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PHENOTYPIC DETECTION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING *Escherichia coli* FROM SUSPECTED CASES OF URINARY TRACT INFECTION IN KANO METROPOLIS, NIGERIA

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ABSTRACT

Urinary tract infections (UTIs) caused by antimicrobial-resistant bacteria, especially ESBL-producing *Escherichia coli* can be life-threatening as therapeutic options available to treat infected patients are limited. Resistance due to ESBL-producing bacteria poses a peculiar challenge in treating infections because of its association with multidrug resistance. *The aim of this study was thus to determine the susceptibility pattern and phenotypic detection of ESBL-producing E. coli from UTI patients.* Two hundred and forty-six (246) *E.coli* isolates obtained from patients with suspected urinary tract infections were studied. The identity of the isolates was confirmed using standard biochemical tests. Antibiotic susceptibility testing was carried out using the Kirby-Bauer Disc Diffusion Technique. Screening for ESBL production was done using the Clinical Laboratory Standards Institute breakpoint. Suspected ESBL producers were subjected to confirmation using the Double Disc Synergy Test. Standard Discs of Augmentin (AMC 30µG Oxoid England), Ceftazidime (CAZ 30µG, Oxoid England) and Cefotaxime (CTX 30µG, Oxoid England) were used for the screening and confirmation. Multidrug-resistant *E.coli* were found to be 65.4%. Screening for ESBL production showed 67.1% suspected ESBLs producing *E.coli.* The Double Disc Synergy Test showed 22.4% confirmed ESBLs producing *E.coli*. Antimicrobial sensitivity of the ESBLs producing organisms showed 100% resistance to augmentin, ceftriaxone, ceftazidime, ciprofloxacin and cefotaxime while resistance to gentamicin was 91.1%, chloramphenicol 89.2%, nitrofurantoin 78.4%, and cotrimoxazole 94.6%. A 100% sensitivity to imipenem was also observed. ESBL-producing *E.coli* are present in Kano metropolis and are resistant to commonly prescribed antibiotics. We, therefore, suggest screening and confirmation for ESBL, to prevent treatment failure.

Keywords: UTI, *Escherichia coli*, Extended-spectrum beta-lactamase, 3rd Generation Cephalosporins, Antimicrobial susceptibility, Imipenem

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common infectious diseases worldwide with about 250 million people being diagnosed with UTIs each year (Ronald *et al*., 2001). The causative agents in UTIs are majorly Gram-negative pathogens, primarily Enterobacteriaceae with *Escherichia coli* being the most predominant, followed by *Klebsiella pneumonia* and Proteus *mirabilis* (Tenney *et al*., 2018). Several studies demonstrate how the use of antibiotics influences both the development and emergence of resistant microorganisms. Uropathogenic *E. coli* has shown increasing antimicrobial resistance to most antibiotics, particularly cephalosporins due to the production of enzymes known as Extended spectrum β- lactamases (Miranda *et al*., 2004; Al janaby *et al*., 2017; Khalid *et al*., 2017).

ESBLs are β-lactamases which can hydrolyze oxyimino-cephalosporins and are inhibited by beta-lactamase inhibitors (Bust *et al*., 1995). The overuse and misuse of antibiotics have led to an increase in the incidence of ESBL-producing *E. coli* globally (Chauhan *et al*., 2015). In many parts of the world, 10%-40% of strains of *E. coli* and *K. pneumoniae* express ESBLs (Rupp and Fey, 2003). In Nigeria, prevalence rates range from 5% to 44.3% as shown by (Olonitola *et al*., 2007; Olowe and Aboderin, 2010; Yushau *et al*., 2010; Akujobi and Ewuru, 2010; Ogefere *et al*., 2015; Mohammed *et al*., 2016). With the change in the aetiology of UTI and the antibiotic resistance pattern of uropathogens over the past years, we are left with limited therapeutic options to treat Urinary Tract Infections.

Therefore, this study was carried out to determine the frequency, and resistance pattern of ESBL-producing *E.coli* from suspected cases of UTIs in Kano metropolis using phenotypic techniques. The current study will be extremely helpful for the empirical treatment and prudent management of UTI patients. Additionally, it will support the relevant authorities in enhanced antibiotic prescribing strategy planning.

MATERIALS AND METHODS

Sample collection and analysis

The study was conducted on 246 isolated uropathogenic *E.coli* collected from Aminu Kano Teaching Hospital, Murtala Muhammad Specialist Hospital and Muhammad Abdullahi Wase Teaching Hospital from patients with suspected UTIs between January and July 2017 following approval from the Aminu Kano Teaching Hospital Ethical Committee and Kano State Ministry of Health.

Urine microscopy was carried out using a drop of uncentrifuged urine to determine significant pyuria. The samples were inoculated on Cysteine Lactose Electrolyte Deficient Agar and incubated at 37^0C for 18-24 hours. Discrete colonies were picked, and Gram staining was carried out. All isolates were identified at the species level using standard biochemical tests (Cheesebrough, 2000).

Antibiotic susceptibility testing

This was carried out using the Kirby-Bauer-CLSI modified Disc Agar Diffusion technique (DAD). One millilitre (1.0 ml)

of a standardized overnight culture of each isolate (containing 10^6 CFU/ml) was used to flood the surface of Mueller Hinton Agar (MHA) plates and excess drained off and dried while the Petri dish lid was in place. The standard antibiotic discs were then aseptically placed at reasonable equidistance on the inoculated MHA plates and allowed to stand for 1 h. The plates (prepared in duplicates for each isolate) were then incubated at 37°C for 18 hours. The diameter of the zones of inhibition produced by each antibiotic disc was measured and recorded (CLSI, 2010)

The following oxoid antibiotic discs were used; Amoxicillin/Clavulanic acid (20/10µg), Ceftriaxone CRO (30µg), Cefotaxime CTX (30µg) Ceftazidime CAZ (30µg), Ciprofloxacin CIP (10µg), Chloramphenicol C (30µg), Gentamicin CN (10µg), Cotrimoxazole STX (1.25/23.75µg), Imipenem (10µg), Nitrofurantoin (300µg).

Extended Spectrum Beta Lactamase Screening Test

Screening for ESBL production by disc diffusion test

Resistance to cefotaxime ceftriaxone and ceftazidime was detected by disc diffusion test as recommended by (CLSI, 2016). From the pure cultures of bacteria grown overnight on MacConkey agar, a suspension matching 0.5 McFarland standard (1.5 x 10^8CFU/ml) was made in nutrient broth. Using sterile cotton swab, the bacteria was spread on Mueller Hinton agar to obtain a lawn culture. After allowing the plate to dry, the antibiotic discs were placed on the surface and the plates incubated at 37ºC for 18-24 hours. Following growth, the diameter of the zone of inhibition around the discs were measured and recorded. The disc potency and zone diameters for inferring resistance were as follows; cefotaxime (30µg) ≤27mm, ceftriaxone (30 µg) ≤25 mm, ceftazidime (30 µg). Resistance to at least one of the antibiotics will be considered positive in the screening test for possible ESBL production (CLSI, 2016)

Confirmation of ESBL production by Double Disc Synergy Test (DDST)

Double Disc Synergy Test was carried out using 3 antibiotics, namely amoxicillin-clavulanic acid (20/10µg), cefotaxime (30µg) and ceftazidime (30µg). The discs were placed 25mm (centre to centre of the discs) from amoxicillin-clavulanic acid on Mueller Hinton Agar. Enhancement of the zone of inhibition towards the clavulanate disc after 24 hours of incubation at 37°C was considered indicative of a potential ESBL producer (NCCLS, 2000)

Statistical analysis

The Chi-square test was used to compare differences for categorical data by using SPSS statistics 128 (version 17) program (IBM Corporation, NY, USA). A P-value of < 0.05 was considered indicative of statistical significance

RESULTS AND DISCUSSION

A total of one thousand five hundred (1500) suspected urine samples were collected from three hospitals in Kano metropolis; Aminu Kano Teaching Hospital (AKTH), Muhammad Abdullahi Wase Teaching Hospital (MAWSH), Murtala Muhammad Specialist Hospital (MMSH). Out of the 1500 samples, 500 were obtained each from AKTH, MMSH and MAWSH.

Table 1 shows the distribution of samples across the hospitals based on gender. There is a significant difference between genders at the different sampling sites (p value= 0.0178) with the female population having a higher rate of suspected UTIs.

Table 1: Distribution of samples across the hospitals based on gender

	Sampling site			
Gender	**AKTH**	**MMSH**	**MAWSH**	**Total**
Male	106 (21.2%)	153 (30.6%)	127 (25.4%)	386
Female	394 (78.8%)	347 (69.4%)	373 (74.6%)	1114
Subtotal	500	500	500	1500
P<0.05				

From the 1500 samples collected, 621 samples showed growth of organisms. A total of 246 *E.coli* isolates where obtained from the different sampling sites. Murtala Muhammad Specialist Hospital had the highest number of E. coli isolates 127(51.6%). Table 2 below shows the distribution of *E.coli* isolates across the sampling sites.

Table 2: Distribution of *E.coli* isolates across the sampling sites

	Sampling site			
	AKTH (%)	**MMSH (%)**	**MAWTH (%)**	**Total**
Organism				
E. coli	71(29.9%)	127(51.6%)	48(19.5%)	246

Based on the CLSI breakpoint for screening ESBL production using ceftriaxone, ceftazidime and cefotaxime, Table 3 below shows the distribution on the isolates as suspected ESBLs and non-ESBLs with 67.1% of *E. coli* being suspected ESBL producers.

Table 3: Distribution of Suspected ESBLs producing *E.coli* based on CLSI breakpoint

Isolates	Number of isolates (%)
Suspected ESBLs producers	165 (67.1)
Non ESBLs producers	81 (32.9)
Total	246

Table 4 shows percentage distribution of confirmed ESBL producers using the Double Disc Synergy Test. ESBL production was confirmed in 22.4% of *E. coli* isolates.

Table 4: Distribution of Confirmed ESBLs and Non-ESBLs *E. coli* using Double Disc Synergy Test

Isolates	Number of isolates (%)
Confirmed ESBLs producers	37 (22.4)
Non ESBLs producers	128(77.6)
Total	165

The percentage distribution of confirmed ESBLs across the three hospitals is shown in Table 5 with AKTH having the highest percentage of ESBLs producing *E. coli* isolates

Table 5: Percentage distribution of ESBLs across the different hospitals

Organism	**Sampling site**			
	AKTH (%)	**MMSH (%)**	**MAWTH (%)**	**Total**
E. coli	30 (81.1%)	0 (0%)	7 (18.9%)	37

The antimicrobial susceptibility pattern of the ESBLs-producing *E. coli* is shown in Table 6 with 100% of the isolates being resistant to ceftazidime, cefotaxime, augmentin and ceftriaxone. All the isolates were sensitive to imipenem. Resistance to gentamicin, chloramphenicol, nitrofurantoin, ciprofloxacin and cotrimoxazole was 91.9%, 89.2%, 78.4%, 97.3% and 94.6% respectively.

Table 6: Antimicrobial Susceptibility Pattern of ESBLs producing *E. coli* (n=37)

	Organism	
	E.coli	
Antibiotic	Sensitive (%)	Resistant (%)
Gentamicin (10µg)	3(8.1)	34(91.9)
Ceftazidime (30µg)	0 (0)	37 (100)
Chloramphenicol(30µg)	4 (10.8)	33(89.2)
Cefotaxime(30µg)	0 (0)	37(100)
Nitrofurantoin(300µg)	12 (32.4)	29(78.4)
Ciprofloxacin (5µg)	1 (2.7)	36(97.3)
Augmentin (30µg)	0(0)	37(100)
Imipenem (10µg)	37 (100)	0 (0)
Ceftriaxone(30µg)	0 (0)	37(100)
Cotrimoxazole(1.25/23.75µg)	2 (5.4)	35(94.6)

Discussion

In this study, a total of 1500 urine samples were collected from 3 hospitals in Kano metropolis, 386 from males and 1114 from females, out of which 621 showed bacterial growth. There was a significant difference between samples collected from males and females. This finding correlates with other reports which showed that females are more prone to UTIs than males (Griebling 2007; Randrianirina *et al*., 2007; Fauci *et al*., 2008; El Mahmood, 2009; Habte *et al*., 2009; Yismaw *et al*., 2012; Ahmad 2012; Anejo-okopi *et al*., 2015). The higher frequency in females has been attributed to the shorter female urethra and its proximity to the gastrointestinal outlet hence making it easier for enteric flora to colonize the area (Salvatore *et al*., 2011). Other contributing factors may include the use of contraceptives, childbirth and menopause.

E. coli dominated the uropathogens seen in this study; similar results have been reported (Ehinmidu 2003; El Mahmood 2009; Mansour *et al*., 2009; Pondei *et al*., 2012; Iregbu and Nwajiobi-Princewill 2013 Alanazi *et al*., 2018) and Adabara *et al*.,2020).

In this study, the distribution of confirmed ESBLs producing *E.coli* was 22.4% as opposed to the suspected number of 67.1%. A similar drop in the number of suspected and confirmed ESBLs production has been reported by Yushau *et al* (2010) which could be due to the production of multiple β lactamases leading to an interference with the results (Yushau *et al*., 2010). A higher occurrence was reported by Yushau *et al* (2010) where *E. coli* was reported to be 42.6% In another study in Pakistan, 56.9% of isolates of *E. coli* were ESBL positive (Ullah *et al*., 2009) and in a study in India, nearly 40% of urinary isolates of *E. coli* were ESBL positive (Babypadmini and Appalaraju, 2004). Mekki *et al* (2010) reported ESBL producing *E.coli* was 53% from the patients suffering from Urinary Tract Infections. Ejaz *et al* (2011) reported ESBL-producing *E. coli* to be 57.4% in Pakistan. The differences in the results could be due to different numbers of samples, recent antibiotic usage and hospitalization

Distribution of ESBLs across the hospitals showed that AKTH had the highest occurrence of ESBLs producing *E. coli* at 81.1%. The spread of an ESBL variant can be facilitated by a referral system where the presence of a single ESBL variant in a different centre may be imported by a patient on referral to another centre (Nordmann *et al*., 2009). This situation could hold for AKTH because it is a tertiary referral centre receiving patients from different parts of northwestern Nigeria.

In this study, ESBL-producing isolates were highly resistant to antibiotics (both β lactams and non β lactams). This suggests cross-resistance induced by the ESBL enzymes to the isolates against non β lactam antibiotics. This suggests cross-resistance induced by the ESBL enzymes to the isolates against non β lactam antibiotics such as gentamicin, nitrofurantoin, cotrimoxazole and ciprofloxacin (Bonnet, 2004). Imipenem was the only antibiotic that showed activity against 100% of the ESBLs producing *E.coli*, followed by nitrofurantoin which had activity against 32.4% of the isolates. A similar activity has been reported by Chaturvedi *et al*., (2020).

CONCLUSION

This study has shown a higher occurrence of UTIs in females than males with *Escherichia coli* being the most frequently isolated organism. A high resistance among *E. coli* isolates from UTIs to commonly prescribed antibiotics in Kano metropolis has been shown. AKTH had the highest number of ESBL-positive isolates. Imipenem has excellent performance against ESBL positive isolates and is therefore recommended as a treatment of choice for confirmed ESBL producers. Furthermore, a high rate of resistance has been developed in uropathogens with empiric antibiotic treatment, including cotrimoxazole, fluoroquinolones, amoxyclav and broad spectrum cephalosporins. Therefore, proper screening is needed for ESBLs detection in laboratories. It is of utmost importance that antibiogram report must mention clearly whether the isolate is a doubtful or a confirmed ESBL producer. The report should also state that irrespective of their in vitro susceptibility, production of ESBL can render β-lactam antibiotics therapeutically ineffective. Efficient infection control measures like barrier precautions and hand washing should be made mandatory to lessen the pervasiveness of antimicrobial-resistant pathogens, including ESBL producing *E. coli*.

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