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Pattern of Bacterial Contamination of Urine amongst Asymptomatic Students of a Tertiary Institution in Port Harcourt, Rivers State, Nigeria

Barinua, D. N*., Sampson, T., and Peekate, L. P.
Department of Microbiology, Rivers State University,
Port Harcourt, Nigeria.
***Corresponding Author:** *barinuadandy484@gmail.com*

ABSTRACT

Urinary tract infection (UTI) can be symptomatic or asymptomatic depending on factors such as etiological agent, severity of infection and health status of the infected individual. This study was aimed at determining the prevalence of asymptomatic UTI amongst students of Rivers State University. Eighty (80) urine specimens from male and female students were subjected to standard bacteriological procedures. Data obtained for age-specific total heterotrophic bacterial population (THBC) ranged from 1.26±0.04 $\times10^5$CFU/ml to 3.07±0.01 $\times10^5$CFU/ml. There was a significant difference ($p<0.05$) between the THBC for the age groups sampled. Result showed that the male gender had the higher mean value (2.56±0.01 $\times10^5$CFU/ml) than the female gender that recorded 1.83±0.01 $\times10^5$ CFU/ml, for THBC. There was no significant difference ($p>0.05$) in THBC between the genders sampled. Mean values of bacteriuria population of THBC of on-campus students were higher (2.35±0.00 $\times10^5$ CFU/ml) than mean values of off-campus students (1.52±0.01 $\times10^4$ CFU/ml). There was however no significant difference ($p>0.05$) in THBC between the places of residence. The THB population for the levels of study ranged from 1.70±0.14 $\times10^5$ CFU/ml to 2.43±0.03 $\times10^5$ CFU/ml for 100 and 400 levels, respectively. A total of 80 bacterial isolates were obtained with *Staphylococcus aureus* having the highest prevalence of 63.74%, followed by *Bacillus* sp (30%), *E. coli* (4%) and *Klebsiella* sp (1.25%). Though the prevalence of THB, and total coliform were higher in males, the prevalence and diversity of phenotypically identified bacterial isolates was higher in the females. The study has shown that a high proportion of students with bacteriuria are asyptomatic, and at risk of urinary tract infection (UTI). Personal hygiene, proper diagnosis and efficient treatment protocols are therefore required to check the incidence of UTI amongst students of tertiary institutions.

Keywords: Asymptomatic, bacteriuria, prevalence, tertiary institution, students.

Introduction

Asymptomatic urinary tract infections is a condition that is best described as a presence of bacteria in the properly collected urine of a patient that has no signs or symptoms of a urinary tract infection. This is a common occurrence in many societies, as several studies have shown. Urinary tract infection (UTI) is a common bacterial illness that affects many sections of the urinary system, and it can affect both men and women (CDC, 2021). Urinary tract infections can be symptomatic or asymptomatic, depending on several factors such as etiologic agent, severity of infection as well as the health status of the infected individual (Flores-Mireles *et al*., 2015).

Asymptomatic UTI has been described as a condition where bacteria penetrate the bladder without creating symptoms in humans (Nwankwo *et al*., 2017). When infections are only present in a human host for a brief period of time, they are generally removed by host defense systems. Asymptomatic bacteriuria has also been defined as the presence of at least 10^5 colony forming units (CFU) of bacteria per millilitre of urine, in the absence of signs of infection in the urinary tract (Cortes-Penfield *et al*., 2017). Although Urinary tract infection is a common infection that affects both men and women, women are more sensitive, owing to their physiology, anatomy and reproductive system.

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Bacterial invasion of the urinary system, comprising the lower and upper urinary tract, is the most common cause of infection (Hickling *et al*., 2015). The most prevalent type of bacterial infection, according to research, is urinary tract infection. Although bacteria are the most common cause of urinary tract infection (UTI) in humans, some fungi and viruses can cause urinary tract infection. UTI caused by a virus or fungus is however considered an uncommon occurrence (Flores-Mireles *et al*., 2015). Poor diagnosis can lead to urinary tract infection, which is the most frequent hospital-acquired illness (Flores-Mireles *et al*., 2015). The infection comprises a wide range of clinical syndromes and disorders, each with its own epidemiology, origin, and severity (Behzadi *et al*., 2019). Aside from the aforementioned variables, it varies in terms of stated local symptoms, frequency of recurrence, level of damage produced, existence of aggravating conditions, and risk of recurrence (Storme *et al*., 2019).

Despite the fact that *Escherichia coli* is responsible for 80% of UTI, the presence of other bacteria cannot be discounted, and Gram-positive cocci are one of the culprits responsible for UTI (Flores-Mireles *et al*., 2015; Minardi *et al*., 2011). Gram-positive cocci are rising in popularity across the world, with *Staphylococcus* species being the most frequent (Sizar and unakal, 2022).

Pathogens linked to UTIs are known to have a feature known as biofilm production, which is responsible for infection (Delcaru *et al*., 2016). Another critical and probable factor promoting Urinary tract infection is the use of public rest rooms and conveniences (Storme *et al*., 2019; Poisson *et al*., 2010). Examples of these public toilets include those found in hotels, places of worship, restaurants and schools (Sampson *et al*., 2020). It is easier for UTIs to spread to several individuals through these public conveniences (Otokunefor *et al*., 2020).

Due to the rareness of complications resulting from asymptomatic urinary tract infections, people tend to ignore the risk associated. Studies have shown that some individuals can have increased risk of developing kidney infection with asymptomatic bacteriuria. Transmission of UTI from an asymptomatic individual to his/her sexual partner is a major problem that the public tend to ignore.

There is therefore need to improve awareness on the asymptomatic bacteriuria. Hence, the study targeted the epidemiology of asymptomatic urinary tract infection amongst tertiary institution students, to ascertain the prevalence of asymptomatic bacteriuria amongst university students.

Materials and Methods

Study Design and Duration

The research was a cross-sectional study with specimens obtained by a simple random sampling technique. The study was conducted between June and August 2020.

Description of the Study Area

This study was carried out in a university campus within, Port Harcourt, Rivers State, Nigeria, with GPS coordinate of 4°47'46.1" N 6°58'45.8" E for the institution. This institution was chosen for this study because of its large student population.

Sample Size determination

The sample size was calculated based on the formula,
$n = Z^2 P (1 - P)/d^2$ (Naing *et al.*, 2006).
At a 95% level of confidence,
Where n = sample size,
Z = degree of confidence Z statistic,
P = predicted prevalence or proportion, and
d = precision.
Therefore, P = 5.6% (P = 5.6/100 = 0.056),
d = 0.05,
Z = 1.96 (at a level of confidence of 95%, which is conventional) (Naing *et al.*, 2006).
Thus, $n = 1.96^2*0.056(1-0.056)/0.05^2$, n = 80.

Sample Collection

Well-structured Questionnaires was distributed to all students who consented to the study and to be sampled prior to the collection of urine specimens for laboratory assessment. Demographic data collected included sex, age, level of study, urinary tract infection history, description of toilet facility and their places of residence. In this investigation, a total of 80 samples were obtained randomly from male and female students of the institution.

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Preparation of Urine Specimens

Urine samples were collected using sterile, screw cap bottles and labeled appropriately. Students were advised to wash their hands before collecting urine samples, to collect early morning "mid-stream" urine, and to wash their hands thoroughly after covering the urine filled bottles with its cap. The collected samples from the students were placed in an ice-chest and transported to the Department of Microbiology Laboratory, Rivers State University where all laboratory assessment was carried out.

Microbiological Analysis

Culturing of Urine Samples

Samples were slightly agitated, and subjected to a ten-fold serial dilution. Diluted samples were inoculated on bacteriological media using spread plate technique. The culture media used included; Nutrient agar, Mannitol salt agar, MacConkey agar and Eosin-methylene blue (EMB) agar. The inoculated media plates were incubated at 37ºC for 24 hours. After 24 hours of incubation the bacterial colonies were counted, and the counts used to calculate the number of bacterial colonies present per millimetre of urine. A significant bacteria count was taken as any count equal or in excess of 10,000 cfu/ml. Urine samples with counts of 10^5 and above were indicative of asymptomatic bacteriuria while samples with counts below 10^5 were negative for asymptomatic bacteriuria (Cheesbrough, 2005).

Enumeration, Characterization and Identification of the Bacterial Isolates

Bacterial isolates were identified phenotypically based on cultural, morphological, biochemical characteristics and Gram staining reports. Following a serial tenfold dilution of homogenized urine samples, 0.1ml of appropriate dilutions were spread in duplicates onto Nutrient Agar, Mannitol Salt Agar, and MacConkey Agar plates. The plates were incubated at 37ºC for 24 hours. The colonies formed on the plates were counted and described morphologically. Isolate from total heterotrophic bacterial count which is greater than 10^5 indicated possible sign of infection. While total heterotrophic bacterial count less than 10^5 indicated that there were no significant bacteria in the urine (bacteriuria).

Isolation and preservation of Pure Cultures

Pure cultures of the bacterial isolates were obtained by aseptically streaking (in a back and forth manner) representative colonies of different morphological types which appeared on the cultured plates onto freshly prepared nutrient agar plates and incubating at 37ºC for 18 to 24 hours, which then served as stock cultures as described by Obire and Hakam (2015). The pure cultures of bacterial isolates were stored in 10% (v/v) glycerol suspension at -4ºC as a cryopreservative agent to prevent the damage of the pure cultures for further analysis.

Statistical Analysis

Descriptive statistics was used to summarize all data obtained. Analysis of variance (ANOVA) was carried out to test for significant difference in the total heterotrophic bacteria count, total Staphylococcal counts and total coliform counts. Where there was a significant difference, Duncan Multiple Range Test (DMRT) was used to separate the means (Bewick *et al*., 2004).

Results

The result of the population of bacteria in urine specimens for the various age groups as presented in Table 1. The highest total heterotrophic bacterial count ($3.07\pm0.01 \times 10^5$ CFU/ml) was reported for age group 36-Above while age group 31-35 years had the least count ($1.26\pm0.04 \times 10^5$ CFU/ml). There was however a significant difference ($p<0.05$) in the total heterotrophic bacterial counts of the age groups sampled. The total staphylococcal count ranged from $2.67\pm0.01 \times 10^4$ CFU/ml to $3.28\pm0.01 \times 10^4$ CFU/ml for age groups 15-20 and 31-35, respectively. The total coliform count ranged from $2.19\pm0.01 \times 10^4$ CFU/ml to $5.43\pm0.03 \times 10^4$CFU/ml for age groups 36-Above and 26-30, respectively. There were significant differences ($p<0.05$) in the Total Staphylococcal count and total coliform count across the different age groups sampled.

The gender-based bacterial population is presented in Table 2. The total heterotrophic bacterial counts for the males had higher mean of $2.56\pm0.01 \times 10^5$CFU/ml while the females had the least count of $1.83\pm0.01 \times 10^5$CFU/ml.

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There was no significant difference ($p>0.05$) in the total heterotrophic bacterial count between the male and female gender sampled. The total staphylococcal counts obtained from the male gender were however higher (3.30±0.14 $x10^4$ CFU/ml) than that obtained from the female gender (2.57±0.01 $x10^4$ CFU/ml). The total coliform count revealed urine specimens from the male subjects had the highest mean value (3.09±0.01 $x10^4$ CFU/ml) while that from the female subjects had the least (2.55±0.01 $x10^4$ CFU/ml). While there was a significant difference ($p<0.05$) in the total staphylococcal count, no significant difference ($p>0.05$) in the total coliform count was observed across the different genders sampled.

The result of the bacterial urinary population based on residence of students is presented in Table 3. The total heterotrophic bacterial count revealed that campus students had the highest mean value (2.35±0.00 $x10^5$ CFU/ml) while off-campus students had the least mean value (1.52±0.01 $x10^4$ CFU/ml). There was no significant difference ($p>0.05$) in the total heterotrophic bacterial count between the places of residence sampled. Total staphylococcal count of on-campus students (3.53±0.02 $x10^4$CFU/ml) were higher than of off-campus students (2.25±0.07 $x10^4$ CFU/ml).

The total coliform count revealed that on-campus students had the highest mean value of (3.25±0.07 $x10^4$ CFU/ml) while off-campus students had the least mean value (2.33±0.02 $x10^4$ CFU/ml). There were also no significant differences ($p>0.05$) in the total staphylococcal and the total coliform counts across the both residences sampled.

The result of the bacterial population for the levels of study of students from the urine specimens is presented in Table 4. The total heterotrophic bacterial count ranged from (1.70±0.14 $x10^5$ CFU/ml) to (2.43±0.03 $x10^5$ CFU/ml) for 400 and 100 level students, respectively. There was a significant difference ($p<0.05$) in the total heterotrophic bacterial count between the different levels sampled.

Total staphylococcal count ranged from 2.42±0.01 $x10^4$ CFU/ml to 4.35±0.01 $x10^4$ CFU/ml for 300 and 500 levels, respectively. The total coliform count ranged from 2.13±0.02 $x10^4$ CFU/ml to 3.18±0.01 $x10^4$CFU/ml for 400 and 300 levels, respectively. There were significant differences ($p<0.05$) in both the Total staphylococcal and total coliform counts across the different levels of study sampled.

Table 1: Bacterial population in urine specimen of different age groups from students

Age Range (Years)	Bacterial Population (CFU/ml)		
	THBC (×10^5)	TSC (×10^4)	TCC (×10^4)
15-20	2.18±0.01^c	3.28±0.01^e	3.24±0.01^c
21-25	2.23±0.02^d	2.79±0.01^b	3.42±0.02^d
26-30	2.07±0.01^b	2.83±0.02^c	2.19±0.01^a
31-35	1.26±0.04^a	2.67±0.01^a	2.59±0.01^b
36-above	3.07±0.01^e	2.98±0.01^d	5.43±0.03^e
P-value	0.000	0.000	0.000

Legend: THBC-Total heterotrophic bacterial count; TSC-Total staphylococcal count; TCC-Total coliform count

Table 2: Gender-based bacterial count in urine of students

Gender	Bacterial count (CFU/ml)		
	THBC (×10^5)	TSC (×10^4)	TCC (×10^4)
Female	1.83±0.01	2.57±0.01	2.55±0.01
Male	2.56±0.01	3.30±0.14	3.09±0.01
P-value	0.000	0.018	0.000

Legend: THBC-Total heterotrophic bacterial count; TSC-Total staphylococcal count; TCC-Total coliform count

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Table 3: Population of bacteriuria of on-campus and off-campus students

Residence Location	Population of bacteriuria (CFU/ml)		
	THBC (x10^5)	TSC (x10^4)	TCC (x10^4)
Off-campus	1.52±0.01	2.25±0.07	2.33±0.02
On-campus	2.35±0.00	3.53±0.02	3.25±0.07
P-value	0.000	0.002	0.003

Legend: THBC-Total heterotrophic bacterial count; TSC-Total staphylococcal count; TCC-Total coliform count

Table 4: Population of bacteriuria for the different levels of study of the students

Level of Study	Population of bacteriuria (CFU/ml)		
	THBC ×10^5	TSC (×10^4)	TCC (×10^4)
100	1.70±0.14^a	2.48±0.02^b	2.84±0.03^b
200	2.25±0.01^{bc}	2.94±0.03^c	2.84±0.01^b
300	2.14±0.02^b	4.35±0.01^d	2.13±0.02^a
400	2.43±0.03^d	2.94±0.01^c	3.18±0.01^d
500	2.33±0.01^{cd}	2.42±0.01^a	2.95±0.01^c
P-value	0.001	0.001	0.000

Legend: THBC-Total heterotrophic bacterial count; TSC-Total Staphylococcal count; TCC-Total coliform count

Result of the prevalence of the various bacterial species in the different population of students studied is as presented in Figure 1. The prevalence of *Staphylococcus* species, *Escherichia coli*, *Bacillus* spp and *Klebsiella* spp was higher in urine samples of the female students compared to that of the male students. The result indicated that *Staphylococcus* species had the highest bacteriuria prevalence of 72.5% and 55% among the female and male students, respectively.

The study recorded higher prevalence of *Staphylococcus aureus* (71.43%), *E. coli* (7.14%) and *Klebsiella* spp. (1.92%) for off-campus students than On-campus dwellers that had a prevalence of 59.62%, 3.85% and 0% for *Staphylococcus aureus*, *E. coli* and *Klebsiella* spp., respectively. The prevalence of *Bacillus* species was however higher among campus dwellers (34.61%) than off-campus students (21.43%) (Figure 2).

The overall prevalence of the bacterial species as recorded in Figure 3 revealed that *Staphylococcus aureus* (63.75%) was the predominant, while *Klebsiella* spp was the least occurring (1%) bacteria species in the urine specimens.

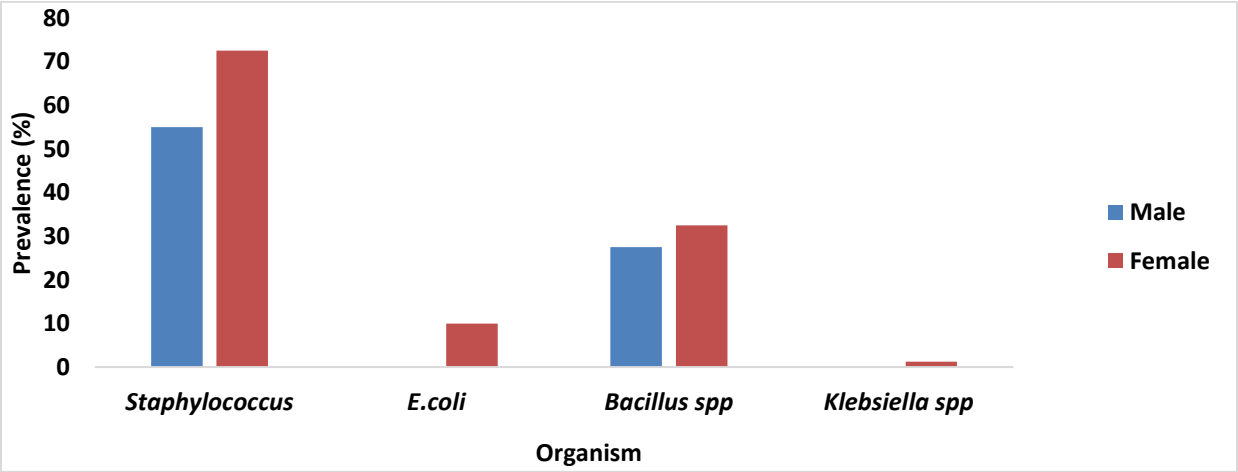

Fig. 1: Prevalence of bacterial Isolates in urine specimens of gender

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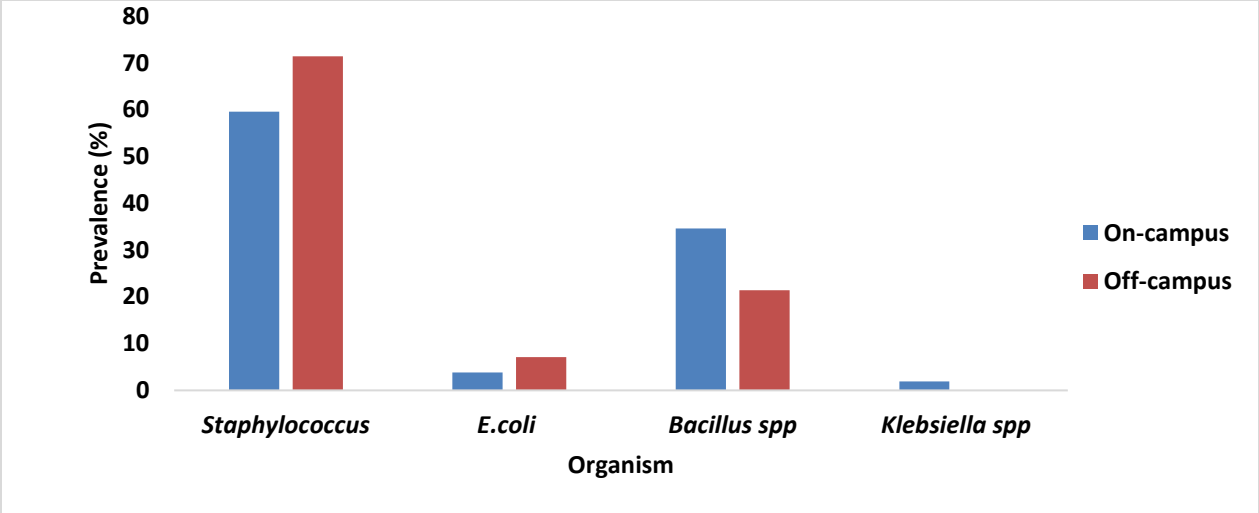

Fig. 2: Prevalence of Bacterial Isolates in urine specimens and students' location

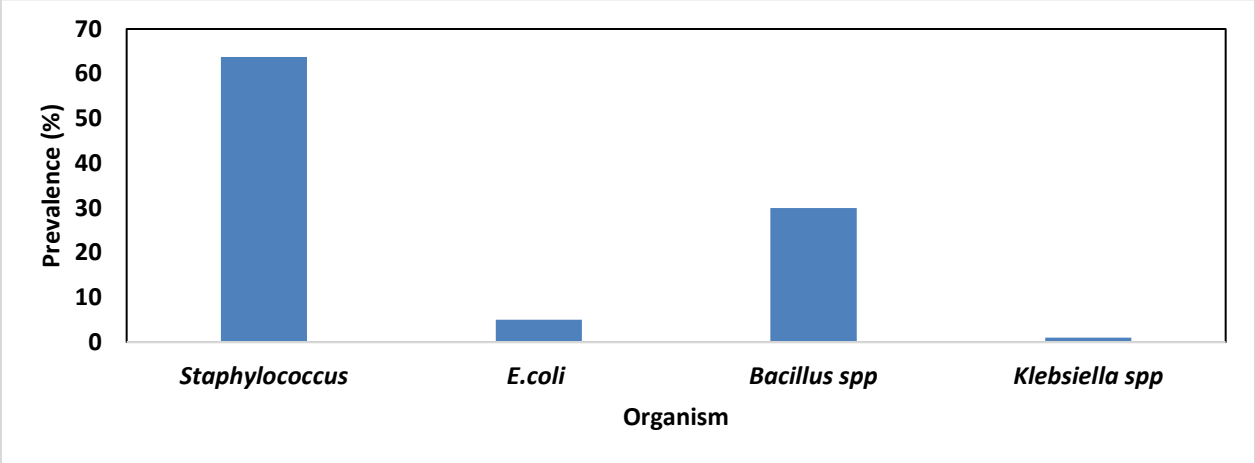

Fig. 3: Overall prevalence of bacteria isolated from urine specimen

Discussion

The diagnostics importance of bacterial population in urine is well known as it will help to ascertain the level of significance of the bacteriuria. This study reported the population dynamics of the bacterial species recovered from the urine of asymptomatic students population enrolled in the study. A significantly high bacteria count was recorded for most of the categories of students studied.

A similar high number of bacteria in urine had been reported elsewhere (Andreu *et al*. 2008; Galvin *et al*., 2010). These previous researchers had reported that the total heterotrophic bacterial counts in their study were high in all urine samples obtained.

The total heterotrophic bacterial count, total staphylococcal count, and total coliform count in this study were higher in the males than in the female students. The higher bacterial counts were associated with students at the extremes of age, male gender, campus dwellers and higher levels of study. This has therefore indicated the factors influencing the prevalence of bacteriuria in students. The higher prevalence of bacteriuria among the students at the extremes of age is attributable of immunological variations, as the level of immune responses is known to vary with age. Previous researchers had reported the role of toilet facilities in dormitories as a sink for potential pathogenic bacteria species (Sampson and Emelogu, 2022). The higher prevalence recorded for campus dwellers can be attributed to personal hygiene and sanitary conditions of the places of residence.

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The implication of these findings is serious, as the result indicates that the campus dwellers are more at risk of UTI compared to the off-campus students. This therefore calls for more sanitary measures as well as hygienic behaviour among campus hostel dwellers (Flores-Mireles *et al.*, 2015).

The study phenotypically identified the isolates to be *Klebsiella* sp, *Staphylococcus aureus*, *Bacillus* sp., and *Escherichia coli.* Although the total heterotrophic bacterial count, total staphylococcal count, and total coliform count in this study were higher in males than in females, the prevalence and diversity of these phenotypically identified bacterial isolates were significantly higher in the females than in the males. the prevalence of the bacterial species was noted to be significantly high. and were also higher among off-campus students and females. The higher prevalence of bateriuria in the females is attributed to anatomical differences with the males. Females are more susceptible than males, due to their short urethra and easy contamination of urinary tract with fecal flora (Tadesse *et al*., 2018).

These findings are comparable to that of Ferreira *et al*. (2010), who also found a high prevalence of urinary tract pathogens from urine samples. Among the other strains of bacterial isolates found, *Klebsiella* sp. was observed in this study but had a very low prevalence of 1.25%. This finding is consistent with the findings of Caneiras *et al.*, 2019, which indicated a low rate of *Klebsiella pneumoniae* association with uninary tract infections. The predominantly high prevalence of *Staphylococcus aureus* in this study is attributable to the organism being a normal flora of the human skin. These bacteria may lurk around the urinary tract openings and may gain entrance as a result of social behaviours including the use of sex toys (Ogbonna *et al*., 2023), as well as poor hygienic practices.

In conclusion, this present study has shown the role of age, gender, level of study and place of residence in the epidemiology of bacteriuria. It can be inferred from this study that a high proportion of these groups of students with bacteriuria are asymptomatic, thus accentuating the need for frequent screening in order to check the incidence of bacterial-mediated urinary tract infections.

The study reported more bacterial species in the female gender than the male, indicating that the females are more prone to urinary tract infection.

Also, it was noted that *Staphylococcus aureus* was the predominant bacterial species among the university students presenting asymptomatic urinary tract infection. Therefore the need for hygienic and sanitary practices by students can never be over emphasized. Efficient prevention and control protocols as well as proper diagnosis are highly recommended.

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