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Antimicrobial Susceptibility Profile of *Escherichia coli* Isolated from the Urine of Asymptomatic Students in a University in South-Western Nigeria

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Abstract

Asymptomatic bacteriuria is a common occurrence among healthy students, with a tendency to develop into symptomatic urinary tract infections (UTIs). This study was carried out to investigate the occurrence and antimicrobial susceptibility profile of *Escherichia coli* from the urine of asymptomatic students. A total of 180 mid-stream urine samples were collected from apparently healthy students, whose consents were sought. *E. coli* was isolated and tested for their susceptibility to commonly used antimicrobial agents using the disk diffusion protocol. Result analysis was done using one way analysis of variance (ANOVA) with the help of SPSS and level of significance taken at $p<0.05$. The isolates showed resistance against 90% of the antibiotics used in this study with 93.3% of the isolates resistant to cefuroxime and 35 % resistant to nitrofurantoin. Multidrug resistance (MDR) was also observed where the isolates showed resistance to at least three classes of the antimicrobial agents used in this study. A multiple antibiotic resistance index greater than 0.2 was observed in over 80 % of the isolates. This study showed a rare occurrence in which almost all the *Escherichia coli* isolates showed resistance to over 90% of the antibiotics used in this study with the exception of nitrofurantoin which seems to be the most effective antibiotics against all the isolates. This situation portends grave danger to public health; hence, the need for high level surveillance and monitoring of the use of antibiotics in therapy as well as the molecular identification of the prevalent resistant strains.

Key words: Antimicrobial, Asymptomatic, *Escherichia coli*, Susceptibility profile, Urine

1. Introduction

Urinary tract infections (UTIs) have been defined as conditions in which the urinary tract becomes successfully colonized by pathogenic microorganisms, leading to the presentation of clinical symptoms of UTIs such as dysuria, fever, frequency and urgency of urination that may be bloody or contain pus in severe cases as well as supra pubic pain in women with normal genitourinary tract. In some cases, however, the microbial colonization does not result in any noticeable symptoms and this is referred to as asymptomatic bacteriuria, not requiring any antimicrobial intervention [1]. Asymptomatic bacteriuria (or asymptomatic urinary tract infection) is said to be significant when urine contains a bacterial count of $\geq 10^5$ cfu/mL in voided midstream clean catch urine, aseptically collected from the volunteer subject without symptoms of UTI [2]. It is said to be very common but varies widely with age, sex and the presence of genitourinary abnormalities [2]. A number of studies showed that people harboring bacteriuria had greater chances of having symptomatic urinary tract infections than those without asymptomatic bacteriuria [3, 4]. The global occurrence of UTI had been put at about 150 to 250 million people [5]. This may be on the rise in areas where there are improper waste disposal systems and very poor sanitary conditions generally. UTI has a higher preponderance in females than in males due to their shortened urethra (about 4 cm), thus opening nearer to the anus compared to the males with a much longer urethra (about 20 cm) and opening farther away from the anus [1]. Asymptomatic bacteriuria is said to be rare among healthy young men [6]. However, the occurrence in men rises significantly after 60 years of age, presumably because of obstructive uropathy and voiding dysfunction associated with prostatic hypertrophy [6]. The UTI is believed to be caused chiefly by poor personal hygiene [7]. Other predisposing factors to UTI include pregnancy, prolonged period of catheterization and sexual intercourse [8]. The outcome of several researches had implicated *Escherichia coli* as the most prominent pathogenic microorganism isolated from both symptomatic and asymptomatic bacteriuric subjects [2, 9]. However, other less implicated pathogenic organisms such as *Klebsiella pneumonia*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Proteus mirabilis* can also cause urinary tract infection [7,2,10]. The antimicrobial susceptibility profile of bacteria seems to favor resistance, especially those isolated from symptomatic bacteriuria, known to be more virulent [2] and capable of acquiring multidrug resistance to antimicrobials, posing serious public health concerns. In addition, there is the proliferation of substandard or counterfeit drugs in many developing countries, with its attendant chaotic drug distribution

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channels and indiscriminate prescription as well as poor infection control strategies, thus further increasing the development of multidrug resistant pathogens [11, 5].

There are limited data on the occurrence of *E. coli* asymptomatic bacteriuria as well as their antimicrobial susceptibility pattern among undergraduate students on the Sagamu campus of Olabisi Onabanjo University, Ogun State, Nigeria. The aim of this study, therefore, is to determine the susceptibility profile of *E. coli* bacteriuria in apparently healthy undergraduate students to some antimicrobial agents.

2. Material and Methods

2.1 Study Area, Study Population, Exclusion Criteria and Collection

This study was carried out on the Sagamu Campus of Olabisi Onabanjo University in Ogun State Nigeria, comprising the Faculty of Pharmacy and the Faculty of Basic Medical Sciences. Sagamu is about 52 km from Lagos, the former capital city of Nigeria. A total of 180 mid-stream clean catch urine samples (90 from males and 90 from females) were collected between May and June 2019 from apparently healthy undergraduate students, whose consents were sought. Students of the age bracket of 18-30 years, who were not on antimicrobial chemotherapy at the time of sample collection or had not taken antibiotics two (2) weeks prior to sampling and are not pregnant, were considered for this study. The early morning mid-stream clean catch urine samples were collected in batches using sterile sample bottles, appropriately labeled and transported to the Pharmaceutical Microbiology Laboratory of Olabisi Onabanjo University for further investigations.

2.2 Isolation and Identification of Bacteria Isolates

The urine samples were inoculated on Mac Conkey agar (MCA) (Oxoid) within 24 h of collection in order to isolate the lactose fermenters, which appear as pink colonies on this medium. The pink colonies were sub-cultured onto Eosin methylene blue agar (EMB) (Oxoid) in order to differentiate *Escherichia coli* isolates from other lactose fermenters, evidenced by their greenish metallic sheen on EMB agar. The presumptive *E. coli* isolates were further confirmed microscopically to be Gram negative motile rod and biochemically by Indole $^{(+)}$, Methyl red $^{(+)}$, Catalase $^{(+)}$ and oxidase $^{(-)}$ tests.

2.3 Antimicrobial susceptibility testing

The antimicrobial susceptibility profiles of the isolates were determined by a modified method of the disc diffusion technique (Kirby-Bauer method) according to the set criteria provided by the Clinical and Laboratory Standards Institute (CLSI) [12]. Briefly, each of the isolate maintained in a nutrient agar slant were transferred into tubes containing Mueller Hinton broth (MHB) and then incubated at 37 ^{0}C for 24 hours. The bacteria suspensions were then standardized to 0.5 McFarland's standard and then streaked on the Mueller Hinton agar (MHA) plates. This was followed by placing the Gram-negative multiple discs (Rapid lab) containing ampicillin (10 µg), ceftaxidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), nitrofurantoin (300 µg) and amoxicillin/clavulanate (Augmentin®) (25 µg) aseptically on MHA plates and allowed to stand for 30 min for proper diffusion before it was incubated in inverted position at 37 ^{o}C for 24 h. The inhibitory zone diameters (IZDs) were finally measured using a ruler to the nearest millimeters (mm). The control organism, *E. coli* ATCC 25922, was used to validate the accuracy of this test. The isolates were then classified as resistant (R), intermediate (I) or susceptible (S) based on the standard interpretation guidelines provided by CLSI.

2.4 Statistical Analysis

The results obtained were analyzed using the Statistical package for Social Sciences (SPSS) version 16 by one way analysis of variance (ANOVA) and significance taken at $p<0.05$

3. Results and Discussion

3.1 Results

The results obtained are as presented on Tables 3.1 - 3.6.

Table 3.1: Isolation Rate of *E. coli*

Urine Screened	**Samples**	**Positive *E. Coli***	**Isolation Rate (%)**
Gender	Number		25
Male	90	19	
Female	90	26	
Total	180	45	

Table 3.2: Antibiogram of the *E. coli* Isolates

Isolates	Inhibitory Zone diameter (mm)															
	AMP			CAZ			AUG			CPR			NIT			CRX			OFL			GEN		
	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S
AS_{22}		16			10			22			12			23			10			07			08	
AS_{24}		22			14			20			08			23			12			08			12	
AS_{27}		09			18			21			08			15			00			09			10	
AS_{34}		14			10			14			09			12			10			08			13	
AS_{35}		07			08			07			12			05			00			00			00	
AS_{47}		09			07			05			08			08			05			05			00	
AS_{60}		00			00			00			00			17			00			00			00	
AS_{61}		07			18			09			21			21			10			12			15	
AS_{64}		07			10			10			16			20			15			00			10	
AS_{67}		00			00			23			08			20			09			07			10	
AS_{69}		08			00			20			12			25			07			09			00	
AS_{72}		00			05			12			00			19			00			00			10	
AS_{73}		18			00			22			21			25			15			20			24	
AS_{75}		15			14			22			21			24			19			19			22	
AS_{76}		00			00			00			00			17			00			00			00	
AS_{81}		10			05			13			02			15			00			00			00	
AS_{83}		08			05			10			04			10			00			00			04	
AS_{85}		00			10			15			00			25			00			14			00	
AS_{88}		00			00			00			00			00			00			00			00	
B_{7}		00			00			00			00			13			05			08			07	
B_{9}		00			14			00			00			19			00			00			00	
B_{10}		00			16			04			00			15			00			00			00	
B_{13}		00			00			08			00			17			00			00			00	
B_{14}		00			00			00			21			18			00			00			02	
B_{16}		00			00			00			00			00			00			00			00	
B_{20}		00			06			00			00			19			07			00			00	
B_{21}		00			00			00			00			14			00			00			00	
B_{25}		00			18			00			21			00			00			18			14	
B_{38}		00			00			00			00			14			00			00			00	
B_{40}		00			00			00			00			17			00			00			00	
B_{41}		00			00			08			08			15			00			00			00	
B_{48}		00			09			00			00			17			00			00			00	
B_{57}		10			00			12			00			13			00			00			07	
B_{61}		00			00			00			00			15			00			00			00	
B_{62}		00			00			00			00			14			00			00			00	
B_{65}		00			00			00			00			14			00			00			00	
B_{70}		00			18			00			21			19			00			20			15	
B_{73}		07			00			09			00			15			00			00			00	
B_{75}		00			18			00			22			16			00			20			15	
B_{79}		00			00			00			00			13			00			00			00	
B_{83}		00			00			00			00			15			08			09			00	
B_{84}		00			14			00			21			00			00			20			14	
B_{87}		00			00			00			00			15			00			20			00	
B_{90}		00			18			10			22			00			00			19			15	
B_{91}		00			15			15			21			05			08			19			15	

Key:
R-Resistance; I-Intermediate; S-susceptible; AMP-Ampicillin; CAZ-Ceftazidine; CRX-Cefuroxime; GEN-Gentamicin; AUG-Augmentin; CPR-Ciprofloxacin; NIT-Nitrofurantoin; OFL-Ofloxacin

Table 3.3: Multi Drug Resistant (MDR) Isolates

No. of Antibiotic Classes to Which Isolate is Resistant to	No. (%) of Isolates with Multiple Resistance E. Coli (n=180)
3	15(8.3)
4	22(12.2)

Table 3.4: Multiple Antibiotic Resistant (MAR) Index

Number of Antibiotics to which the Isolate Showed Resistance (a)	Total Number of Antibiotics to which Isolates had been Exposed to (b)	MAR= a/b
1	8	0.1
3	8	0.4
4	8	0.5
5	8	0.6
6	8	0.8
7	8	0.9
8	8	1.0

Table 3.5: Percentage Susceptibilities of *E. coli* Isolate to the Antibiotics

Antibiotics	Disk content (µg)	Number Susceptible to Drugs *E. coli* (n=45)	Percentage
Ampicillin	10	2	4.4
Ceftazidine	30	0	0.0
Cefuroxime	30	1	2.2
Gentamicin	10	6	13.3
Augmentin	30	7	15.6
Ciprofloxacin	5	10	22.2
Nitrofurantoin	300	19	42.2
Ofloxacin	5	9	20.0

Table 3.6: Percentage Resistances of *E. coli* Isolate to the Antibiotics

Antibiotics	Disk content (µg)	Number Resistance to Drugs *E. coli* (n=45)	Percentage
Ampicillin	10	40	88.9
Ceftazidine	30	39	86.7
Cefuroxime	30	42	93.3
Gentamicin	10	35	77.8
Augmentin	30	35	77.8
Ciprofloxacin	5	34	75.6
Nitrofurantoin	300	16	35.6
Ofloxacin	5	35	77.8

3.2 Discussion

From the results obtained, isolation rate was low and the isolates obtained were largely resistant strains. The study showed the antimicrobial susceptibility profile of *Escherichia coli* isolates from the urine of asymptomatic male and female students against four common classes of antimicrobial agents including penicillin (Ampicillin, Augmentin®), Cephalosporin (Cefuroxime, Ceftaxidine), Aminoglycosides (Gentamicin) and Quinolones (Ciprofloxacin, Nitrofurantoin and Ofloxacin). Although, the occurrence was very low (Table 3.1), which might be due to the strict exclusion criteria employed in the process of sample collection, excluding conditions that may be great source of clinical isolates. Also, it has been proven in the earlier researches that the occurrence is higher in females than in males and this research is in agreement with that [1, 13]. The reason given for this was due to the short urethra in females (about 5 cm) giving it a relatively close proximity to the anus as against the male urethra that is farther away from the anus (about 22 cm). The *E. coli* isolates showed resistance against almost all the antimicrobial agents used in this study (Table 3.6) with 93.3 % of the isolates resistant to cefuroxime and 35 % resistant to nitrofurantoin. This is a rare occurrence which has not been reported in our sampling location, but in a town 90 km away [15-16]. This may be due to the indiscriminate use of these antibiotics often times dispensed without prescription, as well as the preponderance of substandard antibiotics for therapy. Multidrug resistance (MDR) was also observed where the isolates showed resistance to at least three classes of the antimicrobial agents used in this

study. A total of 37 isolates showed multidrug resistant of which 15 of the isolates showed MDR against 3 classes of the antibiotic used, representing 8.3 %, while 22 isolates showed MDR against 4 of the classes of antimicrobial agents employed in this study, representing 12.2 % (Table 3.3). These isolates also showed multiple antibiotic resistant (MAR) index greater than 0.2 in over 80 % of the isolates evaluated (Table 3.4). This high MAR index has been suggested by earlier workers to be due to an earlier exposure of the multidrug resistant isolates to these antimicrobial agents [14]. This implies that there is already a spread of these resistant strains in the sampling location. As per the performance of the individual antibiotics, the highest percentage susceptibility was elicited by nitrofurantoin (42.2 %) and the least susceptibility by ceftazidine (0.0 %) (Table 3.5). It may be because nitrofurantoin is hardly abused or rarely prescribed in the sample area. Hence the somewhat potent antimicrobial activity against the E. coli isolates. Conversely, the highest percentage resistance was given by Cefuroxime (93.3 %) while nitrofurantoin showed the least percentage resistance (35.6 %) (Table 3.6). This high resistance could be attributable to the indiscriminate use of this chemotherapeutic agent; especially due to the erroneous belief around the sampling location and a little beyond that it confers emergency contraception.

4. Conclusion

The present study showed a rare occurrence in which almost all the *Escherichia coli* isolates elicit resistance to over 90% of the antimicrobial agents used in this study with the exception of nitrofurantoin which seems to be the most effective antibiotics against the isolates. This situation portends a grave danger to public health especially because multidrug resistance had been reported in a town with close proximity to our study location hence, the need for high level surveillance and monitoring of the use of antibiotics in therapy. Antibiotics should therefore not be used for prophylaxis or for some form of emergency contraception. A further study on the molecular identification of the prevalent resistant strains as well as the rationale behind the MDR observed is therefore imperative to curtail this dangerous onslaught on the insufficient antibiotics for therapy. There is also the dire need to discover new antimicrobial moieties in order to enrich the armamentarium of antimicrobial chemotherapy.

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Conflict of Interest

The authors declare no conflict of interest. No funding was received for this study.

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