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Phenotypic Detection of Extended Spectrum Beta-Lactamase (ESBL) Produced by Gram-Negative Uropathogens in Sokoto Metropolis

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Abstract

Extended spectrum beta-lactamases (ESBL) produced by Gram-negative uropathogens constitute an emerging public-health concern. The aim of the study was to determine the prevalence of ESBL producing enterobacteriaceae uropathogens in Sokoto. The study was undertaken at Specialist Hospital Sokoto (SHS) over a 2-month period. Ninety-one urine samples were collected. The isolates collected were identified using biochemical tests. All isolates were tested for susceptibility to eight different antibiotics by the modified Kirby Baur disc diffusion. Screening and confirmatory tests for ESBL production were done according to Clinical Laboratory Standard Institute (CLSI) guidelines. A total of 25 isolates were Gram-negative which accounted for 41% prevalence, while 59% accounted for Gram-positive. *E. coli* was the most common isolates (44%), followed by *Proteus mirabilis* (20%), *Klebsiella spp* (16%), *Enterobacter spp* (12%), and *Salmonella spp* (8%). All isolates displayed multi-drug resistance. The overall production of ESBL-producing isolates was 16%. Our study confirms the prevalence of ESBL-producing uropathogens in Sokoto.

Keywords: Phenotypic detection, ESBL, Uropathogens

Introduction

Bacteria are the major causative organisms and are responsible for more than 95% of UTI cases (Nuhu *et al.*, 2015 and Khosbakt *et al.*, 2013). About 80-85% of UTIs are caused by Gram-negative bacteria (Nuhu *et al.*, 2015 and Venkatadri *et al.*, 2014). Treatment of UTIs caused by *E. coli* is becoming difficult due to antibiotic resistance (Giske *et al.*, 2009).

The widespread and inappropriate use of antibiotics is recognized as a significant contributing factor for the spread of bacterial resistance and the development of resistance to antimicrobial agents (Kurutepe *et al.*, 2005; Hryniewicz *et al.*, 2001; Mincey and Parkulo, 2001 and Tenever and McGowan, 1996). For most bacteria, there is evidence that increased usage of a particular antimicrobial correlates with increased levels of bacterial resistance to that agent (Lamido *et al.*, 2010).

Antimicrobial resistance among pathogenic bacteria is increasing worldwide especially against beta-lactam drugs, due to the development of beta-lactamase enzymes (National Nosocomial Infections Surveillance, NNIS, 2003) which destroy the beta-lactam ring of these antibiotics, thus preventing the action of penicillin binding proteins (PBPs) (Livermore, 1995). In the battle against beta-



lactamase mediated bacterial resistance, development of third generation cephalosporins in the early 1980s was a major breakthrough. But soon, a new plasmid encoded beta-lactamase capable of hydrolyzing the extended spectrum cephalosporins was reported (Heritage *et al.*, 1999; Jacoby and Medeiros, 1991 and Knothe *et al.*, 1983). These enzymes mediate resistance to oxymino-cephalosporins (ceftriaxone, ceftazidime, cefotaxime, cefepime) and monobactams (aztreonam) with the exception of carbapenems. The first beta-lactamase with extended spectrum was detected in *Klebsiella pneumoniae* in Germany in 1983 (Knothe *et al.*, 1983) and later in the western parts of Europe, probably because extended-spectrum beta-lactam antibiotics were first used clinically. They occur predominantly in members of the enterobacteriaceae with *Klebsiella pneumoniae* and *E. coli* being the most commonly reported worldwide. A commonly used working definition is that, ESBLs are beta-lactamases capable of conferring bacterial resistance to the penicillins; first, second and third generation cephalosporins; and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by beta-lactamase inhibitors such as clavulanic acid (Paterson and Bonomo, 2005).

The detection of ESBL-mediated resistance in Gram-negative bacilli is of paramount importance because of its clinical implications and the limited therapeutic options (Hassan and Abdalhamid, 2014; Ramphal and Ambrose, 2006; Paterson and Bonomo 2005 and Sturenberg and Mack, 2003). It has been observed that conventional methods for resistance detection rely on phenotypic identification based on bacterial growth inhibition in disk diffusion or dilution tests, which are cost-effective but which usually require two days to complete (Hassan and Abdalhamid, 2014 and M'Zhali *et al.*, 2000).

Laboratory detection of these enzymes, proper reporting, awareness creation and necessary precautions to avoid their spread is lacking, despite the fact that it is a significant health problem. Although ESBL-producing members of *Enterobacteriaceae* have been reported in Sokoto

(Nuhu *et al.*, 2015). The study was conducted to determine the distribution, antibiotic susceptibility and phenotypic detection of ESBL producers in Gram-negative uropathogens. This is with the aim of creating more awareness on the prevalence of these enzymes in Sokoto Metropolis.

Materials and Methods

Study Area

Approval to carry out this study was obtained from the ethics committee of the Specialist Hospital Sokoto (SHS) and informed consent was obtained from each study participant. The site for sample collection is Specialist Hospital Sokoto (SHS). SHS is a 300- bed referral hospital with modern equipment and facilities providing health care services. SHS is patronized by people from within and outside Sokoto Metropolis and even neighboring Niger Republic.

Sample and Sampling Technique

A total of twenty-five (25) non-repetitive isolates of Gram-negative bacteria comprising of *E. coli* (n=11), *Proteus mirabilis* (n=5), *Klebsiella* spp (n=4), *Enterobacter* spp (n=3) and *Salmonella* spp (n=2) from ninety-one (91) urine samples were collected at SHS Microbiology Laboratory from April to June, 2016. The isolates were from different patients from the General Out Patient Clinic (GOPC) which comprised of both male and female with age ≥ 18 years.

Antibiotic Susceptibility Testing

Susceptibility was determined using the modified Kirby Baur disc diffusion method. Commercial antibiotic discs (Oxoid, UK) used include: Gentamicin (CN, 30 μ g), Nalidixic acid (NA, 30 μ g), Cefpodixime (CPD, 10 μ g), Cefotaxime (CTX, 30 μ g), Ceftazidime (CAZ, 30 μ g), Augmentin (AMC, 30 μ g), Erythromycin (E, 15 μ g) and Norfloxacin (NOR, 10 μ g). The plates were incubated at 37°C for 24 hours. The inhibition zone diameters around the discs were measured and interpreted according to CLSI guideline.

Screening and Phenotypic Detection of ESBL

The isolates were screened for ESBL production. Thereafter, phenotypic confirmatory test was done using CLSI guidelines 2014.

Confirmation of ESBL phenotype was performed by Double Disc Synergy Test (DDST) using antibiotic discs containing two 3rd generation cephalosporin (CPD 10 µg and CTX 30 µg) and Amoxicillin/Clavulanic acid (Augmentin, 30 µg). Using a sterile needle, CPD 10 µg and CTX 30 µg were placed on the agar at a distance of 15 – 20 mm center to center from Augmentin 30 µg. The plates were incubated at 37°C for 24 hours and were examined for an extension of the edge of zone of inhibition of antibiotic discs towards the disc containing Augmentin 30 µg i.e. after incubation. Enhancement of zone of inhibition of either or both the CPD and CTX discs towards the AMC disc is indicative of ESBL production. This is interpreted as synergy and considered positive for ESBL production.

Results

A total of 25 Gram-negative uropathogens were isolated out of the 91 urine samples collected in the study period. This account for 27.5 % prevalence. Among the patients with Gram-negative uropathogens, female patients were predominant

17/25 (68 %), while male were 8/25 (32 %) (Figure 1). Male to female ratio in this study was 1:2.12.

Of the 25 isolates, 11 were *E. coli* (44%), 3 *Enterobacter spp* (12%), 4 *Klebsiella spp* (16%), 5 *Proteus mirabilis* (20%) and 2 *Salmonella spp* (8%) as shown in Figure 2. The isolates were highly resistant (84 – 100%) to all the antibiotics used, with the exception of amoxicillin clavulanate (56%). Highest rate of resistant was recorded in ceftazidime and nalidixic acid (100%) each. This was followed by erythromycin, cefpodoxime and gentamicin (96%) each, then cefotaxime (92%), norfloxacin (84%) and amoxicillin clavulanate (56%) as shown in Table 1.

Since all isolates have displayed Multiple Drug Resistance (MDR), the 25 isolates were screened for ESBL production. A total of 23 which accounted for 92% out of the 25 were potential ESBL producers (Figure 3). Upon analyzing the 23 isolates for phenotypic ESBL confirmation using the DDST, only 4 (17.0%) were confirmed to be ESBL producers as shown in Figure 4. Expansion of indicator cephalosporin inhibition zone towards antibiotic with clavulanic acid indicates ESBL activity. The frequency for ESBL production among the Gram negative uropathogens showed that *E. coli* were the only ESBL producers.

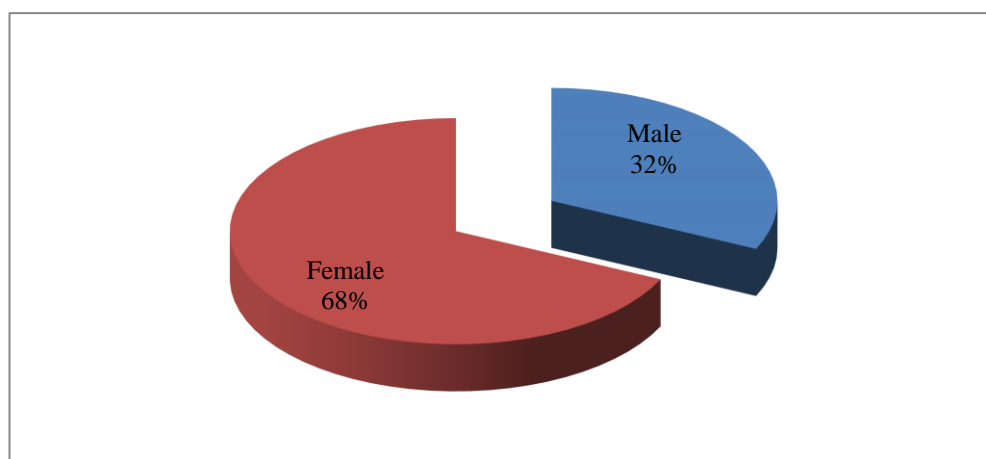


Figure 1: Distribution of isolates based on gender

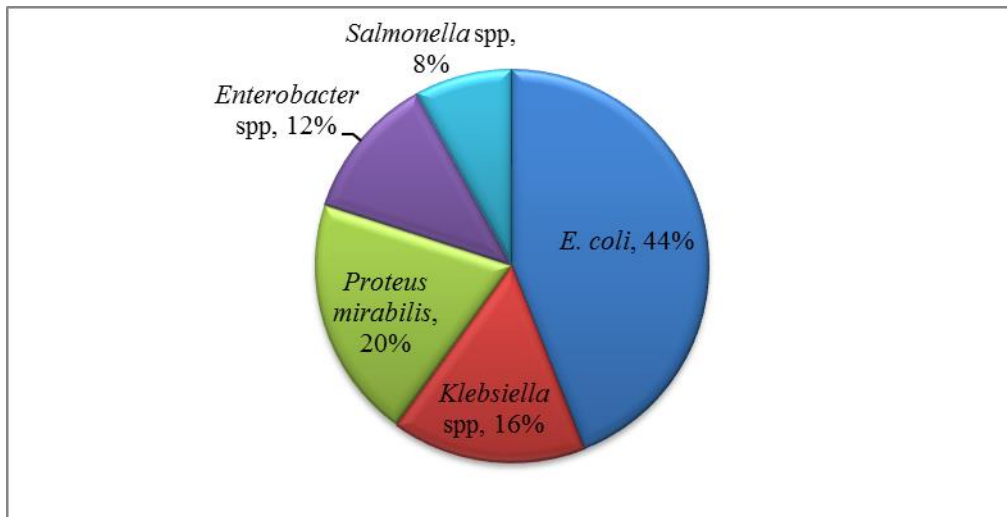


Figure 2: Percentage distribution of Gram negative uropathogens

Table 1: Antibiotic resistance profile of Gram negative uropathogens at SHS

Antibiotic	Potency (µg)	Gram negative uropathogens (%)	
		Resistant	Sensitive
Nalidixic acid	30	25 (100)	0
Cefpodoxime	10	24 (96)	1 (4)
Cefotaxime	30	23 (92)	2 (8)
Ceftazidime	30	25 (100)	0
Erythromycin	15	24 (96)	1 (4)
Gentamicin	30	24 (96)	1 (4)
Amoxicillin clavulanate	30	14 (56)	11 (44)
Norfloxacin	10	21 (84)	4 (16)

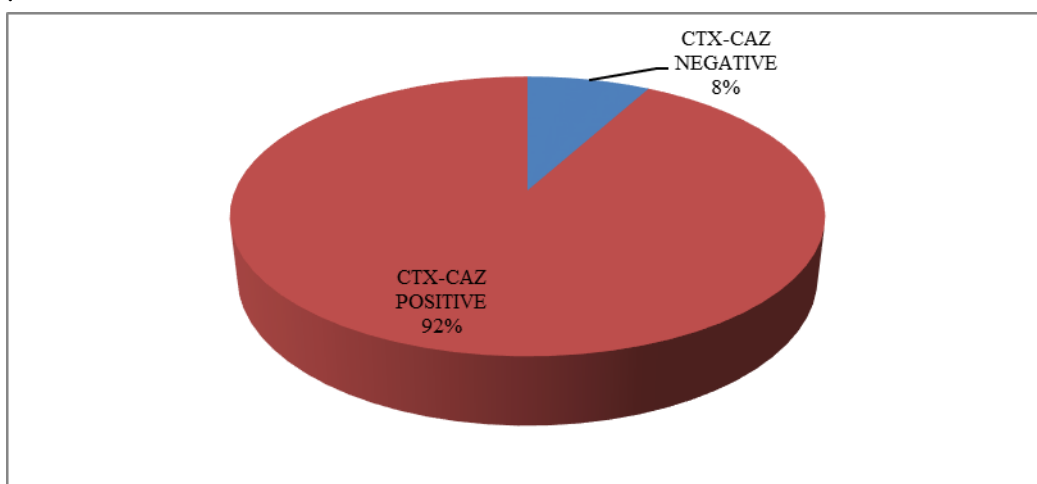


Figure 3: Results of screening for ESBL production in Gram negative isolates using cefotaxime and ceftazidime

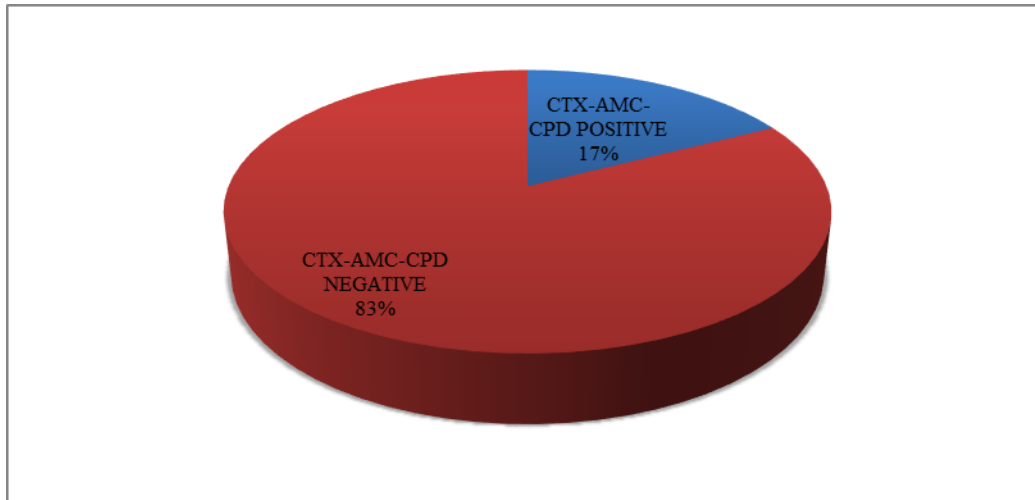


Figure 4: Phenotypic confirmation for ESBL production in Gram negative isolates using DDST

Discussion

Most studies revealed that females were more susceptible to UTI than males during youth and adulthood (Yabaya *et al.*, 2012, Khoshbakt *et al.*, 2013), because the urethra is shorter and more readily transversed by microorganisms and they lack the bacteriostatic properties of prostatic secretions (Al-Jebouri and Mdish, 2013). This study showed that females have a higher prevalence of UTI than males. This is line with most studies where indicated that females are statistically more prone to UTIs than males (Khameneh and Afshar, 2009).

The most frequently isolated urinary pathogens in this study was *E. coli*. The predominance of *E. coli* is in line with results from most studies (Manikandan *et al.*, 2011; Muhammad *et al.*, 2014), identifying *E. coli* as the most prevalent causative organism of UTI. Also, *E. coli* as a commonest cause of UTI may be due to certain virulence factors like haemolysin production and presence of fimbriae (Al-Jebouri and Mdish, 2013).

Faisal *et al.*, 2012 stated that 'there is variation in prevalence of ESBL producing organisms from one place to another and even over time for a given place' (Nuhu *et al.*, 2015). Our study showed that out of 25 bacteria isolates, 17% were ESBL producers. The prevalence of ESBL producing organisms observed in this study was slightly higher than that observed in a previous study carried out in Kano (North-West Nigeria), where ESBL accounted

for 15.4% (Tijjani *et al.*, 2012). Similarly, Osazuwa and Osazuwa (2011) in Benin (South-South Nigeria) reported a prevalence of 16.41% in 2011.

Our findings, also showed the highest frequency of ESBL production in *E. coli* (17%). Tijjani *et al.* (2012), reported a higher prevalence of *E. coli* with 23.3%. Faisal *et al.* (2012), also reported a higher frequency of ESBL production in *E. coli* (68.55%). Venkatadri *et al.* (2014), reported ESBL production in *E. coli* (50.9%). These were far higher than our findings in Sokoto.

Conclusion and Recommendation

Our study confirms ESBL-producing uropathogens in Sokoto like other studies. It is important for Laboratory Scientist to be trained to have the ability to detect and report ESBLs production in clinical isolates. It is also vital that Clinical Microbiologist and Clinicians be updated to effectively manage patients with ESBL-producing uropathogens. When there is adequate detection of ESBL, it will aid in selecting appropriate antimicrobial therapy. This will go a long way in reducing the cost of treatment and threat of resistance.

Conflict of Interest

The authors declare no conflict of interest.



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