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Extended Spectrum Beta-Lactamase Production in *Escherichia coli* From Urine of Symptomatic and Asymptomatic Subjects in Keffi, Nigeria

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Abstract: *Escherichia coli are frequently isolated in symptomatic and asymptomatic bacteriuria. The detection of extended spectrum beta-lactamase (ESBL) production in E. coli isolates from urine of symptomatic and asymptomatic subjects in Keffi was investigated. Urine samples were collected from patients with cases of UTIs attending two health facilities in Keffi and asymptomatic volunteering students, staff and others within and around Nasarawa State University, Keffi main campus. Escherichia coli were isolated from the samples using standard cultural, microscopical and biochemical methods. Antibiotic susceptibility testing and minimum inhibitory concentrations were evaluated as described by the Clinical and Laboratory Standards Institute (CLSI). In addition, the detection and confirmation of ESBL production was carried out by modification of double disc synergy test (DDST). A total of hundred (100) isolates (50 from urine of symptomatic and 50 from urine of asymptomatic) were isolated from the urine samples. Symptomatic isolates had susceptibility which decreased as follows: ciprofloxacin (82.0%) and gentamicin (82.0%) > ampicillin (62.0%) > streptomycin (60.0%) > septrin (52.0%) > augmentin (48.0%) and perfoxacin (48.0%) > cephalexin (44.0%) > ofloxacin (40.0%) > nalidixic acid (22.0%). Asymptomatic isolates had susceptibility which decreased as follows: ciprofloxacin (68.0%) > streptomycin (66.0%) > ampicillin (60.0%) > septrin (58.0%) > gentamicin (54.0%) > ofloxacin (50.0%) > augmentin (46.0%) and perfloxacin (46.0%) > cephalexin (38.0%) and nalidixic acid (38.0%). The commonest antibiotic resistance phenotypes were AU-SXT-PN-CEP-OFX-NA-PEF (symptomatic, 8.3%) and PN-CEP-NA-CN-SXT (asymptomatic, 9.1%). Multiple antibiotic resistance (MAR) was observed in 80 (86.95%) of the combined isolates distributed as follows: 40 (83.3%) of symptomatic and 40 (90.9%) of asymptomatic isolates. Irrespective of the source of isolates, most (79, 98.8%) had MAR indices ≥ 0.2 which suggested that the isolates originated from environments where antibiotics were freely abused/misused. The commonest indices were 0.4 and 0.5 (22.5%) for symptomatic and 0.5 (37.5%) for asymptomatic isolates. Most (85.7%) symptomatic isolates jointly resistant to cefotaxime and ceftazidime were ESBL producers; but only 42.9% of asymptomatic isolates jointly resistant to both cefotaxime and ceftazidime were ESBL producers. The correlation of Minimum Inhibitory Concentration (MIC) and antibiotic susceptibility irrespective of the source of the isolates were insignificant (P>0.05). Overall, most of the E. coli isolates jointly resistant to cefotaxime and ceftazidime were positive for ESBL. Molecular characterization of the ESBL genes in these ESBL producing isolates in Keffi is on-going.*

Keywords: *Escherichia coli; Antibiotic Susceptibility; Extended Spectrum beta-lactamase; Symptomatic; Asymptomatic; Urine*

1. INTRODUCTION

Escherichia coli are the predominant facultative anaerobe of the human colonic microflora. Most *E. coli* strains are harmless to humans, but pathogenic strains can cause gastroenteritis, urinary tract infections and neonatal meningitis; and in rare cases, hemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicemia and gram-negative pneumonia (Todar, 2008).

Resistance to cephalosporin has become widespread throughout the world and many types of extended spectrum beta-lactamases (ESBLs) have been detected in some bacterial species (Shah *et al*., 2004). Extended spectrum beta-lactamase producing *E. coli* are resistant to all penicillins, cephalosporin as well as to aztreonam (CLSI, 2006 and 2008). The ESBLs are a group of plasmid-mediated bacterial enzymes that confer significant resistance to oxyimino-cephalosporin and

monobactam antimicrobials (Canton *et al*, 2008; Ahmed *et al*., 2013). Extended-spectrum beta-lactamase arises mainly due to mutations in beta-lactamases encoded by the *bla*SHV, *bla*TEM, and *bla*CTX-M genes and these parent enzymes are commonly found in Gram negative bacteria, particularly Enterobacteriaceae (Bradford, 2001). More the 300 different ESBL variants have been described (Pertson *et al*., 2005). Although TEM and SHV variants are the most common ESBLs, during the past years strains expressing CTX-M ESBLs have emerged in many countries.

Escherichia coli are one of the most frequently isolated bacteria in asymptomatic bacteriuria and UTIs (Ngwai *et al.*, 2010; Todar, 2008; Stamm, 1994). Antimicrobial resistance in *E. coli* increased worldwide and its susceptible patterns show substantial geographic variation as well as difference in population and environment (Baum *et al*., 2000; Aghazadeh *et al*., 2015). Resistance to cephalosporin has become widespread throughout the world and many types of extended spectrum beta-lactamases (ESBLs) have been detected in some bacterial species (Shah *et al*., 2004). This study aimed at isolation and detection of extended spectrum beta-lactamase producing *Escherichia coli* from urine of symptomatic and asymptomatic subjects in Keffi, Nigeria.

2. MATERIAL AND METHODS

2.1. Materials

Media used were: MacConkey agar (MCA: Oxoid Ltd, England), Eosin-Methylene Blue Agar (EMB: Merck KGaA, Germany) and Nutrient Agar (NA: Oxoid Ltd, England), Mueller-Hinton Agar (MHA: Oxoid Ltd, England), Peptone Water (PW: Oxoid Ltd, England**)** Glucose Phosphate Broth (GPB: Fluka Chemic AG, Germany) and Simmons Citrate agar (SCA: Oxoid Ltd, England**)**. All media were prepared in accordance with manufacturer's instructions.

Chemicals used were: Kovac's Indole (HiMedia Laboratories PVT Ltd, India), D (+) Glucose (Fluka Chemic AG, Germany). Antibiotic disks used, which were products of Optun Lab. Ltd (Nigeria), include: Streptomycin (30 µg), Ciprofloxacin (5 µg), Ofloxacin (10 µg), Gentamicin (10 µg), Perfloxacin (10 µg), Augmentin (10 µg), Septrin (30 µg), Cephalexin (30 µg), Ampicillin (30 µg) and Nalidixic Acid (30 µg).

2.2. Sample collection

A total of 400 urine samples were collected- 200 from patients with UTI symptoms attending Federal Medical Center, Keffi and South Atlantic Petroleum Medical Center, Nasarawa State University, Keffi; 200 from volunteering asymptomatic students, staff and others within and around Nasarawa State University, Keffi. The samples were transported to Microbiology Laboratory, Nasarawa State University, Keffi for analysis.

2.3. Isolation of *Escherichia coli*

Escherichia coli were isolated from urine as follows; a loopful of urine was streaked on MCA plate and incubated at 37°C for 24 h. A pinkish colony that grew on MCA was further streaked on EMB plate and further incubated at 37°C for 24 h. Greenish metallic sheen colonies that grew on EMB plate was presumptively selected as *E. coli.*

2.4. Identification of *Escherichia coli*

Escherichia coli were further identified by Gram staining of the greenish metallic sheen colonies from EMB and the minimum biochemical tests for *E. coli* identification namely: Indole, Methyl red, Voges-Proskeuer and Citrate tests (IMViC) as earlier described (Cheesbrough, 2006).

2.5. Antibiotic Susceptibility Testing

Antibiotic susceptibility testing for *E. coli* isolates was carried out using Kirby-Bauer disc diffusion method as modified by Clinical and Laboratory Standards Institute (CLSI, 2007). Briefly four (4) colonies of the isolates were transferred into 5 ml of sterile normal saline in a tube such that the turbidity of the bacterial suspension is equivalent to 0.5 McFarland Standard. The sterile swab was dipped in the bacterial suspension and streaked on Muller-Hinton agar and each antibiotic disc was aseptically placed with a sterile pair of forceps on the surface of the inoculated MHA plate. The plate was incubated at 37ºC for 24 h. The diameter of the zone of inhibition was measured using meter rule and the result was interpreted in accordance with the susceptibility break point as earlier described (CLSI, 2007).

2.6. Determination of Minimum Inhibitory Concentration (MIC)

The MICs of ceftazidime and cefotaxime against the beta-Lactam (ampicilin or amoxicillin) resistant *E. coli* isolates were evaluated using agar dilution method as earlier described (CLSI, 2007). Briefly, two-fold concentration agar dilutions of ceftazidime or cefotaxime in MHA were prepared and inoculated with approximately 1 x 10^4 colony-forming units (CFU) from an adjusted suspension of a test organism. Results were observed and registered after 24-h incubation at 37°C. MIC was defined as the lowest concentration that inhibited visible growth.

2.7. Confirmatory Test for Extended Spectrum beta-lactamase (ESBL) production

The confirmatory test for extended spectrum beta-lactamase production by *E. coli* isolates jointly resistant to both ceftazidime and cefotaxime antibiotics was carried out with isolates whose MICs were ≥ 2.0 µg/ml using Double Antibiotic Synergy Test (DAST) being a modification of Double Disk Synergy Test (DDST) (Jarlier *et al*.,1988). Briefly, the swab stick was soaked in standardized suspension of an *E. coli* isolate (10^5 Cfu/ml) and streaked on a MHA plate. Three wells of 6 mm size were bored on the inoculated MHA plate with a cork borer- one at the center and other two 20 mm away from the centre; 0.1 ml of augmentin (20 µg amoxicillin and 10 µg clavulanate) was dispensed into the well at the center and 0.1 ml containing 30µg of cefotaxime and 0.1 ml containing 30 µg ceftazidime were each dispensed into the other two wells 20 mm away from the well at the center of the MHA plate. The plate was allowed to stand for 1 h for pre-diffusion before they were incubated at 37°C for 24 h. Any isolate with increase in zone of inhibition of both ceftazidime and cefotaxime towards augmentin was confirmed as extended spectrum beta-lactamase producer.

2.8. Statistical Analyses

The data obtained from this study were analyzed by One way Analysis of Variance (ANOVA) using Smith Statistical Package (SSP), version 2.8 (September 26, 2005, copyright ©1995-2005 by Gary Smith, Pomona College, Claremont, California); and the significance of differences was determined at 5% probability.

3. RESULTS

3.1. Antibiotics Susceptibility

The antibiotic susceptibilities of the isolates from urine of symptomatic and asymptomatic subjects are as shown in Table 1. Susceptibility of symptomatic isolates decreased as follows: ciprofloxacin (82.0%) and gentamicin (82.0%) > ampicillin (62.0%) > streptomycin (60.0%) > septrin (52.0%) > augmentin (48.0%) and perfoxacin (48.0%) > cephalexin (44.0%) > ofloxacin (40.0%) > nalidixic acid (22.0%). Susceptibility of asymptomatic isolates decreased as follows: ciprofloxacin (68.0%) > streptomycin (66.0%) > ampicillin (60.0%) > septrin (58.0%) > gentamicin (54.0%) > ofloxacin (50.0%) > augmentin (46.0%) and perfloxacin (46.0%) > cephalexin (38.0%) and nalidixic acid (38.0%). The differences in the susceptibilities of symptomatic and asymptomatic *E*. coli isolates to the antibiotics tested was insignificant (P>0.05).

Table 1. *Antibiotic susceptibility of Escherichia coli isolates from urine of symptomatic and asymptomatic subjects in Keffi, Nigeria*

Antibiotics	Disc content (µg)	No. (%) Susceptibility of *E. coli*	
		Symptomatic (n=50)	Asymptomatic (n=50)
Streptomycin (S)	30	30(60.0)	33(66.0)
Ciprofloxacin(CPX)	5	41(82.0)	34(68.0)
Ofloxacin (OFX)	10	20(40.0)	25(50.0)
Gentamicin (CN)	10	41(82.0)	27(54.0)
Perfloxacin (PEF)	10	24(48.0)	23(46.0)
Augmentin (AU)	10	24(48.0)	23(46.0)
Septrin (SXT)	30	26(52.0)	29(58.0)
Cephalexin (CEP)	30	22(44.0)	19(38.0)
Ampicillin (PN)	30	31(62.0)	30(60.0)
Nalidixic Acid (NA)	30	11(22.0)	19(38.0)

3.2. Antibiotic resistant phenotypes of the isolates

The antibiotic resistance phenotypes of the isolates are as shown in Table 2. For symptomatic isolates, the commonest resistance phenotypes were AU-SXT-PN-CEP-OFX-NA-PEF and NA at 8.3% and

6.3% respectively. For asymptomatic isolates the commonest resistant phenotypes were; PN-CEP-NA-CN-SXT and CEP at 9.1% and 6.8% respectively.

Table 2. *Antibiotic Resistance Phenotypes of Escherichia coli from urine of symptomatic and asymptomatic subjects in Keffi, Nigeria*

Antibiotic Resistance phenotypes	Frequency (%) Resistance phenotypes	
	Symptomatic (n=48)	Asymptomatic (n=44)
CPX	1(2.1)	0(0)
NA	3(6.3)	0(0)
PN	0(0)	1(2.3)
CEP	0(0)	3(6.8)
OFX	2(4.2)	0(0)
PEF	1(2.1)	1(2.3)
SXT	1(2.1)	1(2.3)
PEF-CN	1(2.1)	1(2.3)
SXT-NA	1(2.1)	0(0)
OFX,NA	1(2.1)	0(0)
NA-PEF	0(0)	1(2.3)
CPX-NA	1(2.1)	0(0)
CEP-NA-AU	0(0)	1(2.3)
NA-CPX-S	1(2.1)	1(2.3)
AU-CPX-SXT	1(2.1)	0(0)
OFX-NA-PEF	2(4.2)	0(0)
PN-NA-CN-AU	0(0)	1(2.3)
PN-NA-AU-SXT	0(0)	1(2.3)
NA-PEF-CN-AU	0(0)	1(2.3)
PN-CEP-OFX-NA	2(4.2)	0(0)
CEP-OFX-NA-PEF	1(2.1)	1(2.3)
AU-CPX-NA-CN	1(2.1)	0(0)
AU-S-CEP-NA	1(2.1)	0(0)
AU-CEP-OFX-NA	1(2.1)	1(2.3)
AU-PN-CEP-OFX	1(2.1)	0(0)
AU-CEP-NA-PEF	2(4.2)	1(2.1)
PN-CEP-NA-CEP-S	0(0)	0(0)
OFX-NA-AU-CPX-S	0(0)	1(2.3)
OFX-PEF-CN-AU-SXT	0(0)	1(2.3)
NA-PEF-CN-CPX-S	1(2.1)	1(2.3)
PN-CEP-NA-PEF-CPX	0(0)	1(2.3)
PN-CEP-OFX-AU-CPX	0(0)	1(2.3)
OFX-NA-PEF-CN-CPX	0(0)	1(2.3)
PN-CEP-NA-AU-SXT	1(2.1)	1(2.3)
PN-CEP-NA-CN-SXT	0(0)	4(9.1)
AU-OFX-NA-PEF-CN	1(2.1)	0(0)
CPX-SXT-PN-CEP-PEF	1(2.1)	0(0)
AU-PN-CEP-OFX-NA	1(2.1)	0(0)
AU-CPX-SXT-NA-PEF	1(2.1)	1(2.3)
PN-CEP-NA-CPX-S	0(0)	1(2.3)
OFX-NA-AU-CPX-S	1(0)	1(2.3)
AU-SXT-CEP-NA-PEF	1(2.1)	0(0)
SXT-CEP-NA-PEF-CN	1(2.1)	1(2.3)
PN-NA-PEF-AU-CPX-S	0(0)	1(2.3)
OFX-NA-PEF-CPX-SXT-S	0(0)	1(2.3)
CEP-OFX-NA-CEP-CPX-SXT	0(0)	1(2.3)
SXT-PN-CEP-OFX-NA-CN	1(2.1)	0(0)
AU-SXT-CEP-OFX-NA-PEF	1(2.1)	0(0)
PN-CEP-NA-PEF-CN-AU-CPX	0(0)	1(2.3)
CEP-OFX-NA-CN-AU-SXT-S	0(0)	1(2.3)
PN-CEP-OFX-NA-AU-CPX-SXT	0(0)	1(2.3)
CPX-SXT-S-PN-CEP-OFX-PEF	1(2.1)	0(0)

CPX-SXT-S-PN-CEP-NA-CN	1(2.1)	0(0)
CEP-OFX-NA-PEF-CN-AU-CPX	0(0)	2(4.5)
CEP-OFX-NA-CN-AU-SXT-S	0(0)	1(2.3)
AU-SXT-PN-CEP-OFX-NA-PEF	4(8.3)	0(0)
CEP-OFX-NA-PEF-CN-AU-S	0(0)	1(2.3)
AU-CPX-SXT-S-CEP-OFX-NA-PEF	2(4.2)	0(0)
AU-CPX-SXT-PN-CEP-OFX-NA-PEF	1(2.1)	1(2.3)
PN-CEP-OFX-NA-PEF-CN-AU-CPX	0(0)	1(2.3)
AU-CPX-SXT-S-PN-CEP-OFX-NA-PEF	1(2.1)	0(0)
AU-CPX-SXT-S-CEP-OFX-NA-PEF-CN	1(2.1)	1(2.3)
AU-CPX-SXT-PN-CEP-OFX-NA-PEF-CN	1(2.1)	2(4.5)

S = Streptomycin; CPX = Ciprofloxacin; OFX = Ofloxacin; CN = Gentamicin; PEF = Perfloxacin; AU = Augmentin; SXT = Septrin; CEP = Cephalexin; PN = Ampicillin; NA = Nalidixic Acid

3.3. Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance (MAR), defined here as resistance to at least two antibiotics was observed in 80 (86.95%) of the combined isolates distributed as follows: 40 (83.3%) of symptomatic and 40 (90.9%) of asymptomatic isolates. The MAR indices of the isolates are as shown in Table 3. Whether from symptomatic or asymptomatic subjects, most (79, 98.8%) isolates had MAR indices ≥0.2 which suggested that the isolates originated from environments where antibiotics were freely abused/misused (Krumpermann, 1983). For symptomatic isolates, the commonest indices were: 0.4 and 0.5 (22.5%) and 0.7 (15.0%). For asymptomatic isolates, the commonest indices were: 0.5 (37.5%) and 0.7 (17.5%).

Table 3. *Multiple Antibiotic Resistance (MAR) Indices of Escherichia coli from urine of symptomatic and asymptomatic subjects in Keffi, Nigeria*

No. of antibiotics MAR isolate is resistant to (a)	No. of antibiotic tested (b)	MAR indices (a/b)	No. (%) MAR isolates	
			Symptomatic (n = 40)	Asymptomatic (n = 40)
9	10	0.9	3(7.5)	3 (7.5)
8	10	0.8	3(7.5)	2 (5.0)
7	10	0.7	6(15.0)	7 (17.5)
6	10	0.6	2(5.0)	3 (7.5)
5	10	0.5	9(22.5)	15 (37.5)
4	10	0.4	9(22.5)	6(15.0)
3	10	0.3	4(10.0)	2(5.0)
2	10	0.2	4(10.0)	2 (5.0)

3.4. Minimum Inhibitory Concentrations (MICs) of Ceftazidime and Cefotaxime

The minimum inhibitory concentrations of ceftazidime and cefotaxime against ampicillin and cephalexin resistant *E. coli* isolates are as shown in Table 4. For symptomatic ampicillin and cephalexin resistant isolates, more were resistant to cefotaxime (87.5%) than ceftazidime (31.3%). Similarly, more ampicillin and cephalexin resistant isolates were resistant to cefotaxime (33.3%) than ceftazidime (26.7%) for asymptomatic isolates. The differences in MICs of ceftazidime and cefotaxime against ampicillin and cephalexin resistant isolates from urine of both symptomatic and asymptomatic subjects were insignificant (P> 0.05).

Table4. Minimum Inhibitory Concentration of ceftazidime and cefotaxime against ampicillin and cephalexin Resistant Escherichia coli from urine of symptomatic and asymptomatic subjects in Keffi, Nigeria

Antibiotics	MIC Ranges (µg/ml)		No. (%) resistance Isolates	
	Symptomatic	Asymptomatic	Symptomatic (n=16)	Asymptomatic (n=15)
Cefotaxime	1 – 64	≤ 0.25-16.0	14(87.5)	5 (33.3)
Ceftazidime	2 – 256	≤ 0.25-12.80	5(31.3)	4(26.7)

3.5. Confirmation of Extended Spectrum beta-Lactamase (ESBL) Production

Production of ESBL was observed in 6(85.7%) of 7 isolates symptomatic isolates jointly resistant to both cefotaxime and ceftazidime; but only in 3(42.9%) of 3 asymptomatic isolates jointly resistant to both cefotaxime and ceftazidime.

4. DISCUSSION

Escherichia coli are one of the most important causes of community-acquired and nosocomial infections (Aghazadeh *et al*., 2015). This organism can be isolated from urine of both symptomatic and asymptomatic individuals (Scholes *et al*., 2010; Ngwai *et al*., 2012). Antimicrobial resistance in *E. coli* increased worldwide and it's susceptible patterns show substantial geographic variation as well as difference in population and environment (Baum *et al*., 2000; Aghazadeh *et al*., 2015).

The high susceptibility of the *E. coli* isolates to streptomycin, ampicillin and septrin observed in this study is different from that from other earlier studies (Ngwai *et al*., 2012; Mbata, 2007; Aghazadeh *et al*., 2015). This high susceptibility pattern justifies the fact of using those drugs which are usually very cheap and readily available as first line drugs for treatment of UTIs. It is possible that these antibiotics may not have been abused within the environment as others despite their being readily available and very cheap.

The high susceptibility of the *E. coli* isolates to gentamicin and streptomycin was expected and these antibiotics are administered parentrally and therefore not likely misused due to the discomfort of infection (Ngwai *et al.*, 2012). In contrast, ciprofloxacin are relatively costly in Nigeria and this also limits their misuse (Ngwai *et al*., 2012).

The high susceptibility of ampicillin and cephalexin resistant *E. coli* isolate to third generation cephalosporin (cefotaxime and ceftazidime) was expected and the MIC value of ceftazidime and cefotaxime observed from these studies is not different from other studies reported by Ngwai *et al*. (2013). The production of ESBL by cefotaxime and ceftazidine resistant *E. coli* isolates was expected and is consistent with previous studies earlier described (Jacoby and Han, 1996). Although the molecular detection of ESBL gene was not evaluated in this study, a previous study (Bradford, 2012) has shown that isolates resistant to cefotaxime and ceftazidime produce SHV, TEM and CTM-X beta-lactamases.

In conclusion, the *E. coli* isolated from urine of symptomatic and asymptomatic subjects were more susceptible to streptomycin, gentamicin, ampicillin, ciprofloxacin and septrin. In addition, most of the *E. coli* isolates jointly resistant to cefotaxime and ceftazidime were confirmed as for ESBL producers. Further studies on molecular detection of genes that code for the production of these ESBLs should be carried out.

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