



Antibiotic Susceptibility Profile of Gram-negative Enterobacteriaceae from Women Attending Selected Hospitals in Sokoto, North Western Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Authors MA and NT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MA managed the analyses of the study. Authors MA and NT managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of our study is to isolate, characterize as well as determine the antibiotic susceptibility pattern of Gram-negative *Enterobacteriaceae* from women attending some selected hospitals in Sokoto, North western Nigeria.

Study Design: The study was designed to isolate and characterize Gram-negative organisms isolated from women attending some selected hospitals in Sokoto state. Only women of age 18 years and above were included. Each of the participants gave a written and verbal consent of their willingness to participate in the study.

Place and Duration of Study: The study was conducted in a tertiary specialist hospital and 3 other secondary health facilities within Sokoto metropolis for a period of 5 months (March to August, 2018).

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Methodology: Urine samples were inoculated on prepared CLED agar. Samples count up to and greater than 10^6 cfu/ml were considered positive. Microgen GN-ID was used to identify the bacterial isolates based on manufacturer's instruction. Antibiotic susceptibility testing was determined against 8 antibiotics using the modified Kirby-Bauer disc diffusion method on Mueller Hinton agar. Results of AST were interpreted using the CLSI guideline.

Results: A total of 411 urine samples were analyzed during the period. Out of the 411 samples, 73 (17.8%) were Gram negative isolates. The AST showed that the Gram-negative isolates were highly sensitive to piperacillin/tazobactam (100.0%), followed by imipenem (98.1%), then ciprofloxacin (93.1%). norfloxacin showed 72.6% sensitivity, while gentamicin and nalidixic acid showed sensitivity of 68.5% and 56.2% respectively. Majority of the isolates were resistant to ampicillin and co-trimoxazole.

Conclusion: *E. coli* was the most prevalent among the uropathogens investigated. The high resistance encountered with co-trimoxazole and ampicillin underscores the need for continuous monitoring of antibiotic susceptibility result before the commencement of treatment. This can be complimented with antibiotic stewardship if possible in these hospitals.

Keywords: AST; gram-negative; Enterobacteriaceae; women.

1. INTRODUCTION

The presence or growth of microorganisms in the urinary tract is the cause of urinary tract infection (UTI) [1]. Several reports have indicated that UTI can occur in both male and female [2], with many reported cases of higher prevalence in women than men [3]. Wagle et al. [2] also reported that UTI needs empirical therapy for uncomplicated cystitis in healthy adults [4] before availability of microbiological results, which is based on the local susceptibility patterns. UTI may be asymptomatic in many cases.

The efficacy of antibiotics has been severely compromised due to global spread of resistant bacteria. Antibiotic resistance occurs when bacteria lose their sensitivity to antibiotics. Resistance develops among microorganisms by spontaneous mutations in existing genes or by the acquisition of extraneous genes [5]. Multiple drug resistance is caused by the interplay of multiple resistance mechanisms that emerge via the acquisition of extraneous resistance determinants or spontaneous mutations/gene mutations, horizontal gene transfer and recombination or changes in the regulation of gene expression, which can influence the construction of the bacterial wall or produce antibacterial proteins.

The development of the population of antibiotic-resistant bacteria and their worldwide distribution are the consequence of long years of selective pressure on the grounds of antibiotics underuse, overuse, as well as misuse [6]. Antimicrobial resistance in *Enterobacteriaceae* has emerged as a major clinical problem in recent years [7,8].

Drug resistance among this group of bacteria is mainly caused by the emergence and proliferation of extended spectrum beta lactamases [9], fluoroquinolone resistance [10] and the dissemination of multiple drugs resistant (MDR) carbapenem resistant strains [11]. Hence detection of bacterial isolates is indispensable for establishing the effective antibiotic policies and infection control strategies not only in the hospital setting, but in general clinical practice.

The aim of our study is to isolate, characterize as well as determine the antibiotic susceptibility testing of Gram-negative *Enterobacteriaceae* from women attending some selected hospitals in Sokoto, North western Nigeria.

2. MATERIALS AND METHODS

The study was hospital based. Information on comorbid diseases (surgical intervention, renal diseases), presence of previous antibiotic use, urinary catheters, ICU admission, previous hospitalization, and length of hospital stay were recorded. The study obtained clearance from the hospitals' ethics committee. Only women of age 18 years and above who were sent to microbiology laboratory for investigation as determined by the physician and consented and volunteered to be part of the study were recruited for the study. Urine samples were collected in a sterile urine container. Prior to collection of urine samples, participants were counseled on how to aseptically collect mid-stream urine.

2.1 Culturing of Urine Samples

Urine samples were inoculated on prepared Cystein Lactose Electrolyte Deficient (CLED)

agar in a sterile petri dishes. The plates were allowed to stay for some minutes and incubated in aerobic condition for 24 hours at 37°C. Samples with counts up to and greater than 10⁶cfu/ml were counted microscopically and considered positive.

2.2 Characterization of Isolates

Isolates were purified by single colony isolation onto Nutrient Agar plates and incubated at 37°C for 18-24 hours. Isolates from pure culture were characterized by Gram staining.

2.3 Identification of Bacteria Isolates

The Microgen GN-ID system was used to identify the bacterial isolates based on the manufacturer's instruction. It comprises of microwell strips GN A. The GN A microwell strip is intended for the identification of oxidase negative, nitrate positive, glucose fermenters comprising the most commonly occurring genera of the family Enterobacteriaceae.

2.4 Antibiotic Susceptibility Testing

The antibiotic susceptibility of the isolates was determined against eight (8) panels of antibiotics

using the modified Kirby Bauer disc agar diffusion on a fresh sub-cultured isolates on Mueller-Hinton Agar (MHA). The antibiotics discs were ampicillin (10 µg), imipenem (10 µg), ciprofloxacin (5 µg), piperacillin/tazobactam (100/10 µg), gentamicin (10 µg), nalidixic acid (30 µg), co-trimoxazole (25 µg), and norfloxacin (10 µg). The discs were placed with the aid of a disc dispenser. Plates were incubated at 37°C for 24 hours. Following incubation, the diameter of the zones of growth inhibition produced was measured to the nearest millimeter using a ruler. Results were interpreted using the CLSI guidelines.

3. RESULTS AND DISCUSSION

A total of 411 urine samples were analyzed for a period of 5 months from the hospitals. Out of the 411 samples analyzed, 73 accounting for 17.8% were Gram negative isolates. The distribution of Gram negative isolates is shown in Table 1.

The antibiotic susceptibility pattern showed that the Gram-negative isolates are highly sensitive to piperacillin/tazobactam (100%). This was followed by imipenem (98.1%), then ciprofloxacin (93.1%) as shown in Table 2.

Table 1. Distribution of gram negative isolates from women in the hospitals

S/No	Uropathogen isolated	Frequency of isolates	Prevalence (%)
1	<i>E. coli</i>	48	65.7
2	<i>Klebsiella oxytoca</i>	5	6.8
3	<i>Klebsiella pneumonia</i>	8	11.0
4	<i>Proteus mirabilis</i>	3	4.1
5	<i>Citrobacter freundii</i>	5	6.8
6	<i>Enterobacter gergoviae</i>	4	5.5
Total		73	

Table 2. Percentage susceptibility of gram negative uropathogens from the women

S/No	Antibiotic	Susceptibility	Resistance	% Susceptibility	% Resistance
1	Ampicillin	16	57	21.9	78.1
2	Imipenem	72	1	98.6	1.4
3	Ciprofloxacin	68	5	93.1	6.8
4	Pip/tazobactam	73	0	100.0	0.0
5	Gentamicin	50	23	68.5	31.5
6	Nalidixic acid	41	32	56.2	43.8
7	Cotrimoxazole	12	61	16.4	83.6
8	Norfloxacin	53	20	72.6	27.4

Key: Pip/tazobactam = Piperacillin/tazobactam

The prevalence of Gram negative uropathogens in this study was 17.8%. Higher prevalence was reported in Sokoto by Nuhu et al. [12] with 27.5%. Similar results were obtained by Nuhu et al. [3] with prevalence rate of 17.5%. Although the two studies carried out conducted [3,12], considered both male and female patients. A study by Ojo and Anibijuwon [13] have shown a much higher (65.0%) prevalence of uropathogens in females students of University of Ado Ekiti. In their study, they include both Gram positive and Gram negative organisms.

E. coli is the most frequently isolated uropathogen in this study. The prevalence rate was 65.7%. This is in line with results of most studies [3,12,13,14] were 29.7%, 44.0%, 32.7% and 24.6% respectively were reported. These studies are however lower than what was obtained in our study. Al-Jebouri and Mdish [15] reported that certain virulence factors like haemolysin production and presence of fimbriae may be the cause of high prevalence of *E. coli* in UTIs [12]. Nicolle [16] also reported that the higher prevalence of *E. coli* in UTIs may be due to faecal contamination, the predilection of the organisms from the toilets and the shortness of the female urethra [13].

The result of AST against these uropathogens revealed a relatively higher susceptibility of these organisms to piperacillin/tazobactam (100.0%), followed by imipenem (98.6%), and then ciprofloxacin (93.1%). With respect to ciprofloxacin and gentamicin, it has been reported to have a low susceptibility to Gram-negative isolates of as low as 35.0% and 30.0% respectively [17]. The least was however observed with ampicillin (21.9%) and co-trimoxazole (16.4%). These results were similarly obtained in study carried out by [2,18]. It was reported by Abejew et al. [19] that ciprofloxacin to be very active against uropathogens [14]. Majority of the isolates were resistant to ampicillin and co-trimoxazole. This high level of resistance may be attributed to the irrational use of these drugs in the study area. Availability and easy access to these antibiotics may likely be a reason why patients can decide to opt for self medication even without doctors' prescription. Our findings correlate with most studies reported on co-trimoxazole [3,20]. The resistance rate of uropathogens to co-trimoxazole as reported by Ejikeugwu et al. [21] was 89.0%, which is almost similar to our study (83.6%).

4. CONCLUSION

E. coli was the most prevalent among the uropathogens investigated. The high resistance encountered with co-trimoxazole and ampicillin underscores the need for continuous monitoring of antibiotic susceptibility result before the commencement of treatment. This can be complimented with antibiotic stewardship if possible in these hospitals.

CONSENT

Verbal and written Informed consent was sought from the participants of this study.

ETHICAL APPROVAL

Ethical approval for this study was obtained from the hospital ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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