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Bacteriuria and Antimicrobial Susceptibility of *Escherichia coli* Isolated From Urine of Asymptomatic University Students in Keffi, Nigeria

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ABSTRACT

Background: Asymptomatic bacteriuria can develop into symptomatic urinary tract infection.
Objectives: This study investigated asymptomatic *Escherichia coli* bacteriuria among undergraduate students of Nasarawa State University, Keffi, Nigeria, and the antimicrobial susceptibility of bacterial isolates from these subjects.
Patients and Methods: Four hundred urine samples were collected from consenting healthy male and female students. The bacterial load of each sample was determined by spread plate count on nutrient agar. *E. coli* was isolated and antimicrobial susceptibility of the isolates to common antibiotics was evaluated by the disc-diffusion method.
Results: Of the urine samples, 80 (20%) showed significant bacteriuria, with a higher prevalence in females (25%) than in males (15%). While 60% of *E. coli* isolates from male samples were susceptible to pefloxacin or gentamicin, 3.3% were susceptible to amoxicillin/clavulanic acid. Twenty-seven (90%) *E. coli* isolates from male samples had multiple antibiotic resistance (MAR), with 37% being resistant to 5 antibiotics and possessing MAR indices of 0.5. Forty-nine (98%) of the *E. coli* isolates from female samples had MAR, with 13 (26.5%) being resistant to 6 antimicrobial agents and possessing MAR indices of 0.6.
Conclusions: Significant bacteriuria is observed among the students of Nasarawa State University, with a higher prevalence in females than in males. Pefloxacin, ofloxacin, and gentamicin are effective against *E. coli* isolates from the urine of these students.

▸ *Implication for health policy/practice/research/medical education:*
This study has highlighted the need to include asymptomatic bacteriuria investigation as part of the medical laboratory examination for students especially new entrants, in tertiary institutions as we have observed that they could be incubating asymptomatic infection and ordinarily would not go to see a doctor. Finally, the study also provides information on the likely choice of antibiotics to treat infections that might arise from these organisms.

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1. Background

Escherichia coli is a bacterium commonly found in the large intestine of humans and other warm-blooded animals. Depending upon the particular strain that is present, it can provide resistance against pathogenic organisms or can itself be pathogenic, causing disease at intestinal and extra-intestinal sites (1, 2). *E. coli* strains that cause disease within the intestinal tract are referred to as diarrheagenic *E. coli*, and the major pathotypes are enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAggEC), and diffusely adherent *E. coli* (DAEC) (3). Strains of *E. coli* that cause disease outside of the gastrointestinal tract are referred to as extra-intestinal pathogenic *E. coli* (ExPEC) and are divided into uropathogenic *E. coli* (UPEC), strains causing neonatal meningitis, and septicemic *E. coli* (4-

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6). UPEC is the most common pathotype of ExPEC and is found in patients with urinary tract infections (UTIs) (7). UTIs are among the most common bacterial infections acquired in the community and in hospitals worldwide (8).

UTI is defined as the presence of significant numbers of pathogenic bacteria, or other organisms, in the urinary system (9, 10). When these bacteria are present in the urinary tract (UT) of patients who do not exhibit symptoms normally associated with UTI (e.g., pain, frequency, and urgency), it is termed bacteriuria (11). Under these conditions, *E. coli* strains exist in an asymptomatic carrier state without any obvious symptoms of UTI (12). Bacteriuria may result from contamination during or after collection of urine, or it may indicate the presence of bacteria in the urinary bladder. Bacteriuria is said to be significant when urine contains a bacterial count of $\geq 10^5$ CFU/mL in voided midstream urine, aseptically collected from an individual without symptoms of UTI (13). Asymptomatic bacteriuria occurs more frequently in females than in males and is a major criterion of UTI (14).

2. Objectives

E. coli is among the most common bacterial causes of UTIs (15-25). Hence, in order to determine antibiotics that may be used against this organism, it is necessary to periodically investigate the prevalence of asymptomatic *E. coli* bacteriuria and to isolate and test the bacterium for its susceptibility to antimicrobial agents. Therefore, this study aimed to evaluate *E. coli* bacteriuria in healthy undergraduate students of Nasarawa State University, Keffi, Nigeria.

3. Patients and Methods

3.1. Study Area

The study was carried out in Nasarawa State University, Keffi, in Nasarawa State of Nigeria. Nasarawa State shares boundaries with Kaduna, Plateau, and Kogi States, and Federal Capital Territory (FCT) Abuja. Keffi is approximately 68 km from the municipal town of Abuja and 128 km from Lafia, the Nasarawa State capital. The town lies between latitude 8° 5´ N of the equator and longitude 7° 5´ E of the Greenwich meridian at an altitude of 850 m above sea level (26).

3.2. Sample Population, Exclusion Criteria, and Collection

The study population included 400 (200 males and 200 females) undergraduate students of the Nasarawa State University, Keffi. Volunteers, who gave their informed consent, included non-pregnant students and those who were not on antimicrobial therapy at the time of sample collection, or who had not taken antimicrobials within 2 weeks prior to sampling. Samples of early morning, mid-stream, and clean-catch urine were collected in 15- to 20-mL volumes in batches using sterile, wide-necked, leak-proof universal bottles. The urine samples were labeled and immediately transported to the Microbiology Laboratory of Nasarawa State University for analysis.

3.3. Bacterial Burden of Urine

One milliliter of each urine sample was diluted serially to a ratio of $1:10^6$ with sterile distilled water. Aliquots of each diluted sample (0.1 mL) were spread on the surface

Table 1. Bacterial Burden in the Urine of Asymptomatic Male and Female Undergraduate Students of Nasarawa State University, Keffi, Nigeria

Total Viable Bacteria (×106) CFU/mL	Samples With Load, No		Frequency of Occurrence, %	
	Male	Female	Male	Female
50–59	20	15	10.0	7.5
60–69	19	10	9.5	5.0
70–79	10	13	5.0	6.5
80–89	10	9	5.0	4.5
90–99	12	8	6.0	4.0
100–109	8	17	4.0	8.5
110–119	20	10	10.0	5.0
120–129	19	18	9.5	9.0
130–139	15	13	7.5	6.5
140–149	10	12	5.0	6.0
150–159	15	18	7.5	9.0
160–169	12	10	6.0	5.0
170–179	5	4	2.5	2.0
180–189	10	7	5.0	3.5
190–199	5	8	2.5	4.0
200–209	10	20	5.0	10.0
210–219	-	8	-	4.0

Table 2. The Occurrence of Bacteriuria Among Asymptomatic Male and Female Students of Nasarawa State University, Keffi, Nigeria

	Samples with Significant Bacteriuria, No. (%)	Samples with Non-Significant Bacteriuria, No. (%)	Samples with No Growth, No. (%)
Male	30 (15.0)	110 (55.0)	60 (30.0)
Female	50 (25.0)	90 (45.0)	60 (30.0)
Total	80 (20.0)	200 (50.0)	120 (30.0)

of dried sterile nutrient agar (NA; Merck KGaA, Darmstadt, Germany) and the plates were then incubated at 37°C for 18 h. Colonies were counted and only plates showing 50–300 CFU were selected for computation of the bacterial load (CFU/mL) of urine samples.

3.3. Isolation and Identification of Bacteria From Samples

Samples were first cultured on MacConkey agar (MAC; Sigma-Aldrich Chemie GmbH, Germany) to isolate lactose fermenters (pink or red colonies). Pink colonies were then transferred to eosin methylene blue agar (EMB; BIOTEC Laboratories Ltd, Ipswich, UK) to differentiate *E. coli* (on the basis of its metallic sheen when grown on EMB) from other lactose fermenters. Suspected *E. coli* colonies were further confirmed biochemically by "IMViC" (Indole, Methyl Red, Voges-Proskauer, and Citrate) tests. Pink or red colonies that grew with a metallic sheen on EMB and that were indole-positive, methyl red-positive, Voges-Proskauer-negative, and citrate-negative were identified as being *E. coli*. Isolates were either used immediately or were maintained on slants of NA (Merck KGaA) at 4°C for future use. All chemicals used were from BDH Laboratory Supplies, Poole, England.

3.4. Antibiotic Susceptibility Testing

Susceptibility testing of all isolates was carried out by the disc-diffusion technique in accordance with Clinical and Laboratory Standards Institute criteria (27) using gram-negative multi-antibiotic discs (Abidec Company, England) containing the following antimicrobials and disc content (in μg): amoxicillin (30 μg), sparfloxacin

Table 3. Rate of Susceptibility of *Escherichia coli* Isolated From Asymptomatic Male Students of Nasarawa State University, Keffi, Nigeria, to Common Antimicrobial Agents

Antimicrobial Agents	Disc Content, μg	Number of Isolates (% Susceptibility)	
		Male (n = 30)	Female (n = 50)
Sulfamethoxazole/Trimethoprim (SXT)	30	6 (20.0)	15 (30.0)
Amoxicillin (AMX)	30	2 (6.7)	3 (6.0)
Pefloxacin (PEF)	30	18 (60.0)	33 (66.0)
Amoxicillin/Clavulanic acid (AUG)	30	1 (3.3)	3 (6.0)
Chloramphenicol (CH)	30	6 (20.0)	10 (20.0)
Gentamicin (GEN)	10	18 (60.0)	35 (70.0)
Streptomycin (STR)	30	10 (33.3)	20 (40.0)
Ciprofloxacin (CIP)	10	8 (26.7)	9 (18.0)
Ofloxacin (OFX)	10	16 (53.3)	31 (62.0)
Sparfloxacin (SPX)	10	8 (26.7)	15 (30.0)

Table 4. Multiple Antibiotic Resistances in *Escherichia coli* Isolated From Asymptomatic Male and Female Undergraduate Students of Nasarawa State University, Keffi, Nigeria

Resistant to Antimicrobial Agents, No.	MAR index [a]	Isolates With MAR, No. (%)	
		Male (n = 27)	Female (n = 49)
3	0.3	3 (11.1)	2 (4.1)
4	0.4	5 (18.5)	4 (8.2)
5	0.5	10 (37.0)	4 (8.2)
6	0.6	8 (29.6)	13 (26.5)
7	0.7	5 (18.5)	8 (16.3)
8	0.8	6 (22.2)	9 (18.4)
9	0.9	4 (14.8)	5 (10.2)
10	1.0	-	4 (8.2)

[a] MAR index, No. of antimicrobial agents the isolate is resistant to/No. of antimicrobial agents tested

(10 μg), gentamicin (10 μg), pefloxacin (30 μg), chloramphenicol (30 μg), streptomycin (10 μg), ciprofloxacin (10 μg), sulfamethoxazole/trimethoprim (30 μg), ofloxacin (10 μg), and amoxicillin/clavulanic acid (30 μg). *E. coli* ATCC 25922 was used as an internal control.

4. Results

The bacterial load of urine samples is shown in *Table 1*. For urine samples from males, the most frequent bacterial load ranges were 50–59 ($\times 10^6$) CFU/mL and 110–119 ($\times 10^6$) CFU/mL, each of which was obtained in 10% of the male samples examined. The least frequent ranges were 170–179 ($\times 10^6$) CFU/mL and 190–199 ($\times 10^6$) CFU/mL, each of which was obtained in 3% of the male samples tested. In female samples, the most frequent bacterial load ranges were 200–209 ($\times 10^6$) CFU/mL, obtained in 10% of samples, and the least frequent was 170–179 ($\times 10^6$) CFU/mL obtained in 2% of samples.

The percentage of bacteriuria among the students was as shown in *Table 2*. The overall percentage of significant bacteriuria was 20%, with a higher prevalence of *E. coli* in females (25%) than in males (15%). The susceptibility of isolated bacteria to different antimicrobial agents is shown in *Table 3*. For male samples, 18 of the 30 (60%) *E. coli* isolates recovered were susceptible to pefloxacin and gentamicin, while only 1 isolate (3.3%) was susceptible to amoxicillin/clavulanic acid. For female samples, 33 (66%) of the 50 *E. coli* isolates recovered were susceptible to pefloxacin, 31 (62%) were sensitive to ofloxacin, and 35 (70%) to gentamicin, but only 3 (6%) were susceptible to amoxicillin or amoxicillin/clavulanic acid.

In this study, we defined multiple antibiotic resistance (MAR) as resistance to at least 3 antimicrobial agents (27). The percentage frequency of MAR was as shown in *Table 4*. In male samples, 27 (90%) of 30 *E. coli* isolates recovered had MAR, with 10 isolates (37%) being resistant to 5 antimicrobial agents and possessing MAR indices of 0.5. In female samples, 49 (98%) of the 50 *E. coli* isolates recovered had MAR, with 13 (26.5%) being resistant to 6 antimicrobial agents and possessing MAR indices of 0.6. MAR indices > 0.2 indicated that such isolates originated from environments where antimicrobial agents are freely available, leading to a high potential for misuse (28).

5. Discussion

Asymptomatic bacteriuria occurs frequently and is a major cause of UTI (14). This is because under favorable conditions, asymptomatic bacteriuria progresses to symptomatic (clinical) UTI (10, 29). Bacteria that colonize the UT may ascend towards the bladder to cause cystitis, which is usually associated with the classic symptoms of UTI (i.e., pain, frequency, and urgency). UTIs can proceed from the bladder, via the ureters, to the kidneys, where it can cause pyelonephritis, which may lead to irreversible kidney damage, renal failure, and death (30). The presence of bacteriuria among the students observed in this study is in agreement with previous findings that have demonstrated the presence of bacteria in urine without the occurrence of physical symptoms of UTI (10-12, 31-35).

The low percentage of significant *E. coli* bacteriuria observed among the students, despite bacterial growth occurring in 50% of the urine samples, could be attributed to contaminants from procedural error or the presence of vaginal feces or perennial skin of the volunteers. The higher level of significant *E. coli* bacteriuria in females than in males is consistent with the findings of previous reports (14, 36). In addition, clinical UTIs are more common among females, with up to 60% of women having at least 1 episode in their lifetime (37). Factors such as shortness of urethra, sexual activity, contraceptive use, estrogen deficiency, diabetes, obstructing lesions, and genetic factors, such as blood group secretor status, increase the likelihood of women contracting a UTI (10, 29).

The generally similar antibiotic susceptibility pattern of the isolates from both male and female volunteers indicates that antimicrobial treatment can be achieved in both sexes with similar drugs. The low susceptibility (i.e., higher resistance) of the isolates to the common and cheap orally administered antibiotics such as amoxicillin, amoxicillin/clavulanic acid, chloramphenicol, and ciprofloxacin is not surprising because these drugs are more commonly misused, thereby leading to the development of resistance, as previously reported (18). The higher susceptibility of isolates to other antibiotics such as gentamicin, ofloxacin, and pefloxacin was expected, as this has been reported previously (20).

Gentamicin is administered parenterally and, therefore, due to the discomfort of injection, it is less likely to be misused than oral drugs. In contrast, ofloxacin and pefloxacin are relatively costly in Nigeria, and this limits their misuse. A MAR index > 0.2 indicates that bacteria originate from an environment where antibiotics are freely available and are misused (28). The findings of this study have highlighted the need to include assessments of asymptomatic bacteriuria as part of the medical examination for students, especially new entrants, in tertiary institutions. Ordinarily such students would not attend a clinic, although as we have observed, they could be carrying an asymptomatic infection. Finally, our study also provides important information on the best choice of antibiotics to treat infections that might arise from these organisms.

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