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Antibiotic Resistance and Phynotypic Detection of AmpC Beta-Lactamase Producing *Escherichia coli* from Urine of Students Attending Fulafia Clinic

Usman S. Okposhi*, Kabiru A. Shuaibu, Chuku Aleruchi, Fatima A. Yusuf, Naja'atu S. Hadi

Microbiology Department, Federal University of Lafia, Lafia, Nigeria
Email: *okposhiusman@gmail.com

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Abstract

Escherichia coli also known as *E. coli* are gram-negative facultative anaerobic, rod-shaped, Coliform bacteria, commonly found in the lower intestine of warm-blooded organisms. This study was designed to determine the antibiotic susceptibility and the phenotypic detection of AmpC beta-lactamase producing *Escherichia coli* from the urine of students attending FuLafia Clinic, Nasarawa State, Nigeria. A total of 22 urine samples were collected from the students. Eleven (50%) *Escherichia coli* isolates were recovered and identified by standard Microbiological methods. The antibiotic susceptibility of *Escherichia coli* from the urine of students showed that the isolates were susceptible to gentamycin (27%), streptomycin (22.7%), chloramphenicol (18%), sparfloxacin (13.6%), tarivid (9%), augumentin and septrin with (4.5%) each, while none was susceptible to amoxicillin/clavulanic acid, pefloxacin and ciprofloxacin respectively. The *E. coli* isolated from the urine of the students showed varying antibiotic-resistant phenotypes with SXTCH-SP-CPX-AM-AU-CN-PEF-OFX-S as the most common; with a percentage occurrence of 27.3%. The commonest Multiple Antibiotic Resistance indexes (MAR) of these isolates was 0.9 and the frequency of occurrence was 11 (50%). The *E. coli* isolated from the urine showed that 5 (45.5%) were resistant to cefoxitin, as 3 (60.0%) out of which were confirmed to be AmpC beta-lactamase producing *E. coli.*

Subject Areas

Microbiology

Keywords

Urine, *Escherichia coli*, Antibiotic, AmpC, Beta-Lactamase

1. Background of the Study

Escherichia coli (*E. coli*) is gram-negative, facultative anaerobic, rod-shaped, Coliform bacteria belonging to the genus *Escherichia* that is commonly found in the lower intestine of both humans and animals (Tenaillon *et al.* 2010) [1]. Most *Escherichia coli* exist as normal flora in the intestine (harmless), but some serotypes when consumed with food cause food poisoning and sometimes call for product recall as a result of food contamination (CDC, 2012) [2]. The virulent strains can cause gastroenteritis, Urinary tract infection, and Neonatal meningitis.

The non-virulent strains form part of the normal flora of the gut, and are beneficial to their host organisms by producing vitamin k_2 (Bentley and Meganathan, 1982) [3] and also prevent colonization of the intestine with pathogenic bacteria (Hudault *et al.* 2001) [4].

Escherichia coli are expelled into the environment within fecal matter. The bacterium grows massively in the fresh fecal matter under aerobic conditions for 3 days, but its number decline slowly afterward (Russell and Javis, 2001) [5], *Escherichia coli* and other facultative anaerobes constitute about 0.1% of gut flora (Eckburg *et al.* 2005) [6] and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside for a limited amount of time, which makes them a potential indicator organism to test environmental samples for fecal contamination (Thompson and Andrea, 2007) [7]. A growing body of research, though, has examined environmentally persistent *E. coli* which can survive for an extended period outside of a host (Ishii and Sadowsky, 2007) [8].

The bacterium can be cultured easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. *Escherichia coli* are a chemoheterotroph whose chemically defined medium must include a source of carbon and energy (Tortora and Gerard, 2010) [9].

The mainstay of treatment is the assessment of the dehydration and replacement of fluid and electrolytes. Administration of antibiotics has been shown to shorten the course of illness and duration of excretion of enterotoxigenic *E. coli* (ETEC) in adults in endemic areas and traveler's diarrhea, though the rate of resistance to commonly used antibiotics is increasing and they are generally not recommended (CDC, 2016) [10]. Antibiotics use depends upon the susceptibility patterns in the particular geographical region. Currently, the antibiotics of choice are Fluoroquinolones or Azithromycin, with an emerging role of Rifaximin.

E. coli that vaccine development efforts have been focused on are the ETEC. Other proven prevention methods for *E. coli* transmission include hand washing and improving sanitation and drinking water, as transmission occurs through fecal contamination of food and water.

2. Materials and Methods

2.1. Materials

Materials used include: Media, Chemicals/Reagents, Glassware, Equipment, and Antibiotic Disks.

2.2. Methods

2.2.1. Study Area

This study was carried out at Federal University Lafia, Nasarawa State, Nigeria.

2.2.2. Ethical Approval

Ethical approval was obtained from Federal University Lafia, Health centre, Lafia, Nasarawa State, Nigeria.

2.2.3. Sample Collection

Urine samples were collected from the laboratory department of Federal University Lafia, Health centre, Lafia. The samples were transported in an ice box to Microbiology Laboratory, Federal University Lafia, for microbiological analysis.

2.2.4. Media Preparation and Sterilization

Media preparation was done according to the manufacturers specifications. Appropriate gram of the powder agar was weighed and dissolved in appropriate amount of water, followed by shacking and/or heat boil to dissolve completely.

2.2.5. Preparation of McFarland Standard

The McFarland's standard was prepared as follows: 0.5 ml of 1.172% (w/v) $BaCl_2.H_2O$ was added to 99.5 ml of 1% (v/v) H_2SO_4.

2.2.6. Sterilization

Media and other materials such as Petri dishes, conical flask, and other glass wares are sterilized by autoclaving (moist heat sterilization at 121˚C for 15 minutes) or by Hot air Oven (Dry heat sterilization) for the plastics.

2.2.7. Isolation of *E. coli*

Escherichia coli (*E. coli*) were isolated from urine samples of students attending Federal University Lafia, Health centre, as follows, A loopful of the urine was streaked on McConkey agar plates and incubated at 37˚C for 24 hours, pinkish colonies suspected to be *E. coli* on McConkey agar were subculture onto Eosine Methylene Blue (EMB) agar, and incubated at 37˚C for 24 hours. Colonies growing with Greenish metallic sheen on EMB agar plates, after 24 hours incubation was selected to be suspected *E. coli.*

2.2.8. Identification of *Escherichia coli*

Cultural identification: *E. coli* grows as a pinkish colony on McConkey agar while on Eosine Methylene Blue agar; *E. coli* has a greenish metallic sheen colony. Morphological identification: *E. coli* is a gram negative rod shaped bacteria as seen under the light microscope during the gram staining. Biochemical test used for the identification of *E. coli* include: Gram staining, Indole test, Methyl-red/Voges proskeur test and Citrate utilization test.

2.2.9. Antibiotic Susceptibility Test

The antibiotic susceptibility testing was carried out using Kirby-Bauer disc diffusion method modified by CLSI (2014) [11]. Exactly, four (4) variants colonies

of *E. coli* isolates were inoculated into 5 ml of sterile normal saline in a test-tube, and the turbidity of the bacteria suspension were adjusted equivalents to turbidity of 0.5 McFarland's standard. Using sterile swab stick soaked in the adjusted *E. coli* suspension, streak on Mueller Hinton agar (MHA) plates and antibiotic disc were placed aseptically at equidistance on Mueller-Hinton agar plates inoculated with the *E. coli* isolates. The plates were allowed to stand for 1 hour for pre-diffusion at room temperature before they were incubated at 37˚C for 24 hours. The diameter zone of inhibition (mm) was determined using meter rule and the results were interpreted in accordance with CLSI (2014) [11]. Antibiotics disks used include: gentamicin (10 µg), cefuroxime (30 µg), amoxicillin-clavulanate (30 µg), perfloxacin (10 µg), streptomycin (30 µg), ampicillin (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), sulphonamide/sulphomethoxazole (30 µg), and cefoxitin (30 µg).

2.2.10. Phenotypic Detection of AmpC Beta-Lactamase Production

A lawn culture of *E. coli* was prepared on Mueller Hinton Agar plate. Sterile disk (6 mm) were moistened with sterile saline (20 µl) and inoculated with several colonies of test organism. The inoculated disk was then placed beside a cefoxitin disk (almost touching) on the inoculated plate. The plates were incubated overnight at 35˚C. A positive test appears as a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk. A negative test was undistorted zone (Black *et al.* 2003) [12].

3. Result

3.1. Isolation and Identification of *Escherichia coli*

The cultural, morphological and biochemical characteristics of *E. coli* isolated from Urine of students attending Federal university of Lafia, Health Centre is as given in Table 1.

3.2. Rate of Occurrence of *Escherichia coli*

The occurrence of *Escherichia coli* isolated from the urineof students attending Federal University of Lafia, Health centre, shows that out of the 22 urine samples

Table 1. Cultural, Morphological and Biochemical characteristics of *Escherichia coli* isolated from urine of students attending Federal Unversity of Lafia, Health centre.

Cultural Characteristics	Morphological Characteristics		Biochemical Characteristics				Inference
	Gram Stain	Morphology	IND	MR	VP	CT	
Pinkish colonies on MacConkey agar and greenish metallic sheen on EMB agar	−	Rod shape	+	+	−	−	*E. coli*

Key: CT = Citrate Test; IND = Indole Test; MR = Methyl Red Test; VP = Voges-Proskauer Test.

obtained from students, the frequency of occurrence of *E. coli* in the urine was 11 (50%) as given in Table 2.

3.3. Antibiotics Susceptibility

The antibiotic susceptibility of *Escherichia coli* from urine of students attending Federal University of Lafia, Health centre, is as shown in Table 3. The *E. coli* isolated from the urine were less susceptible to gentamicin (27%), streptomycin (22.7%), chloramphenicol (18%), sparfloxacin (13.6%), ofloxacin (9%), augmentin and septrin (4.5%). But none was susceptible to amoxicillin/clavulanic acid, pefloxacin and ciprofloxacin respectively. The differences in the susceptibility of *E. coli* isolates from urine of students attending Federal University of Lafia, Health centre, Lafia, Nigeria were statistically insignificant ($p > 0.05$).

3.4. Antibiotic Resistance Phenotypes

The antibiotic resistance phenotypes of *E. coli* isolated from urine of students attending Federal University of Lafia, health centre is as shown in Table 4. The *E. coli* isolates are distributed into different antibiotic resistant phenotype and the most common antibiotic resistance phenotype is SXT-CH-SP-CPX-AM-AU-CN-PEF-OFX-S the percentage occurrence was 27.3% as shown in Table 4. The differences in the occurrence of antibiotic phenotypes of *E. coli* from urine were statistically significant.

Table 2. Rate of occurrence of *Escherichia coli* isolated from urine of students attending Federal University of Lafia, Health centre.

Sample	No examined	No of *E. coli* (%)
Urine	22	11 (50.0%)
Total	22	11 (50%)

Table 3. Antibiotics Susceptibility of *Escherichia coli* isolated from urine of students attending Federal Univesity of Lafia, Health centre, Lafia.

Antibiotics	Disc contents	Urine (n = 22)
Septrin	30 µg	1 (4.5)
Chloronpheniocal	30 µg	4 (18)
Sparfloxacin	10 µg	3 (13.6)
Ciprofloxacin	10 µg	0 (0)
Amoxacilin	30 µg	0 (0)
Augmentin	30 µg	1 (4.5)
Gentamycin	10 µg	6 (27)
Pefloxacin	30 µg	0 (0)
Ofloxacin	10 µg	2 (9)
Streptomycim	30 µg	5 (22.7)

3.5. Multiply Antibiotics Resistance (MAR) Index

The MAR index of the *E. coli* isolates were MAR isolates with MAR Index of ≥0.4 as given in Table 5. The commonest MAR index in the urine isolates was 0.9 and the frequency of it occurrence was 11 (50%) as given in Table 5.

3.6. AmpC Beta-Lactamase Production

The result of phenotypic confirmatory of AmpC beta-lactamase producing *E. coli* isolated from the urine of students attending Federal University of Lafia, Health centre is as given in Table 6. From the 11 *E. coli* isolates from the urine,

Table 4. Antibiotics resistance phenotype of *E. coli* isolates from urine of students attending Federal University of Lafia, Health centre.

Antibiotics resistant phenotype	No (%) resistance Urine (22)
SXT-SP-CPX-AM-AU-CN-PEF	1 (4.5)
SXT-CH-CPX-AM-AU-PEF-OFX-S	1 (4.5)
SXT-CH-SP-CPX-AM-PEF-OFX-S	1 (4.5)
SXT-SP-CPX-AM-AU-PEF-OFX-S	1 (4.5)
CH-SP-CPX-AM-AU-CN-PEF-OFX-S	1 (4.5)
SXT-CH-CPX-AM-AU-CN-PEF-OFX-S	1 (4.5)
SXT-SP-CPX-AM-AU-CN-PEF-OFX-S	1 (4.5)
SXT-CH-SP-CPX-AM-AU-PEF-OFX-S	2 (9.1)
SXT-CH-SP-CPX-AM-AU-PEF-OFX-S	4 (18.2)
SXT-CH-SP-CPX-AM-AU-CN-PEF-OFX	3 (13.6)
SXT-CH-SP-CPX-AM-AU-CN-PEF-OFX-S	6 (27.3)

Table 5. Multiply antibiotics resistance (MAR) index of *E. coli* isolated from urine of students attending Federal University of Lafia, Health centre.

No of antibiotic resistance (a)	No of antibiotic tested (b).	MAR index (a/b)	Frequency (%) Urine (n = 22)
10	10	1.0	6 (27)
9	10	0.9	11 (50)
8	10	0.8	4 (18.2)
7	10	0.7	1 (4.5)
6	10	0.6	0 (0)
5	10	0.5	0 (0)
4	10	0.4	0 (0)

Table 6. Phenotypic confirmatory of AmpC beta-lactamase producing *E. coli* isolated from urine of students attending Federal University of Lafia, Health centre.

Organism	No (%) resistance to cefoxitin	No (%)AmpC beta-lactamase Producers
E. coli (n = 11)	5 (45.4%)	3 (60.0%)

5 (45.4%) were resistance to cefoxitin. While, 3 (60.0%) of the resistance *E. coli* isolates from the urine were confirmed to be AmpC beta-lactamase producing *E. coli.*

4. Discussion

The growing frequency of AmpC beta-lactamase producing bacteria in clinical settings is causing treatment failure and greater Hospital costs due to infection caused by this bacterium (Tschudin-Sutter *et al.* 2010) [13]. The presence of AmpC beta-lactmase production in many *E. coli* strains are of serious concern, since these organism are the most common cause of different human infection (Nathisuwan *et al.* 2001) [14]. AmpC beta-lactamase producing *E. coli* are becoming a great challenge and an increasing problem for hospitals worldwide (Nathisuwan *et al.* 2001) [14].

Study on AmpC β-lactamas production in *E. coli* isolated from Urine of Students attending FuLafia Clinic was carried out. From this study we observed that the percentage occurrence of *E. coli* from the urine was high and this is in agreement with the study earlier reported by CDC (2013) [15], Islam *et al.* (2015) [16] and Ngwai *et al.* (2014) [17]. Though, the occurrence of *E. coli* from urine was not surprising and is in agreement with the study earlier described by Ajayi and Ekozien, (2014) [18] that *E. coli* is one of the common etiological agents of UTIs. The percentage occurrence of *E. coli* from urine of the students observed in this study (52.0%) was higher than the study earlier reported by Ngwai *et al.* (2014) [17].

The less susceptibility of *E. coli* to gentamycin, streptomycin, tarivid and chloronpheniocal observed in this study was expected and this may be due to indiscriminate use of this antibiotic without prescription by physicians (Ngwai *et al.* 2011 [19]; Okeke *et al.* 2007 [20]). The susceptibility of *E. coli* isolated from urine to gentamycin, ofloxacin and streptomycin contradicts the study's high susceptibility of *E. coli* to gentamycin, streptomycin, and ofloxacin as earlier described by Ngwai *et al.* (2014) [17]. The low susceptibility of *E. coli* to antibiotics mentioned may be due to misuse or abuse of the antibiotics (Ngawi *et al.* 2011 [19]; Okeke *et al.* 2007 [20]). The low susceptibility of *E. coli* to antibiotics such as ofloxacin, ciprofloxacin, amoxicillin, pefloxacin, and augmentin observed in this study seem to be in agreement with the study earlier reported by Ngwai *et al.* (2011) [19]. The production of AmpC β-lactamase by *E. coli* isolates resistant to cefoxitin observed in this study was not surprising and this finding is in agreement with the study earlier described by Wiegand *et al.* (2007) [21] and Jacobsy (2009) [22]. The percentage occurrence of AmpC β-lactamase producing *E. coli* isolates observed in this study was higher than the study as earlier described by Ogbolu *et al.* (2013) [23] and Al-Agamy *et al.* (2016) [24]. The high occurrence of AmpC β-lactamase producing *E. coli* in the urine of students is an indication of indiscriminate use of cefoxitin antibiotics in the clinical setup.

5. Conclusion

The *E. coli* isolates from the urine of the students were less susceptibility to gen-

tamicin, streptomycin, ofloxacin, and chloronpheniocal. Most of the *E. coli* isolates were Multiple Antibiotic Resistance (MAR) isolates. In addition, some of the *E. coli* isolated from the urine of students attending FuLafia Clinic were AmpC β-lactamase producers.

6. Recommendations

Based on the findings of this study, the following are recommended:

There should be mass education and public awareness programmes on the importance of proper personal hygiene and good environmental sanitation habits. Since antimicrobial-resistant patterns are constantly evolving, and present global public health problems there is a need for constant antimicrobial susceptibility surveillance. This will help clinicians provide safe and effective empiric therapies. There is a need for controlling the spread of multidrug-resistant organisms by providing sufficient personnel and resources for infection control in all healthcare facilities.

Judicious use of antimicrobials *i.e.* using the appropriate antibiotics at the appropriate dosage and for the appropriate duration through the appropriate route of administration is an important means of reducing the selective pressure that helps resistant organisms emerge.

Conflicts of Interest

The authors declare no conflicts of interest.

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