Antibiotic Resistance and Plasmid Profile of *Escherichia coli* Isolated from Pregnant Women in Abia State

Ngoka D. I.[1*] Nwankwo E. O.[2] Okey-Kalu E. U.[3]

1. Department of Microbiology, Abia State University, Uturu, Nigeria
2. Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
3. Department of Applied Microbiology and Brewery, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

* Corresponding author: dorismariaifeoma@gmail.com

Abstract: This study was undertaken to determine the antibiotic resistance and plasmid profile of *Escherichia coli* isolated from pregnant women in Umuahia. A total of 250 midstream urine samples from pregnant women were analyzed and cultured on cystein lactose electrolyte deficient (CLED) agar and MacConkey agar. Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method. Plasmid profiling was also carried out on highly resistant isolates. *E. coli* isolated from the pregnant women showed 100% resistance to amoxicillin and tetracycline. Thirty, 30 (50.8%) of the *E. coli* isolates showed potential to produce biofilm. Extended spectrum beta-lactamase (ESBL) production among *E. coli* isolates was found to be 12 (20.3%) while 2 (3.4%) isolates produced chromosomal ambler C (AmpC). Imipenem and gentamicin should be the antibiotic of choice for treating UTI in the study area because they showed very high sensitivity to the antibiotics used. Screening of pregnant women for possible UTI at early stage of pregnancy should be considered an essential care in the study environment to avoid complications in pregnancy.

Key word: Antibiotic resistance, *E. coli*, plasmid, pregnant women

INTRODUCTION

Pregnancy causes numerous changes in a woman's body. These changes along with an already short urethra allow bacteria quick access into the bladder. Infections of the upper urinary tract (pyelonephritis), lower urinary tract (cystitis) and asymptomatic bacteriuria are the three different types of bacteriuria that can occur during pregnancy. Bacteriuria of the lower tract increases the likelihood of pyelonephritis during pregnancy, a condition linked to unfavorable mother and foetal results (Glaser and Schaeffer, 2015). The World Health Organization (WHO) defines antimicrobial resistance as a microorganism's resistance to an antimicrobial drug that was once able to treat an infection caused by that microorganism (WHO, 2014). The result is, infections can linger and spread when routine therapies are no longer effective. Certain features such as production of extended-spectrum beta-lactamases (ESBLs), presence of plasmid, generation of biofilm, can confer resistance to an organism.; Every pregnancy-related bacteriuria need to be dealt with and antimicrobial decision in pregnancy need to reflect wellbeing for the mother as well as the foetus (Glaser and Schaeffer, 2015). Numerous strains of *E. coli* are usually harmless and live in the gut of healthy people. In any case, a few strains are a reason for normal diseases such as urine infections (McNally *et al*., 2019). Recently, there has been concern that some strains of *E. coli* can produce small proteins (enzymes) called extended-spectrum beta-lactamases (ESBLs) (Bubpamala *et al*., 2018). ESBLs are chemicals (enzymes) that can be made by some groups of bacteria. They mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem or imipenem). These chemicals are critical in light of the fact that, when they are created by microorganisms, they can make the bacteria resistant to certain regularly utilized anti-microbial drugs. ESBLs can make a few antibiotics ineffective. This means that the bacteria can continue to multiply, causing more severe infection and

becoming more difficult to treat. ESBL-producing bacteria is another case of a quickly developing issue of antibiotics being inadequate in treating certain infections. Expectant women with urinary tract contaminations ought to be monitored closely after treatment on the grounds that a good number will encounter a repeat.

MATERIALS AND METHODS

Study area: Pregnant women who attended prenatal sessions in Ubakala primary health centre, Umuahia, Old Umuahia primary health centre, Umuahia, Nwachinemere maternity home Ihie Ndume, Umuahia and Elshaddai hospital, Umuahia, Abia State, were the subjects for sample collection.

Ethical consideration: Ethical clearance for this study was obtained from the Research and Ethics Committee of the different medical centres used for the study.

Sample collection and processing: A total of 250 midstream urine (MSU) samples were collected inside sterile disposable universal bottles from pregnant women of different age groups. The urine specimens were transported in ice pack to the Microbiology laboratory of Michael Okpara University of Agriculture, Umudike for further examination.

Isolation and identification of bacteria: The urine specimens were inoculated on MacConkey agar and CLED agar using streak plate method as described by Cheesbrough (2000) with calibrated wire loop. It was then incubated at 37^0C for 24 hours. Using a sterile wire loop, cells picked from pink coloured colonies on MacConkey agar and yellow coloured colonies on CLED plates were sub-cultured in peptone water and nutrient agar plates and then incubated at 37^0C for 24 hours. Smears prepared from the subculture were put through Gram staining and examined microscopically. Indole test was conducted on Gram negative rods that were motile. Indole positive isolates were further cultured on Kligler iron agar to identify *E. coli.*

Antibiotic susceptibility test: The Kirby-Bauer disc diffusion technique was used to tests for antibiotic susceptibility. These antibiotic discs were used: ceftriaxone (30 µg), cotrimoxazole (30 µg), chloramphenicol (30 µg), amoxycillin/clavulanate (30 µg), gentamicin (10 µg), ofloxacin (10 µg), streptomycin (30 µg), ciprofloxacin (5 µg), amoxicillin (10 µg), tetracycline (30 µg), ampicillin (10 µg), nalidixic acid (30 µg), imipenem (5 µg), levofloxacine (5 µg). A suspension of *E. coli* was produced by picking 4-5 colonies from nutrient agar plate and mixed in 4 ml of normal saline in sterile bijou bottle. To meet 0.5 McFarland turbidity standard, the bacterial suspension's turbidity was adjusted. Utilising a sterile swab stick, the isolates were introduced onto the surface of Mueller-Hinton agar plates using the spread plate method and left to stand for 10 minutes to allow the agar soak in the bacteria. Using sterile forceps, antibiotic discs were aseptically inserted on the agar plates. Plates were labeled and incubated aerobically at 37^0C for 18 hrs. The diameter zone of inhibition surrounding the disc was measured in millimeters using a transparent plastic ruler (Cheesbrough, 2000).

Extended Spectrum Beta Lactamase (ESBL) screening and confirmatory test: The resistant isolates were further tested for the production of Extended Spectrum Beta Lactamases (ESBL) against various antibiotics, such as penicillin, first, second and third generation cephalosporins using the Clinical Laboratory Standard Institute (CLSI) recommended WHO modified Kirby Bauer disc diffusion method (CLSI, 2011). Zone diameters were interpreted using the revised CLSI standard (CLSI, 2002). Phenotypic confirmation test was carried out using Double Disc Synergy test (DDST). Ceftazidime (30 µg) disc was placed on agar 15 mm away from the center of amoxicillin-clavulanic acid (20 µg/10 µg) disc. Extension of zone of inhibition towards amoxicillin-clavulanic acid was interpreted as ESBL producer (Chaudhary *et al*., 2008).

Biofilm production: Modified Congo red agar comprising 4 g blood agar base, 1 g glucose and 0.4 g Congo red dye was used.

The organisms were inoculated using streak plate method and incubated at 37°C for 24 hours. Black or grey-black pigment indicated biofilm production while a pink colour indicates negative results (Pragyan *et al*., 2016), (Hassan *et al*., 2011).

Detection of Chromosomal Ambler C (AmpC): All the isolates that showed a synergistic effect with cephalosporin group only with DDST were further tested for the AmpC enzyme production by AmpC disc test after a preliminary screening with a cefoxitin (30 µg) disc. A 30 µg cefoxitin disc was positioned over the inoculate surface of the MHA. A sterile plain disc (6 mm) which was inoculated with several colonies of the organism under test was placed beside the cefoxitin disc, almost touching it. After an overnight incubation at 37^0C, the plates were examined for either an indentation or a flattening of the zone of inhibition, which indicates enzymatic inactivation of cefoxitin (positive result) or an absence of distortion which indicates cefoxitin was not significantly inactivated (negative result) (Black *et al*., 2005).

Plasmid analysis: First, plasmid DNA was extracted using a method described by Ehrenfeld and Clewell (1987). Agarose gel electrophoresis was carried out on the extracted plasmid. This was performed on a 0.8% agarose gel in a 1X concentration of Tris Borate EDTA (TBE) buffer in an electrophoresis tank. Thereafter, plasmid DNA band was visualized by florescence of bound ethidium bromide in a UV transilluminator and the photograph was taken using a photo documentation system. To ascertain where the drug resistance maker is located, curing (elimination) experiment was done using acridine orange (Mbim *et al*., 2016). Treatment of the multidrug resistant (MDR) strains with prepared dilutions of acridine orange was done using a modified method of Bryon *et al*, (2003). Using a sterile Pasteur pipette, 0.5 ml aliquot of each diluted overnight broth culture of MDR strain was added to 4.5 ml sterile molten nutrient agar. Already prepared dilutions of acridine orange was then added in 0.5 ml volume and mixed. The set up for each dilution was poured on top of sterile hardened or set 2% nutrient agar plate and left to set. The same antibiotic discs used before treatment was then picked with sterile forceps and impregnated on the set agar overlay plates. Plates were incubated at 37°C for 24 hours. Measurement of diameter zone of inhibition was recorded according to CLSI (2002).

Statistical analysis: Data entry and analysis was done using IBM SPSS version 20. Simple descriptive statistics such as frequency and percentages were carried out. Statistical significance was considered at $P<0.05$.

RESULTS

The antimicrobial susceptibility profile of *E. coli* isolates is presented in Table 1. Fifty nine (59) of the isolates identified as *E. coli* were exposed to 14 different antibiotics. The isolates were most sensitive to Imipenem and Levofloxacine with 54 (91.5%) sensitivity each and just 5 (8.5%) resistance. This was followed by gentamycin with 44 (74.5%) sensitivity. The isolates showed moderate (42.4%) sensitivity to ceftriaxone, but were 100% resistant to amoxycillin and tetracycline.

Table 2 shows biofilm, ESBL and Chromosomal Ambler C (AmpC) production among *E. coli* isolates. From the 59 positive *E. coli* isolates, 30 (50.8%) showed potential for production of biofilm, while 12 representing 20.3% produced ESBL. Only 2 *E. coli* isolates were positive for iAmpC. Table 3 shows the presence of plasmid. Plasmids weighing 9.416 kbp were found in four isolates of highly resistant *E. coli*. In the plasmid analysis shown in Plate 1, four isolates had resistant genes present in their plasmid. After treatment with acridine orange, the isolates became sensitive to some previously resistant antibiotics (Table 4).

Table 1: Antibiotic susceptibility pattern of *Escherichia coli* (N=59)

Antimicrobial Agent	Disc Potency	Code	Number of Sensitive	Number of Resistant
Ceftriaxone	30	CRO	25(42.4)	34(57.6)
Cotrimoxazole	30	SXT	8(13.6)	51(86.4)
Chloramphenicol	30	CHL	8(13.6)	51(86.4)
Amoxycillin/clavulanate	30	AU	20(33.9)	39(66.1)
Gentamicin	10	CN	44(74.6)	15(25.4)
Ofloxacin	10	OFX	46(78)	13(22)
Streptomycin	30	S	40(67.8)	19(32.2)
Ciprofloxacin	5	CPX	45(76.3)	14(23.7)
Amoxycillin	10	AM	0	59(100)
Tetracycline	30	TE	0	59(100)
Ampicillin	10	PN	4(6.8)	55(93.2)
Nalidixic acid	30	NA	8(13.6)	51(86.4)
Imipenem	5	IPM	54(91.5)	5(8.5)
Levofloxacin	5	LEV	54(91.5)	5(8.5)

Key: N= Number of positive *E. coli* isolates; NB: All values in parentheses represent percentage

Table 2: Biofilm, extended spectrum Beta-lactamase (ESBL) and chromosomal ambler C (AmpC) production among isolates

Characteristics	*E. coli* (N=59)	Percentage
Potential for Biofilm Production	30	50.8
ESBL	12	20.3
AmpC	2	3.4
Total	44	74.5

Table 3: Presence of plasmid among *Escherichia coli* isolates

S/N	Sample code	Molecular weight (kbp)	Number of plasmid present
1	D1	9.416	1
2	D2	9.416	1
3	D3	-	-
4	D4	9.416	1
5	D5	9.416	1
6	D6	-	-
7	D7	-	-
8	D8	-	-
9	D9	-	-
10	D10	-	-

Table 4: Distribution of cured plasmids

Plasmid	Resistant Antibiotics Before Curing	Sensitive Antibiotics after Curing
D1	CRO,SXT,CHL,CN,OFX,CPX,AM,TE,PN,NA,IPM,LEV	IPM,LEV,CN,OFX,PN
D2	CRO,SXT,CHL,AU,CN,OFX, CPX,AM,TE,PN,NA,LEV	CN,OFX,LEV,CPX,PN
D4	CRO,SXT,CHL,AU,OFX, CPX,AM,TE,PN,NA	
D5	CRO,SXT,CHL,AU, OFX,CPX,AM,TE,PN,NA	OFX,CPX,PN,NA

KEY: Ceftriaxone (CRO), cotrimoxazole (SXT), chloramphenicol (CHL), amoxycillin/clavulanate (AU), gentamicin (CN), ofloxacin (OFX), streptomycin (S), ciprofloxacin (CPX), amoxicillin (AN), tetracycline (TE), ampicillin (PN), nalidixic acid (NA), imipenem (IPM), levofloxacine (LEV). Previously resistant antibiotics became sensitive after treatment with acridine orange.

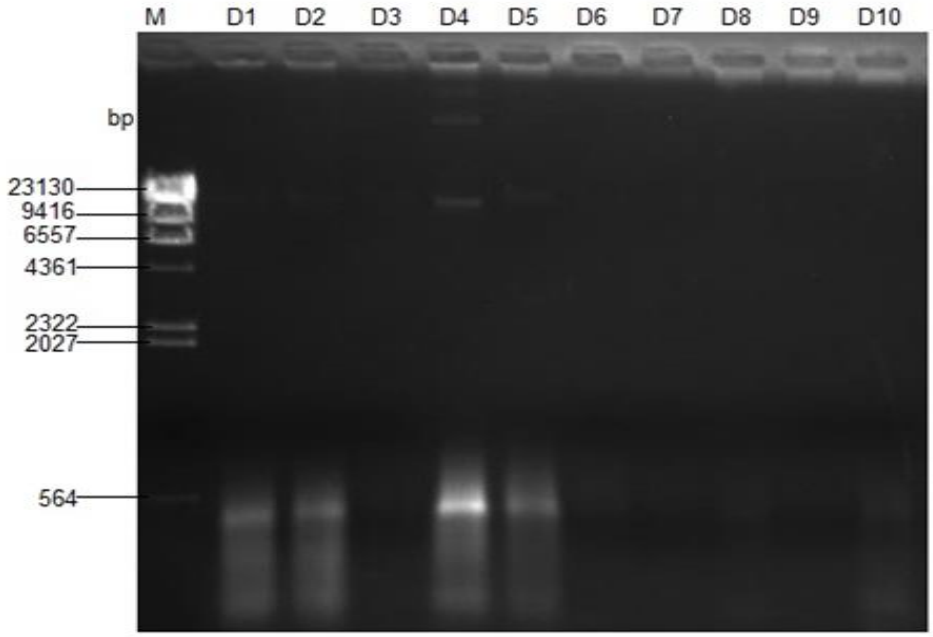

Plate 1: Agarose gel electrophoresis of *E. coli* isolates showing plasmids

DISCUSSION

All *E. coli* isolates in this research were resistant to tetracycline and amoxicillin, meaning that pregnant women should exercise caution when using these antibiotics to treat UTIs. Olowo *et al.* (2007) reported that in different areas of the world resistance of *E. coli* to penicillins group of antibiotics have been on higher side and is increasing daily, but there are just few reports which indicates 100% resistance to penicillins. Resistance to the combination of amoxicillin and a beta lactamase inhibitor (augmentin 66.1%) was also on the higher side. Comparable results, where beta lactamase inhibitors inhibitors increase the efficiency of penicillin group of antibiotic against *E. coli*, has been cited in previous studies (Drawz and Bonomo, 2010). Among the cephalosporins, this study made use of ceftriaxone. It gave resistance of 57.6%, showing extremely high resistance. This findings is at variance with the outcomes of Abayneh *et al.* (2018) which recorded 100% resistance of *E. coli* isolates to ceftriaxone. In the penicillins, ampicillin gave extremely high resistance of 93.2% which is greater than the 59.3% recorded by Sevanan *et al.* (2011). Difference in geographical location and usage of antibiotic might be linked to this variation. Resistance of *E. coli* penicillins and cephalosporins in this study does not indicate that this group of antibiotics is not used in some parts of world to treat UTIs caused by *E. coli* Nijssen *et al.* (2004) stated that a larger percentage of *E. coli* strains were discovered to be susceptible to cephalosporins or penicillins in European nations.

The quinolones gave favourable results for sensitivity (levofloxacine, 91.5%), but because they are linked to teratogenicity

during a woman's first trimester and are released in breast milk, they are not the best option for pregnant women (Muhammed, 2015).

In this research, Imipenem antibiotic gave sensitivity of 91.5% which compares favourably with findings from Suman *et al*. (2017) and Abayneh *et al.* (2018) who observed 94.4% and 100% sensitivity in their various investigations conducted at Ethiopia and Nepal respectively. The high sensitivity of imipenem antibiotic in this research makes it the antibiotic preferred option for treating UTI in pregnancy in the study area. Recently the problem of *E. coli* resistance has been amplified by frequency of extended spectrum beta lactamases (ESBL) and multidrug resistant (MDR) *E. coli*. This investigation showed that 30 (50.8%), 12 (20.3%), 2 (3.4%), of the isolated *E. coli* produced Biofilm, ESBL and AmpC respectively. Shayan and Bokaein (2015) reported 62.7% ESBL production and 5% AmpC production amongst *E. coli* isolates. Sevanan *et al.* (2011) recorded 84.3% biofilm production while Seema *et al.* (2015) observed 13.5% biofilm production in their separate studies. Biofilm protects isolates against the action of antibiotics administered to treat infection. *E. coli*, maybe the most researched microbe, has been discovered to have different kinds of plasmid types, including those linked to virulence (Kaper *et al*., 2004). Drug resistance, virulence, and the metabolism of uncommon chemicals are just a few of the features that naturally existing plasmids can help spread. In this study, four isolates had plasmid present in their DNA. Onwuezobe and Orok (2015) in their study of 300 women attending antenatal clinics in Uyo, Nigeria recorded a significant number of isolates that produced plasmid. The resistance that was shown in this investigation could result from the existence of plasmid in some of the isolates.

CONCLUSION

Several changes in physiology happen in pregnancy that cause otherwise healthy women to become susceptible to infections. The combination of mechanical, hormonal and physiologic changes contributes to significant modifications to the urinary tract which has profound influence on the acquisition and natural history of bacteriuria throughout pregnancy. *E. coli* identified from this study was found to possess features such as ability to produce ESBL and AmpC, generation of biofilm and presence of plasmid. These features helped to increase their resistance level to routinely used antibiotics. *E. coli* was susceptible to imipenem and gentamicin and should be considered the recommended antibiotic to be administered during pregnancy.

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