the antibiotics agrees with the reports of Khoshbakht *et al.* and Tula *et al.* [30,31]. This could be attributed to indiscriminate use of these agents by the general physicians. Strict control of antibiotic use and prophylaxis could reverse the situation. This is also applicable to other bacteria isolated. Most of them were resistant to commonly used antibiotics.

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Administration of fake drugs in treating cases has been a problem of effective treatment in Nigeria. The circulation of fake drugs should be checked if emergence and resurgence of resistant strains of these isolated pathogens were to be stemmed. Therefore, government support and subvention to National Agency for Food and Drug Administration Control (NAFDAC) must be sustained.

CONCLUSION

Gram-negative bacilli (Enterobacteracea) were mainly responsible for urinary tract infections and most of the strains were multi-drugs resistant. The most common isolated bacteria from urinary tract infections was *E. coli* and the most effective antimicrobial agents against GNB were imipenem, gentamicin, ofloxacin, nitrofurantoin, tetracycline and ciprofloxacin against Gram-negative bacilli. Augmentin, erythromycin, chloramphenicol and vancomycin were most effective for Gram-positive organisms. These justify the necessity to treat UTI based on antimicrobial susceptibility in order to prevent evolution of resistant mutant strains.

Since UTI has a large socio-economic impact and many factors may contribute to the emergence of bacterial resistance in different places with time, the following are strongly recommended: periodic surveillance of antibiotic susceptibility in a systematic manner under supervision of a joint scientific experts and physicians.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

INFORMED CONSENT

The purpose of this work was explained to the clients before they voluntarily consented to participate in the research. The consent forms were appropriated filled by the investigators after which each client signed their corresponding forms.

ETHICAL APPROVAL

Ethical approval for this study was obtained from the ethical and human research committees of the Ladoke Akintola University of Technology Teaching Hospital and Obafemi Awolowo University Teaching Hospital before embarking on the research.

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PROFILE OF INFECTIONS IN INTENSIVE CARE UNIT (ICU) IN A CENTRAL NIGERIA TERTIARY HOSPITAL

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Abstract

Background: Intensive Care Units (ICUs) accommodate the most seriously ill patients in a relatively confined environment. Increased duration of stay, increased number of indwelling and invasive devices and prolonged or inappropriate use of antibiotics are common features of ICUs, with consequent or associated increase in selection of multi-resistant pathogens, morbidity and mortality.

Objectives: To determine the identity and antimicrobial resistance pattern of organisms commonly associated with infections in the ICU of the hospital.

Method: A retrospective study of Intensive Care Units (ICU) infections in NHA over a three-year period January 1st, 2010 to December 31st, 2012 was conducted through review and analysis of laboratory data.

Results: Data for 79 specimens were fully analysed; 35 (44%) from urine, 17 (22%) from blood, 6 (8%) from tracheal specimens and 8 (10%) from wound. Forty-one (52%) of the specimens yielded growth; 16 (20%) from urine, 8 (10%) from wound, 6 (8%) from tracheal specimens, 3 (4%) from blood and others 8 (10%). 14 (34%) out of the 41 isolates were *Escherichia coli*, 8 (20%) *Pseudomonas aeruginosa*, 6 (15%) were *Staphylococcus aureus* and 6 (15%) *Klebsiella pneumoniae*. Three (4%) of the specimens yielded mixed growths while another 3 (4%) yielded *Candida* species. Sensitivity of *E. coli* to third generation cephalosporins ranged from 62-72% and 90% to imipenem. For *Klebsiella pneumoniae* it was 67-75% to third generation cephalosporins and 100% to imipenem. *Pseudomonas aeruginosa* was 71% and 83% sensitive to ceftazidime and imipenem respectively. *Staphylococcus aureus* was 67% and 83% sensitive to amoxicillin-clavulanate and imipenem respectively. Susceptibility of all these isolates to fluoroquinolones and aminoglycosides remained poor.

Conclusion: The isolates from the ICU were same as common in clinical specimens. There was wide variability in resistance with a tendency to increase over time. This trend needs to be monitored while antibiotic stewardship should be emphasised.

Key words: Intensive care units (ICU), nosocomial Infections, antibiotic susceptibility.

PROFILE D'INFECTIONS DANS LE SERVICE DE SOINS INTENSIFS DANS UN HOPITAL TERTIAIRE CENTRAL DU NIGERIA

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RÉSUMÉ

Contexte: Les services de soins intensifs accueillent les patients les plus gravement malades dans un environnement relativement confiné. Une durée accrue du séjour, un nombre accru de demeures et d'appareils et une utilisation prolongée ou inappropriée d'antibiotiques sont des caractéristiques communes de soins intensifs, avec l'augmentation conséquente ou associée de la sélection des agents pathogènes multi-résistants, de la morbidité et de la mortalité.

Méthodes: Une étude rétrospective des infections de services de soins intensifs dans l'Hôpital National d'Abuja a été menée pour l'examen et l'analyse des données de laboratoire sur une période de trois ans allant du 1^{er} Janvier 2010 au 31 Décembre 2012.

Résultats: Les données de 79 échantillons ont été entièrement analysés; 35 (44%) échantillons d'urine, 17 (22%) échantillons du sang, 6 (8%) à partir d'échantillons trachéaux et 8 (10%) à partir de la plaie. Quarante-et-un (52%) des échantillons ont produit une croissance: 16 (20%) d'urine, 8 (10%) de la plaie, 6 (8%) à partir d'échantillons trachéaux, 3 (4%) à partir du sang et 8 (10%) à partir d'autres types d'échantillons. 14 (34%) des 41 isolats étaient

de *Escherichia coli*, 8 (20%) *Pseudomonas aeruginosa*, six (15%) étaient des *Staphylococcus aureus* et 6 (15%) *Klebsiella pneumoniae*. Trois (4%) des spécimens ont montré une croissance mixte tandis que les trois autres (4%) ont donné les espèces de *Candida*. La sensibilité de *E. coli* aux céphalosporines de troisième génération variait de 62 à 72% et 90% pour l'imipénème. Pour *Klebsiella pneumoniae*, 67-75% de souches étaient sensibles aux céphalosporines de troisième génération et 100% à l'imipénème. 71% et 83% de *Pseudomonas aeruginosa* ont été sensibles respectivement à la ceftazidime et à l'imipénème. 67% et 83% de *Staphylococcus aureus* étaient sensibles respectivement à l'amoxicilline+ acide clavulanique et à l'imipénème. La sensibilité de toutes ces souches aux fluoroquinolones et aux aminoglycosides est restée médiocre.

Conclusion: Les souches de services de soins intensifs étaient les mêmes que les principales souches cliniques. Il y avait une large variabilité dans la résistance tendant à augmenter avec le temps. Cette tendance doit être surveillée pendant que la gestion d'antibiotique devrait être accentuée.

Mots clés: Services de Soins Intensifs, Infections nosocomiales, sensibilité d'antibiotique.

INTRODUCTION

Many tertiary hospitals in Nigeria have developed critical care facilities for the care of the critically ill patient(1). The intensive care unit (ICU) of the National Hospital, Abuja (NHA) is an eight bed facility in a 200-bedded centre. This represents 4% of the total bed space in the hospital. This percentage varies from one country to another, ranging from 1-2% in the UK, 1.7% in New Zealand to 6-11% in the USA (2-4)

Infection is often the cause of ICU admissions (5-6), and four types account for most ICU infections; namely pneumonia (usually ventilator associated), urinary tract infection (UTI) (usually catheter associated), primary bloodstream infection (BSI) (usually associated with the use of an intravascular device) and surgical site infections(SSI)(7)

The common pathogens include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida* species, *Escherichia coli* and *Klebsiella* species, often predisposed to by invasive applications and devices, and patients' clinical conditions (8,9). These infections account substantially for the high mortality in ICUs.

Antimicrobial resistance constitutes a major challenge in the management of ICU infections, and these often emerged from selective pressure due to increased, and sometimes inappropriate antibiotic use and transmission via health workers. . Greater than 60% of ICU patients receive broad spectrum antibiotics at some time during their hospitalization, and up to 60% of antibiotic use in hospitals is inappropriate or unnecessary (10,11). Microbiological cultures and antibiotic sensitivity testing are central to rapid and accurate diagnosis and treatment, which improves outcomes and reduces resistance. It also guides empiric antibiotic therapy.

Prevention of infection in the ICU is fundamental and can be achieved through good antimicrobial use and infection control practices, including hand hygiene (12) It is therefore important that physicians strike a balance between patients' safety, and the desire to reduce mortality and antibiotics resistance. In our environment the profile of the common organisms associated with infections in the ICU is not clear. This study was therefore, designed to determine the

common pathogens in our environment and their antibiotics susceptibility profiles so as to guide therapy and achieve reduction in mortality and resistance development.

METHOD

The NHA is a tertiary medical centre providing medical services to Abuja and its surrounding states of Kogi, Nasarawa, Niger, Kaduna and Benue. A retrospective study of adult Intensive Care Units (ICU) infections over three years in NHA was conducted for the period of 1st January 2010 to 31st December 2012. The record of all specimens submitted to the medical microbiology laboratory within this period were analysed including evaluation of their microbial susceptibility pattern. Those with incomplete record were excluded.

Data obtained for analysis included; age, sex, specimen type, isolated organisms and their corresponding antibiotic susceptibility pattern.

Briefly, in our laboratory clinical specimens are collected in sterile plastic containers, swabs or others as appropriate for the relevant anatomic sites and are usually processed as soon as they are received. The macroscopic appearances of the samples are noted and then inoculated onto appropriate culture media. Where necessary direct smears of specimen are made on clean glass slide, fixed and stained by Gram's standard method (13). The plates are then incubated at 37°C in ambient air for 16-24hours, but chocolate agar plates are incubated in a candle light jar. Cultures are examined for growth thereafter. In some cases where there is no growth, cultures may be re-incubated for another 24 hours before they are discarded as having no growth. The colonies are identified using standard methods^{13,14}. Antimicrobial drug susceptibility of the isolates are usually tested by the modified Kirby-Bauer technique and results interpreted according to the Clinical Laboratory Standards Institute-CLSI Guideline ¹⁵.

RESULTS

79 specimen were fully analysed; 35(44%) from urine, 17 (22%) from blood, 6 (8%) from tracheal specimens and 8 (10%) from wound. 41(52%) of the specimens yielded growth; 16 (20%) from urine, 8 (10%) from wound, 6 (8%) from tracheal specimens and 3(4%)

from blood. 14 (34%) out of the 41 isolates were *Escherichia coli*, 8 (20%) *Pseudomonas aeruginosa*, 6 (15%) were *Staphylococcus aureus* and 6 (15%) *Klebsiella pneumoniae*. Three (3%) of the specimens yielded mixed growths while another 3 (4%) yielded *Candida* species (Table 1). One of the blood isolates was *Salmonella typhi*. The ages of patients in this study ranged from 37-68, with a mean of 51 years. There were 44(56%) males and 34(44%) females.

TABLE 1: SPECIMENS DISTRIBUTION

Specimens	No	No of Isolates
Blood	17 (22%)	3(4%)
Urine	35(44%)	16(20%)
Wound	8 (10%)	8 (10%)
Tracheal	6 (8%)	6 (8%)
High Vaginal Swab	4(5%)	2 (3%)
Other	9(11%)	6 (7%)
Total	79(100)	41(52%)

Sensitivity of *E. coli* to third generation cephalosporins ranges from 62-72% and 90% to imipenem. For *Klebsiella pneumoniae* it was 67-75% to third generation cephalosporins and 100% to imipenem. *Pseudomonas aeruginosa* was 71% and 83% sensitive to ceftazidime and imipenem respectively. *Staphylococcus aureus* was 67% and 83% sensitive to amoxicillin-clavulanate and imipenem respectively. Sensitivity of all these isolates to fluoroquinolones and aminoglycosides were poor (Table 2).

DISCUSSION

The National Hospital Abuja has an eight bedded, well equipped ICU, which partly explains the relatively low number of samples received in the laboratory from the unit. The other prominent factor is connected with the financial inability of the majority of the patients admitted into the unit to afford the cost of investigations. The 79 samples included in the study yielded 41 isolates over a period of three years. As established in previous studies, the small numbers of isolates recovered from the ICU specimens tend to limit objective discussions due to its potential to overstate ICU resistance problems based on a small number of resistant isolates¹³⁻¹⁵. This appears to be the trend in most ICUs as most patients presenting to the unit would have had substantial doses of variable antibiotics.

Notwithstanding these limitations, it is worthy of note that most specimens processed in this study were urine, blood, wound and respiratory, corresponding to the most common infections encountered in the ICU, namely urinary tract, blood stream, wound and respiratory infections (16). Together they yielded over 80% of the isolates, dominated by organisms previously identified to be associated with nosocomial infections and invasive procedures (8,9), both of which are common in the ICU.

The two main *Enterobacteriaceae*, *Klebsiella pneumoniae* and *Escherichia coli*, isolated in our ICU demonstrated high and moderate sensitivity to imipenem and the third generation cephalosporins/amikacin respectively, and high resistance to amoxicillin-clavulanate, fluoroquinolones, second generation cephalosporin and gentamicin. This pattern suggests high prevalence of extended spectrum β -lactamase (ESBL) production and low level carbapenemase production among the isolates. *Pseudomonas aeruginosa* isolates only showed moderate sensitivity to imipenem and ceftazidime, similar to similar finding in a previous study (17); the likely implication of this pattern is that while the choice for empiric therapy may be substantially limited, there may be a tendency to over-use the carbapenems, which will further compromise their use as drugs of last resort in our environment at the moment. In all, the predominance of *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* amongst all isolates from the ICU is similar to the profile seen in a previous study (18).

The choice of chemotherapy for staphylococcal infections may appear even more challenging as the isolated strains displayed only moderate sensitivity to amoxicillin-clavulanate and imipenem. There was also isolation of *Enterococcus* and *Candida* spp from urine, in agreement with result of a previous study¹⁸. Generally, the moderate to high resistance and multidrug resistance seen in the ICU isolates, subject to the limitations earlier mentioned, is in agreement with what had been observed with ICU isolates (8,19). This is due to the over-use of antibiotics, which invariably puts the organisms on selective pressure. To address this and reduce the rate of resistance development choice of antibiotics must be based on established guidelines and sound knowledge of pharmacokinetics and pharmacodynamics of the drugs. Since synchronized adherence to locally evolved guidelines within a hospital has been associated with stable antibiotic resistance patterns²⁰, antibiotics stewardship should be practiced, while further studies are needed to determine the prevalence of ESBL and carbapenemase production as well as prevalence of methicillin resistant *staphylococcus aureus* (MRSA) in the ICU.

TABLE 2: FOUR MOST COMMON ICU INFECTIONS ISOLATES AND ANTIBIOTICS SENSITIVITY PATTERN

Antibiotics	Isolates							
	Escherichia coli		Klebsiella pneumoniae		Pseudomonas aeruginosa		Staphylococcus aureus	
	No Tested	% Sensitive	No Tested	% Sensitive	No Tested	% Sensitive	No Tested	% Sensitive
AMC	13	31	0	0	Nil	nil	6	67
CTX	12	67	6	67	7	14	6	17
CAZ	11	72	4	75	7	71	5	0
CRO	13	69	6	67	Nil	Nil	4	0
XM	14	29	2	0	Nil	Nil	6	50
GN	14	36	5	60	6	43	6	33
AK	8	75	6	83	7	57	6	50
CIP	13	43	5	60	7	57	5	40
OFX	9	44	nil	Nil	Nil	Nil	2	50
IMP	10	90	5	100	8	86	6	83

AMC: amoxicillin-clavulanate; CTX: cefotaxime; CAZ: ceftazidime; CRO: ceftriaxone; XM: cefuroxime; GN: gentamicin; AK:

amikacin; CIP: ciprofloxacin; OFX: ofloxacin; IMP: imipenem

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PSEUDOMONAS AERUGINOSA INFECTIONS IN A TERTIARY HOSPITAL IN NIGERIA.

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ABSTRACT

Background: *Pseudomonas aeruginosa* is a known opportunistic pathogen frequently causing serious infections. It exhibits innate resistance to a wide range of antibiotics thus causing high rates of morbidity and mortality worldwide.
Objective: This study was done to determine the distribution and the antibiotic susceptibility pattern in clinical isolates of *Pseudomonas aeruginosa* in NHA.
Method: Laboratory data on 265 *Pseudomonas aeruginosa* isolates from a total of 30,384 clinical specimens processed over a 3 year period (January 1st 2010 to December 31st 2012) were analyzed.
Results: A total 30,384 samples were submitted for bacteriologic analysis, 265 (1%) yielded *Pseudomonas aeruginosa* of which 195 (74%) were from in-patient and 70 (26%) from out-patients. 185 (70%) isolates were from adults while 80 (30%) were from children. 87% of the isolates were susceptible to imipenem, 77% to amikacin, while 34% were resistant to ciprofloxacin and 46% resistant to ceftazidime.
Conclusion: The relatively high proportion of resistance to ciprofloxacin and ceftazidime, and the emerging resistance to amikacin and imipenem are worrisome and calls for rational antibiotic use and institution of effective resistance surveillance and infection control measures. .

Keywords: *Pseudomonas aeruginosa*, National Hospital Abuja, Susceptibility

INFECTIONS A PSEUDOMONAS AERUGINOSA DANS UN HOPITAL TERTIAIRE AU NIGERIA.

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RESUME

Contexte: *Pseudomonas aeruginosa* est un germe pathogène opportuniste reconnu causant fréquemment des sévères infections. Il présente à la résistance innée à une large gamme d'antibiotiques provoquant de taux élevés de morbidité et de mortalité dans le monde.
Objectif: Cette étude a été réalisée pour déterminer la répartition et la sensibilité d'antibiotiques de souches cliniques de *Pseudomonas aeruginosa* à l'hôpital National d'Abuja.
Méthodes: Les données de laboratoire de 265 souches de *Pseudomonas aeruginosae* isolées à partir de 30 384 d'échantillons cliniques traités sur une période de 3 ans (1^{er} Janvier 2010 au 31 Décembre 2012) ont été analysées.
Résultats: Au total, 30 384 échantillons ont été soumis pour l'analyse bactériologique: *Pseudomonas aeruginosa* ont été obtenus dans 265 (1%) échantillons dont 195 (74%) étaient des patients hospitalisés et 70 (26%) patients externes. 185 (70%) souches ont été isolées chez les adultes alors que 80 (30%) étaient chez les enfants. 87% de souches étaient sensibles à l'imipénème, 77% à l'amikacine, pendant que 34% étaient résistantes à la ciprofloxacine et 46% à la ceftazidime.
Conclusion: La proportion relativement élevée de la résistance à la ciprofloxacine et à la ceftazidime, et l'émergence d'une résistance à l'amikacine et à l'imipénème est inquiétante, et les appels à usage rationnel de l'antibiotique et une institution de surveillance efficace de la résistance et les mesures du contrôle de l'infection.

Mots clés: *Pseudomonas aeruginosa*, Hôpital National d'Abuja, Sensibilité

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative aerobic rod that causes severe nosocomial infections, associated with increased mortality (1,2). It has a predilection for moist environments and can be found in water and soil and on plants, including fruits, vegetables, and flowers (3).

The organism is rarely found as part of the microbial flora of healthy people, but may occasionally colonize healthy individuals; the sites of colonization include the gastrointestinal tract and moist body sites, such as the throat, nasal mucosa, axillary skin and perineum (3). Aqueous solutions used in medical care such as disinfectants, soaps, irrigation fluids, eye drops, and dialysis fluids may all become contaminated with *P. aeruginosa* (4).

Nosocomial infections caused by *P. aeruginosa* have been recognized as an acute problem in hospitals due to its intrinsic resistance to many classes of antibiotics and its ability to acquire resistance to all effective antibiotics (5). These infections among others include surgical site infections, urinary tract infections, pneumonia, and bloodstream infections (BSI) (6).

Effective management of *P. aeruginosa* infections requires good background knowledge of the prevailing antimicrobial susceptibility patterns of the organism. There is paucity of data on *Pseudomonas aeruginosa* infections in Abuja. This study was therefore, carried out to determine the distribution and the antibiotic susceptibility pattern of *Pseudomonas aeruginosa* in clinical isolates at the National Hospital Abuja, to serve as a guide for doctors managing patients with *Pseudomonas aeruginosa* infections.

METHOD

The data studied were from the archival records of the department of medical microbiology of National Hospital Abuja, a 200-bedded referral tertiary hospital located in the heart of the Federal Capital Territory of Nigeria, over a three-year period stretching from 1st January 2010 to 31st December 2012.

Records of *Pseudomonas aeruginosa* isolates were analyzed for gender, age, ward, sample type and antibiotic susceptibility using Microsoft excel 2007 software. Isolates with no age, gender, ward and antibiotic susceptibility were excluded (N= 42).

All samples were processed and identifications carried out using established procedures⁷.

RESULTS

A total 30,384 samples were submitted for bacteriologic analysis, 265(1%) yielded

Pseudomonas aeruginosa. Of these 185 (70%) were from adults, while 80 (30%) were from children. 140 (53%) were males while 125 (47%) were females (Table 1).

TABLE 1: AGE AND GENDER DISTRIBUTION OF PATIENTS WITH *PSEUDOMONAS AERUGINOSA* ISOLATES

AGE	n	%
Neonate (<28days)	16	6.0
Infants (>29days -1yr)	29	11.0
Children(>1 -15yrs)	35	13.2
Adult (>15 - 64yrs)	167	63.0
Elderly (>65yrs)	18	6.8
TOTAL	265	100
GENDER	N	%
Male	140	52.8
Female	125	47.2
Total	265	100

195(74%) isolates were from in-patients and 70(26%) from out-patients. Surgery & paediatrics had higher isolates from the inpatient wards, 61(31%) and 49(25%) respectively, while the intensive care unit (ICU) and the accident and emergency (A&E) unit specimens respectively yielded 9(5%) and 26(14%) of the isolates (Table 2).

Pseudomonas aeruginosa was sensitive to imipenem 87%(176/203), amikacin 77%(142/185), but resistant to ciprofloxacin 34%(59/175), gentamicin 44%(70/160), ofloxacin 45%(29/64) and ceftazidime 46%(85/191). (Table 3). Resistance in ICU appears higher than in other locations (Table 4).

TABLE 2; PSEUDOMONAS AERUGINOSA ISOLATES IN SELECTED LOCATIONS

Wards	No.	%
Out-Patients	70	26
In-Patients		
Medical	37	20
Surgical	61	32
Gynaecology	13	7
ICU	9	5
A &E	26	14
Paediatrics	49	25
Total In-patients	195	74
Grand Total	265	100

TABLE 3: SENSITIVITY PATTERN OF PSEUDOMONAS AERUGINOSA

Antibiotics	No tested	% Sensitivity
Imipenem	176 (203)	87
Amikacin	142 (185)	77
Ciprofloxacin	116 (175)	66
Gentamicin	90 (160)	56
Ofloxacin	35 (64)	55
Ceftazidime	106(191)	56

TABLE 4. PERCENTAGE SENSITIVITY OF PSEUDOMONAS ISOLATES BY LOCATION

Wards	Surgical	Medical	ICU	Paediatric	A& E	Gynae	Outpatient
Antibiotics							
Imipenem	93 (42/45)	90 (18/20)	83 (5/6)	88 (28/32)	80 (12/15)	71(5/7)	89(41/46)
Amikacin	82 (37/45)	77 (17/22)	66.7 (2/3)	81 (17/21)	71 (10/14)	71 (5/7)	78(28/36)
Ciprofloxacin	69 (29/42)	26 (5/19)	43 (3/7)	88 (21/24)	79 (11/14)	83 (5/6)	63(22/35)
Gentamicin	63 (27/43)	50 (10/20)	50 (1/2)	69 (16/23)	46 (6/13)	100 (4/4)	55 (16/29)
Ofloxacin	42 (5/12)	100 (3/3)	100 (1/1)	83(10/12)	57 (4/7)	43 (3/7)	38 (5/13)
Ceftazidime	72 (28/39)	24(5/21)	25 (1/4)	54 (19/35)	47 (7/15)	60 (3/5)	67 (28/42)

DISCUSSION

This study revealed that *Pseudomonas aeruginosa* infections were more common in adults than in children, but higher in children fifteen years and below than in the elderly; there is a slight male preponderance. These results agree with those of

similar studies carried out in Enugu, Nigeria and India (8,9). Surgery ward had the highest number of isolates. This may be because surgical patients stay relatively long in the hospital, have a breach of their protective skin and the fact that many patients going in for major surgery tend to get catheterised.

The high resistance profile of the isolates to ciprofloxacin and ceftazidime most probably reflects the extensive and inappropriate use of these antibiotics as revealed in previous studies (9,10). The resistance to ceftazidime is particularly worrisome as this is the only third generation

cephalosporin to which *Pseudomonas aeruginosa* is naturally sensitive. The relatively and moderate sensitivity to imipenem and amikacin again mirrors the not too frequent use of these drugs in our facility. With increasing resistance to ceftazidime and ciprofloxacin there is tendency that more selective pressure will bear on these drugs as therapeutic alternatives, with rising resistance. The pattern of antibiotic sensitivity may vary in different hospitals, and in different sections of the same hospital depending on such factors such as antibiotic prescribing policy, types of patients, level of hygiene and infections control practices.

The sensitivity pattern seen in this study shows that inappropriate antibiotic treatment for *Pseudomonas aeruginosa* infections, and indeed other infections, may lead to increased morbidity and mortality in the hospital. There is therefore, need to maintain regular antibiotic surveillance and to adhere to rational antibiotic prescribing guidelines.

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OCCURRENCE OF EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCING ENTEROBACTERIACEAE ISOLATES IN COMMUNAL WATER SOURCES IN OGUN STATE, NIGERIA

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RUNNING TITLE: EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCING ENTEROBACTERIACEAE ISOLATE IN COMMUNAL WATER SOURCES

ABSTRACT

The role of *Enterobacteriaceae* in dissemination and reservoir of antibiotic resistance genes in outbreaks of disease and infections are pressing public health concern. This study is aimed at investigating the antibiotic resistance patterns and ESBL production in water-borne *Enterobacteriaceae* recovered from some selected drinking water sources among 6 towns in Ogun State (Nigeria). Sixty water samples were collected from boreholes and well waters sources, of these samples 68 *Enterobacteriaceae* including *Enterobacter* spp, *Escherichia coli*, *Klebsiella* spp, *Salmonella* spp, *Citrobacter freundii*, *Serratia* spp were recovered and identified presumptively using standard microbiological and biochemical methods. The susceptibilities of the isolates to nine antibiotics were carried out by disk diffusion method and determination of ESBL production was by double-disk synergy method. Of the 68 isolated strains tested, 1 (1.47%) was susceptible to all the antibiotics, 98.5% of the isolates were resistant to ≥ 1 antibiotics and 75% were found to be resistant to ≥ 2 classes of antibiotics. Incidence of water-borne ESBL-producing enteric in this study was 7.14%. The increasing unabated spread of *Enterobacteriaceae* in public water supply harboring resistance genes portends a high risk for communal outbreaks. This necessitates an urgent precautionary and antibiotics surveillance measures in Nigeria.

Keywords: *Enterobacteriaceae*, antibiotic resistance, ESBL

APPARITION DES ENTEROBACTERIES PRODUCTRICES DE BETA-LACTAMASES A SPECTRE ELARGI ISOLEES DANS LES EAUX DE SOURCES DANS L'ETAT D'OGUN, NIGERIA

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TITRE COURANT: ENTEROBACTERIES PRODUCTRICES DE BETA-LACTAMASES A SPECTRE ELARGI ISOLEES DANS LES EAUX DE SOURCES

RÉSUMÉ

Le rôle des *Enterobacteriaceae* dans la dissémination et le stockage des gènes de résistance aux antibiotiques durant les épidémies et infections est un sérieux problème de santé publique. Cette étude a pour objectif, d'examiner les différents modèles de résistance aux antibiotiques et de quantifier la production ESBL chez les *Enterobacteriaceae* d'origine hydrique présents dans quelques points d'eau potable de 6 villes de l'état d'Ogun (Nigeria). Soixante échantillons d'eau ont été prélevés des forages et des puits. De ceux-ci, 68 *Enterobacteriaceae* comprenant *Enterobacter* spp, *Escherichia coli*, *Klebsiella* spp, *Salmonella* spp, *Citrobacter freundii*, *Serratia* spp ont été retrouvés et identifiés partiellement, au moyen de méthodes microbiologiques et biochimiques. Les tests de susceptibilités des isolats vis-à-vis de neuf antibiotiques ont été évalués par la méthode de diffusion par disque tandis que le taux de production ESBL a été déterminé par la méthode de synergie double-disque. Des 68 souches isolées et testées, 1 souche (1.47%) était sensible à tous les antibiotiques, 98.5% des isolats étaient résistants à au moins 1 antibiotique et 75% étaient résistants à plus de 2 antibiotiques. L'incidence des bactéries entériques d'origine hydrique et productrices d'ESBL était de 7.14% dans la présente étude. Cette propagation sans cesse croissante des *Enterobacteriaceae* porteuses de gènes de résistance dans les points d'approvisionnement publics en eau potable augure un risque accru d'épidémies communales. Ceci nécessite des mesures urgentes de prévention et de surveillance des antibiotiques au Nigeria.

Mots clés: *Enterobacteriaceae*, résistance aux antibiotiques, ESBL

INTRODUCTION

Enterobacteriaceae is a family of Gram-negative rod shaped bacteria most of which are pathogenic in nature. Notable members of *Enterobacteriaceae* that are commensals of human origin are of clinical significance due to their disease causing abilities [1]. They exhibit a remarkable adaptive capability for acquisition and dissemination of resistance gene such as extended-spectrum beta-lactamase (ESBL) that constitutes one of the currently most widely disseminated and important resistance mechanism that confer resistance to several beta-lactam drugs such as cephalosporins, carbapenems and monobactams [2]. In this current era, antibiotic resistance by bacteria is a leading factor to global therapeutic failure, which is seen as an imminent threat to life and economic loss. Contributory role of enteric bacteria as reservoir to resistance gene and dissemination has been widely reported [3,4]. Colonization of healthy individuals with antibiotic resistant *Enterobacteriaceae* is an instrument in the increase of resistant bacteria at community and hospital level [5]. The presence of enteric bacteria, especially *E. coli* in drinking water regarded as an index of bacteriological quality of water, is an indication of fecal contamination [6]. Studies across Africa have shown that boreholes and well water sources are harboring bacteria of multiple resistance profiles [7,8]. In Nigeria, portable water source are not readily available all over the country. Hence most people depend on alternative water supply from the ground such as boreholes, wells and streams for drinking, cooking and other domestic use. However, while several studies on microbial diversity and total coliform counts from Nigeria underground water supplies have shown that these water sources did not meet international drinking water standards [9–15]. There is insufficient information indicating water-borne enterics as potential reservoirs for antimicrobial resistance genes in this country. This study was therefore carried out to determine the antimicrobial resistance profile and production of ESBL among *Enterobacteriaceae* obtained from various drinking water sources in some towns from Ogun state, Nigeria.

MATERIAL AND METHODS

Study Area
The chosen areas for the study are Ago-Iwoye, Ijebu- Ode, Ilisan, Ikenne, Iperu and Sagamu located at the North-eastern part of Ogun state Nigeria. The areas are characterized by warm frost-free subtropical climate with temperature range of 28 – 33°C and moderate rainfall. Each town is averagely populated with a number of urban development and occupation such as farming, trading and other commercial activities. Available

water supply in each place consists of either pipe-borne, borehole, well and streams water or all of the above.

Bacterial

Isolate

Sixty water samples consisting of borehole and well waters were collected over a period of 3 months in a sterile sample bottles from the 6 towns (n=10 for each town) in Ogun State. Each collected water samples were cultured directly on MacConkey agar (Lab M Ltd UK) and Eosine Methylene Blue agar (EMB) (Lab M Ltd UK). Presumptive *Enterobacteriaceae* isolates (oxidase-negative facultative aerobic Gram-negative rods) were selected for further studies.

Characterization and Identification of isolates

Further identification of the isolates were carried out by investigation into their various colonial morphology and biochemical reactions such as coagulase, indole, urease, methyl red, voges-prokauer, citrate utilization and sugar fermentation tests as previously described [16] and results were interpreted according to The Bergey's Manual of determinative bacteriology [17,18].

Antimicrobial

Susceptibility

Antimicrobial susceptibilities to beta-lactam and non-beta-lactams antibiotics were performed using disc diffusion on Mueller-Hinton agar (Lab M Ltd UK) according to the CLSI guideline [19]. *E. coli* ATCC 25922 was used as a control. The antibiotic disks included amoxycillin/clavulanic acid (30 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefuroxime (30 µg), ofloxacin (5 µg), gentamicin (10 µg) and cefixime (5 µg) (Rapids Labs Ltd UK).

Double Disk Synergy Test (DDST)

The isolates with diameter zones of ≤ 18 mm for ceftazidime ($n=28$) were further screened for ESBL production by DDST method on Mueller Hinton agar as described by (Jarlier *et al.*, [20] using amoxicillin/clavulanic acid as beta-lactamase inhibitor. Disk containing 30 µg of aztreonam, ceftazidime, ceftriaxone, and cefotaxime, were placed 20 mm apart (center to center) consecutively from the beta-lactamase inhibitor and incubated for 18 - 24 h at 37°C. Synergy between the disks towards the beta-lactamase inhibitor was regarded as presumptive ESBL production [20].

RESULTS

A total of 68 *Enterobacteriaceae* including *Enterobacter* spp (22.06%), *Escherichia coli* (14.71%), *Klebsiella* spp (22.05%),

Citrobacterfreundii (8.82%), *Salmonella* spp (4.41), fermenter oxidase negative Gram-negative bacilli were distributed in water sources across the 6 towns with each organisms showing different antibiotic susceptibilities profiles. Table 1 shows the percentage distribution and respective locations of the *Enterobacteriaceae* isolated in this study.

Ninety-eight and a half per cent (98.5%) were resistant to ≥ 1 antibiotics while 75% of the isolates were resistant to 2 or more classes of antibiotics tested and the rates of resistance were highest for amoxicillin/clavulanic acid (89.71%) and cefotaxime (76.47%), while highest susceptibilities of 89.70% and 75.00% for most of the isolates was recorded for ofloxacin and ciprofloxacin respectively (Table 2).

Serratiaspp (1.47) and 26.47% unidentified lactose

TABLE 1: DISTRIBUTION OF ENTEROBACTERIACEAE ISOLATES RECOVERED FROM VARIOUS WATER SAMPLES AND LOCATIONS

Locations	No of samples	No of isolates	Percentage (%)
Ago - Iwoye	10	17	25.00
Ijebu Ode	10	11	16.20
Ilishan	10	7	10.20
Ikenne	10	11	16.20
Iperu	10	11	16.20
Sagamu	10	11	16.20
Total	60	68	100

The proportion of water-borne ESBL-producing enteric was 7.14% (2 out of 28 isolates), corresponding to samples recovered from Sagamu borehole water and Ijebu-Ode well water respectively.

TABLE 2: THE SUSCEPTIBILITY PATTERNS OF 68 ENTEROBACTERIACEAE AND THE PERCENTAGE (%) RESISTANCE TO 9 ANTIBIOTICS

Antibiotics (Disc potency, μ g)	No.and Sensitive (%)	No.and (%) Intermediate	No. and (%) Resistant
Amoxicillin/clavulanic acid (20/10)	7 (10.29)	NA	61 (89.71)
Cefixime (5)	34 (50.00)	1(1.47)	33 (48.53)
Cefotaxime (30)	1(1.47)	15 (22.06)	52 (76.47)
Ceftazidime (30)	15 (22.06)	25 (36.76)	28 (41.08)
Cefuroxime (30)	3 (4.41)	49 (72.06)	16 (23.53)
Ciprofloxacin (5)	51 (75.00)	12 (17.65)	5 (7.35)
Gentamicin (10)	40 (58.83)	7 (10.29)	21 (30.88)
Nitrofurantoin (300)	48 (70.59)	NA	20 (29.41)
Ofloxacin (5)	61 (89.70)	3 (4.41)	4 (5.88)

DISCUSSION

Microbiological assessment of drinking water is the most important because it accounts for the microbial quality and level of contamination that can render the water fit or unfit for human consumption [21]. According to World Health Organization and Nigerian Standard for Drinking Water Quality [6,22] drinking water must not contain any fecal coliform bacteria. In this present study, high percentage members of

highest percentage frequency of occurrence (22.06%) followed by *E. coli* (14.70%), *C. freundii* (8.82%), *Salmonella* spp (4.41%), *Serratiaspp* (1.47%). There are 18 (26.47%) of the isolated bacteria showing similar biochemical characteristic of the family *Enterobacteriaceae* belonging to different genera that we were unable to identify due to our limited resources. Similar findings on the presence of enteric bacteria of above genera from well water

Enterobacteriaceae were detected in the water sources of the towns investigated, suggesting that the available waters in the investigated regions are heavily contaminated with bacteria, which are possibly of fecal origin and could pose a potential public health hazard.

In this study, 7 genera of *Enterobacteriaceae* were detected of which *Enterobacterspp* and *Klebsiella* spp had the

and other water sources have been previously reported in Nigeria [10,12,23,24]. Incidence of these bacteria in portable water is unsafe without treatments and calls for urgent attention because these bacteria are pathogens that have been reported to be associated with serious human infections globally.

From the antimicrobial susceptibility studies, 98.5% of the isolates were resistant to ≥ 1 antibiotics and 75% were found to be resistant to ≥ 2 classes of antibiotics. Previous studies on water enteric bacteria from Nigeria and other African countries also showed high antibiotics resistance profiles and similar rates of antibiotic resistance with those found in this study. For instance, a study in Nigeria found 92.3% of the organisms tested to be resistant to ≥ 1 antibiotic while 77.3 % were resistant to ≥ 2 antibiotics [23]. In another study by Lin *et al.* [7] from South Africa, 94.7% water-borne enteric isolates were found to be resistant to ≥ 1 antibiotic and 75.2% of the isolates were multidrug resistant suggesting water-borne enterics may have a high level of antibiotic resistance. High-level resistance to beta-lactam drugs (cephalosporins and cepheims) was observed among the isolates investigated. Resistance to beta-lactam drugs especially the third-generation cephalosporins and the cepheims has been attributed to the presence of extended-spectrum beta-lactamase which are either transferable through mobile genetic elements or could be chromosomally mediated [25]. Studies in Nigeria have shown the consequence of clinical isolates acting as reservoir for transferable antibiotics resistance genes in plasmids and integrons among Gram-negative bacteria [26,27]. The resistance to amoxicillin/clavulanic acid, cefotaxime, cefixime and ceftazidime by the isolated being 89.71%, 79.47%, 48.53% and 41.08% respectively, suggested the presence of ESBL production among the isolates. Although high resistance to ceftazidime and cefotaxime drug was, the rate of carriage of ESBL by these pathogens by phenotypic assay was low (7.14%). This is in agreement with low ESBL production (5.3%) among

Enterobacteriaceae isolated from drinking water in Central Africa previously reported [28]. In contrast, Soge *et al.* [8] reported a non-resistance to cefotaxime and ceftazidime by water-borne multidrug resistant Gram-negative bacteria isolate from Uganda. Since the genetic basis of resistance to both ceftazidime and cefotaxime was not investigated in this study it is difficult to conclusively suggest the reason for differences in result. However, global reports on outbreaks have established that transmission of pathogenic bacteria by drinking water remains a significant cause of illness. Presence of *E. coli* and other coliform bacteria isolated in this study may have been due to contamination from human fecal discharges as widely observed elsewhere.

In conclusion, presence of pathogenic bacteria suspected to harbor resistance genes in drinking water samples investigated in this study pose a greater risk of community acquired infections which could lead to disease outbreaks and/or transfer of drug resistant bacteria to human. Consumption of such water could contribute to the dissemination and persistence of antibiotic resistant bacteria constituting a public health hazard. The low level detection of ESBL among the cephalosporin resistant isolates suggests an interplay of other resistance genes especially ESBL that are not easily detected using conventional DDST method as used in this study. Further studies to determine the types of resistance genes present in the isolates are ongoing in our laboratory.

Conflicts of Interest

The authors declared no conflict of interest.

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PREVALENCE OF INTESTINAL PROTOZOAN PARASITES INFECTION AMONG PRIMARY SCHOOL PUPILS IN BOSSO LOCAL GOVERNMENT AREA, NIGER STATE, NIGERIA.

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ABSTRACT:

The study was carried out to determine the prevalence of *Entamoeba histolytica* and *Giardia lamblia* among primary school pupils in four communities of Bosso Local Government Area in Niger State, Nigeria. Stool samples from 250 pupils were collected and examined for *Entamoeba histolytica* and *Giardia lamblia* using formol ether concentration technique. Out of the 250 samples analyzed, 115 (46%) for either *Entamoeba histolytica* or *Giardia lamblia* or both. Ninety (36.0%) subjects were positive for *Giardia lamblia* while 46 (18.4%) subjects had *Entamoeba histolytica*. Single species infection was seen in 78 (67.8%) of the infected pupils whereas 47 (40.9%) were infected with both parasites. The age group (9-10) years had the highest rates of infection of 14 (21.5%) and 37 (56.9%) for *G. Lamblia* and *E. histolytica* respectively. Males had the highest rate of infection (53.5%) compared to the females with (46.3%). Poverty, ignorance and poor environmental sanitation were factors found to be associated with the high prevalence rates recorded.

Keywords: Protozoan, Parasites, Infections, Pupils, Bosso, Niger State, Nigeria.

PREVALENCES DES INFECTIONS INTESTINALES DE PARASITES PROTOZOAIRES PARMIS LES ELEVES DE L'ECOLE PRIMAIRE DE BOSSO, CHEF LIEU DE GOUVERNEMENT LOCAL DE L'ETAT DU NIGER, NIGERIA

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RÉSUMÉ

L'étude a été effectuée afin de déterminer la prévalence de *Entamoeba histolytica* et de *Giardia lamblia* chez les élèves des écoles primaires dans quatre communautés de Bosso, chef lieu de gouvernement local de l'Etat du Niger, Nigéria. Des échantillons de selles de 250 élèves ont été recueillis et analysés pour *Entamoeba histolytica* et *Giardia lamblia*, en utilisant la technique de la concentration de l'éther du formol. Sur les 250 échantillons analysés, 115 (46%) soit pour *Entamoeba histolytica* ou *Giardia lamblia* ou les deux à la fois. Quarante-vingt dix (36,0%) sujets étaient positifs pour *Giardia lamblia* tandis que 46 (18,4%) sujets avaient *Entamoeba histolytica*. Une infection d'une seule espèce a été observée dans 78 (67,8%) élèves infectés, tandis que 47 (40,9%) étaient infectés par les deux parasites. Le groupe d'âge (9-10 ans) avait le plus haut taux d'infection de 14 (21,5%) et 37 (56,9%) respectivement pour *G. lamblia* et *E. histolytica*. Les hommes avaient le plus haut taux d'infection (53,5%) par rapport aux femmes (46,3%). La pauvreté, l'ignorance et le manque d'hygiène de l'environnement ont été des facteurs associés à des taux enregistrés de prévalence élevée.

Mots clés: Protozoaires, Parasites, Infections, élèves, Bosso, Etat du Niger, Nigéria

INTRODUCTION

Intestinal parasitic infections (IPI) constitute a global health burden causing clinical morbidity in 450 million people, many of which are women of reproductive age and children in developing countries (1). Numerous protozoa inhabit the gastrointestinal tract of humans; the majority of them are non-pathogenic commensals. The pathogenic

protozoa such as *Giardia lamblia*, *Entamoeba histolytica* and *Blastocystis hominis* can cause severe disease under certain conditions such as severe diarrhea. *G.lamblia* and *E. histolytica* are the most common intestinal protozoa in temperate and tropical countries especially among children (2). The main modes of transmission include faecally contaminated

water, food and person to person especially with poor basic hygiene or lack of sanitation (3).

Intestinal parasitic infections have been linked with increased malnutritional anaemia, protein-energy malnutrition and growth deficit in children (4, 5). Intestinal protozoan's infestation is most common among school age children and tends to occur in high intensities among this group. This infection saps energy and lowers the receptivity of the school children thereby constituting not just a health challenge but a socioeconomic one as well. It is therefore important to evolve a surveillance mechanism that will help in the management of those that are already infected as well as monitor prospective infections in the interest of public health. This study was undertaken to investigate the prevalence of *E.histolytica* and *G. Lamblia* among primary school pupils in selected primary schools in Bosso LGA of Niger State, Nigeria hoping to assist the health policy makers to improve the control measures in order to reduce the prevalence of these infections.

MATERIALS AND METHODS

Study Area

The study area comprises of selected primary schools in Makunkele, Rafin-yashi, Tudun-fulani and Bosso all in Bosso LGA, Niger State

Study Population

The study population consist of primary school pupils (n=250) aged between 8-12 years in Bosso town, Niger State. Participants were chosen randomly from each of the selected primary schools and their bio-data were collected using a structured questionnaire.

Sample Collection

Stool samples were collected from subjects in screw capped containers and taken to the laboratory immediately for analysis. Informed consent was taken from subjects through the schools management.

Sample Analysis.

Analysis of the stool samples was done in the laboratory to check for *G.lamblia* and *E.histolytica* using formol-ether concentration technique as previously described (6).

Statistical Analysis

The data generated was analyzed for significant difference between the rate of intestinal protozoan parasites infection recorded between males and females tested during the study using chi-square test (7).

RESULTS

A total of 250 primary school pupils comprising of 135(54%) males and 115(46%) females were investigated for infection with intestinal protozoa. One hundred and thirty six (54.4%) subjects out of the 250 subjects investigated were found to be infected with single species infection was seen in 78 (67.8%) of the infected pupils, whereas 47(40.9%) were co-infected with two or more species of helminths.

The prevalence rates of infection in both males and females were highest in the age group (8-10) years with 53.5% and 46.3% respectively. These were however not statistically significantly different ($p<0.005$).

The distributions of infections in the four selected primary schools in relation to sex and age are shown in Tables 1-3 below.

TABLE 1: OVERALL PREVALENCE OF *E.HISTOLYTICA* AND *G.LAMBLIA* AMONG AGE GROUP AND SEX

Age (Years)	Total Number Tested	Males	Number of Male Positive (%)	Females	Number of Female Positive (%)
8-9	56	32	17(53.1)	24	11(45.8)
9-10	83	45	35(77.8)	38	26(68.4)
10-11	55	30	19(63.3)	25	10(40)
11-12	56	28	13(46.4)	28	5(17.8)
Total	250	135	84(62.4)	115	52(45.2)

TABLE 2: TOTAL NUMBER OF SINGLE INFECTION FOR *G.LAMBLIA* AND *E. HISTOLYTICA*

Age(Years)	Total Number Tested	Number Positive for <i>G. lamblia</i>	Number Positive for <i>E.histolytica</i>
8-9	56	8	24
9-10	83	14	37
10-11	55	12	23
11-12	56	6	6
Total	250	46 (18.4 %)	90 (36 %)

TABLE 3: PREVALENCE OF *E.HISTOLYTICA* AND *G.LAMBLIA* CO-INFECTION IN THE PUPILS STUDIED

Age(Years)	Total Tested	Number Infected with both Parasites
8-9	56	5
9-10	83	8
10-11	55	6
11-12	56	3
Total	250	22(8.8%)

DISCUSSION

The intestinal protozoa are widely distributed and infections usually vary according to immunity, region and age. An increase in the incidence of these infections is evident in low socio-economic communities with poor sanitation (8).

One hundred and thirty six (54.4%) subjects out of the 250 subjects investigated were found to be infected with either *E. histolytica* or *G. Lamblia*. The breakdown of the result revealed 90 (36.0%) and 46 (18.4%) cases of *E. histolytica* and *G.lamblia* respectively. This result is lower than the findings earlier reported (9) in Maiduguri and (10) in India for *G. lamblia* (41.4%) and (79%) respectively. *E.histolytica* however, is higher than earlier findings by (9) in Maiduguri which was about (17.6%). This may be due to the environmental conditions in India and Maiduguri and their high level of orientation about *G.lamblia*.

The high level of *E.histolytica* may be due to the high level of ignorance about this infection in the study area.

The distribution of infection on the basis of age revealed that age 8-9 years 17(53.1%) and 11(14.5%), 10-11 years 19(63.3%) and 10 (40%) for male and female respectively is more infected with this parasitic infection, it was observed in the questionnaire that this age group is ignorant of this disease, with age 9-10 (77.7% and 68.4%) having the highest number of infection. This may be due to the fact that they are the most active among these children and involved more in outdoor activities than the other age groups. Age 11-12 (46.4% and 17.8%) was not highly infected with this diseases, this may be because they are matured than the other groups and do not involve much in outdoor activities than the other age groups. It was also established from the questionnaires that this age groups are knowledgeable of this disease hence the low infectivity rate.

In this study it was observed that males had 84 (62.2%) cases of infection compared to 52 (45.2%) recorded among the females. this could be attributed to the fact that males are more involved in outdoor activities such as playing, fishing etc and are more exposed to this infection than females. This is in line with previous findings in Maiduguri (8 Bui and Adam, 2008) with males having a total of 43(70.5) and 11(18.0) and females 38 (80.9) and 8 (17.0) for *E.histolytica* and *G.lamblia* respectively. This study showed that there was difference in the prevalence rate observed among the different age groups and gender which agrees with the previous findings of 11-15

The result of this study highlights the public health challenge represented by intestinal parasitism in the study area in particular and the nation in general, and the needs to be addressed to decrease its burden on health care. The high prevalence can be attributed to poor environmental management, poor personal hygiene and lack of public health education. Public health education and improved sanitations conditions in our environment are key success to the prevention of spread of intestinal protozoan infections. In this regards the finding of this study can serve as a basis for developing strategies and preventive programs targeting group at risk of intestinal protozoan infections like school children.

CONCLUSION

It can be concluded based on this research that an overall 54% prevalence rate was found and male pupils are infected with the two protozoan parasites (62.2%) than females (45.2%).

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SEROPREVALENCE OF CYTOMEGALOVIRUS INFECTION AMONGST PREGNANT WOMEN IN KADUNA STATE, NIGERIA

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ABSTRACT

Cytomegalovirus (CMV) is a major public health problem throughout the world. It is the leading cause of congenital infections leading to neurological disabilities in children that result to severe sequelae such as sensor neural hearing loss, neuro-developmental delay and blindness. This study was conducted to determine the seroprevalence of human cytomegalovirus among pregnant women in Kaduna State, Nigeria. Three hundred and sixty three (363) blood samples were collected from 330 pregnant women attending antenatal clinics and 33 non pregnant women attending the Outpatient Department in three selected General Hospitals in Kaduna State. Serum obtained from the blood samples were examined for the presence of CMV IgG antibodies by IFA method. About 94.8% of pregnant women tested positive to CMV IgG antibodies while all (100%) of the non pregnant women tested positive. There was no statistically significant association between CMV and pregnancy ($\chi^2=1.784$, $df=2$, $p=0.182$). Infection with CMV was strongly associated with zone location ($\chi^2=15.381$, $df=2$, $p=0.000$) and employment status of the women ($\chi^2=5.519$, $df=1$, $p=0.018$). There was no significant association between CMV infection and age, gravida, gestation age, education, number of marriages and history of previous transfusion. The high prevalence of CMV infection observed in this study indicates that the virus is prevalent in the study area and it is therefore advisable that routine screening of CMV infection be implemented for all antenatal women in the State.

Keywords: Seroprevalence, Cytomegalovirus, IgG, Pregnant women, Kaduna, Nigeria

SEROPREVALENCE D'INFECTION A CYTOMEGALOVIRUS PARMI LES FEMMES ENCEINTES D'ETAT DE KADUNA, NIGERIA

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RÉSUMÉ

Le cytomégalovirus humain est un problème majeur de santé publique dans le monde entier. Il est la principale cause des infections congénitales conduisant à des séquelles neurologiques chez les enfants résultant des séquelles graves tels que la perte sensorielle de neurones auditifs, le retard de développement neurologique, la cécité. Cette étude a été menée pour déterminer la séroprévalence de cytomégalovirus humain parmi les femmes enceintes de l'Etat de Kaduna au Nigéria. Trois cents soixante trois (363) échantillons du sang étaient collectés chez 330 femmes enceintes en consultation prénatale cliniques, 33 femmes non enceintes fréquentant le service de consultation externe de trois hôpitaux généraux sélectionnés dans l'Etat de Kaduna. Les sérums obtenus des échantillons sanguins ont été analysés par la méthode IFA pour déterminer la présence des anticorps IgG anti-CMV. Environ 94,8% de femmes enceintes ont été testées positives aux anticorps anti-CMV tandis que toutes les femmes non enceintes étaient positives (100%). Il n'y avait pas une association statistiquement significative entre le cytomégalovirus humain (HCMV) et la grossesse ($\chi^2=1,784$, $ddl=2$, $p=0,182$). Les infections au cytomégalovirus ont été fortement associées à l'emplacement de la zone ($\chi^2=15,381$, $ddl=2$, $p=0,000$) et le statut d'emploi de femmes ($\chi^2=5,519$, $ddl=1$, $p=0,018$). Il n'y avait pas d'association significative entre l'infection à cytomégalovirus humain (HCMV) et l'âge, gravida, l'âge de gestation, l'éducation, le nombre de mariages et l'antécédent de la transfusion précédente. La forte prévalence de l'infection à CMV observée dans cette étude indique que le virus est répandu dans la zone d'étude et il est donc souhaitable que le dépistage systématique de l'infection à cytomégalovirus doit être mis en œuvre pour toutes les femmes en consultations prénatales dans l'Etat.

Mots clés: cytomégalovirus (CMV), IgG, femmes enceintes

INTRODUCTION

Cytomegalovirus (CMV) is a member of a family of 8 human herpesviruses (HHV) designated as HHV type 5 [1]. It belongs to the subfamily *Betaherpesvirinae* and the family *Herpesviridae*. Other members of *Betaherpesvirinae* include HHV types 6 and 7 which share common clinical characteristics with CMV [2]. Cytomegalovirus shares many attributes with other herpesviruses including genome, virion structures and ability to cause latent and persistent infection. It has a double stranded DNA with 162 hexagonal capsomeres surrounded by a lipid layer [2].

Cytomegalovirus is mainly a problem for certain high risk groups which include unborn babies whose mothers become infected with CMV during pregnancy and children or adults whose immune systems have been weakened by disease or drug treatment such as organ transplant recipients or people infected with HIV [1,3]. It is a common opportunistic infection among Human immunodeficiency virus (HIV) infected individuals, a major source of viral complication among organ transplant recipients and a leading cause of hearing loss, vision loss and mental retardation among congenitally infected children. More children suffer disabilities caused by congenital CMV than by several better known childhood maladies such as Down's syndrome or foetal alcohol syndrome [4]. Each year about 1 in 150 babies is born with congenital CMV infection and about 8,000 children develop lasting disabilities caused by congenital CMV infection [5]. The virus is the leading cause of congenital infection affecting between 0.3-0.6% of all live births in Europe [6].

Cytomegalovirus is usually an asymptomatic infection. In immunocompetent individuals, symptomatic disease usually manifest as infectious mononucleosis. It is characterised by malaise, headache, sore throat and fatigue. Fever is also commonly present and may persist for weeks in 98% of cases of cytomegalovirus mononucleosis. Lymphadenopathy, pharyngitis and splenomegaly are each seen in 30% of CMV patients [6].

Other clinical abnormalities of CMV infection in normal host include Guillain-Barre, retinitis, thrombocytopenia, gastrointestinal ulceration, hepatitis and pneumonia [1,6]. In few cases, there are symptoms at birth which include premature delivery, being small for gestational age, jaundice, rashes and feeding difficulties [4].

Cytomegalovirus is spread through close personal contact with people who excrete the virus in body fluids (e.g. saliva, urine, breast milk cervico-vaginal secretions and semen), by vertical transmission, through organ transplantation or via blood transfusion [6-8]. Mother to child transmission occurs by three routes described by Pass [6] which include; transplacental, intra-uterine and breast milk transmission.

Serological surveys have shown CMV infections in virtually every population that have been tested [9], with seropositivity ranging from 40 - 100% in different parts of the world [8,10]. For example, a serological survey of over 20,000 women in London found 54.4% of these women were seropositive for CMV [11]. It has been reported that African Continent has the highest prevalence of CMV IgG antibodies. Seroprevalence of 72.2% and 96.0% have been reported in Western Sudan and Egypt [12]. In Asian countries such as Malaysia, the seroprevalence of CMV IgG antibodies among pregnant women was reported to be 84.0% [13]. In Nigeria, serological survey conducted in Bida, Lagos and Sokoto among pregnant women found a seroprevalence of 84.2%, 97.2% and 98.7% respectively [9, 14,15]. These results shows that CMV is on the rise in Nigeria.

The prevalence of CMV has been studied in relation to other causes of congenital infections such as *Toxoplasma gondii* and rubella (TORCH agents) among pregnant women. In one of these comparative studies conducted in Turkey, CMV was found to be leading in prevalence with a seroprevalence of 96.4% for IgG, 0.7% for IgM and 1.9% for IgG + IgM [16]. Similarly, a study in Turkey found higher CMV seroprevalence of 94.4% for IgG and 0.4% for IgM [17].

Cytomegalovirus infection has been studied in relation to other infections in Nigeria. In a serological study carried out in Ibadan, Nigeria using complement fixation test (CFT), a seroprevalence of 86.6% among tuberculosis (Tb) patients, 50.6% among patients other than Tb patients and 54.6% among healthy blood donors was recorded. In general, a seroprevalence of 68.3% was obtained among all patients, with Tb patients having the highest rate [18]. Seroprevalence of CMV among blood donors in Nigeria is also high. Seroprevalence of 92.0% CMV IgG antibodies has been observed among blood donors in Jos [19]. Similarly, seroprevalence of CMV among paid and unpaid blood donors in Tirana, Albania gave an overall prevalence of 83% [10].

A case was reported in 2004, of three siblings from a monogamous family in Osun State, Nigeria who presented with a history of visual impairment/blindness due to CMV [20].

Primary infections occur between 1-4% in seronegative women during pregnancy and may be transmitted to foetus in 40% of cases [21-23]. Infected infants can develop hearing, vision, neurological and developmental problems over time. In a few cases, there are symptoms at birth which include premature delivery, being small for gestational age, jaundice, enlarged liver and spleen,

microcephaly, seizure, rashes and feeding difficulties [3,7].

Nevertheless, there is little awareness of CMV among medical personnel and the general public [7]. Research indicates that fewer than half of the obstetricians in U.S talk to their pregnant women about CMV infection [5]. In the same vein, Nigerian medical personnel are hardly aware of the presence of this virus and the damage it cause to the unborn foetuses, nor do they talk to their pregnant women about this virus. So seroprevalence studies of CMV in Nigeria are necessary to raise awareness of CMV infection and inform on appropriate and rational interventions in the country.

MATERIALS AND METHODS

A cross sectional study was carried out amongst 330 pregnant women attending antenatal clinic in selected hospitals from each of the three geographical zones of Kaduna State. The hospitals were Hajiya Gambo Sawaba Hospital (HGS), Zaria from the North zone, Yusuf Dan Tsoho Memorial Hospital (YDMH), Kaduna from the Central zone and General Hospital Kafanchan (GHK), from the South zone.

Thirty three (33) female patients visiting the hospitals other than pregnant women were used as control population. From each woman, five ml (5ml) of blood was collected in plain vacuutainers, centrifuged on same day and the serum stored at -20°C. All samples were screened for CMV IgG antibodies using Immunofluorescence Antibody (IFA) technique CMV IgG kit (Diagnostic Automation, Inc., Calabasas USA).

Principles of Test

The DAI fluorescent CMV-IgG antibody test system is designed to detect circulating CMV-IgG antibodies in human sera. The system employs CMV infected substrate cells and FITC-labeled goat anti-human IgG (γ chain specific) adjusted for optimum use dilution and free of nonspecific background staining. The reaction occurs in two steps: The first step is the interaction of CMV antibodies in patient sera with CMV infected substrate cells. The second is the interaction of FITC-labeled anti-human IgG (γ chain) with the CMV-IgG antibody attached to the CMV localized in the nucleus of the infected cells.

Data Collection and Analysis

Consenting participants were instructed to fill a questionnaire to obtain information on demography, risk factors, and reproductive characteristic. This information included age, town of residence, gravida, gestational age, educational status, occupation, marital status, number of marriages, history of blood transfusion and history of congenital deformity.

Result and data from questionnaires were analysed using SPSS version 16 and the Pearson Chi square

test at 95% confidence interval and a significance level of 0.05 was used to determine the relationships between the variables and seroprevalence rates. Consenting women were recruited between the months of January and April 2011. Ethical approval was obtained from the Ethical Committee of Kaduna State Ministry of Health.

RESULTS

The prevalence of Cytomegalovirus was found to be 94.8% (313/330) among pregnant women, and all (100%: 33/33) the non pregnant women had antibodies to CMV (Table 1). There was no significant difference in the prevalence of CMV infection between the pregnant and non pregnant women ($\chi^2=1.784$, $df=1$, $P=0.182$)

Further analysis of the data based on geographical zoning, showed that there was a very strong significant difference in the distribution of CMV among the pregnant women by zonal location in Kaduna State ($\chi^2=15.381$, $df=2$, $P=0.000$). Women attending HGS, Zaria from the North zone had the highest prevalence (99.1%: 109/110) while those attending YDMH, Kaduna from the Central zone had the lowest prevalence (88.2% : 97/110) (Table 2).

Table 1: Seroprevalence of Cytomegalovirus among Pregnant and Non Pregnant Women in Kaduna State

Subjects	Positive (%)	Negative (%)	Total
Pregnant women	313 (94.8)	17 (5.2)	330
Non-pregnant women	33 (100.0)	0 (0.0)	33
Total	346 (95.3)	17 (4.7)	363

$\chi^2=1.784$, $df=1$, $p=0.182$

Table 2: Seroprevalence of Cytomegalovirus among Women in Selected Hospitals in Kaduna State

Hospital	Positive (%)	Negative (%)	Total
GHK	107 (97.3)	3 (2.7)	110
HGS	109 (99.1)	1 (0.9)	110
YDMH	97 (88.2)	13 (11.8)	110
TOTAL	313 (94.8)	17 (15.5)	330

$\chi^2=15.381$, $df=2$, $p=0.000$

Key: YDMH = Yusuf Dan-Tsoho Memorial Hospital.

HGS= Hajiya Gambo Sawaba Hospital.

GHK= General Hospital Kafanchan.

Age as a risk factors for CMV infection was considered in this study and classified into seven groups. The result shows that there was no significant difference between age group and CMV

infection ($\chi^2=8.790$, $df=6$, $p = 0.186$). All the women in both age group 15-19 and >44 years had highest IgG antibodies to CMV while women in age group 40-44 had the lowest prevalence (85.7%: 6/7) (Table 3).

Table 3: Seroprevalence of Cytomegalovirus among Pregnant Women in Kaduna State by Age Group

Age group (years)	Positive (%)	Negative (%)	Total
15-19	24 (100.0)	0 (0.0)	24
20-24	116 (98.3)	2 (1.8)	118
25-29	79 (90.8)	8 (9.2)	87
30-34	60 (93.8)	4 (6.3)	64
35-39	26 (92.9)	2 (7.1)	28
40-44	6 (85.7)	1 (14.3)	7
>44	2 (100.0)	0 (0.0)	2
Total	313 (94.8)	17 (5.4)	330

$\chi^2=8.790$, $df=6$, $p=0.186$

Analysis of the result based on gravida shows that there was no significant difference between CMV infection and gravida ($\chi^2=0.889$, $df=4$, $p= 0.927$). All women with gravida above 8 had antibodies to CMV (100%: 15/15) and the lowest prevalence rate was recorded among women with gravida 7-8 (94.3%: 33/35) (Table 4).

Table 4: Seroprevalence of CMV among Pregnant Women in Kaduna State by Number of Gravida

Gravida	Positive (%)	Negative (%)	Total
1-2	111 (94.9)	6 (5.1)	117
3-4	85 (94.4)	5 (5.6)	90
5-6	69 (94.5)	4 (5.5)	73
7-8	33 (94.3)	2 (5.7)	35
>8	15 (100.0)	0 (0.0)	15
Total	313 (94.8)	17 (5.2)	330

$\chi^2=0.884$, $df=4$, $P=0.927$

Further, analysis of risk factors for CMV infection among the pregnant women based on gestation period showed that there was no significant difference between gestation and CMV infection ($\chi^2=1.661$, $df=2$, $p=0.436$). Highest prevalence was however recorded in the second trimester (95.8%: 207/216) while the lowest was recorded in the third trimester (Table 5).

Analysis of the the prevalence obtained based on blood transfusion in this study also showed that there was no association between transfusion and CMV infection ($\chi^2=0.074$, $df=1$, $p=0.789$). A higher prevalence rate of 94.9% (299/315) was however recorded among women that had never undergone transfusion against those who had (93.3%: 14/15) (Table 6)

Further analysis of the results based on socio-demographic data such as employment status, educational status and number of marriages shows that there was no statistically significant association between the virus and educational status and number of marriages the women had been in ($p>0.05$). There was however a strong association between CMV infection and employment ($p=0.0180$). Highest seroprevalence of CMV was obtained among women with no formal education (95.9%: 70/73) and the lowest among women who reached tertiary levels of education (90.3%: 28/33) (Table 7)

Table 5: Seroprevalence of CMV among Pregnant Women in Kaduna State by Gestational Age

Gestational age (months)	Positive (%)	Negative (%)	Total
First trimester	22 (95.7)	1 (4.3)	23
Second trimester	207 (95.8)	9 (4.2)	216
Third trimester	84 (92.3)	7 (7.7)	91
Total	313 (94.8)	17 (5.2)	330

$\chi^2=1.661$, $df=2$, $P=0.436$

Table 6: Seroprevalence of Cytomegalovirus Infection in Pregnant Women in Relation to History of Blood Transfusion in Kaduna State

Transfusion History	Positive (%)	Negative (%)	Total
Not transfused	299 (94.9)	16 (5.1)	315
Transfused	14 (93.3)	1 (6.7)	15
Total	313 (94.8)	17 (5.2)	330

$\chi^2=0.074$, $df=1$, $P=0.789$

DISCUSSION

The results obtained from this study show that the seroprevalence rate of CMV infection among pregnant women in Kaduna State is high. A seroprevalence of 94.8% was obtained in this study. This result is similar to the seroprevalence of 84.2% among pregnant women reported in Bida, Nigeria, 97.2% among pregnant women reported in Lagos, Nigeria and 98.7% among pregnant women

reported in Sokoto State, Nigeria [9,14,15]. This prevalence agrees with the assertion of Saraswathy et al. [13], that African continent has the highest prevalence rate of CMV IgG antibodies.

There was no significant association between the seroprevalence of CMV in pregnant and non pregnant women. This could be due to the low sample size of non pregnant women compared to that of pregnant women. However the result is similar to that reported in Southeast Brazil [24]. The reason for the high prevalence of CMV infection among pregnant women in Kaduna State may not be unconnected to the depreciating socioeconomic standard, poor hygienic practices and low standard of education among these women.

High seroprevalence of CMV infection in Kaduna State may have serious consequences on children in Kaduna State. It has been reported, that the incidence of congenital CMV infection depends on

epidemiological characteristics of a population, in particular the maternal CMV seroprevalence [25]. High rates of congenital CMV infection have been consistently demonstrated in populations with high CMV seroprevalence [25]. Hence, the high prevalence rate of CMV infection among pregnant women in Kaduna state implies there might be a corresponding high incidence of congenital CMV infection among infants born in the state. In the study carried out by Mussi-Pinhata et al. [25], it was found that congenital CMV disease occurs in populations with high seroprevalence rates, with a similar incidence of CMV-related hearing loss. This also implies that there may be increased incidence of congenital CMV infection among infants in Kaduna state as a result of high seroprevalence of CMV infection in pregnant women; This might also lead to a similar increase in the incidence of CMV-related hearing and vision loss in children in the state.

Table 7: Seroprevalence of Cytomegalovirus among Pregnant Women in Kaduna State by Sociodemographic Data

Variable	No Positive (%)	No Negative (%)	Total	p-value
No of marriages				
1	290 (95.1)	15 (4.9)	305	0.746
2	22 (91.7)	2 (8.3)	24	
3	1 (100)	0 (0.0)	1	
Employment				
No	200 (97.1)	6 (2.9)	206	0.018
Yes	113 (91.1)	11 (8.9)	124	
Education				
No formal education	70 (95.9)	3 (4.1)	73	0.682
Primary	79 (95.2)	4 (4.8)	83	
Secondary	136 (95.1)	7 (4.9)	143	
Tertiary	28 (90.3)	3 (9.7)	31	

There was a strong association between the seroprevalence of CMV infection and zone of location of the women; with Zaria zone having the highest seroprevalence rate and Kaduna zone having the lowest. Most of the women enrolled in the study from Zaria zone were resident in Zaria City where there is overcrowding and congestion of settlement leading to poor hygiene and condition of living. It was also noticed that majority of women from Zaria zone have low education and are non employed which could possibly account for the highest seroprevalence [12]. The lowest prevalence rate obtained in Kaduna zone might be as a result of enlightenment as the women are more educated and have better hygienic environments and condition of living. This result agrees with the report of Ludwig and Hengel [7] who asserted that seroprevalence of CMV differs between countries and regions.

There was no association between women who had been transfused and those that were not and seroprevalence was higher among those that were not transfused than those that had been transfused. This result disagrees with the report of Matos et al. [8] where blood transfusion was shown to be a risk factor for transmission of CMV infection. The reason for the disagreement here could be as a result of the disproportionate size of women who were transfused to those who were not transfused enrolled in the study.

Analysis of the result by age shows that there was no significant association between CMV and age contrary to previous report [9, 26] that used ELISA method. There was no predictable pattern between seroprevalence and age, even though the youngest and oldest age gave the highest prevalence. The highest seroprevalence among the youngest age group could be explained by increase sexual activities of this age group as the virus can be transmitted sexually while longer duration of exposure to the virus might be responsible for the highest prevalence seen in the older age group.

Even though the seroprevalence of CMV was highest in the second trimester, there was no significant difference between the seroprevalence and gestation period. This result agrees with previous findings [9,15] and could be due to the fact that most pregnant women report for antenatal in their second trimester than in the first and third trimester.

Similarly, there was no significant association between gravida and CMV infection in this study and this agrees with the findings of Okwori et al. [9]. The pattern of change of seroprevalence with There is no licenced vaccine so far against Cytomegalovirus infection except those currently under investigation [12]. Hence, the only means to prevent transmission of CMV among pregnant

gravida was not clear in this study, though the highest prevalence rate was found in gravida >8. This result agrees with the findings of Hamdan et al. [12] that reported high gravida to be a significant risk factor for CMV infection. This could be so as increase in gravida could imply increase in parity which also implies increase in age, which according to previous reports [7, 26] is a significant predictor of CMV infection.

Seroprevalence of CMV infection by employment status which is one of the predictors of socioeconomic status was higher among the non employed. There was a strong association between employment status and CMV infection in this study. Socioeconomic status has been shown to be a risk factor for CMV infection [27]. The reason for this is probably because, high socioeconomic status implies ability to acquire education and afford better and healthy living conditions which decreases exposure to the virus.

The distribution of seroprevalence rates of CMV infection among pregnant women by number of marriages shows that women who had been involved in up to three marriages in their life time had the highest seroprevalence rate. However this difference was not statistically significant. This result disagrees with previous findings [26]. This observed non statistical difference may have arisen from the disproportionate composition of women in the various groups.

In this study, the seroprevalence rate decreased insignificantly with increase in education. The decrease in seroprevalence agrees with a previous report [12] that showed that illiterate women are at higher risk of CMV infection due to contact with contagious secretions from their own children and poor hygienic practice.

CONCLUSION

The seroprevalence of 94.8% obtained in this study shows that CMV is highly associated with pregnant women in Kaduna State. The implies that there might be a corresponding increase in the incidence of congenital CMV infection hence similar increase in the incidence of CMV-related hearing loss, vision loss, microcephaly and poor mental development among children in Kaduna State. Zone location and employment status were statistically associated with CMV infection. Other risk factors such as history of blood transfusion, gestation, parity, number of marriages and educational status did not show any significant association.

RECOMMENDATIONS

women is to create awareness through health talk during antenatal on the transmission, consequences of infection on foetus and its control and preventive measures.

Routine screening of pregnant women for CMV should be adopted in all health care facilities and blood should be properly screened for CMV before transfusion to pregnant women.

Babies born to seropositive mothers should be screened and examined immediately after delivery for possible signs of hearing and vision defects for early management.

More studies are required in order to ascertain the incidence rate of CMV infection among pregnant women in Kaduna State through determination of IgM and IgG avidity test.

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LIMITATIONS

There were no sufficient funds to have expanded this study in order to include the determination of IgM antibody for recent infections. Access to pregnant and non pregnant women was difficult as medical officers in the hospitals were not willing to assist. There was also the problem of getting true information from the women as many of them would not want to disclose certain information because of confidentiality.

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