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Antibiotic susceptibility pattern of isolates from urine of students in Benue State Polytechnic, Ugbokolo, Nigeria

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Abstract

Background: Urinary Tract Infections (UTIs) remains one of the most common infectious diseases diagnosed in developing countries. The widespread use of antibiotics against uro-pathogens has led to the emergence of antibiotic resistant species.

Objectives: The study aimed at determining the antibiotic susceptibility pattern of isolates from urine of students in Benue State Polytechnic, Ugbokolo, Nigeria.

Materials and methods: Three hundred and twenty seven (327) midstream urine samples from 137 (41.9%) males and 190 (58.1%) females were collected from students in hostels of Benue State Polytechnic, Ugbokolo. The urine samples were inoculated into the dried surfaces of Blood agar, MacConkey agar, Eosine Methylene Blue agar and Cysteine Lactose Electrolyte-deficient agar using a calibrated loop. The inoculated plates were aerobically incubated at 37oC for 24h. Colonies were sub-cultured repeatedly to obtain a pure culture. *E.coli* (ATCC 25922) and *S. aureus* (ATCC 25923) standard reference strain were used as control. The isolates were identified using cultural and biochemical characteristics such as Gram stain, Triple Sugar Iron agar test, methyl red, Voges-Proskauer, citrate utilization, catalase, oxidase, urease and coaulase test. The identified species were then exposed to selected antibiotics to test for their susceptibility.

Results: A high prevalence of 15.9% (n=327) was recorded in the study. Significant differences (P<0.05) were observed in the age, sex and number of isolates. Isolation rate showed female (63.5%, n=33) preponderance over male (36.5%, n=19). Isolation rate was highest in students aged 22-26 (44.2%, n=23). *E. coli* was the most frequently isolated organism. The Gram negative bacterial isolates showed the highest level of resistance to amoxicillin (92.3%, n=36) and SXT (92.3%, n=36) while the Gram positive exhibited the highest level of resistance to SXT (87.5%, n=7). All the isolates were however susceptible to imipenen and gentamycin.

Conclusion: The high prevalence showed by this study suggests the existence of a public health concern among the students. The fact that most of these isolates are resistant to one or more of the commonly used antibiotics calls for con

Keywords: Prevalence; Infection; Antibiotic resistance; Diagnose; Susceptibility.

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1. Introduction

Urinary tract infections (UTIs) are the most common infections in humans that can affect any part of the urinary system including kidneys, ureters, bladder or urethra [1]. It is estimated that approximately 150 million cases occur globally every year [2]. UTIs remain one of the most common infectious diseases diagnosed in outpatients [3]. It is most often caused by bacteria but may also be caused by fungal and viruses [4] [3]. Dadi *et al*. [5] opined that Gram-negative bacteria causes 90% of UTI cases while Gram-positive cause only 10% of the cases.

Numerous risk factors can increase the likelihood of developing a UTI, including female gender, sexual intercourse, poor personal hygiene, diabetes, obesity, vaginal infections and heavy use of antibiotics [6] [1]. Amdekar *et al*., [4] reported that the incidence is quite high among women due to the anatomy and physiology of their reproductive organ. The relative frequency of uro-pathogens varies depending on age, sex, catheterization and previous exposure of antimicrobials [3]. The leading pathogens of UTIs are *Escherichia coli*, *Klebsiella pneumoniae, Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida* spp. [2] [7] [8].

The emergence of antibiotic resistance in the management of UTIs is a serious public health issue; particularly in the developing countries characterize by high level of poverty, illiteracy and poor hygienic practices [9]. There are also high cases of drugs of questionable quality in circulation. The easy availability in the community without prescription makes the drug subject to abuse [10]. Knowledge of the local bacterial etiology and susceptibility patterns will be a useful guide in the effective and timely treatment of UTIs. Antibiotic therapy in the mainstay is the treatment of UTIs but unfortunately the emergence of multi-drug resistant strains of the causative organisms has become a major challenge of epidemiological importance [4] [11] [10]. The aim of the study was to determine the prevalence of uro-pathogens and *in vitro* susceptibility pattern to commonly used antibiotics among patients in health centres within Ugbokolo, Benue State.

2. Material and methods

2.1. Specimen collection

10ml of midstream clean urine specimens were collected in sterile containers from 327 students in different hostels of Benue State Polytechnic and taken to the microbiology laboratory of the institution for analysis and culturing on appropriate bacteriological media as suggested by Ochei and Kolhatker, [12] [8].

2.2. Bacteria Culture and Identification

0.01mL of mid-stream urine (MSU) specimen were inoculated onto the dried surfaces of Blood agar, MacConkey agar, Eosine methylene blue agar (EMBA) and Cystine lactose electrolyte-deficient agar (CLED) using a calibrated loop. The inoculated plates were aerobically incubated at 37°C for 24h. The incubation period was extended for a further 24 h in some cases. Colonies were sub-cultured repeatedly to obtain a pure culture. *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) standard reference strains were used as control for culture media. The sterility of culture media was checked in line with laboratory protocol. Bacterial identification was based on standard culture and biochemical characteristics of isolates such as Gram stain, triple sugar-iron agar, methyl-red, Voges-Proskauer, citrate utilization, catalase production, oxisase reaction, urease, coagulase and motility tests as suggested by Cheesbrough [13], Ochei and Kolhatker [12] and Arora and Arora [14].

2.3. Antibiotic Susceptibility Test

The isolates were subjected to antimicrobial susceptibility test using the standard Kirby-Bauer disc diffusion method as described by Arora and Arora [14]. Results were interpreted using the criteria of the Clinical Laboratory Standards Institute [15]. Overnight cultures of the isolates were inoculated into a test tube containing 5 ml of phosphate buffered saline (PBS). The turbidity of each inoculum was adjusted to 0.5 McFarland standard prepared by mixing given amounts of Barium chloride and sulphuric acid.

The standardized culture was evenly spread over the entire surface of Mueller-Hinton agar (Oxoid, CM 0337) plates using sterile swab stick. Sterile forceps was used to carefully pick and gently place the antibiotic discs of known concentrations on the dried but inoculated surface of the Mueller-Hinton agar plates. The discs were gently pressed onto the medium surface with a sterilized forceps to ensure firm contact. The plates were incubated at 37°C for 24 h.

The antibiotic impregnated discs (Oxoid Ltd) used were Amoxicillin/clavulanic (30µg), ceftazidime (30µg), ceftriaxone (30µg), amoxicillin (10µg), sulphamethoxazole/trimethoprim (25µg), chloramphenicol (30µg), ciprofloxacin (5µg), azithromycin (15µg), gentamycin (30µg) and imipenen (10µg).

Diameters of zones of inhibition (if any) around the antibiotic disc were measured to the nearest millimetre using a ruler and results reported as sensitive, intermediate or resistant for each antibiotics used.

2.4. Data Analysis

Statistical analyses were done using Statistical Package for Social Sciences (SPSS) version 17 (2008). Pearson's chi-square test was used to determine associations between variables at 95% confidence level. A p value less than or equal to 0.05 was considered to be indicative of a statistically significant relationship. Analysis of variance (ANOVA) was used to determine associations between variables (age, sex, locations, seasonal variation and rate of multi-drug resistance).

3. Results and discussion

Table 1 Prevalence of UTIs with Respect to Age.

Age (Years)	Positive (%)	Negative (%)
17-21	7 (13.5)	33 (12.0)
22-26	23 (44.2)	124 (45.1)
27-31	16 (30.8)	92 (33.5)
32-36	4 (7.7)	16 (5.8)
≥37	2 (3.8)	10 (3.6)
Total	52 (100)	275 (100)

$\chi=26.063$, df=4, P<0.05.

Table 2 Prevalence of UTIs with Respect to Sex

Sex	Positive (%)	Negative (%)
Male	19 (36.5)	118 (42.9)
Female	33 (63.5)	157 (57.1)
Total	52 (100)	275 (100)

$\chi=10.371$, df=1, P<0.0

Table 3 Frequency of Bacterial Isolates

Bacterial Isolates	Frequency (%)
Escherichia coli	22 (46.8)
Klebsiella pneumoniae	14 (29.7)
Staphylococcus aureus	6 (12.8)
Proteus mirabilis	3 (6.4)
Streptococcus species	2 (4.3)
Total	47 (100)

$\chi=54.615$, df=4, P<0.05.

Table 4 Antibiotic susceptibility pattern of gram-negative bacteria isolates among students of Benue State Polytechnic, Ugbokolo.

Bact. isolates	n	Pattern	CIP	AML	CRO	CAZ	SXT	AMC	C	GN	IMP
E. coli	22	S	18 (81.8)	0 (0)	16(72.7)	15(68.2)	1(4.5)	20(90.0	2(9.1)	20(90.0	22(100)
		I	1(4.5)	1(4.5)	4(18.2)	6(27.3)	0(0)	0(0)	1(4.5)	2(9.1)	0(0)
		R	3(13.6)	21(95.5)	2(9.1)	1(4.5)	21(95.5)	2(9.1)	19(86.4)	0(0)	0(0)
K. pneumoniae	14	S	10(71.4	0(0)	8(57.1)	0(0)	0(0)	11(78.6	0(0)	14(100)	14(100)
		I	1(7.1)	1(7.1)	0(0)	3(21.4)	0(0)	1(7.1)	1(7.1)	0(0)	0(0)
		R	3(21.4)	13(92.9)	6(42.9)	11(78.6)	14(100)	2(14.2)	13(92.9)	0(0)	0(0)
P. mirabilis	3	S	2(66.7)	0(0)	2(66.7)	1(33.3)	0(0)	2(66.7)	0(0)	0(0)	3(100)
		I	1(33.3)	1(33.3)	0(0)	1(33.3)	2(66.7)	0(0)	2(66.7)	0(0)	0(0)
		R	0(0)	2(66.7)	1(33.3)	1(33.3)	1(33.3)	1(33.3)	1(33.3)	3(100)	0(0)
Total	39	S	30(76.9	0(0)	26(66.7)	16(41.0)	1(2.6)	33(84.6	2(5.1)	34(87.2	39(100)
		I	3(7.7)	3(7.7)	4(10.3)	10(25.6)	2(5.1)	1(2.6)	4(10.3)	2(5.1)	0(0)
		R	6(15.4)	36(92.3)	9(23.0)	13(33.3)	36(92.3)	5(12.8)	33(84.6)	3(7.7)	0(0)

Table 5 Antibiotic susceptibility pattern of gram-positive bacteria isolates among students of Benue State Polytechnic, Ugbokolo.

Bact. isolates	n	Pattern	CIP	AML	CRO	CAZ	SXT	AMC	C	GN	IMP
S. aureus	6	S	4 (66.7)	0 (0)	0(0)	4(66.7)	1(16.7)	3(50.0)	2(33.3)	4(66.7)	6(100)
		I	0(0)	1(16.7)	2(33.3)	0(0)	0(0)	1(16.7)	1(16.7)	2(33.3)	0(0)
		R	2(33.3)	5(83.3)	4(66.7)	2(33.3)	5(83.3)	2(33.3)	3(50.0)	0(0)	0(0)
S. spp	2	S	1(50.0)	0(0)	1(50.0)	0(0)	0(0)	1(50.0)	0(0)	1(50.0)	2(100)
		I	0(0)	1(50.0)	1(50.0)	0(0)	0(0)	1(50.0)	0(0)	1(50.0)	0(0)
		R	1(50.0)	1(50.0)	0()	2(100)	2(100)	0(0)	2(100)	0(0)	0(0)
Total	8	S	5(62.5)	0(0)	1(12.5)	4(50.0)	1(12.5)	4(50.0)	2(25.0)	5(62.5)	8(100)
		I	0(0)	2(25.0)	3(37.5)	0(0)	0(0)	2(25.0)	1(12.5)	3(37.5)	0(0)
		R	3(37.5)	6(75.0)	4(50.0)	4(50.0)	7(87.5)	2(25.0)	5(62.5)	0(0)	0(0)

4. Discussion

The prevalence and antibiotic susceptibility pattern of uropathogens from urine of students of Benue State Polytechnic, Ugbokolo was determined. Results of the study showed the overall prevalence of isolates to be 15.9% (n=52). This is consistent with the findings of Ehinmidu, [16], Marwan *et al.* [1] and Ahmed *et al.* [8]. The high prevalence of UTIs in the present study suggests the existence of a significant public health hazard in the study area.

Students within the age range of 22-26 (44.2%, n=23) had the highest prevalence of isolates. This agrees with the findings of Dadi *et al.* [5]. The high prevalence within the age group could be attributable to high sexual activity within this age group as reported by Singh *et al.* [17]. The implication of this finding is that the education of the youth who form the major workforce of the population, and the farming activities which is the main source of economic livelihood will be affected.

Isolation rate showed female (63.5%, n=33) preponderance over male (36.5%, n=19) students. This agrees with the findings of Singh *et al.* [17], Dadi *et al.* [5]. Other supporting earlier studies are those of Ibssa and Solomon, [9] and Wubalem and Alemayehu, [3].This could be due to the anatomical differences of urogenital organs between the two sexes. Gupta *et al.* [18] and Wubalem and Alemayehu, [3] opined that poor hygiene practice may result in direct faecal contamination of urinary tract from the anus in females, consequently providing easier access to the pathogens overgrowth and ascent to the bladder.

Among the isolates, Gram negative bacteria constitute 82.9% (n=39) while Gram positive bacteria constitute 17.1% (n=8). The higher prevalence of Gram negative bacteria in this study agrees with that reported by Mizarzi, [19]; and Wubalem and Alemayehu, [3]. The prevalence of *E. coli* (46.8%, n=22) in this study corroborates that of Ehinmidu, [16] and Wubalem and Alemayehu, [3] in Zaria, Nigeria and Ethiopia respectively. It is however higher than that of Ullah *et al.* [20]; and Beyene and Tsegaye, [21]. The prevalence of *S. aureus* (12.8%, n=6) in this study is consistent with the findings of Ibssa and Solomon, [9] and Wubalem and Alemayehu, [3]. The other isolates ranged between 5 to 10% which agrees with findings of Mohammad *et al.* [7], Singh *et al.* [17] and Dadi *et al.* [5].

The results of the finding also showed that all the Gram negative isolates were susceptible to imipenen (100%, n=39), followed by Gentamicin. They however showed a high level of resistance to Amoxycillin (92.3%, n=36) and SXT (92.3%, n=36). Similarly, the study revealed the most effective antibiotic against the Gram positive isolates to be Imipenen (100%, n=8). They however showed a high level of resistance to SXT (87.5%, n=7). This agrees with the findings of Harriet and Nandita, [11], Mohammad *et al.* [7] and Wubalem and Alemayehu, [3].

5. Conclusion

The prevalence and antibiotic susceptibility pattern of isolates from urine of students of Benue State Polytechnic, Ugbokolo was determined. The study showed a prevalence of 19.3% in the studied area. The most frequently isolated organisms are *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus mirabilis* and *Streptococcus* spp. Isolation rate showed female preponderance over male and age range 22-26 recorded the highest. All the isolates were susceptible to Imipenen followed by Gentamycin. They however showed high resistance to amoxicillin and sulponamide/trimetripine.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest between the authors. All authors of the manuscript have read and agreed to its content and are accountable for all aspects of the accuracy and integrity of the manuscript.

Author's contributions

A.P., M.E. and E.E.T. designed the study, carried out the bench work and data acquisition for analysis. A.J. did the data analysis and interpretation of results. A.P. did the drafting of the manuscript. Revision of the manuscript for critical intellectual content was done by A.P. and O.G.E. All authors gave final approval of the version to be published.

Statement of informed consent

Consent form was administered and filled by each participant.

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