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Prevalence and Antibacterial Susceptibility of Selected Bacteria in the Urine of Biology Students at Tai Solarin University of Education

***Sebiomo Adewole & Ojo Folasade Tinuade**

Department Of Biological Sciences, Tai Solarin University of Education Ijagun, Ijebu-Ode
*rev20032002@yahoo.com;

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Abstract: Urinary tract infection is a condition where one or more urinary structures become infected by the presence and growth of microorganisms that overcome the structures. This research work investigated the prevalence and antibacterial susceptibility of selected bacteria in the urine of biology students of Tai Solarin University of Education, Ijagun, Ogun State, Nigeria. Demographic survey of the students was determined. A 30 morse gauge, 3.26 mm calibrated wire loop capable of delivering 0.001 ml of urine was used for culturing on Blood Agar and MacConkey agar. The culture plates were incubated aerobically at 37˚C for 24 hours. Antibiotic susceptibility testing of the isolated test organisms was determined. Twenty-two percent of the respondents' used antibiotic in the past 14 days while 78% of the respondents did not use antibiotics in the past 14 days. All the twenty-five female students tested for the presence of *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* in their urine tested positive to these pathogenic bacteria, although some in asymptomatic form. The highest *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, counts of $2.41 \pm 0.00 \times 10^5$ CFU/ml, $5.59 \pm 0.00 \times 10^5$ CFU/ml and 1.86 ± 0.00 10^5 CFU/ml respectively were obtained in the urine samples of the female and male students. The highest Total Bacteria count of $4.77 \pm 0.00 \times 10^5$ CFU/ml was obtained in the female urine samples. Gentamicin showed highest zone of inhibition in *K. pneumonia* culture plateswith a mean value of 2.83 mm followed by erythromycin (2.51 mm) and ampicillin (2.48 mm).

Keywords: Susceptibility, Antibiotics, Inhibition, Urine, Bacteria

Introduction

The urinary tract is made up of organs (such as kidneys, ureters, bladder and urethra) that collects stores and releases urine from the body. Urinary tract infection is a condition where one or more urinary structures become infected by the presence and growth of microorganisms that overcome the structures. Urinary tract infections are one of the most common bacterial infections in the human urinary system including lower urinary tract such as bladder and urethra [1].

Diagnosis therefore cannot be made without bacteriological analysis of urine. Urinary tract infections (UTIs) such as cystitis and pyelonephritis are the most common infectious diseases in children. Bacteria causing urinary tract infection (UTI) is the most common found in developing countries like Nigeria where proper sanitization is not adequately considered. It has been reported that more than 150 million people are affected by UTI globally and it has also been estimated that about 30,000 UTI patients are treated in clinical wards [1].

Acute and uncomplicated UTI are most commonly found in women and it has been estimated that more than 60% women have UTI at least once in their lifetime. It has also been reported that the rate of causing UTI is 10.57% higher in sexually active females and teenage girls than males and the most common bacteria involved are *Escherichia coli* (32.8%), *Klebsiella pneumonia* (22.4%) and *Staphylococcus aureus* (15.1%). *E. coli* is the most common bacteria implicated in UTI infections. It causes 75-90% uncomplicated UTI [2].

Microbial invasion being the basis of urinary tract infection could be seen in various clinical manifestations resulting in various disease conditions in both males and females of all ages. Females are most at risk of bacteriuria. For example, in the United States, UTIs result in approximately 8 million physician visits and more than 100, 000 hospital admissions per year of sexually active women treated annually for UTIs. Up to 95% of the UTI cases in the U.S are treated with antibiotics such as cotrimoxazole without bacteriological investigation since these infections are routinely encountered in medical practice. This kind of indiscriminate use of antibiotics is however fraught with the problem of pathogen resistance to antibiotics [3]. Asymptomatic UTIs occur when urinary tract pathogens enter into the bladder without causing apparent symptoms. Typically, the pathogens are usually eliminated by host defense factors when they persist only for a short time in the human host. However, when such pathogens stay in the urinary system for a long time symptomatic urinary tract infection result [3].

Antimicrobial resistance among urinary tract isolates have recently been reported with an increased frequency all over the world. Several studies also have reported that the incidence of UTI has been increasing recently and its treatment has become more complicated because of the emergence of pathogens with increasing resistance to antimicrobial agents. It is necessary to identify the causative agent and spectrum of its antimicrobial susceptibilities in order to treat UTI. Since this spectrum may vary among geographical locations, hospitals and also in different age groups, each institution should carefully plan their antibiotic therapy.

Hence there is need to investigate the prevalence and antibacterial susceptibility of selected bacteria in the urine of biology students.

Materials and Methods

Collection of Samples

Mid-stream urine was collected from apparently healthy undergraduates of TASUED, (25 males and 25 females). The samples were collected into sterile plastic disposable bottles, refrigerated and examined within 2 - 4 hours of collection. The University student population was well known to be a high-risk segment of the community due to age, sex, and sexual activities among other predisposing factors. Students on antibiotic treatment within one week of the study were avoided.

Demographic Survey

A Demographic survey of all the students were determined to include age, sex, use of toilets, use of antibiotics and changes observed after antibiotics use. All these information were collected via the distribution of questionnaires to all the fifty students involved in this research.

Bacteriology

Samples were examined using standard methods as adopted by Ayoade [3]. A 30 Morse gauge, 3.26 mm calibrated wire loop capable of delivering 0.001 ml of urine was used for culturing on Blood Agar and MacConkey agar. The culture plates were incubated aerobically at 37˚C for 24 hours. Culture plates without visible growth were further incubated for an additional 24 hours before being discarded. The number and types of colonies grown on the medium were recorded as being insignificant when samples gave a colony count of less than 10^4 CFU/ml. Samples with colony count equal to or greater than approximately 10^5 CFU/ml of the urine samples was considered to have significant bacteriuria. Bacterial isolates were identified based on a combination of cultural, morphological and biochemical characteristics.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing of the isolated test organisms, was carried out using the following antibiotics: Penicillin, Chloramphenicol, Ampicillin, Gentamycin and Erythromycin,. The nutrient broth was prepared according to the manufacturer's specification and 10 ml was dispensed into sterile test tubes. A sterile loop was used to pick a pure colony of the test isolate and emulsified in the nutrient broth. The nutrient agar was then incubated at 37˚C for 18 - 24 hours. Normal saline was prepared using sodium chloride and distilled water and the normal saline was sterilized using the autoclave for 15 minutes at 121˚C then allowed to cool. Then the test organism was placed in 10 ml of the normal saline in a test tube then incubated for 2 hours. Turbidity standard of 0.5 Mc Farland was used, this is barium sulphate standard against which the turbidity of the test and control inocula was compared, when matched with the standard the inocula gives almost confluent growth.

A sterile swab was used to inoculate the Mueller Hinton agar previously prepared by putting the sterile swab into the inocula and rotating the swab against the side of the test tube above the level of the suspension. The swab was used to streak the surface of the Mueller Hinton Agar plates in three directions, rotating the plates in approximately 60˚C to ensure even distribution with a sterile forceps. The approximate antimicrobial sterile discs were placed evenly distributed on the inoculated plates. The plates were inverted and incubated aerobically at 37˚C and examined after 18 - 24 hours incubation and from the underside of the plates. A ruler was used to measure the diameter of the zone of inhibition in millimetres. The reaction of the test organism to each antibiotic was reported as sensitive, intermediate or resistant on the basis of zone of inhibition.

Data Analysis

The data were statistically analysed, with SPSS version 20 software, using a one-way analysis of variance (ANOVA)

Results

Table 1 shows the age and sex distribution of students used for the study. The table shows that 25 males and 25 females partook in the study. The table also shows that age group of 15-19 (25) partook of the study than other age group

Table 1: A Summary of the Age and sex distribution of students used for the study

Age distribution	No of Males examined	No. of Females examined	Frequency of individual by Age-group
15-19	12	13	25
20-24	9	10	19
25 and above	4	2	6

Table 2 shows the type of toilet used by the respondents. The table showed that 84% of the respondents use water closet while 16% of the respondents use pit latrine. The result shows that majority of the respondents use water closet.

Table 2: Summary of the toilet used by the respondents

Type of toilet	Frequency	Percentage
Pit Latrine	8	16%
Water Closet	42	84%

Table 3 shows that 22% of the respondents' used antibiotic in the past 14 days while 78% of the respondents did not use antibiotics in the past 14 days.

Table 3: Summary of antibiotics in the past 14 days.

Antibiotic for the past 14 days	Frequency	Percentage
Yes	11	22%
No	39	78%

Table 4 shows the summary of changes after taking antibiotics. The table shows that 100% of the respondents were of the view that there are changes after taking the antibiotics.

Table 4: Summary of changes after taking antibiotics

Changes after taking Antibiotics	Frequency	Percentage
Yes	10	100%
No	-	0%

Table 5, shows the total count of *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* obtained from the urine samples of all the female students tested. All the twenty-five female students tested for the presence of *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*in their urine tested positive to these pathogenic bacteria, although some in asymptomatic form, showing the carrier status of these female students. These shows that the organisms tested were 100% prevalent in all the female urine samples tested. Similarly, in Table 6, all the twenty-five male students tested showed 100% prevalence of *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*in their urine samples. The highest *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, counts of $2.41\pm0.00 \times 10^5$ CFU/ml,$5.59\pm0.00 \times 10^5$ CFU/ml and 1.86 ± 0.00 10^5 CFU/ml respectively were obtained in the urine samples of the female and male students. The highest Total Bacteria count of $4.77\pm0.00 \times 10^5$ CFU/ml was obtained in the female urine samples (Table, 5), while the highest Total Bacteria Count of$4.13\pm0.00 \times 10^5$ CFU/ml was obtained in the male urine samples (Table 6).

Table 5: The Total Bacterial, *E. coli*, *S. aureus* and *K. pnneumoniae* counts ($\times 10^5$ CFU/ml) in female urine samples

S/N	Sample	Total count	*E.coli*	*Staphylococcus auerues*	*K.pneumoniae*
1	F_{11}	3.83 ± 0.00^a	1.75 ± 0.10^b	2.95 ± 0.10^b	$1.16\pm.000^a$
2	F_{26}	3.90 ± 0.00^a	1.77 ± 0.10^b	3.54 ± 0.10^b	1.30 ± 0.00^a
3	F_{22}	3.93 ± 0.00^a	$1.93\pm.0.10^b$	3.69 ± 0.10^b	1.33 ± 0.17^c
4	F_{40}	4.10 ± 0.00^a	1.95 ± 0.10^b	4.40 ± 0.81^c	134 ± 0.90^d
5	F_{35}	4.19 ± 0.77^d	1.96 ± 0.10^b	4.64 ± 0.00^a	1.36 ± 0.12^c
6	F_5	4.30 ± 0.67^c	1.97 ± 0.10^a	4.06 ± 0.10^a	1.39 ± 0.15^b
7	F_{20}	4.31 ± 0.00^a	1.97 ± 0.10^b	4.50 ± 0.10^b	1.41 ± 0.47^c
8	F_{49}	4.32±0.00a	1.80 ± 0.10^b	4.07 ± 0.10^b	1.46 ± 0.10^b
9	F_{19}	4.35 ± 0.00^a	2.10 ± 0.10^b	4.07 ± 0.00^a	1.49 ± 0.10^b
10	F_{28}	4.39 ± 0.11^c	2.12 ± 0.10^b	4.08 ± 0.00^a	1.53 ± 0.15^d
11	F_{14}	4.40 ± 0.11^c	2.18 ± 0.10^b	4.03 ± 0.10^b	1.59 ± 0.00^a
12	F_{16}	4.41 ± 0.97^d	2.20 ± 0.10^b	4.08 ± 0.00^a	1.62 ± 0.20^c
13	F_{13}	$4.46\ \pm0.63^c$	2.22 ± 0.10^b	4.12 ± 0.00^a	1.64 ± 0.73^d
14	F_9	4.50 ± 0.14^b	2.22 ± 0.19^c	4.14 ± 0.10^a	1.73 ± 0.51^d
15	F_{50}	4.02 ± 0.57^c	2.22 ± 0.19^b	4.27 ± 0.00^a	1.75 ± 0.00^a
16	F_6	4.55 ± 0.00^a	$2.24\pm.010^b$	4.69 ± 0.00^a	1.76 ± 0.00^a
17	F_{30}	4.59 ± 0.00^a	2.25 ± 0.10^c	4.75 ± 0.10^c	1.80 ± 0.01^b
18	F_4	4.60 ± 0.01^a	2.25 ± 0.30^b	4.83 ± 0.01^a	1.81 ± 0.51^c
19	F_{34}	4.62 ± 0.00^a	2.25 ± 0.10^b	4.94 ± 0.00^a	1.82 ± 0.00^a
20	F_{29}	4.66 ± 0.21^d	2.26 ± 0.01^b	5.07 ± 0.15^c	1.83 ± 0.00^a
21	F_{27}	4.70 ± 0.00^a	2.28 ± 0.00^a	5.22 ± 0.47^c	1.84 ± 0.01^b
22	F_3	4.70 ± 0.00^a	2.37 ± 0.01^b	5.46 ± 0.11^c	1.84 ± 0.51^d
23	F_{12}	4.74 ± 0.00^a	$2.38\pm.0.00^a$	5.47 ± 0.11^b	1.85 ± 0.00^a
24	F_{15}	4.77 ± 0.00^a	2.40 ± 0.00^a	5.48 ± 0.10^b	1.86 ± 0.00^a

25	F_{10}	4.77±0.00^{a}	2.41±0.00^{a}	5.59±0.00^{a}	1.80±0.01^{b}

Mean with the same superscripts in the same column or row are not significantly different but mean with different superscripts are significantly different using DMRT at (P<0.05)

Table 6: The Total Bacterial, *E. coli*, *S. aureus* and *K. pnneumoniae* counts (× 10^5 CFU/ml) in male urine samples

S/N	Sample	Total count	*E.coli*	*Staphylococcus aureus*	*K.pneumonia*
1	M_8	3.20±0.00^{a}	1.22±0.10^{b}	2.85±0.10^{b}	1.19±.000^{a}
2	M_{13}	3.30±0.00^{a}	1.41±0.10^{b}	2.90±0.10^{b}	1.23±0.00^{a}
3	M_9	3.50±0.00^{a}	1.48±.0.10^{b}	3.19±0.10^{b}	1.33±0.17^{c}
4	M_{15}	3.50±0.00^{a}	1.60±0.10^{b}	3.26±0.81^{c}	134±0.90^{d}
5	M_7	3.56±0.77^{d}	1.65±0.10^{b}	3.30±0.00^{a}	1.36±0.12^{c}
6	M_6	3.61±0.67^{c}	1.70±0.10^{a}	3.33±0.10^{a}	1.39±0.15^{b}
7	M_1	3.64±0.00^{a}	1.73±0.10^{b}	3.50±0.10^{b}	1.41±0.47^{c}
8	M_{20}	3.70±0.00^{a}	1.80±0.10^{b}	3.70±0.10^{b}	1.46±0.10^{b}
9	M_5	3.37±0.00^{a}	1.82±0.10^{b}	3.77±0.00^{a}	1.49±0.10^{b}
10	M_{21}	3.77±0.11^{c}	1.83±0.10^{b}	3.81±0.00^{a}	1.53±0.15^{d}
11	M_{22}	3.81±0.11^{c}	1.85±0.10^{b}	3.93±0.10^{b}	1.59±0.00^{a}
12	M_{18}	3.86±0.97^{d}	1.90±0.10^{b}	4.08±0.00^{a}	1.62±0.20^{c}
13	M_{25}	3.97 ±0.63^{c}	1.97±0.10^{b}	4.12±0.00^{a}	1.64±0.73^{d}
14	M_{14}	3.99±0.14^{b}	2.00±0.19^{c}	4.14±0.10^{a}	1.73±0.51^{d}
15	M_{24}	4.02±0.57^{c}	2.06±0.19^{b}	4.27±0.00^{a}	1.75±0.00^{a}
16	M_{23}	4.02±0.00^{a}	2.10±.010^{b}	4.69±0.00^{a}	1.76±0.00^{a}
17	M_{10}	4.11±0.00^{a}	2.12±0.10^{c}	4.75±0.10^{c}	1.80±0.01^{b}
18	M_2	4.10±0.01^{a}	2.15±0.30	4.83±0.01^{a}	1.81±0.51^{b}
19	M_{11}	4.12±0.00^{a}	2.22±0.10^{b}	4.94±0.00^{a}	1.82±0.00^{a}
20	M_3	4.11±0.21^{d}	2.36±0.10^{b}	5.07±0.15^{c}	1.83±0.00^{a}
21	M_{17}	4.12±0.00^{a}	2.37±0.00^{a}	5.22±0.47^{b}	1.84±0.01^{a}
22	M_{16}	4.13±0.00^{a}	2.37±0.01^{b}	5.46±0.11^{c}	1.84±0.51^{d}
23	M_{12}	4.12±0.00^{a}	2.38±.0.00^{a}	5.47±0.11^{b}	1.85±0.00^{a}
24	M_{19}	4.10±0.00^{a}	2.40±0.00^{a}	5.48±0.10^{b}	1.86±0.00^{a}
25	M_4	4.13±0.00^{a}	2.41±0.00^{a}	5.59±0.00a	1.80±0.01^{d}

Mean with the same superscripts in the same column or row are not significantly different but mean with different superscripts are significantly different using DMRT at (P<0.05)

Table 7 shows the zones of inhibition of three bacteria. Gentamicin showed highest zone of inhibition in *K. pneumonia* culture plates with a mean value of 2.83 mm followed by erythromycin (2.51 mm) and ampicillin (2.48 mm). Penicillin showed the lowest zone of inhibition with a mean value of 1.55 mm. Also, Erythromycin showed the highest zone of inhibition in *Esherichia coli* culture plates with a mean value of 3.60 mm followed by Gentamicin (3.30 mm) and ampicillin (3.16 mm). Chloramphenicol shows the lowest zone of inhibition with a mean value of 2.20 mm. Also, Erythromycin shows highest zone of inhibition in the treatment of *Staphylococcus aureus* with a mean value of 5.67 mm followed by Gentamicin with a mean value of 5.58 mm, followed by Chloramphenicol with a mean value of 4.40 mm. Ampicillin showed the lowest zone of inhibition with a mean value of 4.20 mm.

Table 7: The zones of inhibition (mm) of various antibiotics of three bacteria

Bacteria	Penicillin	Chloramphenicol	Ampicillin	Gentamicin	Erythromycin
K. pneumonia	1.55[a]	1.80[a]	2.48[a]	2.83[a]	2.51[a]
Esherichia coli	2.77[b]	2.20[b]	3.16[b]	3.30[b]	3.60[b]
Staphylococcus aureus	4.50[c]	4.40[c]	4.20[c]	5.58[c]	5.67[c]

Discussion

Urinary tract infection caused by microorganisms is one of the most common infections in the world. Increasing resistance to broad spectrum antibiotics of urine pathogens especially *E. coli* and *K. pneumonia* as the prevalent UTIs pathogens is alarming. The rapid and correct choice of the antibiotic enables rapid cure of the patient, and sometimes even to save the patient's life. So, it is very important to determine the antibiotic resistance patterns of UTIs pathogens. It was discovered in this study that the infection subsided after the use of antibiotics. Widespread inappropriate use of antimicrobial agents in Nigeria is also due to the possibility of buying antibiotics from pharmacy stores with or without prescription; this is a significant contributing factor to the development of resistance to antimicrobial agent

Five antibiotics, Penicillin, Chloramphenicol, Ampicillin, Gentamycin and Erythromycin were found to be all effective for all the Gram-negative organisms isolated in this study particularly *E. coli* and *Staphylococcus aureus* while Gentamycin was found to be effective for the gram-positive organisms. Abejew [1] reported that Pseudomonas and Proteus species were resistant to almost all antibiotics except Gentamycin.

The result of the study indicates that *E. coli, S. aureus*and *K. pneumoniae* are among the most common types of bacteria found in the urine sample of undergraduates causing UTIs. This result agrees with other reports that indicate that *E. coli* is the commonest pathogen isolated in patients with UTI [4,5]. *Staphylococcus aureus* was observed to be sensitive to almost all the antibiotics tested, though to varying degrees. The results in this study which showed that *E. coli* wassusceptible toerythromycin, Gentamycin, ampicillin, chloramphenicol and penicillin, at inhibition zone values of 3.60 mm 3.30 mm, 3.16 mm, 2.20 mm and 2.77 mm respectively, were found to be similar to the observations of Nwanze *et al*.[6]. Ako-Nai *et al*.[7] presented a report in which 67.3% of *E.coli* isolates were resistant to ampicillin, 64.9% resistant to amoxicillin, 51.8% to penicillin, 70.2% to tetracycline, 48.8% to erythromycin, 36.9% to Gentamycin, 11.9% to chloramphenicol and 1.8% to gentamycin in a study Ibadan, Nigeria. S. *faecalis* had a profile of 70.7% (ofloxacin), 70.4% (augmentin), 60.3% (erythromycin)and a susceptibility of equal to or higher than 50.0% to chloramphenicol, pefloxacin, nitrofurantoin, colistin and streptomycin, while a susceptibility of less than 50.0 % to gentamycin, nalidixic acid, tetracycline and cotrimoxazole similar to the data presented by Nwanze *et al.*[6].

Recommendations

- Regular monitoring is required to establish reliable information about resistance pattern of urinary pathogens for optimal empirical therapy of patients with UTIs.
- Empirical antibiotic selection should be based on the knowledge of local prevalence of bacterial organisms and antibiotic sensitivities rather than on universal guidelines.
- Periodic epidemiological studies such as the one being reported here will also help in identifying the important UTI pathogens with a view to developing an effective and proper treatment model. Moreover, infection from UTI-causing pathogens can be drastically reduced when intermediate hosts of UTI-causing parasites are eradicated and by improved personal hygiene.
- Furthermore, public health education should be given to the public concerning the prudent use of antibiotics so as to avoid the problem of antibiotic resistance. Additionally, legislation is required to enforce proper use of animal and human medicines to minimize cross-transmission of resistant genes from animals to humans and vice versa.
- There is need to monitor the profile of etiological bacteria of UTIs and the antimicrobial resistance regularly. This would show emergence of resistance to newer therapeutic agents as well as keep track of effectiveness of serving therapeutic agents.
- Health workers should mobilize patients to ensure that they have duly completed the prescribed antimicrobial therapy as not finishing it leads to resistance.
- Parents should not assume their children automatically need antibiotics and should not routinely ask for them when their children are sick this will greatly reduce antimicrobial resistance instead they should seek a physician’s advice.

Conclusion

The study has shown an overview of the most common pathogenic bacteria found among undergraduates compared to other studies. *Escherichia coli* and *Klebsiella pneumoniae* were the most predominant strains in urine tract infection (UTI).

Suggestion for further studies

Several future research gaps worth filling is recommended. There is need to establish the influence of resistance of recurrent UTIs, and the risk factors involved in infection with resistant bacteria strain. Elaboration of the in vivo effectiveness of the commonly used antimicrobial agents and investigation of the endogenous nature of the identified uropathogenic isolates are other fruitful future research areas.

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