Retrospective Analysis of Gram-Negative Bacteria Isolated at a Tertiary Hospital in Maiduguri, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KOO, STB and YBJ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors UMA and TMI involved in the analyses of the study. Authors CUA and PEG managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Gram negative bacteria accounts for significant proportion of hospital and community associated infections responsible for significant proportion of hospital admission, and associated increased level of antibiotic resistance pattern. Based on this information, we retrospectively analyzed the prevalence and resistance pattern of gram negative bacteria isolated from clinical specimens submitted in a tertiary hospital in

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Methodology: Bacteriological data of gram negative bacteria isolates recovered from clinical specimens submitted to medical microbiology laboratory of University of Maiduguri Teaching Hospital (UMTH) between 2007-2011were extracted and analyzed. A total of 36,800clinical specimens were examined.

Results: The prevalence level of gram-negative bacteria isolates was 24.09% (8865/36,800), majority (29.16%, n=2585) of the isolates were recovered from wound specimens. *Escherichia coli* accounted for 31.8% (n=2823) of the total isolates. High susceptibility was observed with fluoroquinolones, aminoglycosides and cephalosporin tested, and resistance with cotrimoxazole and chloramphenicol. Overall, 7.6% (n=671) of the gram negative isolates exhibited multidrug resistance pattern, *Escherichia coli* accounted for 39.9% (268/671) of the multidrug resistant isolates.

Conclusion: The study highlights epidemiological characteristics of the gram-negative bacteria isolated in our hospital, with prevalence level of 24.09% and diverse isolation pattern which affirmed gram-negative bacteria clinical implication in hospital and community associated infections. In addition, the multidrug resistance pattern level of 7.6% is an indication for laboratory personnel to be aware of possible emergence of multidrug resistant strain among gram-negative isolated in the hospital.

1. INTRODUCTION

Antimicrobial resistance is a global phenomenon, which is evident in hospital-and community associated infections responsible for increased morbidity and mortality rate, medical expenses resulting from treatment failure and prolonged hospitalization [1]. Gram negative bacteria accounts for significant proportion of hospital admission, particularly in the intensive care unit [2]. These isolates possess propensity to acquire and developed resistance to variety of classes of antimicrobial agents over period of time, with potential of multidrug resistant pattern[3].Emerging reports revealed increased prevalence of multidrug resistant gram-negative bacteria isolates worldwide [4,5].

In Europe and US, comprehensive epidemiological data on antimicrobial resistance pattern of bacterial pathogens are published annually by agencies like European Antimicrobial Resistance Surveillance study (EARSS) and Centre for Disease Control (CDC) [1,6]. In sub Saharan Africa, such valuable data are `lacking thus created an epidemiological information gap, on the relative state of resistance pattern of bacterial pathogens associated with bacterial infection in our hospitals. Although, studies have reported increased prevalence of 'superbugs' bacteria like methicillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta lactamase producing *E. coli* and *Klebsiella pneumoniae*in hospital setting in Nigeria [7-9]. The report of such studies might be just be a pointer to a bigger problem that is being overlook as relate to resistance of bacterial pathogens in our hospital. The situation reports are furthered compounded by lack of basic facilities necessary for definitive isolation and identification of bacterial pathogens, a common norm in resource-limited laboratories. Epidemiological data generated from local hospital laboratory on these bacterial pathogens based on available facilities, tends to providebaseline information. Based on this knowledge, we undertake a 5-years retrospective analysis of gram negative bacteria isolates and their antimicrobial susceptibility pattern in Maiduguri, Nigeria.

2. MATERIALS AND METHODS

This retrospective study analyzed bacteriological data of gram-negative bacteria isolated from clinical specimens submitted to the Medical Microbiology laboratory of University of Maiduguri Teaching Hospital Maiduguri (UMTH) between January 2007 to December 2011. An average of 7360 clinical specimens were received in the laboratory per year.

Criteria of inclusion, the study extracted bacteriological results of gram negative bacteria isolates, non-duplicate isolates and the antimicrobial agents tested throughout the study period. In addition, the following demographic information, age, sex, type of clinical specimens, bacterial pathogens, source of specimen either in-/-outpatient were collated. Clinical specimens analyzed includes, urine, catheter-tip, sputum, throat swab, high vaginal /endocervical swabs, urethral swabs, semen, pus/aspirate, eye swabs, ear swab,   blood culture and stool.

2.1 Isolation and Identification of Bacterial Pathogens

All the clinical specimens analyzed except blood and stool were inoculated on blood and Mac Conkey agar, incubated at 37ºC for 24 hours. Bacterial colonies were identified by standard bacteriological methods which includes, colonial morphology, gram reaction, biochemical tests (urease, oxidase, indole, citrate) and motility [10].

In bloodstream infection, two blood culture bottles (containing 10ml Robertson cooked meat broth medium) inoculated with 5 milliliter venous blood each were incubated at 37°C for up to 7 days or until growth detected. Blood culture bottles were observed macroscopically daily for visible evidence of bacterial growth such as turbidity. The blood cultures bottles were further sub cultured on blood and Mac Conkey agar for growth and identification by biochemical reactions. In suspected case of enteric infection, aloopful of stool specimen was inoculated into Selenite F broth incubated at 37ºC for 24hours. The specimen was further subculture on Deoxychoclate agar and Kliger tube then incubated at 37ºC for 24 hours. Suspected *Salmonella spp* colonies were   identified by biochemical reaction.

2.2 Antimicrobial Susceptibility Testing of Bacterial Isolates

Antimicrobial susceptibility testing were determined by disc diffusion method using Mueller-Hinton agar medium [10]. The following antibiotics discs (manufactured by Optum Nigeria limited)   were   tested,   ofloxacin   (OFX,10µg),ciprofloxacin   (CPX,30µg),   gentamicin (GEN,10µg) pefloxacin (PEF,10µg), cotrimoxazole (SXT,30µg), streptomycin (S,30µg), ceftazidime (CAZ,10µg)and chloramphenicol (CHL,10µg). The zone of growth inhibition diameter was measured and compared to the breakpoint of the antibiotic disc, classified whether sensitive or resistant [10]. Multidrug resistant (MDR) was defined as isolate resistant to >3 antimicrobial classes in the study.

2.1 Data Analysis

Data were analyzed using SPSS version 16.0, values were expressed as means± standard deviation, and percentages, where appropriate. Changes in the resistance pattern in relation to demographic variable were analyzed by chi-square test. Difference considered significance when   p-value is $p < 0.05$.

3. RESULTS

Of 36,800 clinical specimens received and analyzed over the study period. 24.09% (n=8865) were identified as gram-negative bacteria isolates, 80.4% (n=7129) were lactose-fermenting members of the *Enterobacteriaceae*, and 19.6% (n=1736) were non-lactose fermenting gram-negative bacteria, *P. aeruginosa* isolates. The mean age of the patients was 27.54 ± 19.15 years, with gender distribution of 51.8% (n=4588) males and 48.2% (n=4277) females. The source of clinical specimens, 47.3% (n=4193) recovered from inpatients clinical samples and 52.7% (n=4672) out patients. The frequency of occurrence of gram-negative bacteria isolates (Fig. 1), *E. coli* accounted for 31.8% (n=2827), followed by *Klebsiella* spp (28.2%), *P. aeruginosa* (19.6%), *Proteus* spp (16.3%) and the least *Salmonella* spp (4.0%) respectively. Predominate of gram-negative bacteria isolates in clinical specimens examined (Table 1), *P. aeruginosa*in ear swabs(55.7%) and pus/aspirate (31.8%), *E. coli* in urine(63.1%), urethral swabs (32.5%), high vaginal swab/endocervical swab(56.8%), and wounds(30.0%), *Klebsiella* spp in semen(60%), catheter tip (37.4%), throat swabs (88.0%) and sputum (81.4%),*Salmonella spp* in blood culture(49.0%) and stool specimens (52%) respectively ($p<0.001$). Difference was observed in the isolates distribution with gender, more of the isolates (*P. aeruginosa*, *Klebsiella* spp, *Salmonella* spp and *Proteus* spp) were isolated in clinical specimens from males, compared to *E. coli* from female patients ($p<0.05$)(data not shown). Frequency of isolation with the age-group of the patient (Table 2), significant proportion of the isolates were recovered from patient within the age-group 20-29years (25.6%), followed by <10years (22.0%) and 30-39years (20.5%). *Escherichia coli* predominate in all the age-groups except in <10 and 40-49years in which *P. aeruginosa* accounted 6.5% in <10years, and *Klebsiella* spp 3.4% in 40-49 years respectively.

Overall antimicrobial pattern of gram-negative bacteria isolates (Table 3), high resistance was observed with the isolates, cotrimoxazole (76.3-84.8%) and chloramphenicol (31.8-91.1%). High susceptibility patterns were observed with the ofloxacin (3.0-26.0%), pefloxacin (3.7-36.2%),ciprofloxacin (8.2-29.1%), gentamicin (10.4-38.0%), streptomycin (22.5-38.7%) and ceftazidime (16.9-44.8%). Trend of resistance pattern over the study period (Fig. 2), a relatively steady resistance pattern was observed throughout the years except slight variation. In 2008, *E. coli* resistance to pefloxacin was the highest compared to other years, while *P. aeruginosa* resistance to gentamicin in 2008 and 2009was 40.2 and 40.6% compared to 36.2%, 38.15 and 37.7% in 2001, 2004 and 2005 respectively. Increased resistance of *P. aeruginosa* to ceftazidime (28.9%) was observed in 2003.

Of the 8865 isolates, 7.6% (671) gram negative bacteria exhibited multidrug resistant pattern (more than 3 antibiotics). Among these multidrug resistant pathogens, *E. coli*accounted for 40.0% followed by *Klebsiella* spp 24.7% *P. aeruginosa* 18.3% and the least *Salmonella* spp 0.03%.
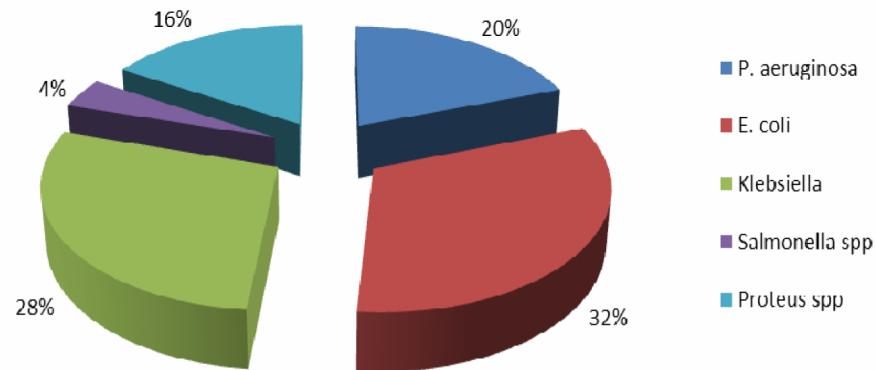
Fig. 1. Frequency of occurrence of gram negative bacteria isolates

Table 1. Frequency of occurrence of gram-negative bacteria isolates with clinical specimens

Clinical specimens	*P. aeruginosa*	*E. coli*	*Klebsiella spp*	*Salmonella spp*	*Proteus spp*	p-value
Urine	28	448	86	0	148	0.001
Urethral swab	77	104	57	0	82	.0001
Blood	28	91	224	330	0	.0001
High vaginal swab/endocervical swab	87	1061	481	1	239	.001
Semen	0	124	243	0	38	.001
Pus /aspirate	42	36	25	0	29	.0001
Ear	692	76	223	0	249	.001
Eye	14	39	8	0	7	.001
Stool	0	16	7	25	0	.002
Wound	685	775	512	0	613	.001
Catheter tip	29	0	43	0	43	.0001
Throat swab	22	21	315	0	0	.001
Sputum	32	32	280	0	0	.001
Total	1736	2823	2504	356	1446	

Table 2. Distribution of gram-negative bacteria isolates according to age-group of patients

Age-group (years)	*P. aeruginosa*	*E. coli*	*Klebsiella spp*	*Salmonella spp*	*Proteus spp*	Total
<10	579(6.5)	356(4.0)	521(5.9)	245(2.8)	253(2.9)	1954(22.0)
10-19	139(1.6)	212(2.4)	169(1.9)	45(0.5)	112(1.2)	667(7.5)
20-29	350(3.9)	922(10.4)	605(6.8)	9(0.1)	383(4.3)	2269(25.6)
30-39	268(3.0)	605(6.8)	500(5.6)	1(.0)	442(5.0)	1816(20.5)
40-49	172(1.9)	294(3.3)	305(3.4)	56(0.6)	122(1.4)	949(10.7)
>50	228(2.6)	434(4.9)	404(4.6)	-	146(1.6)	1212(13.7)
Total	1736(19.6)	2823(31.8)	2504(28.2)	356(4.0)	1448(16.3)	8865(100)

P. aeruginosa

E. coli

Klebsiella spp.

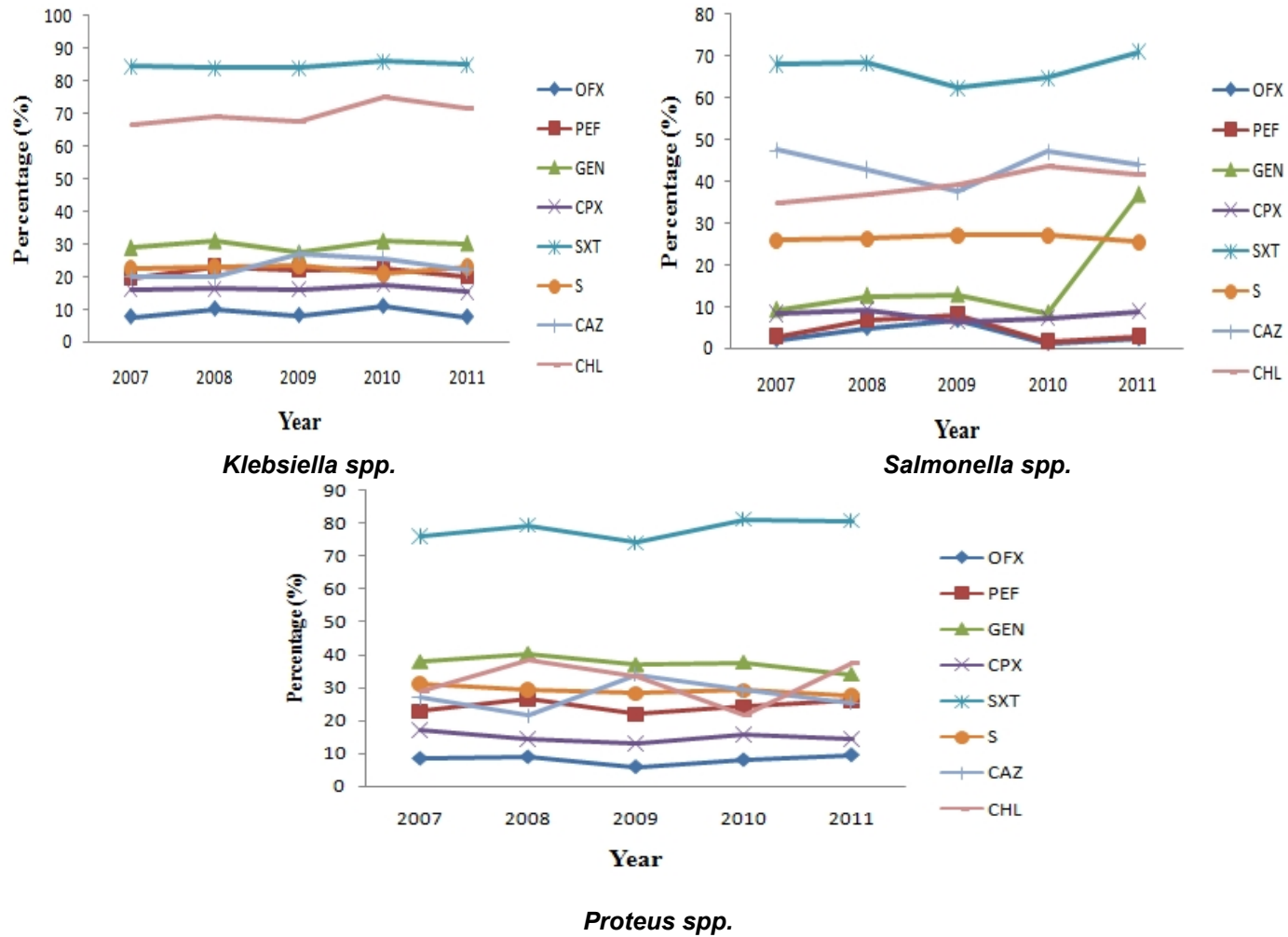**Salmonella spp.**

Proteus spp.
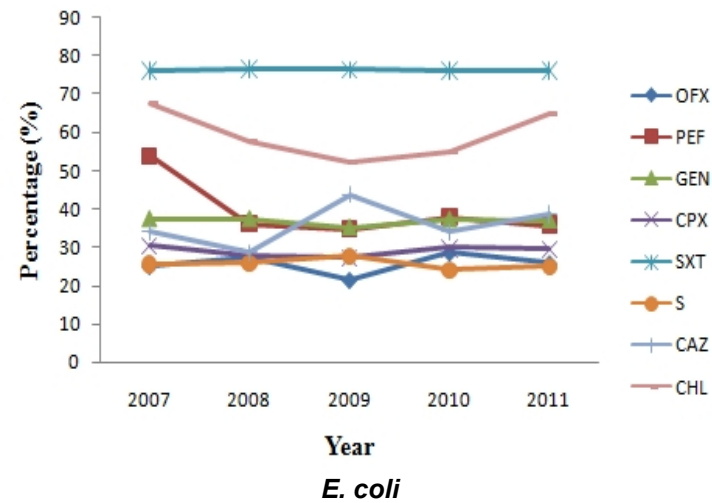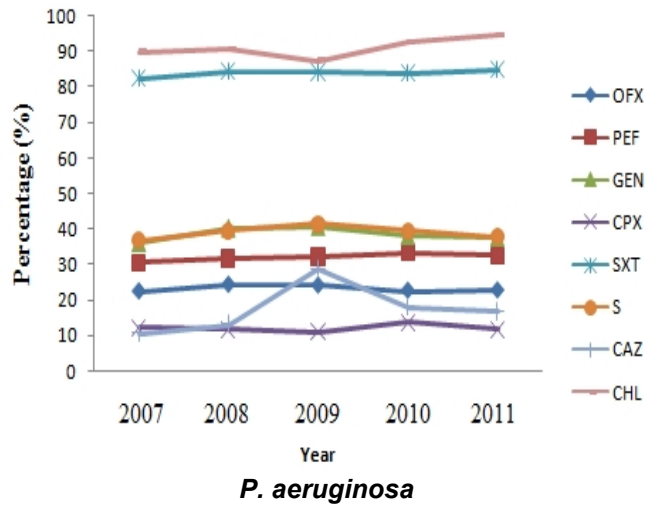
Fig. 2. Trend of antimicrobial resistance pattern of gram-negative bacteriaisolates over the study period

Table 3. Antimicrobial resistance pattern of Gram-negative bacteria isolates

Antimicrobial agents	*P. aeruginosa* (n=1736)	*E. coli* (n=2827)	*Klebsiella spp* (n=2504)	*Salmonella spp* (n=356)	*Proteus spp* (n=1448)
Ofloxacin	23.1	26.0	8.8	3.0	8.2
Pefloxacin	32.0	36.2	21.4	3.7	24.4
Gentamicin	38.3	37.1	29.7	10.4	38.0
Ciprofloxacin	12.2	29.1	16.2	8.2	15.2
Cotrimoxazole	83.8	76.3	84.8	76.6	78.5
Streptomycin	38.7	25.7	22.5	26.1	29.1
Ceftazidime	16.9	35.6	22.6	44.8	27.8
Chloramphenicol	91.1	60.3	69.9	50	31.8

4. DISCUSSION

The emerging epidemiological data of bacterial resistant strain in both hospital and community setting, makes the need for periodic surveillance study imperative, as an intervention measure towards prudent prescription and administration of antibiotics. Unlike, the situation report in developed countries with comprehensive data on resistant pattern of bacterial pathogens. In developing countries, the inability to conduct such comprehensive surveillance studies are limited by lack of adequatere sources, thereby presenting a blurred picture of exact epidemiological situation of bacterial pathogens and their resistance pattern. In this study, the prevalence level of the gram-negative bacteria isolates was 24.1% with 80.4% were members of *Enterobacteriaceae* and 19.6% non-fermenting gram negative bacteria (*P. aeruginosa*).The bacterial isolates were widely distributed within the clinical specimens, age-group and gender. *E. coli* was the most bacterial pathogens, similar to findings reported in other hospital based studies, although the frequency of occurrence differs [11-13]. While in other studies, *Klebsiella* spp and *P. aeruginosa* have been reported as predominate pathogens [14-15]. It is believed that the observed difference in prevalence level of bacterial pathogens may be due to variation in studied population, clinical prognosis and clinical specimens examined [16]. Of the clinical specimens examined, majority of isolates were recovered from wounds infections (2585), followed by genitourinary tract infection (1869), ear infection (1238), respiratory traction infection (704), bloodstream infection (673) and urinary tract infection (710).This affirmed the diversity and clinical implication of gram negative bacteria in both hospital and community-associated infections.

In this study, *E. coli* ranked as the leading pathogen in both urinary tract and genitourinary tract infection, with observed difference in gender pattern as reported in other studies [16,17]. The isolation pattern observed in the respiratory tract infections, showed *Klebsiella spp* as predominate pathogens, while *Pseudomonas aeruginosa* was reported in a study conducted in Saudi Arabia [16] and *Acinetobacter spp* in SENTRY programme among the bacterial pathogens isolated from patients with pneumonia [18].

Blood stream infection is one of the leading causes of hospital admission, and also responsible for high morbidity and mortality particularly among children. Bacterial pathogens implicated in bloodstream infection are diverse, from gram-positive cocci to *Enteriobacteriaceae* and non-fermenting gram-negative bacteria. *Salmonella enterica* (tyhoidal and non-tyhoidalserovar) involvement in bloodstream and gastroenteric diseases have been documented in many studies [19,20] In this study, *Salmonella spp* predominate bacterial pathogens isolated from blood culture and stool specimens. However, serotyping of the *Salmonella spp*, was not routinely carried out in our laboratory. The lack of information on serotyping of the isolates, limits the public health implication of these isolates as to

whether they were of tyhoidal serovar that is associated with enteric fever, a common enteric disease in our community.

Overview of antibiotic pattern and factors responsible for emergence of resistant strains seem to differ with countries. In developed countries, extensive use of antimicrobial agents in hospital setting and their incorporation in animals feeds had been known to be responsible for wide range of highly resistant pathogens documented [21]. In contrary, the relatively high prevalence of resistant strains in developing countries, particularly in sub Saharan Africa, self-medication/substandard agents for wide variety of bacterial infections, remains the major factors [21]. Overall resistance pattern of the isolates revealed two definitive antimicrobial susceptibility pattern, high resistance pattern tocotrimoxazole, and chloramphenicol compared to high susceptibility to streptomycin, ceftazidime, gentamicin, pefloxacin, of loxacin and ciprofloxacin. This similar pattern have been documented in other studies [12,22]. Overall resistance pattern over the study period as shown in Fig. 2, revealed a relatively stable resistance pattern with slight variation. We are of opinion that the resistance pattern might be due to the fact that factors such as over-the-counter drug purchase, self-medication, sub-standard drugs a common norm in most community in sub Saharan Africa could be responsible for the relatively stable resistance seen over the study period.

The high resistance pattern observed with cotrimoxazole seem to be a common pattern in studies conducted in sub Saharan Africa [12,23] and in Europe [24,25]. This pattern might be due to the fact that cotrimoxazole is commonly administered for wide variety of clinical conditions (urinary, respiratory and gastrointestinal tract infections) in addition, the same reason could be attributed to the pattern observed with chloramphenicol.

Ceftazidime, is a third generation cephalosporin drug was introduced into clinical armamentarium due to high resistance by gram-negative bacteria to beta-lactam antibiotics. Its antibacterial activity in this study ranged from 16.9% to 44.8%, with lowest resistance observed with *P. aeruginoasa* isolates. Though ceftazidime, isa known anti-pseudomonal, studies have reported resistance level up to 76.8% in studies conducted in Iran [5] and in Turkey [26]. Of the aminoglycosides commonly prescribed and administered for the treatment of infections due to gram-negative bacteria was gentamicin [27]. In this study, overall resistance level of gentamicin was similar to the report in southwestern Nigeria [12] and in Tanzania [28] However, studies had associated gentamicin resistance, particularly in tertiary hospital to excessive gentamicin usage, especially among immunocomprised patients and in medical practice that involves invasive procedures and surgical manipulation [29]. Overall susceptibility to gentamicin, showed varied pattern with the gram-negative bacteria, with *P. aeruginosa* 38.3%, *E. coli* 37.1%, *Klebsiella spp* 29.7%, *Salmonella spp* 10.7% and *Proteus spp* 38.0% respectively. High susceptibility level to ofloxacin (16.8%) and ciprofloxacin (16.8%) was observed in this study. This signifies that these drugs could serve as alternate option in treatment of bacterial infection due to gram-negative bacteria isolates that might exhibit high resistance to commonly used antibiotics. Although, studies in Iran, Argentine and Brazil had reported high fluoroquinolones resistance level of 65%, 80%and 25.5% respectively [5,30,31].

There are emerging reports of increased prevalence of multidrug resistant gram-negative bacteria worldwide, with attendant consequences such as worse clinical outcome, long hospitalization and difficulty in patient management [32-34]. In Africa, relatively few reports on multidrug resistance and ESBL producing strains have been documented in studies conducted in Nigeria [22,35]. In Gabon, 15.4% extended spectrum beta lactamase producing gram negative bacteria [13], 12% in Cameroon(4), 28.7% in Saudi Arabia [36] and a ranged

of 29.2-50% in Tanzania [28] One unique characteristic of gram-negative bacteria is the propensity to develop and acquire resistance to multiple antimicrobials. In this study, the prevalence level of the gram –negative bacteria isolates demonstrated MDR pattern was 7.6 percent. This level considerably low when compared to other level reported elsewhere, 37% in USA [37], 46% in Thailand(38), 50% in Saudi Arabia [16] and 55% in Iran [39]. Although, this MDR level is low, but it is an indication for laboratory personnel involved in isolation and antimicrobial testing of these pathogens to aware and the need for prompt documentation, as part of patient report and epidemiological data for hospital infection control unit.

Though, data obtained from this study had provided the baseline information on the status of gram negative bacteria and their susceptibility pattern necessary for rational approach to antibiotic usage and infection control program. Nevertheless, there were limitations associated with such similar retrospective study. Firstly, there were possibility of transcription errors in documentation of laboratory results, that will invariably translated to documentation error and bias as applied to this study. Secondly, inherent intrinsic factors like, quality of clinical specimen, and pre-medication can compromised bacterial result outcome. Thirdly, lack of basic facilities is one of the limiting factors in carrying out standard bacteriological procedures in resource-limited medical microbiology laboratories in hospital setting. Such observations are evident in this study, which might invariably influenced the bacteriological results.

5. CONCLUSION

In conclusion, this retrospective study provided epidemiological information on gram-negative bacteria isolates and affirmed their association with hospital and community associated infections. In addition, *E. coli* and *Klebsiella spp*isolates in this study demonstrated multidrug resistance (MDR) pattern, with relatively high prevalence. This MDR pattern is an indication for laboratory personnel involved in bacteriological analysis of these pathogens, to report and document accordingly for patient management and infection control unit in the hospital.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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