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Detection of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* from Urinary Tract Infection in General Hospital, Minna

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Abstract

Possession of virulence factors such as extended spectrum beta lactamase (ESBL) by pathogens generally exacerbates morbidity as well as increases healthcare costs. This study determined the production of ESBL among *Escherichia coli* isolated from the urinary tracts of subjects attending General Hospital, Minna, Nigeria. One hundred and fifty (150) urine samples were collected from subjects and cultured on CLED agar for the isolation of *E. coli.* Antibiotic susceptibility testing was done using Kirby Bauer disk diffusion method, while the phenotypic identification of ESBL-producing strains was carried out using double disk synergy test. All results were interpreted based on Clinical and Laboratory Standards Institute guidelines. In all, 26 (17.33%) out of the 150 urine samples cultured had *E. coli;* out of which 23 (88.5%) were subsequently found to be ESBL producers. Among the *E. coli* isolates, high rates of antibiotic-resistance was observed against nalidixic acid (100%), cefdinir (88.4%), cefotaxime (84.6%) and cefpodoxime (84.6%), while remarkable sensitivity to tarivid (46.1%), ciprofloxacin (38.4%) and gentamicin (34.6%) was also detected. This study has established the involvement of ESBL-producing *E. coli* in urinary tract infections in the study area. Rational prescription of antibiotics against pathogens generally is highly recommended to halt the spread of resistance.

Keywords: Beta-Lactamase, Extended spectrum, Infection, Prescription, Resistance.

Introduction

Urinary tract infection (UTI) which results from the microbial infections of either the lower or the upper urinary tracts known as acute cystitis and pyelonephritis, respectively, remains a public health challenge worldwide (Gupta et al. 2017). UTI is the commonest bacterial infection among females and males. It is expected that about 35% of healthy women experience warning signs of UTIs (Nithyalakshmi 2014). The incidence is far higher in women than men due to the proximity of the vagina to the anus which provides for easy contamination with faecal flora and also as a consequence of pregnancy (Yadav and Prakash 2017). The incidence of UTI in developing countries is quite high and this may not be unconnected with the dearth of social and sanitary infrastructures like toilets and pipe borne

water, poor sanitation, ignorance and poverty. About 95% of cases of UTI worldwide have bacterial aetiology (Ramesh et al. 2008) out of which only about 15–20% are caused by Gram positive bacteria making the Gram negative bacteria as the main aetiology of bacterial UTI (Babu et al. 2014). Among the Gram negative bacteria frequently implicated in UTI, *E. coli* is the most frequently encountered.

In recent years, the threat of antibiotic resistance among *E. coli* and other *Enterobacteriaceae* has generated great concern within the medical community. This development is especially frightening because of a concurrent significant increase in community-acquired infections caused by extended-spectrum β-lactamase (ESBL) or AmpC β-lactamase-producing *E. coli* strains which have also been observed worldwide (Simner et al. 2011). In addition to being resistant to most cephalosporins, these *E. coli* strains are often co-resistant to fluoroquinolones and other first-line antibiotics. For patients infected with these drug-resistant *E. coli* strains, adequate antibacterial therapy easily gets delayed as treatment options for infections caused by these organisms such as urinary tract infections are often limited and the therapy complicated (Voets et al. 2012, Lin et al. 2019).

Extended-spectrum beta-lactamases (ESBLs) are enzymes produced by certain bacteria which confer on them the ability to resist most common beta-lactam antibiotics such as penicillins, cephalosporins and monobactam (Picozzi et al. 2014, Ghafourian et al. 2015). Among the ESBL producing *Enterobacteriaceae*, *E. coli* has emerged again as the most frequently encountered.

Ever since the discovery of the first plasmid mediated beta lactamase among *E.* coli in 1965, ESBL producing *E. coli* has increased worldwide and it is now one of the most common causes of hospital-acquired infections with increased morbidity and mortality rate (Kim et al. 2017). Community acquired urinary tract infections caused by extended spectrum beta lactamase producing organisms is on the rise throughout the world (Dayan et al. 2013). Increasing evidence has suggested that ESBL producing organisms commonly retain resistance factors against other classes of antibiotics, especially the quinolones and aminoglycosides. This could be attributed to the association of multi drug resistance in ESBL producing isolates (Kumar et al. 2014). There are known risk factors such as age (<1 year), children on uro-prophylaxis, recurrent UTI and recent antibiotic usage which can predict the occurrence of the ESBL producers (Balasubramanian et al. 2018).

The rapid identification and characterization of resistant organisms, especially ESBL producing organisms that are evidently associated with greater morbidity and mortality, is therefore an important assignment for laboratories in the fight against microbial resistance. This study attempted to isolate and identify ESBL producing *E. coli* from subjects with urinary tract infections in Minna.

Materials and Methods

Study area

Minna is a city in North central region of Nigeria with an estimated population of 304,113 in 2017.

Ethical consideration

Ethical clearance was obtained from the Ethics and Research Committee of the General Hospital Minna, Niger State, while samples collection from the participants was based on informed consent.

Inclusion and exclusion criteria

Patients with medical history of nausea, painful micturition, haematuria, pelvic inflammation, pain or pressure in back or lower abdomen were included with the exclusion of patients who refused to give their consent.

Collection of samples

Urine samples were collected from a total of 150 patients attending General Hospital Minna, Niger State, Nigeria in sterile sample containers and transported immediately to the Microbiology Laboratory of the Federal University of Technology Minna, Niger State for processing.

Isolation and identification of *E. coli* from urine

Each urine sample collected was aseptically inoculated on Cystine-Lactose-Electrolyte Deficient (CLED) agar using sterile loop and incubated at 37 °C for 24 hours. Distinct colonies on growth from the primary culture were sub-cultured on MacConkey agar and further incubated at 37 °C for another 24 hours to obtain pure culture. Pure cultures obtained were stored in slant bottles and kept in the refrigerator for further tests. The identification of *E. coli* isolates was done using morphological appearance of the colonies, Gram stain reactions and biochemical properties.

Antimicrobial susceptibility testing (AST)

The modified Kirby Bauer disk diffusion method was used to determine the susceptibility of the isolates to the commonly prescribed antibiotics in the General Hospital, Minna, namely ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefixime (5 µg), cefpodoxime (10 µg), cefdinir (5 µg), ciprofloxacin (5 µg), tarvid (5 µg), gentamicin (10 µg) and nalidixic acid (30 µg). The antibiotic disks which were of analytical grade were obtained from high media laboratories. A 5 ml suspension of each isolate was prepared in sterile normal saline to a turbidity of 0.5 McFarland standard (10^{-8} cfu/mL). The suspension was then inoculated aseptically over the surface of Mueller-Hinton agar plate and antimicrobial disks were placed on the plates using sterile forcep. The plates were incubated at 37 °C for 24 hours. The diameter of zones of inhibition were measured and interpreted according to CLSI standard interpretation chart (CLSI 2018).

Phenotypic screening for the production of extended spectrum beta lactamase enzymes

Screening for the production of Extended Spectrum Beta Lactamases was done using double disc synergy test (DDST). A disk containing amoxicillin + clavulanic acid (30 µg/10 µg) was placed centrally on a Mueller-Hinton agar plate previously swabbed with the test isolate, while third-generation cephalosporin antibiotics: ceftazidime (30 µg) and cefotaxime (30 µg) disks were placed 20 mm around the amoxicillin + clavulanic acid disk. The plates were incubated at a temperature of 37 °C overnight. A clear extension of the edge of the zone of inhibition of cephalosporins toward the amoxicillin-clavulanic acid disc was interpreted as positive result for ESBL production.

Results

Prevalence of UTI among suspected respondents

Out of the 150 samples screened comprising 63 and 87 males and females, respectively, 26 (17.3%) were positive for *E. coli* with males having 8 (12.7%) against females 18 (20.7%) as shown in Table 1. The distribution of infections on the basis of age revealed the highest prevalence for *E. coli* among age group 11-20 (24.2%), followed by 51 and above (16.7%). Age groups 31–40, 21–30, and 0–10 had prevalence rates of 16.0%, 15.6%, and 13.3%, respectively, while the least prevalence was observed in the age group 41–50 (12.0%).

Table 1: Gender distribution for *E. coli* isolates

Gender	Number of samples	Number of positive samples	Percentage (%)
Female	87	18	20.7
Male	63	8	12.7

Table 2: Age distribution for *E. coli* isolates

Age (Years)	Number of samples	Number of positive samples	Percentage (%)
0-10	15	2	13.3
11-20	33	8	24.2
21-30	45	7	15.6
31-40	25	4	16.0
41-50	18	3	12.0
51 and above	14	3	16.7

Antibiotic susceptibility pattern of the *E. coli* isolates

The isolates mostly resisted nalixidic acid (100.0%), cefdinir (88.4%), cefpodoxime (84.6%), cefotaxime (84.6%), cefixime (80.7%) and ceftriaxone (76.9%) but exhibited varying sensitivity to tarivid (46.1%). ciprofloxacin (38.4%) and gentamycin (34.6%). The observations are presented in Table 3.

Table 3: Antibiotic susceptibility pattern of *E. coli* isolates

Antibiotics	Sensitive	Percentage of isolates (%) Intermediate	Resistance
Ceftazidime	19.2	19.2	61.5
Ceftriaxone	15.3	7.6	76.9
Cefotaxime	3.8	11.5	84.6
Cefixime	7.6	11.5	80.7
Cefpodoxime	7.6	7.6	84.6
Cefdinir	7.6	3.8	88.4
Ciprofloxacin	38.4	19.2	42.3
Gentamicin	34.6	7.6	56. 9
Tarivid	46.1	15.3	38.4
Nalixidic acid	0.0	0.0	100.0

Presence of ESBL in *E. coli* isolates

Out of the 26 *E. coli* isolates that exhibited resistance against the third generation cephalosporin, 23 (88.5%) were ESBL producers, while 3 (11.5%) were non-ESBL producers as presented in Table 4.

Table 4: Presence of ESBL in *E. coli* Isolates

Isolates	Number of isolates (%)
ESBL-producer	23 (88.5)
Non–ESBL-producer	3 (11.5)
Total	26

Discussion

In this study, a relatively high recovery of *E. coli* isolates was observed among the 150 urine sample screened. This observation is clearly in agreement with the prevailing reports from Mansour et al. (2009) and Alanazi et al. (2018) that the organism is among the most frequently isolated in urine samples. This observation may not be unconnected with the presence of *E. coli* in stool being a resident flora in the gastrointestinal tract as a result of which it does not require sexual activity for its

transmission to occur. It was also observed that the female subjects had higher carriage rate (20.7%) of *E. coli* than males (12.7%). This is in agreement with the earlier findings of Alqasim et al. (2018) and Yadav and Prakash (2017), in which higher carriage rates of *E. coli* were reported in females compared to the males. High prevalence of UTI in women has been generally attributed to the closeness of the urethra to the anus and their complex physiology especially during gestational periods. Certain forms of contraceptives used by females are also believed to encourage the occurrence of UTI. An age dependent distribution of the prevalence of *E. coli* infection revealed that the age groups 11-20 (24.2%) and 41-50 (12.0%) years had the highest and the lowest rates, respectively.

The isolates exhibited varying antibiotic resistance against the antibiotics tested. The highest resistance was recorded in the order: nalixidic acid (100%), cefdinir (88.4%), cefotaxime (84.6%) cefpodoxime (84.6%), and cefixime (80.7%). The highest antibiotic sensitivity was observed with tarivid (46.1%), ciprofloxacin (38.4%) and gentamicin (34.6%). In similar studies, Yadav and Prakash (2017) observed the highest resistance with ceftriaxone (67.47%), ceftazidime (63.41%) and nalidixic acid (73.98%), Fernando et al. (2017) observed the highest resistance with ceftriaxone (100%), ceftazidime (100%) and ciprofloxacin (90.10%), while Hassuna et al. (2020) record 100% resistance to ceftazidime, cefotaxime and cefpodoxime and susceptibility to gentamycin and ciprofloxacin at 73.75% and 86.25%, respectively. The high rate of resistance in this study was ascertained to be due to the production extended spectrum beta lactamase by almost all the *E. coli* isolates.

Out of the 26 *E. coli* isolates, 23 (88.5%) were ESBL-producers, while 3 (11.5%) were non-ESBL producers. Singh et al. (2016), Datta et al. (2014), Kim et al. (2017) and Rajabnia et al. (2019) reported prevalence of ESBL-producer as 46.87%, 82.6%, 21.4% and 37.11%, respectively. In Kano, North-western Nigeria, a prevalence of 15.4% for ESBL producers was earlier reported (Tijjani et al. 2012). The differences in the results could be due to different numbers ofsamples, recent antibiotic usage and hospitalization. However, the findings of this study even though limited by its restriction to a single hospital, has further lend credence to the growing concerns about the spread of ESBL producing bacteria, especially *E. coli* within hospital settings. In a study carried out in Spain, a rise in ESBL by *E. coli* producers from less than 0.36% to 4.8% was recorded only within seven years from 1995 to 2002 (Romero et al. 2005).

Conclusion

While the findings of this study are inadequate to make a generalized statement because of its restriction to a single hospital, a dramatic increase in the prevalence of ESBL-producing *E. coli* was however observed. Given its consequences which include longer hospitalization, increased hospital expenses, reduced rates of therapeutics responses and higher mortality, a national surveillance of their prevalence and antibiotic susceptibility pattern as well as strict compliance with antibiotics policy and rational antibiotics usage are strongly recommended.

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