Elite Journal of Public Health. Volume 2 issue 4(2024), Pp. 31-40
https://epjournals.com/journals/EJPH

Prevalence And Anti-Microbial Susceptibility of Urinary Tract Pathogens among Female Students Visiting Kampala International University Teaching Hospital in Bushenyi, Uganda

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Abstract

The study was done to determine the prevalence of UTI among female students visiting KIU-TH, determine the etiological agents of UTIs among female students visiting KIU-TH and determine the antibiotic susceptibility patterns of bacteria associated with UTIs. The study was descriptive Cross sectional. Purposive sampling and simple randomsampling techniques were used to choose students. The study included all female students visitingKIU-TH and those within the age of 18 to 50 years. It was revealed that the overall prevalence of UTI among KIU female Students attendingKIUTH was at a rate of 35 (22.6%) out of 155 students who participated. The results are shown intable 2 below. The overall prevalence of the uropathogens was 35(22.6%). All the pathogens wereisolated from females with isolation rate of 22.6% and 77.4% who were positive and negative respectively. The highest isolation rate was observed in the age group between 18 to 25 years of age. Different antibiotics were used after bacteria isolation which include Pefloxacin, Cefixime, Amikacin and Ampicillin. It was found out that pefloxacin was the most resistant antibiotic to *E. coli* with the frequency of 22 (100%) followed by Ampicillin with frequency of 16 (72.7%). UTI is high and one

Citation: Uwakwe OS, Abalinda GM, Pius T, Okweny D, Wagana P, Obeagu EI. Prevalence And Anti-Microbial Susceptibility of Urinary Tract Pathogens among Female Students Visiting Kampala International University Teaching Hospital in Bushenyi, Uganda**. Elite Journal of Public Health, 2024; 2 (4): 31-40**

of the most common human bacterial infections among KIUstudents and around the world both in community and hospital settings. The different forms of antibacterial agents are used to treat the UTIs.

Keywords*: Prevalence, Anti-Microbial Susceptibility, Urinary TractPathogens, Female Students*

Introduction

The urinary tract includes the organs that assemble and pile up urine and release it from the body, and these organs are the kidneys, ureters, bladder, urethra and associate structures.[1] Urinary Tract Infection **(**UTI**)** is defined as the microbial colonization of any of the tissues of the urinary tract extending from the renal cortex to the urethral meatus.[2-5] Urinarytract infection and its related complications are the reason of virtually 150 million deaths per year globally.[6] Urinary tract infections characterize one of the most important public health threats faced in medical practice today affecting all categories of people ranging fromthe neonate to the elderly age group. In spite of the extensive accessibility of antibiotics, UTI remains the greatest public health problems among bacterial infections in the human residents.[7] Urinary tract infection (UTI), can affect any place in the urinary tract (urethra, bladder, ureters, or kidneys).

Urinary tract infection (UTI) describes microbial entry or inflammation of the bladder (cystitis), urethra (urethritis), or renal pelvis and kidneys (pyelonephritis).[8] The twotypes of UTIs are lower UTI which is an infection of the lower part of the urinary tract (the bladderand Urethra) and upper UTI which is an infection of the upper part of urinary tract (the kidneys and ureters). The upper UTI is potentially more serious than the lower one because there is a chanceof kidney damage.[9] A study carried out in Uganda in 1963 indicated a prevalence of symptomless bacteriuria among in-patients to be 6% and 8% in men and women respectively on medical wards and *E. coli* caused 41% of the UTIs.[10]

Methodology

Study area

The area of study was at KIU-TH in Bushenyi-Ishaka Municipality. Bushenyi-Ishaka Municipalityis located in Bushenyi district 58 km along Mbarara-Kasese road in western Uganda.

Study design and sampling

The study was descriptive Cross sectional. Purposive sampling was used to select students with UTI symptoms and then simple random sampling was used to choose students to include in the study with strict application of the inclusion criteria. Eligible participants were approached and requested to give a voluntary consent to participate in the study. Inclusion into the study was donesequentially until the required sample size is achieved.

Study population and Sample size determination

The study included all female students visiting KIU-TH and those within the age of 18 to 50 years.It also included students who presents with symptoms of UTI, and those who have not taken antibiotics

in the last 14 days as these would prevent the growth of bacteria. From the target population, a sample size was determined as shown below:

Sample size (n) = n = $\frac{z^2 \times p \times (1-P)}{d^2}$ (Kish, 1965)

$\frac{1.96^2 \times 0.114 \times (1-0.114)}{0.05^2}$

=155

Where D = margin of error of setting a significance level of 0.05 (i.e. 5%).

P= Prevalence of UTIs among non-diabetic patients attending clinics in Bushenyi district of Uganda

Is 11.4% (Odoki *et al.*, 2015).

Z=Level of significance (1.96) for confidence interval of 95%.

Inclusion criteria

The study included all female students visiting KIU-TH and those within the age of 18 to 50 years.It also included students who presents with symptoms of UTI, and those who have not taken antibiotics in the last 14 days as these would prevent the growth of bacteria.

Exclusion criteria

The study did not include female students who refused to consent to participate in the study and also those who are under antibiotics. In addition, female below 18 years of age and without UTI symptoms was excluded.

Laboratory procedures

Collection of urine samples

Midstream urine samples were collected using sterile universal bottles. Students was instructed to collect mid-stream urine into a sterile container up to at least half the capacity and deliver the specimen in the laboratory within an hour.

Culturing urine samples

The cysteine lactose electrolyte deficient (CLED) was used as selective media for isolation. The media was prepared according to the manufacturer's instructions and 0.001 ml of sample was inoculated onto the media using a platinum wire loop. Plates were then incubated for 24 hours at 37^{o}C. The number of pure colony forming units was multiplied by 1000 to determine the numberof micro-organisms per milliliter in the original specimen of urine. Those with more than 105 colonies was selected for further tests. Plates with no growth or tiny colonies was returned to the incubator for another 24 hours before discarding the plates since antimicrobial treatment or otherfactors may inhibit initial growth (WHO, 2003).

Triple sugar iron test (TSI)

This test was used in Gram negative colonies. TSI agar has glucose with a 0.1 % concentration and lactose and sucrose with a concentration of 1 %. Sterile TSI slants with agar was taken from the refrigerator and wiped using a dry cotton wool. The cap was removed and then the neck was flamed.[11] An inoculating straight loop was sterilized in the blue flame of the Bunsenburner and then allowed to cool. A colony of the suspected organism from CLED agar was picked,stabbed into the medium up to the butt of the TSI tube and then it was streaked back and forth along the surface of the slant. Again, the neck of the TSI was flamed, capped and placed in the incubator for 24 hours at a temperature of 37^{o} C. Triple sugar iron agar tube was used to test for the fermentation of only glucose (yellow butt), fermentation of lactose and sucrose (all over yellow), CO2 formation (crack in agar), or ferrous ammonium sulphate produced (black precipitate).[11]

Catalase test

Two drops of 3 % hydrogen peroxide were put onto a clean glass slide usinga dropper; a pure colony of the organism was picked from CLED agar using a wooden applicatorstick.[11] Placing the colony on the hydrogen peroxide on the glass slide; emulsification was done. Observation for bubble formation was done within 30 seconds.[11]

Mannitol Salt (MSA) Agar

It was done following results of catalase test for the confirmation of *S. aureus*. A plate of MSA was inoculated with a discrete colony of the test organism using a sterile wire loop by a streak plate method and incubated at 24-48 hours. The salt tolerant organism fermented mannitol which showed yellow zones around the colonies indicating *Staphylococcus aureus*.

Indole, Methyl Red, Voges-Proskauer and Citrate test (IMVIC) tests

A 1 % Tryptone broth was used during the test.[11] Kovac's reagent was addedto the tryptone broth and if indole is present then a red coloration forms at the top indicating the presence of *E. coli*.[11] A MR-VP broth was used to look for mixed acid and butanediol fermenters in the test organisms.One tube was used for each test. Half of the broth, once incubated, was removed and placed into adifferent tube. Methyl red was added to one tube to see if the pH is neutral (yellow) indicating negative while the development of red colour after addition of methyl red indicates positive, *E. coli*. Barritt's solution (alpha hapthol and potassium hydroxide) was added to the other tube to test the Butanediol fermenters and if the bacteria are butanediol fermenters then the broth turns red indicating *Klebsiella spp.*

Citrate test was used to test for the presence of citrate which is the sole source of carbon for bacteria. An agar slant with synthetic medium containing small amounts of mineral salts (citrate and ammonium) was used to perform the test. Bromothymol blue (pH indicator) was added to the agar slant and if there is growth (presence of citrate) the agar is blue indicating the presence of *Klebsiella spp.* and if there is no growth the agar is green.

Urease test

Urease test was used to determine the bacteria's ability to hydrolyze urea to make ammonia using the enzyme urease. It was conducted by inoculating Urea broth with a sterile inoculating wire loop. Urea broth is a yellow-orange color. The enzyme urease will be used to hydrolyze urea to make ammonia. If ammonia is made, the broth will turn a bright pink color, and is positive indicating the presence of *Proteus spp*. If test is negative, broth will have no color change and no ammonia is made.

Bacterial antibiotic susceptibility assay

The antimicrobial susceptibility tests were performed using the Kirby Bauer disk diffusion technique with commercially available disks on Mueller Hinton agar plates. Antibiotics disks viability was controlled using *E. coli* ATCC 25922. This was performed weekly.The agar was poured to a uniform depth of 4 mm and allowed to cool and solidify according to Clinical and laboratory standards institute and international guidelines. A 0.5 McFarland turbidity standard was prepared according to the method described by Dulczak and Kirk (2005). A solution with 9.95 ml of 1 % chemically

pure sulphuric acid was mixed with 0.05ml of 1.175 % barium chloride to form a barium sulfate precipitate which will cause turbidity. Thisstandard was used to adjust the turbidity of the inoculums for the antimicrobial susceptibility test.Well isolated single colonies were transferred to the tube with sterile saline and suspensions and was compared to 0.5McFarland turbidity. After, the turbidity of the inocula was adjusted, and a sterile cotton swab was dipped into the suspension, and pressed firmly against the inside wall of the tube; the swab was then streaked over the surface of the medium 3 times rotating the plate aftereach application to ensure an even distribution and was allowed to stand at room temperature for 10 minutes.

Antimicrobial disks containing specified concentrations in micrograms was placed on the agar plates after 10 minutes (to allow the agar to dry) using a pair of sterile forceps and then gently pressed down on the agar to ensure contact. The plates were inverted, and was incubated at a temperature of 37^{o}C for 24 hours. *E. coli* ATCC 25922 was used as reference. After incubation the zone diameters with complete inhibition, including the diameter of the disk was measured using a ruler and was recorded in millimeter on the under surface of the plate without opening thelid. The diameter of the zone of inhibition for each antibiotic was measured and interpreted as resistant, intermediate and sensitive according to Clinical Laboratory Standards Institute criteria (2007). The following antibiotics were used; Ampicillin, Gentamycin, Imipenem, Cefixime, Pefloxacin, Amikacin, Oxacillin,Vancomycin, Amoxycillin, Nalidixic acid.

Data analysis

Data collected was labeled appropriately. Patients names were not used, numbers and letters were used to label the samples. The raw data was entered into excel spreadsheets and was later importedto Statistical package for social sciences (SPSS version 20) for analysis. Data was presented as frequencies in tables and graphs.

Ethical considerations

Informed consent and respect for participants

Voluntary recruitment was done and an informed consent was signed. Informed consent from participants was obtained after fully explaining the details of the study to them. Participants were not forced to enroll themselves against their will. Participants were free to withdraw from the studyat any time they wished without coercion or compromise of care they are entitled to.

Approval Procedure

Approval to carry out the study was sought from the department of Medical Laboratory Sciences, School of Allied Health Sciences and finally the Kampala International University Research Ethics Committee (KIU-REC).

Results

From the study, majority of the study population belonged to the age group between 18 and 25 with the frequency of 70 accounting for 45.7% followed by 39 participants accounting for 25.2%who

belonged to the age group between 26 and 30. Other participants belonged to the age group of 31 – 40, 41 – 45, 46 – 50 with the frequencies of 31 (20%), 12 (7.7%) and 3(1.9%) respectively. On the other hand, participants' religion status was also analyzed and the majority of respondentswere found to be Christians with a frequency of 80 (51.6%) followed by Moslems with 61(39.4%)out of the total respondents. Only 4 (2.6%) of the participants belonged to traditional as their religion while 10 participants accounting for 6.4% belonged to other types of religion. About the level of education of the participants, it was revealed that majority of the respondents 59 (38.1%) had certificate as their level of education, 54 (34.5%) had Diplomas as their level of education, 40 (25.8%) had bachelor level of education while only 2 participants had masters as their levels of education. Over 65.2% of the study participants were single. This is because all the study participants were students while 54 respondents were married. None of the respondents was neither in a divorced status nor in widow status. The above results are shown in the table 1 below.

Table 1: Socio-demographic characteristics of study population

Age (years)		**Frequency (N= 155)**	**Percentages (%)**
	18 – 25	70	45.2
	26 – 30	39	25.2
	31 – 40	31	20
	41 – 45	12	7.7
	46 – 50	3	1.9
Religion			
	Moslem	61	39.4
	Christian	80	51.6
	Traditional	4	2.6
	Other	10	6.4
Level of education			
	Certificate	59	38.1
	Diploma	54	34.8
	Bachelor	40	25.8
	Masters	2	1.3
Marital Status			
	Single	101	65.2
	Separated / Divorced	0	0
	Married	54	34.8
	Widow	0	0

During the study, a total of 155 urine samples from suspected UTIs were analyzed for isolation and identification of bacteria and antimicrobial susceptibility testing. The age of the patients ranged from 18 years to 50 years, with mean age of 32.26 (SD=14.45) years. All urines samples (100%) were from female.

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It was revealed that the overall prevalence of UTI among KIU female Students attending KIUTH was at a rate of 35 (22.6%) out of 155 students who participated. The results are shown in table 2 below.

Table 2: The prevalence of UTI among female students visiting KIU-TH

Demographic Characteristics		**Positive No. (%)**	**Negative No. (%)**	**Total (%)**	***P* value**
Age (years)	18 - 25	14(20)	56 (80)	70 (100)	P=0.011
	26 – 30	9(23.1)	30 (76.9)	39 (100)	
	31 – 40	8 (25.8)	23 (74.2)	31 (100)	
	41 – 45	3(25)	9 (75)	12(100)	
	46 - 50	1 (33.3)	2 (66.7)	3 (100)	
Gender	Female	35 (22.6)	120 (77.4)	155 (100)	
Total		35(22.6)	120(77.4)	155	

The overall prevalence of the uropathogens was 35(22.6%). All the pathogens were isolated from females with isolation rate of 22.6% and 77.4% who were positive and negative respectively. The highest isolation rate was observed in the age group between 18 to 25 years of age (Table 2).

E. coli was the most predominant pathogen isolated from urine samples with prevalence of 22 (62.9%), *Klebsiella spp.,* with prevalence of 9 accounted for 25.7% while *S. aureus* with prevalence of 4 which accounted for 11.4% of the isolates (Table 3). Gram negative and Gram positive bacteria were responsible for 88.6% and 11.4% of the isolates, respectively.

Table 3: Bacterial isolates from urine samples of patients with suspected UTI

Bacteria Isolated	**Frequency**	**Percent**	**Total (%)**
Gram negative			**88.6**
E. coli	22	62.9	
Klebsiella spp.	9	25.7	
Gram positive			**11.4**
S. aureus	4	11.4	
Total	35	100	

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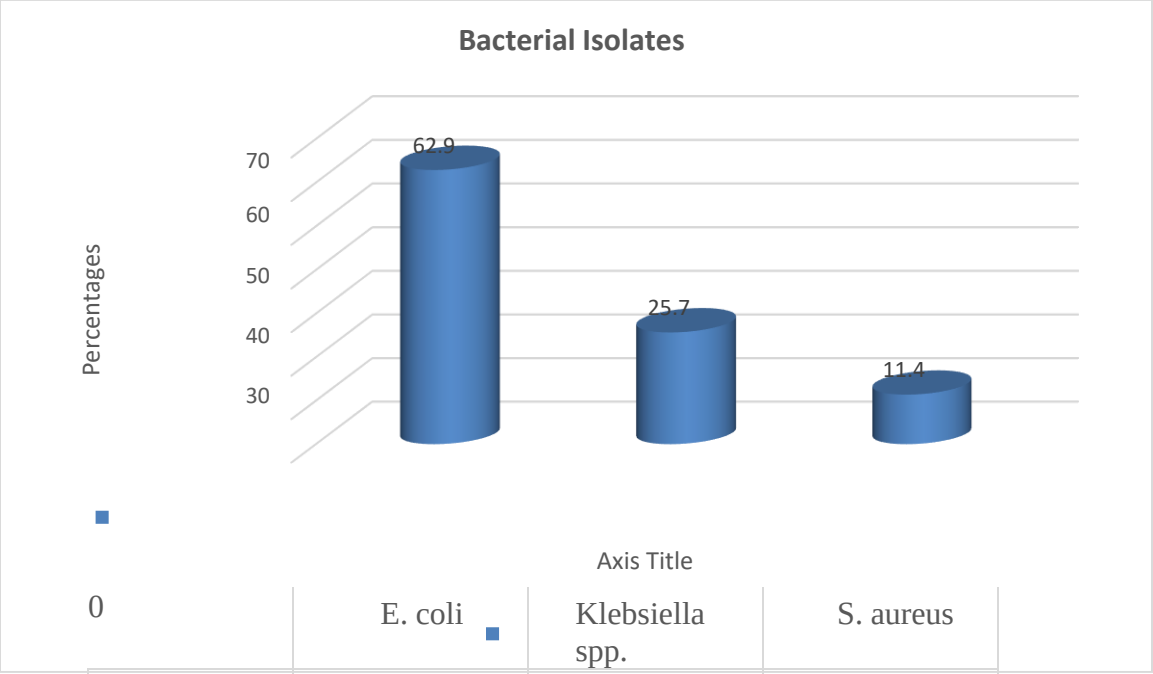

Bacterial Isolates	62.9	25.7	11.4

Figure 1: Bacterial isolates from urine samples of patients with suspected UTI

The figure above shows the three bacterial isolates. The Gram-negative isolates used were *Escherichia coli* and *Klebsiella spp.* while the gram-positive isolate used was *Staphylococcus aureus*. It was found out that *E. coli* was the most predominant pathogen isolated from urine samples.

Different antibiotics were used after bacteria isolation which include Pefloxacin, Cefixime, Amikacin, Gentamycin, vancomycin, Nalidixic acid and Ampicillin. It was found out that pefloxacin was the most resistant antibiotic to *E. coli* with the frequency of 22 (100%) followed by Ampicillin with frequency of 16 (72.7%).

Cefixime was the most sensitive antibiotic with the frequency of 17 (77.3%). 3 (13.6%) of the organism was resistant to cefixime and only 2 (9.1%) intermediately responded to cefixime. The majority isolates of the organism were intermediate to Amikacin as indicated by 10 (83.3%) while only 2 (9.1%) of the organism was sensitive to Amikacin. Nalidixic acid, Vancomycin and Gentamycin were revealed to be sensitive with the frequency of 3(75%), 3(60%) and 2(50%)

respectively. The results are shown in table 4 below.

Table 4: Showing susceptibility of *E. coli* to different antibiotics

	Susceptibility patterns		
	Resistant No. (%)	Intermediate No. (%)	Sensitive No. (%)
Pefloxacin	22 (100)	0(0)	0(0)
Cefixime	3 (13.6)	2 (9.1)	17 (77.3)
Amikacin	0(0)	10 (83.3)	2 (9.1)
Ampicillin	16 (72.7)	2 (11.1)	0 (0)
Nalidixic acid	1(25)	0(0)	3(75)
Gentamicin	2(50)	0(0)	2(50)
Vancomycin	2(40)	0(0)	3(60)
P-value			**0.0001**

According to the study findings, antimicrobial susceptibility test showed that *Klebsiella* was sensitive to Cefixime, Nalidixic acid, Vancomycin and Gentamycin. On the other hand, intermediate reactions were also considered and it was found out that Vancomycin and Amikacin reacted intermediately to 1 (25%) and 10 (100%) of the organism respectively. However, *Klebsiella* was resistant to Pefloxacin and Ampicillin with frequencies of 9 (100%) and 8 (88.9%) respectively (Table 5).

Table 5: Showing susceptibility of *Klebsiella* to different antibiotics

Antimicrobial agents **No. of Antimicrobials tested**		**Susceptibility patterns**		
		Resistant No. (%)	Intermediate No. (%)	Sensitive No. (%)
Pefloxacin		9 (100)	0 (0)	0 (0)
Ampicillin		8 (88.9)	0 (0)	1 (11.1)
Nalidixic Acid		2 (22.2)	0 (0)	7 (77.8)

Amikacin		0(0)	10(100)	0(0)
Cefixime		0(0)	0 (0)	9 (100)
Vancomycin		1 (25)	1 (25)	2 (50)
Gentamycin		0 (0)	0 (0)	5 (100)
P-value		**0.0001**		

On the other hand, Susceptibility of *Staphylococcus aureus* was also determined using different antibiotics which included Nalidixic acid, Amikacin, Cefixime, Vancomycin, Ampicillin and Gentamycin. It was revealed that Nalidixic acid, Vancomycin and Gentamycin were sensitive to Staphylococcus aureus with frequencies of 4(100%), 4 (100%) and 4 (100%) respectively, Cefixime and Amikacin were intermediately susceptible to it while Ampicillin was the most resistant antibiotic to *Staphylococcus aureus* as shown in table 6 below.

Table 6: Table showing susceptibility of *Staphylococcus aureus* to different antibiotics

Antimicrobial agents No. of Antimicrobials tested	**Susceptibility patterns**		
	Resistant No. (%)	Intermediate No. (%)	Sensitive No. (%)
Nalidixic acid	0(0)	0(0)	4(100)
Amikacin	1 (25)	3 (75)	0(0)
Cefixime	0(0)	4(100)	0(0)
Vancomycin	0(0)	0(0)	4 (100)
Ampicillin	4(100)	0(0)	0(0)
Gentamycin	0(0)	0(0)	4 (100)
P-value	**0.0001**		

According to the overall susceptibility profiles of bacterial isolates, it was found out that Pefloxacin had the highest overall resistance of 100%, followed by Ampicillin.

Vancomycin, Amikacin, Cefixime, Nalidixic acid, Vancomycin and Gentamycin had overall resistance rates of 23.1%, 3.8%, 8.6%, 17.6%, 23.1%, and 15.4% respectively (Table 7). Species specific antimicrobial resistance rates are displayed in Tables 4, 5 and 6.

E. coli, the most frequently isolated bacterium, showed high resistance rates of 100% to Pefloxacin and 72.7% to Ampicillin. *Klebsiella* was sensitive to Cefixime, Nalidixic Acid, Vancomycin and Gentamycin. On the other hand, intermediate reactions were also considered and was found out that Vancomycin reacted intermediately to 1 (11.1%) organism. However, *Klebsiella* was resistant to Pefloxacin, Ampicillin, and Nalidixc acid with frequencies of 9 (100%), 8 (88.9%) and 2 (22.2%) respectively (Table 3). Majority (100%) of *E. coli* isolates were susceptibly resistant to Pefloxacin with resistance rate of 100%.

Table 7: Overall antimicrobial susceptibility profiles of bacteria isolate from KIU Students attending KIUTH suspected of UTIs

Antimicrobial agents No. of Antimicrobials tested		**Susceptibility patterns**		
		Resistant No. (%)	Intermediate No. (%)	Sensitive No. (%)
Gentamicin	13	2 (15.4)	0(0)	11 (84.6)
Pefloxacin	31	31 (100)	0(0)	0(0)
Ampicillin	31	28 (90.3)	2 (6.5)	1 (3.2)
Vancomycin	13	3 (23.1)	1 (7.7)	9 (69.2)
Amikacin	26	1 (3.8)	23 (88.46)	2 (7.7)
Cefixime	35	3 (8.6)	6 (17.1)	26 (74.3)
Nalidixic acid	17	3 (17.6)	0(0)	14 (82.4)
P-value		**0.0001**		

According to the overall susceptibility profiles of bacterial isolates, it was found out that Pefloxacin and Ampicillin had the highest overall resistance of 100%. Vancomycin, Amikacin and Cefixime had overall resistance rates of 23.1%, 3.8% and 8.6%, respectively (Table 7). Species specific antimicrobial resistance rates are displayed in Tables 4, 5 and 6. *E. coli*, the most frequently isolated bacterium, showed high resistance rates of 100% to Pefloxacin and 72.7% to Ampicillin. *Klebsiella* was sensitive to Cefixime, Nalidixic Acid, Vancomycin and Gentamycin.

On the other hand, intermediate reactions were also considered and was found out that Vancomycin reacted intermediately to 1 (11.1%) organism respectively. However, *Klebsiella* was resistant to Pefloxacin and Ampicillin with frequencies of 9 (100%) and 3 (33.3%) respectively (Table 3). Majority (100%) of *E. coli* isolates were susceptibly resistant to Pefloxacin with resistance rate of 100%.

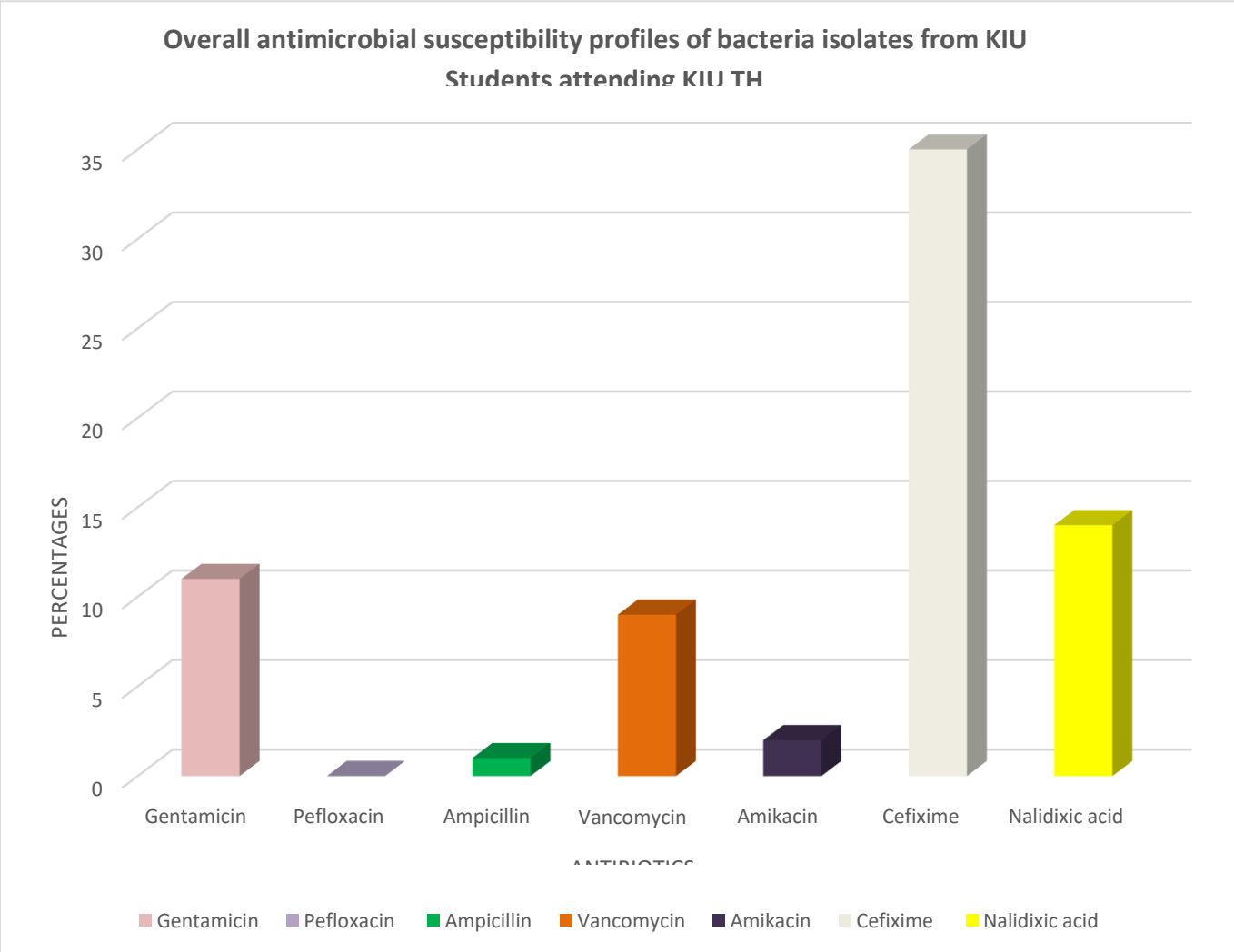

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Figure 2: Overall antimicrobial susceptibility profiles of bacteria isolate from KIUStudents attending KIUTH suspected of UTIs

Discussion

In general, the total prevalence rate of bacteriuria among the female students recruited for this study between July and September 2018, was 22.6% (35 out of 155). Considering the specific age groups of the students sampled; the highest prevalence rate of 20% (was observed instudents within the age group of 18-25 while the least occurrence rate of 33.3% (1 out of 6) was observed in the age group 46-50. Most students in our study population fall within the age group 18-25 (40%) i.e. 14 out of 35. This showed that there is a high prevalence of UTI among female students attending KIUTH. This was in line with study done by Mwaka *et al.* in Mulago Hospital.[12]

UTIs are one of the most common diseases diagnosed worldwide. Availability of new antimicrobials has improved the management of UTIs. However, the management of UTI infections has been jeopardized by increase in immergence of antimicrobial drug resistance. The overall isolation rate of uropathogens in the study was 22.6%. However, according to Kashef, the rate was higher than some other studies.[13]

This study showed that the prevalence of UTI was high in all age groups. The most frequently isolate bacterium was sensitive to Cefixime and the other isolates were sensitive to Nalidixic acidand gentamicin. Nalidixic acid, Gentamicin, Amikacin, Cefixime and Ampicillin are considered as appropriate antimicrobials for empirical treatment of UTI in the area. Periodic monitoring of etiology and antimicrobial susceptibility testing is recommended. The most frequently isolated bacterial isolates were found to be highly resistant to Ampicillin, andPefloxacin but sensitive to Cefixime, Gentamycin, Amikacin, Nalidixic acid and Vancomycin. *Klebsiella spp.*, wasfound to be resistant to Vancomycin and Pefloxacin but sensitive to Cefixime, Ampicillin, Nalidixic Acid, and Gentamycin. Statistically significant resistance rate was demonstrated to be Vancomycin and Pefloxacin ($P<0.001$). Cefixime was found to be effective against *E. coli.* Gentamicin and Nalidixic acid were effective against other isolates. High rates of sensitivity to Cefixime, Nalidixic acid and gentamicin have been documented from earlier studies.In this study, resistance to two and more antimicrobial agents was 74.9%.

Conclusion

The prevalence of UTI among female student attending KIUTH was found high with the frequency of 22.6%. UTI is high and one of the most common human bacterial infections among KIU studentsand around the world both in community and hospital settings. It was revealed that most female students at KIU suffer from UTIs as a result of poor personal hygiene, sharing of public conveniences and through unprotected sex.

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Citation: Uwakwe OS, Abalinda GM, Pius T, Okweny D, Wagana P, Obeagu EI. Prevalence And Anti-Microbial Susceptibility of Urinary Tract Pathogens among Female Students Visiting Kampala International University Teaching Hospital in Bushenyi, Uganda. Elite Journal of Public Health, 2024; 2 (4): 41-55