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Antibiotics susceptibility profile of extended beta-lactamase-producing *Escherichia coli* in urine samples from pregnant women attending Ajikobi Hospital, Ilorin, Kwara state

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Abstract

One of the most frequently acquired infections is urinary tract infection and Escherichia coli is a major causative pathogen. This study aims to determine the prevalence and antibiotic susceptibility of E. coli and extended-spectrum beta-lactamases (ESBLs) producing E. coli in urine samples of antenatal pregnant women attending Ajikobi Hospital in Ilorin, Nigeria. The study was conducted on urine samples from 117 consented pregnant women attending the antenatal clinic between January and October 2022. They were screened for Escherichia coli and Extended-spectrum β-lactamase producing E. coli using standard microbiological procedures such as growth on Eosin methylene blue, Gram staining, and biochemical tests. Antibiotic susceptibility testing was done by the modified Kirby-Bauer protocol while ESBL production was determined by the Double Disk Synergy Test (DDST). A total of 117 samples were screened, 26 (22.2%) showed significant bacteriuria, and 18 (69%) E coli was isolated. The age distribution shows that 15- 20 years has a prevalence rate of 23.1%, 21- 25 years (24.1%), 26- 30 years (25%), 31-35 (21.6%), and women above 35 years (27.3%). Antibiotic sensitivity profile shows that 16.7% were resistant to gentamicin, 66.7% to cefuroxime, 27.8% to meropenem, 11.1 % to ceftriaxone, ceftazidime (33.3%), 16.7% to amoxicillin/clavulanic, 22.2 % to ciprofloxacin and 100% susceptibility to both ofloxacin and nitrofurantoin. The study shows the prevalence of ESBL-producing E. coli and the high susceptibility of nitrofurantoin and ofloxacin.

Keywords: *E. coli*, Extended Beta Lactamases; Ilorin.

INTRODUCTION

Globally urinary tract infections (UTIs) affect both inpatient and outpatient individuals however patients with structural abnormalities or comorbidities including diabetes, advanced age, pregnancy, or immunocompromised condition are more likely to have complicated infections (Medina *et al.,* 2015). Women are more likely to have urinary tract infections because of pathogenic organisms indigenous to the rectum and perineum (Ala-Jaakkola *et al.,* 2022). Additionally, women's urethras are shorter than men's, which increases their vulnerability to UTIs (Zare *et al.,* 2022). Only a few UTIs are caused by blood-borne bacteria and *Escherichia coli* are by far the two most frequent bacteria found in UTIs (Fazly Bazzaz *et al.,* 2021).

The use of a urinary catheter is a significant UTI risk factor, others include sexual activity, the use of spermicides, diaphragm usage, frequent pelvic examinations, vesicoureteral reflux, the use of immunosuppressive medications, diabetes mellitus, and use of antibiotics (Aggarwal and Lotfollahzadeh, 2022; Bono *et al.,* 2022). Although UTI mortality rates are modest however morbidity is very high (Yang *et al.*, 2022). The World Health Organization declared antimicrobial resistance (AMR) as a major global public health problem (Dadgostar, 2019). The dissemination of multidrug-resistant (MDR), extended-spectrum β-lactamase (ESBL)-producing Enterobacterales is of particular concern limiting treatment options for invasive infections to last-line antibiotics (Igbinosa *et al.,* 2023).

Extended-spectrum β-lactamase are enzymes that hydrolyze an extended spectrum of β-lactam antibiotics inclusive of penicillins and oxyimino-cephalosporins, but excluding cephamycins (Estaleva *et al.,* 2021). The most widely circulated ESBL enzymes are CTX-Mtype β-lactamases, which selectively hydrolyze cefotaxime and Plasmid-mediated AmpC β-lactamases (pAmpCs) are also a significant cause of broad spectrum β-lactam resistance in many Gram-negative bacteria (Zeynudin *et al.,* 2018). Furthermore, most Enterobacterales have chromosomally-encoded AmpC-β-lactamases while many AmpC-genes have also been mobilized from their chromosomal origin and may be carried by plasmids (pAmpC), with blaMOX/FOX/DHA/CMY known to be the most widely distributed variants (Estaleva *et al.,* 2021).

This study seeks to determine the prevalence and antibiotics susceptibility profile of extended beta-lactamase-producing *E. coli* in urine samples of antenatal pregnant women at a secondary health center in Ilorin, Nigeria

MATERIALS AND METHODS

Area of Study

The study was conducted at Ajikobi Cottage Hospital, Okekere in Ilorin West Local Government of Kwara State. Ilorin, the study area is approximately on lat 8030'N of the equator and long 4035'E of the Greenwich Meridian has an area of about 100km2.

Ethical consideration

Ethical approval was obtained from the Kwara State Ministry of Health and all participants gave their consent to participate in the study.

Collection of samples

Samples were collected and screened as previously described (Bale *et al.*, 2021). *Escherichia coli* was subsequently identified and confirmed using standard laboratory procedures such as growth on Eosine methylene blue, MacConkey agar, Gram staining, colony morphology on catalase, indole, Methyl Red, but negative for oxidase, Voges-Proskauer, Simmon citrate, Urea, and TSI test.

Escherichia coli were screened and confirmed for extended-spectrum beta-lactamases (ESBLs) activity by Clinical and Laboratory Standards Institute guidelines (CLSI) (Yadav *et al.,* 2015). Initial ESBL activity was determined by screening cefotaxime (CTX 30µg), ceftazidime (CAZ: 30µg), and ceftriaxone (CRO: 30µg) using Mueller Hinton agar (MHA: Oxoid, UK) already inoculated with the isolates (Mofolorunsho *et al.,* 2021).

To improve the sensitivity of ESBL detection, more than one antibiotic disc was used as recommended by CLSI guidelines (Kumar *et al.,* 2014). Freshly grown colonies were suspended into normal saline and the turbidity of the suspension was adjusted at 0.5 McFarland's standard. The suspension was subsequently inoculated onto Mueller Hinton agar (MHA: Oxoid UK) with all three discs placed at a gap of 20mm. Plates were then incubated for 18 hours at 37^{o}C. Isolates with reduced susceptibility to cefotaxime (zone diameter of ≤ 27mm), ceftazidime (zone diameter of ≤ 22mm), and ceftriaxone (zone diameter of ≤ 25mm) around the discs were suspected to be ESBLs producers. The double-disc synergy method was employed for the confirmation of suspected ESBL producers. This was done by testing the following antibiotic discs; cefotaxime (CTX 30µg), ceftazidime (CAZ 30 µg), and amoxycillin+clavulanic acid (AMC 30 µg) on Mueller Hinton agar (MHA: Oxoid, UK). Amoxycillin+clavulanic acid disc was placed in the center of the Mueller Hinton agar plates. Cefotaxime and ceftazidime were placed at a distance of 20mm from the amoxycillin+clavulanic acid disc. Plates were then examined after incubation for 24 hours at 37^{0}C for an expansion of the inhibition zone of the oxyimino-β -lactam caused by the synergy of the clavulanate in the amoxycillin+clavulanic acid disc which was interpreted as ESBLs positive (Teklu *et al.,* 2019).

Antibiotic Susceptibility Test

Susceptibility testing of isolates was performed using the disc diffusion method. Isolates were enriched in peptone water for 24 hours after which 0.1ml was streaked onto Mueller Hinton agar (Oxoid, UK). The following antibiotics were used; ciprofloxacin (CIP 5µg), cefuroxime (CXM 30µg), ceftazidime (CAZ 30µg), gentamicin (GEN 10µg), cefotaxime (CTX 30µg),

amoxycillin+clavulanic acid (AMC 30 µg UK), nitrofurantoin (NIT 50 µg), ofloxacin (30 µg) and meropenem (MEM 10µg). Results were interpreted as resistant or susceptible based on the interpretative standard according to the clinical and laboratory standards institute (Malande *et al.,* 2019; Yarbrough *et al.,* 2020).

RESULTS

Age distribution of participants in the study showed that 11.11 percent of the total participants are between the ages of 15-20 years, 24.78 percent are between 21-25 years, 13.69 percent are 26-30 years of age, 31.62 percent are 31-35 years of age, and 18.8 percent are 35 years and above. This implies that the majority of the participants who participated in this study were between the age of 31-35 years.

Table 1: Age distribution of *E coli* isolated from the urine of antenatal pregnant women

Age group (years)	Total sample	Bacteriuria	*E. coli*
15-20	13	3	1
21-25	29	7	4
26-30	16	4	3
31-35	37	6	6
Above 35	22	6	4

The study revealed that 45.29 % of the respondents were illiterates, 16.23% were primary school students, 29.05% were secondary school students and 9.4% of the respondents are tertiary students. It also shows a high level of lack of western education in the area under study.

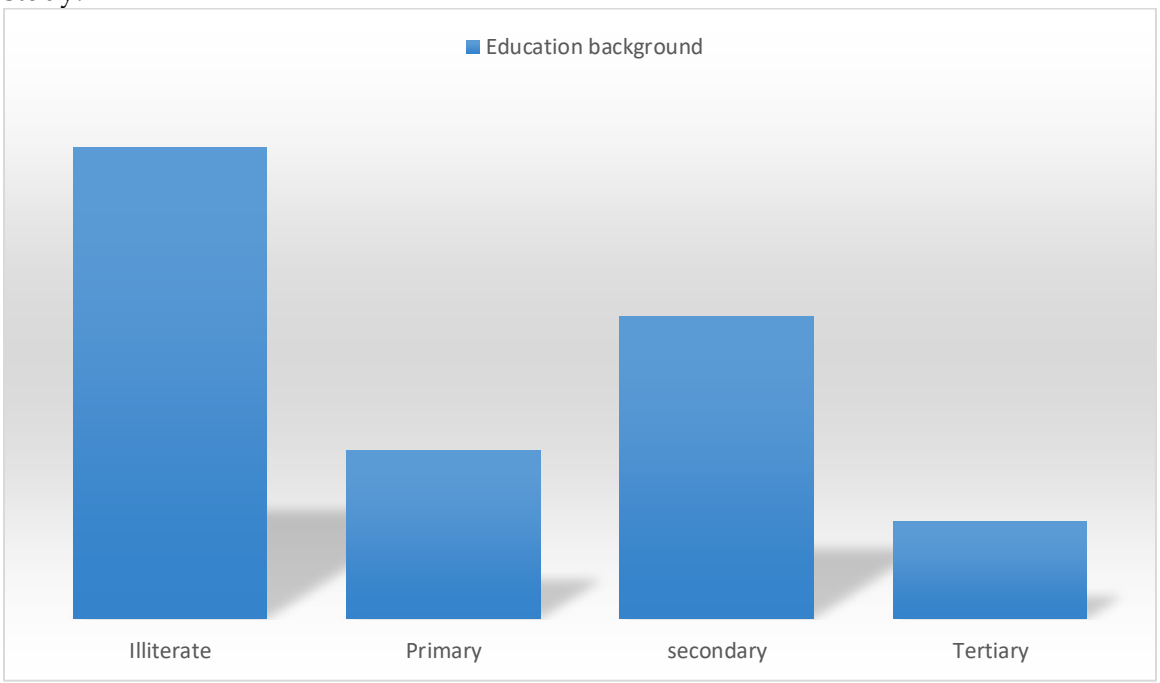

Figure 1: Educational background of participants in the study.

Out of the 18 *E. coli* isolated only 3 are ESBL-producing *E. coli* which depicts 17% of the *E. coli* isolated.

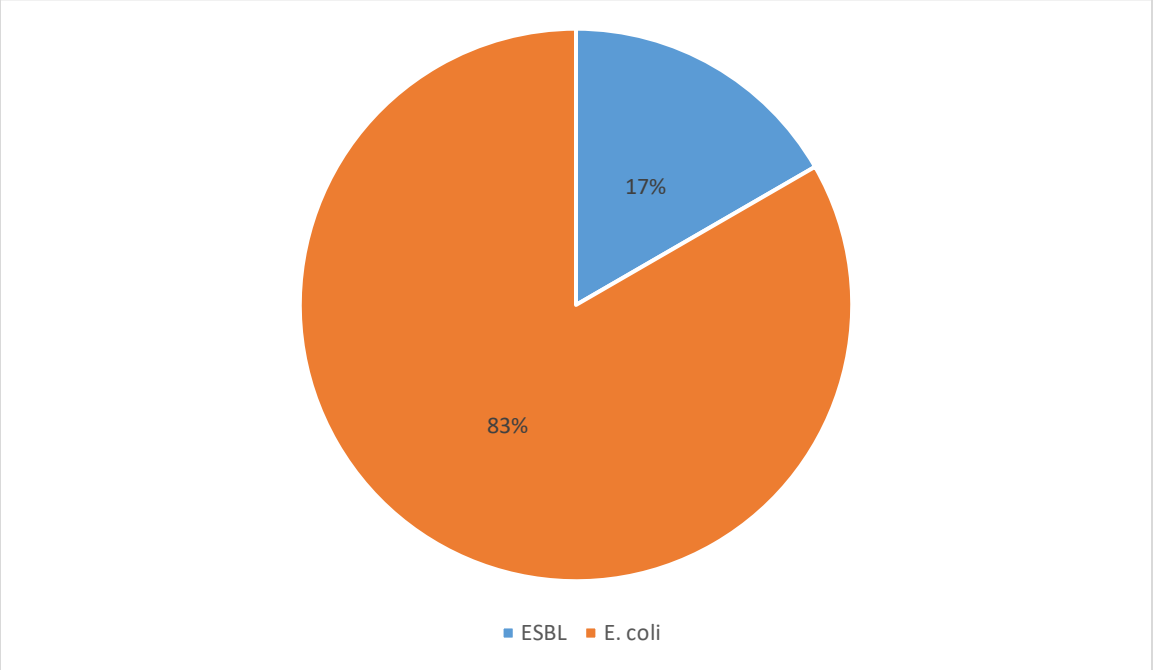

Figure 2. The prevalence of ESBL-producing *E. coli* (%).

E. coli was susceptible to ceftrixone (98.29%), ciprofloxacin (96.58%), cefuroxime (89.74%), meropenem (95.72%), nitrofurantoin (100%), ceftazidime (89.74%), gentamicin (97.43%), augmentin (95.72%), ceftazidime (94.87%), and ofloxacin (100%)

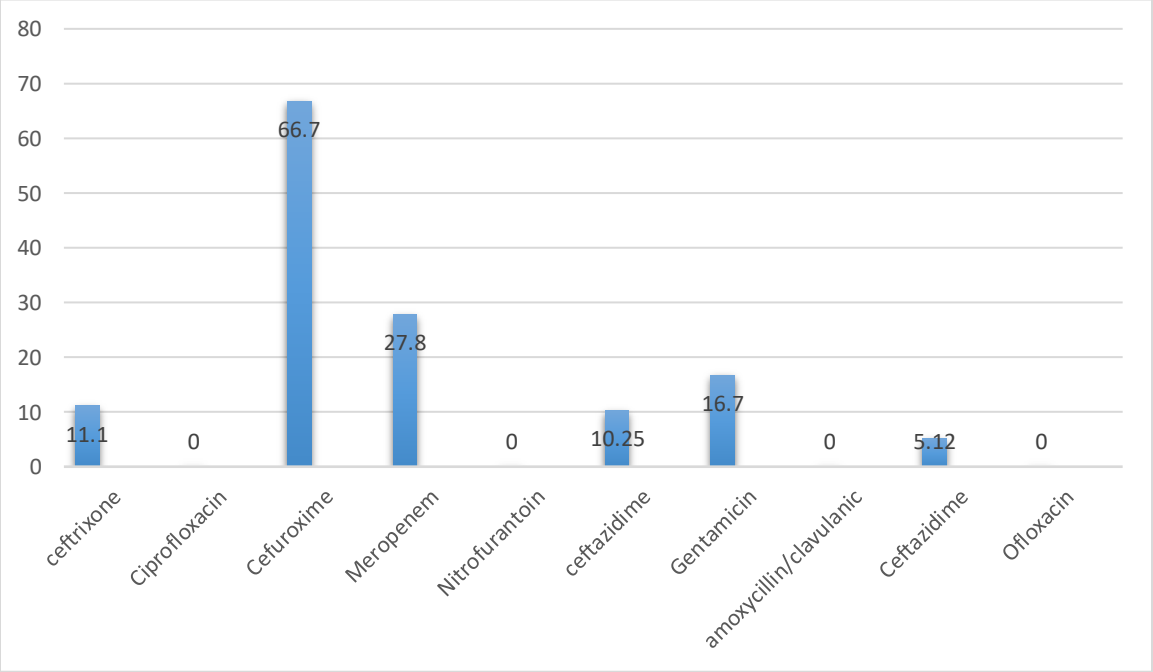

Figure 3: Resistant profile of the *E. coli* isolated from antennal pregnant women

DISCUSSION

Drug-resistant diseases are predicted to cause between 700,000 and 10 million deaths per year, with a global economic cost of up to US$100 trillion by 2050 (Osman *et al.*, 2019). Inadequate data on the molecular epidemiology of infectious diseases in Africa hinders the lack of

knowledge on AMR epidemiology in the African continent. This is because only a few nations have adequate drug resistance surveillance, which leads to inaccurate statistics on the real scope of the issue (Ndihokubwayo *et al.*, 2013). As a result, there were more treatment failures recorded in individuals who had infections brought on by germs that were multi-, extensive-, and multi-drug resistant (Founou *et al.,* 2016). Evidence suggests that AMR is becoming more common in both high- and low income nations. As a result, AMR became a burden with *E. coli* and *K. pneumoniae* being the two most prevalent resistant bacteria, according to a recent WHO monitoring report covering 22 countries (Mansouri *et al.,* 2019). ESBL-producing organisms were formerly thought to be hospital-acquired or healthcare-associated infections, i.e. infecting people who had often visited hospitals or other healthcare institutions. However, ESBL-producing Enterobacteriaceae isolates have moved from hospitals to the community and it has been identified in people in the community who have never previously used the healthcare system (Abayneh *et al.,* 2018).

In this study, a total of 117 urine samples were collected from antenatal pregnant women, 26 yielded positive bacteriuria and 18 *E. coli* were recovered. The ESBL-producing *E.coli* were detected in 16.7% (3/18) of the urinary isolates. Previous studies have shown that female patients suffered more UTIs (Idakwo *et al.,* 2015; Iseghohi *et al.,* 2020). The closeness of the female urethral opening to the anus and the anatomy and reproductive physiology of women may be the major reason and additionally, the female urethra is shorter than the male's thus microorganisms can easily enter the bladder (Abelson *et al.,* 2018). Due to their significant antibiotic resistance, these bacterial diseases have been linked to attributable mortality, and the World Health Organization (WHO) has classified them as important Gram-negative pathogens under monitoring (Tacconelli, 2017).

Also in this study, 11.1% of the total participants were between the ages of 15 and 20 years, 24.8% between 21-25 years, 13.7% were 26-30 years of age, 31.6% were 31-35 years of age, and 18.8% were 35 years and above, and of these, the distribution of significant bacteriuria were 23.1%, 24.1%, 25%, 21.6%, and 18%. This is in agreement with a previous study in Nigeria where 26.5 % and 25.3% were reported 21 -30 and 31 - 40 years respectively (Iseghohi *et al.,* 2020) while 20 - 29 years were most affected in another study (Idakwo *et al.,* 2015). Furthermore, 45.3 % of the respondents lack formal education, 16.2% are primary school students, 29.1% are secondary school students and 9.4% of the respondents are tertiary students.

The resistance pattern of the isolated *E. coli* in this study was 11.1% against ceftriaxone, ciprofloxacin (22.2%), cefuroxime (66.7%), meropenem (27.8%), ceftazidime (33.3%), gentamicin (16.7%), Cefotaxime (66.7%), amoxicillin/clavulanic acid (27.8%), and 100% susceptibility against ofloxacin and nitofurantoin. 16.7% resistance shown against gentamicin is in contrast to the 33% resistance rates shown in a study done in India (Pathak *et al.*, 2012) while 46.7% against gentamicin was shown in another study in Pakistan (Abdullah *et al.*, 2013) and Nepal (Nepal *et al.*, 2017). Gentamicin is less often abused when given parenterally. The high susceptibility of the antibiotics used in this study can also be attributed to the high illiteracy of the respondents, which can decrease the abuse of the antibiotics in the study area. Many factors influencing multiple antibiotic resistance in developing countries include low patient compliance and the threat of substandard antibiotics. Nitrofurantoin is a synthetic nitrofuran antimicrobial agent that has been in use for much longer and studies have shown that ofloxacin and nitrofurantoin are very effective against *E. coli* strains (ESBL inclusive) (Sardar *et al.*, 2017; Pancu *et al.*, 2021). These study results suggest that nitrofurantoin and

ofloxacin were suitable, effective, and cheap alternative drugs in the treatment of ESBL-producing *E. coli*-related UTI.

CONCLUSION

This study documents the prevalence and occurrence of ESBL-producing *E.coli* among antenatal pregnant women. A moderately high resistance to the commonly prescribed antibiotics was also observed. The Physicians are recommended to follow stringent hospital infection control procedures and appropriate antimicrobial usage guidelines. Clinical and diagnostic laboratories must regularly and routinely monitor the presence of clinical isolates that produce ESBLs. Nitrofurantoin and ofloxacin can be used as alternative antibiotics in the treatment of ESBL-producing *E.coli* in the study area.

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