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Bacterial Profile of Asymptomatic Bacteriuria and High Prevalence of *TetA* Resistant Genes among Students in a Tertiary Institution in Nigeria.

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Abstract

Asymptomatic bacteriuria (ASBU) is a condition in which urine culture reveals a significant growth of bacteria that is greater than 10^5 bacteria/ml, but without the patient showing symptoms of urinary tract infection (UTI). In about 70% of the cases, ASBU is a major risk factor for the occurrence of UTI in an individual. This cross-sectional investigation was carried out among 160 healthy students of LAUTECH, Ogbomosho, Nigeria. Data containing information that predisposes the students to the risk of UTI and urine samples were collected. Bacteria were isolated using standard microbiological techniques, antibiotic susceptibility to 11 antibiotics and detection of tetracycline-resistant genes *tet*A, *tet*B and *tet*C using the disc diffusion and PCR methods respectively was done. Bacteria were isolated from 18 (11.3%) samples while 142 (88.7%) samples had no significant growth. *Escherichia coli* and *Klebsiella* species (3.7%) were the most frequently isolated followed by *Staphylococcus aureus* (2.5%) and *Pseudomonas species* (1.3%). The isolates were more susceptible to Gentamicin and Imipenem and were mostly resistant to Tetracycline. Molecular characterization of tetracycline resistance revealed that 5 *E. coli* (83.3%), and 6 *Klebsiella* spp. (100%) isolates resistance was due to the *tetA* gene only, 50% of *Pseudomonas aeruginosa* isolates contained the *tetB* gene while none of the bacteria carried the *tetC* gene. The prevalence of tetracycline resistance is a setback hindering its use in the treatment of infection and a limit to its adoption as a treatment option in combination therapy. Students should be educated on healthy toilet habits and indiscriminate use of antibiotics.

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Introduction

Asymptomatic bacteriuria is a condition in which urine cultures reveal a significant growth of pathogens that is greater than 10^5 bacteria/ml, but without the patient showing symptoms of urinary tract infection. (Bhugra *et al.,* (2021). The prevalence of ASBU among males and females varies across different countries. In Africa, a prevalence of 24.7% was reported from Nigeria Onu *et al.,* (2015) and 5.5% in Ghana Afoakwa *et al*., (2018). 21.2% and 18.8% in North and South Ethiopia Gebremariam *et al*., (2019). In about 70% of the cases, ASBU is a major risk factor for the occurrence of UTI in an individual Ullah *et al.,* (2007).

International studies have shown that UTIs are very common; therefore, one in five adult women experience UTI in her lifetime and it is a clinically apparent, worldwide patient problem Yang *et al.,* (2022) Chieng *et al.,* (2023).

Infections of the urinary tract (UTI) are the most frequently reported bacterial infection in the community coming second to respiratory tract infections the human Thass *et al.,* (2019) and occur in all ages in both men and women. The commonest pathogenic organism isolated in UTI are *E. coli* followed by *Klebsiella pneumoniae*, *Staphylococcus*, *Proteus*, *Pseudomonas*, *Enterococcus*, and *Enterobacter. Escherichia coli* strains amongst others

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are the most isolated bacteria in UTI. They are common bacteria that inhabit human gastrointestinal tract, whilst they are often harmless commensals; they can cause a multitude of infections such as urinary tract infections (UTIs), meningitis, diarrhoea and septicemia amongst others Foster-Nyarko and Pallen (2022). *Escherichia coli* has been implicated in many urinary tract infections whether with symptoms or without (asymptomatic) with increasing reports of resistance to commonly used antibiotics, Kot, (2019); Lee *et al.,* (2018). Their harmless strains can remain commensals as long as they do not acquire genetic elements encoding virulence factors that may eventually result in them causing infections.

UTI are the most common infections in outpatients with a prevalence rate of 50−60% which mostly affects adult women of reproductive age but is also common in males, Medinal and Castillo-Pino, (2019). It accounts for about 25 % of all infections and can occur in any population irrespective of the age group. In the United States, in hospitalized patients, UTIs are associated with an attributed mortality rate of 2.3% and an estimated annual cost of $340 to $450 million Medinal and Castillo-Pino, (2022). The corresponding mortality rate increased by 0.55% annually, which led to the global deaths due to UTIs in 2019 to be 2.4-fold that in 1990 Yang *et al.*, (2022).

One of the biggest challenges in the treatment of UTIs is the development of resistance by the pathogens against antibiotics often referred to as 'the silent tsunami facing modern medicine' Cox (2015). The alarming increase in the rate at which bacteria associated with UTI acquire antibiotic-resistance genes has limited therapeutic options, especially for UTIs for which extensive use of antibiotics has been witnessed in both community and hospital settings. Lee *et al.,* (2018). Despite several efforts and awareness, antibiotic resistance is continuously increasing throughout the world Exner *et al.,* (2017) leading to multi-drug resistance (MDR). Due to the rise of drug resistance by bacteria against antibiotics, it has been recommended by WHO that physicians should obtain laboratory information on local resistance patterns of organisms before treatment and that periodic surveillance should be conducted to monitor changes in the susceptibility of pathogenic bacteria. Mandell *et al.,* (2005).

The study of bacteria pathogens from healthy individuals is of great significance to understand and monitor the prevalence, distribution and carriage of pathogenic traits and antimicrobial resistance. The pathogenic or potentially pathogenic microbes with antimicrobial determinants may spread to other individuals not only humans but also to animals and animal-originated foods. (Olowe *et al.,* (2008), Olowe (2012); Ajayi *et al.,* (2012); Olowe *et al.,* (2013); Adefioye *et al.,* (2020).

Tetracyclines are antibiotics that inhibit bacterial growth by interfering with protein synthesis. It is used to treat many bacterial infections involving the skin, intestine, respiratory tract, genitals and other body systems. Tetracyclines can also be used to treat urinary tract infections. Olowe *et al.,* (2013). The emergence of bacterial resistance to these antibiotics has nowadays limited their use. The most common resistance mechanism in Gram-negative bacteria against tetracycline is the energy-dependent efflux pump system which is encoded by the genes *tet*A, *tet*B, *tet*C, *tet*D, *tet*E, *tet*Y and *tet*I, with *tet*A and *tet*B genes being the most frequently described. Olowe *et al.,* (2013); Grossman, (2016). The main purpose of this study was to investigate the bacterial etiology, resistance profiles, spread of tetracycline resistance genes and risk factors of bacteria-associated bacteriuria among students of a tertiary institution in Nigeria.

Materials and Methods

Study Site, Population and Sample Collection

This study was a cross-sectional investigation carried out among apparently healthy students of the Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria and the samples were analyzed at the Centre for Emerging and Re-Emerging Infectious Diseases (CERID), LAUTECH, Ogbomosho between March and July 2023. A total number of 160 urine samples in sterile universal containers were collected from the healthy consenting subjects selected using the non-probability purposive sampling method. The sample size of 160 was determined by taking the 11.8% prevalence from a previous study. Popoola *et al*., (2019) at a 95% confidence interval and a 5% margin of error. The samples were transported immediately to the laboratory for microbiological and molecular analysis. Demographic, medical history and toilet habits information about the participants were included in the data collection.

Isolation and Identification of Bacteria

The appearance and turbidity of the urine samples were observed and recorded and each sample was examined microscopically after centrifugation at 2000 rpm for 5 min. A loopful of each urine sample was also inoculated on both Blood agar and CLED agar plates (each plate containing 20ml agar) using a flame-sterilized calibrated wire loop of 0.001ml. It was incubated aerobically at 35°c for 24 hours. The deposits were examined microscopically using x10

and x40 objectives. Microscopic findings reported for each sample were: the presence of WBC, RBC, Crystals, Yeast, Epithelial cells, Parasites, etc. Samples with less than or equal to 10 white blood cells/mm3 was regarded as pyuric. Smith and Jone, (2003) Samples were cultured aseptically by inoculating unto blood and CLED agars and incubated at 37 °C for 18–24 h. Growth on blood agar and CLED (Oxoid Ltd., Basingstoke, Hampshire, UK) agar was identified by cultural characteristics, morphological appearance, and biochemical tests

Antibiotic Susceptibility Test

Each isolated and identified colony was submitted to an antibiogram test carried out by the disk diffusion method, as recommended by the National Committee for Clinical Laboratory Standards. Gajic *et al*., (2022) using Kirby-Bauer Disc Diffusion Method on Muller-Hinton Agar. The different classes of antibiotics tested were Augmentin (30µg), Imipenem (10µg), Cefpodoxime (10µg), Cefotaxime (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Nitrofurantoin (300µg), Tetracycline (30µg), Erythromycin (10µg), cefixime (5µg) and Ampicillin-Sulbactam (20µg). All plates were incubated at 37 °C for 24 h. The diameters of inhibition zones were measured to the nearest millimeter using a ruler according to CLSI guidelines and were interpreted as Sensitive (S) or Resistance (R) or Intermediate (IR).

Molecular Detection of Resistant Genes

Pure strains of the significant bacteria ($\geq 10^5$ CFU/ml) isolated from the urine samples had their DNA molecules extracted using the boiling method· Khavandi *et al*., (2022), amplified in a conventional PCR (BioRad®) and quantified using the DeNovix spectrophotometer. 10µl of the extracted DNA of each bacteria sample was amplified along with the *tet* forward and reverse primers (RTD®), $MgCl_2$, dNTPs (Invitrogen®) and Taq polymerase (Invitrogen®) for the presence of tetracycline resistance genes (*tetA, tetB and tetC)* in a multiplex PCR. The amplification conditions in the thermal cycler were as follows: initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 1 minute, and final cycle of amplification at 72°C for 10 minutes. After the completion the amplification, PCR products were loaded in 2% agarose gel stained with 0.1µL of ethidium bromide for gel electrophoresis. The presence of the genes was detected, viewed and photographed in the ultraviolet Azure imaging system for the presence of the known band sizes of the genes.

Table 1: Primers used to amplify genes

Gene	Antimicrobial resistance	Name	*Oligonucleotíde* sequences5'-3'	Size of the amplified product in base pairs
tetA	Tetracycline	tet(A)-F tet(A)-R	GTGAAACCCAACATACCCC GAAGGCAAGCAGGATGTAG	888bp
tetB	Tetracycline	tet(B)-F tet(B)-R	CCTTATCATGCCAGTCTTTTGC ACTGCCGTTTTTTCGCC	774bp
tetC	Tetracycline	tet(C)-F tet (C)-R	ACTTGGAGCCACTATCGAC CTACAATCCATGCCAACCC	880bp

Hedayatianfard *et al.,* (2014)

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences software (SPSS version 24), and statistical significance was set at $p < 0.05$. Data were presented as frequencies and percentages.

Results

Responses on predisposing conditions of UTI

The students were between the ages 15-35 with 94(58.8%) of the respondents being within the 21-25yrs age range. There were more females 85(53.1%) than males. Responses on predisposing conditions of UTI show that more than three quarter 120(80%) of them used water closet as toilet facilities while 2(1.3%) of them used pit latrines. A few 30(18.8%) stated that they used other toilet facilities. While

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93(58.1%) of them cleaned up after urinating,67(41.9%) did not clean up at all after urinating. 64(40%) of the respondents used pipe-borne water while 96(60%) of them used well water for cleanup after urinating/defecating. 25(15.6%) of the respondents used antibiotics recently while 134(84.4%) of them haven't used antibiotics in recent times. 15(9.4%) of them have been treated for UTI while 145 (90.6%) of them haven't been treated for UTI. The responses can be seen in Table 2.

Table 2- - Responses on Risk factors of UTI

What is the source of your water?

Response	Frequency	Valid Percent	Cumulative Percent
Pipe borne	64	40.0	40.0
Well	96	60.0	100.0
Total	160	100.0	

Have you used antibiotics recently?

Response	Frequency	Valid Percent	Cumulative Percent
Yes	25	15.6	15.6
No	135	84.4	100.0
Total	160	100.0	

Have you ever been treated for any UTI?

Response	Frequency	Valid Percent	Cumulative Percent
Yes	15	9.4	9.4
No	145	90.6	100.0
Total	160	100.0	

Are you diabetic?

Response	Frequency	Valid Percent	Cumulative Percent
No	160	100.0	100.0

Toilet facility

Response	Frequency	Valid Percent	Cumulative Percent
Water closet	128	80.0	80.0
Pit latrine	2	1.3	81.3
Others	30	18.8	100.0
Total	160	100.0	

Do you clean up after urinating?

Response	Frequency	Valid Percent	Cumulative Percent
Yes	93	58.1	58.1
No	67	41.9	100.0
Total	160	100.0	

Significant Bacteria Isolated from Urine

Out of the 160 urine samples cultured, 144 (88.8%) had no significant growth. *Escherichia coli* and *Klebsiella species* were the most common causative agents of asymptomatic Urinary Tract Infection among the studied population of students with a prevalence of 3.8% (6 out of 160) each. Next to them was *Staphylococcus aureus* with a prevalence of 2.5% (4 out of 160) and the least was *P. aeruginosa* with a prevalence of 1.3% (2 out of 160). This is shown in Table 3.

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Table 3 -Frequency of Bacterial Species Isolated

Bacteria Isolates	Frequency	Percentage
Escherichia coli	6	3.8
Staphylococcus aureus	4	2.5
Klebsiella species	6	3.8
Pseudomonas aeruginosa	2	1.3
No Significant Growth	142	88.8
Total	160	100.0

Antibiotic Resistance Patterns of the Bacteria Isolates

Only 27.8% of *E. coli* isolates were sensitive to ciprofloxacin, while no isolates were sensitive to imipenem and cefotaxime. The antibiotic susceptibility data for *P.aeruginosa* revealed moderate sensitivity to imipenem (11.1%) and cefotaxime (11.1%). However, the majority of *P.aeruginosa* isolates exhibited resistance to other tested antibiotics. *Staphylococcus aureus* demonstrated good sensitivity to imipenem (22.2%) and gentamicin (22.2%). The data also revealed relatively low sensitivity rates for amoxicillin/clavulanate (AUG) against *Klebsiella* species and *P. aeruginosa* (16.7% and 5.6%, respectively).

Table 4- Antibiotic Susceptibility Pattern in the Urine Isolates

Antibiotics	Category	*E.coli*	*K.spp*	*P.aeruginosa*	*S.aureus*
IMI	S	3(16.7%)	0(0.0%)	2(11.1%)	4(22.2%)
	I	3(16.7%)	0(0.0%)	0(0.0%)	0(0.0%)
	R	0(0.0%)	6(33.3%)	0(0.0%)	0(0.0%)
CIP	S	5(27.8%)	0(0.0%)	2(11.1%)	2(11.1%)
	I	1(5.6%)	0(0.0%)	0(0.0%)	2(11.1%)
	R	0(0.0%)	6(33.3%)	0(0.0%)	0(0.0%)
CTX	S	2(11.1%)	0(0.0%)	2(11.1%)	3(16.7%)
	I	4(22.2%)	0(0.0%)	0(0.0%)	1(5.6%)
	R	0(0.0%)	6(33.3%)	0(0.0%)	0(0.0%)
PX	S	0(0.0%)	6(33.3%)	0(0.0%)	0(0.0%)
	I	0(0.0%)	0(0.0%)	0(0.0%)	2(11.1%)
	R	6(33.3%)	0(0.0%)	2(11.1%)	2(11.1%)
AUG	S	0(0.0%)	3(16.7%)	1(5.6%)	1(5.6%)
	I	0(0.0%)	1(5.6%)	1(5.6%)	1(5.6%)
	R	6(33.3%)	2(11.1%)	0(0.0%)	2(11.1%)
GN	S	6(33.3%)	3(16.7%)	2(11.1%)	4(22.2%)
	I	0(0.0%)	3(16.7%)	0(0.0%)	0(0.0%)
	R	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
TE	S	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
	I	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)

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	R	6(33.3%)	6(33.3%)	2(11.1%)	0(0.0%)
F	S	0(0.0%)	1(5.6%)	0(0%)	2(11.1%)
	I	0 (0.0%)	2(11.1%)	0(0.0%)	2(11.1%)
	R	6(33.3%)	3(16.7%)	1(5.6%)	0(0.0%)
CFM	S	6(33.3%)	0(0.0%)	0(0.0%)	0(0.0%)
	I	0(0.0%)	2(11.1%)	2(11.1%)	0(0.0%)
	R	0(0.0%)	4(22.2%)	0(0.0%)	0(0.0%)
E	S	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
	I	0(0.0%)	0(0.0%)	0(0.0%)	4(22.2%)
	R	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
SAM	S	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
	I	0(0.0%)	0 (0.0%)	0(0.0%)	2(11.1%)
	R	0(0.0%)	0(0.0%)	0(0.0%)	2(11.1%)

Key:S=Sensitive I= Intermediate R= Resistant; Augmentin (AUG), Imipenem (IMI),), Cefepoxime (PX), Cefotaxime (CTX), Ciproflocaxin (CIP), Gentamicin (GN), Nitrofurantoin (F), Tetracycline (TE), Erythromycin (E),Cefixime (CFM), Ampicillin-Sulbactam (SAM).

The Distribution of the Tet Genes in the Tetracycline Resistant Bacteria Isolates

Table 5 depicts the prevalence of the *tet* genes in the urine isolates, *Klebsiella species* had the most presence of the *tetA* gene with a 100% occurrence (6 out of 6) followed by *E.coli* with an 83.3% occurrence (5 out of 6) while *Pseudomonas aeruginosa* did not contain the *tetA* gene.

tetB gene was found in *P. aeruginosa* with a 16.7% occurrence (1 out of 6) whereas *tetB* gene was not detected in *Klebsiella* and *E.coli* . *tet C* gene was not present in any of the isolates.

Table 5- Detection of Resistance Genes

Genes	Antimicrobial agent	*E.coli*	*Klebsiella spp*	*Pseudomonas aeruginosa*
tetA	Tetracycline	5(83.3%)	6(100%)	-
tetB	Tetracycline	-	-	1(16.7%)
tetC	Tetracycline	-	-	-

The gel electrophoresis profiles of *tet*A, *tet*B and *tet*C are presented in **Figure 1**, **Figure 2**, and **Figure 3** respectively.

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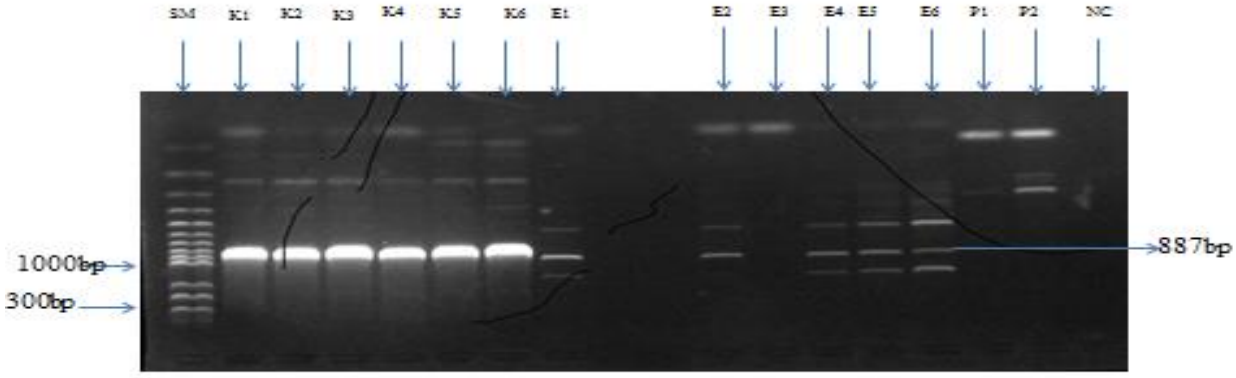

Figure 1 MOLECULAR IDENTIFICATION OF *TET* A GENES

Figure 1- Agarose Gel Electrophoresis of amplified *tetA* gene product (887bp). Lanes 1, 2, 3, 4, 5, 6, 7, 8, 10, 11 and 12 show the presence of *tetA*gene. Lanes 9, 13 and 14 show that *tetA*gene is absent.
SM: size marker; NC: negative control*;* K1*: Klebisella 1;* K2*: Klebisella 2;* K3*: Klebisella 3;* K4*: Klebisella 4;* K5*: Klebisella 5; K6: Klebisella 6;* E1*: E.coli1;* E2*: E.coli2;* E3*: E.coli3;* E4*: E.coli4;* E5*: E.coli5;* E6*: E.coli6;* P1*:Pseudomonas1;* P2*:Pseudomonas2.*

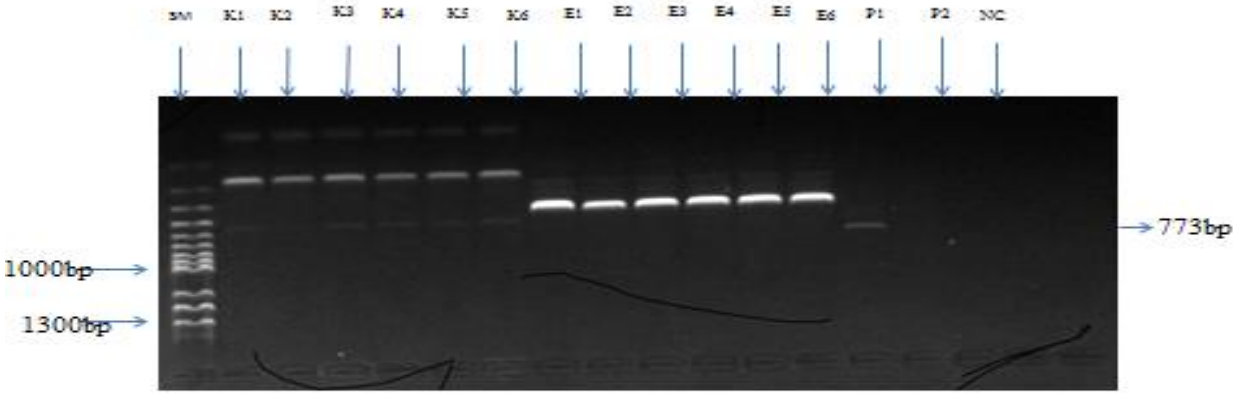

Table 2 MOLECULAR IDENTIFICATION OF *TET* B GENES

Figure 2- Agarose Gel Electrophoresis of amplified *tetB* gene product (773bp). Lanes 13 shows the presence of *tetB*gene while the others show that *tetB*gene is absent.
SM: size marker; NC: negative control; K1*: Klebisella 1;* K2*: Klebisella 2;* K3*: Klebisella*3*;* K4*: Klebisella 4;* K5*: Klebisella 5;* K6*: Klebisella 6;* E1*: E.coli1;* E2*: E.coli2;* E3*: E.coli3;* E4*: E.coli4;* E5*: E.coli5;* E6*: E.coli6;* P1*:Pseudomonas1;* P2*:Pseudomonas2.*

MOLECULAR IDENTIFICATION OF *TETC* GENES

Figure 3: Agarose Gel Electrophoresis of amplified *tetc* gene product (880bp). All the lanes show that *tet C*gene is absent. SM: size marker; NC: negative control.

Discussion

The prevalence of asymptomatic bacteriuria (11.3%) in this study is similar to the one previously conducted Onu *et al*., (2015); Popoola et al., (2019). The data obtained from the susceptibility test provided valuable insights into the effectiveness of different antibiotics against these potentially pathogenic bacteria. The results demonstrated varying degrees of sensitivity and resistance to the tested antibiotics. The observed susceptibility pattern of *E. coli* to ciprofloxacin, imipenem, and cefotaxime reflects a disturbing trend. Only 27.8% of *E. coli* isolates were sensitive to ciprofloxacin, while 3(16.7%) isolates were sensitive to imipenem and 2 (11.1%) cefotaxime. *E.coli* could have been passed into the urinary tract through unhealthy toilet habits. *E.coli* resistance has also been reported in other studies. Roos *et al.,* (2006) and underline the need for judicious use of these antibiotics to prevent further spread of resistance.

The antibiotic susceptibility data for *P. aeruginosa* revealed moderate sensitivity to imipenem (11.1%) and cefotaxime (11.1%). However, the majority of *P. aeruginosa* isolates exhibited resistance to other tested antibiotics, indicating a persisting challenge in treating infections caused by this pathogen Awole and Ibadin (2008).

S. aureus, a leading cause of healthcare-associated and community-acquired infections, demonstrated good sensitivity to imipenem (22.2%) and gentamicin (22.2%). These findings corroborate previous studies indicating the efficacy of imipenem and gentamicin against certain strains of *Staphylococcus aureus* Kidwai *et al.*, (2017). The observed resistance to other antibiotics, including ciprofloxacin and erythromycin, further highlights the importance of prudent antibiotic use.

Among the tested antibiotics, cefepoxime demonstrated notable efficacy against Klebsiella species with 33.3% sensitivity. This result is consistent with previous studies indicating the use of cefpodoxime in the treatment of certain gram-negative infections Brooke, *et al.,* (2017).

The data also revealed relatively low sensitivity rates for amoxicillin/clavulanate (AUG) against *Klebsiella*

species and *P. aeruginosa* (16.7% and 5.6%, respectively). These findings are consistent with studies indicating that *Klebsiella* species and *P. aeruginosa* are increasingly developing resistance to multiple antibiotics Patel *et al.,* (2008). Moreover, the emergence of extended-spectrum beta-lactamase (ESBL) and carbapenemase-producing strains of *Klebsiella* species has raised concerns about the effectiveness of commonly used beta-lactam antibiotics, Koren *et al.,* (2019). Alternative treatment strategies and antimicrobial stewardship programs are essential to address this alarming trend.

All the strains in this study showed resistance against tetracycline. Our study revealed that *tet* genes were found in all the isolates with *tet*A gene being the most prevalent. Several studies have reported the prevalence of *tetA* and other tetracycline resistance genes in various bacterial species. For instance, a study examined the genetic basis of tetracycline resistance in *E. coli*, *Klebsiella* species, *P. aeruginosa* and *S. aureus* from clinical samples and identified the *tetA* gene as one of the major determinants of resistance in their cohort (Olowe *et al.,* 2013; Zhang *et al.,* (2019). Similarly, research conducted by Mendes investigated tetracycline resistance in *Klebsiella* pneumoniae strains isolated from hospital settings and found a high prevalence of *tet*A and other tetracycline resistance genes Zhang *et al.,* (2019); Odewale *et al.,* (2023). In Nigeria, the work using *E. coli* isolates from clinical and non-clinical sources, 89.9% of isolates were found to contain *tet*A genes Olowe *et al.,* (2013); Doubra *et al.,* (2022).

The absence of *tet*A genes in *P. aeruginosa* is expected because tetracycline is hardly used to treat pseudomonas infections. *P. aeruginosa* is intrinsically resistant to tetracyclines and glycylcyclines due to the MexAB/MexXY efflux systems Morita *et al.,* (2014); Akinloye *et al*., (2019) and Ciofu and Tolker-Nielsen (2019). The presence of *tet*C genes might have been acquired through gene transfer from co-infection with other bacteria however more research is needed to confirm this in the study area.

The prevalence of tetracycline resistance is a setback hindering the use of tetracycline in the treatment of infection and could limit its adoption as a treatment option in combination therapy.

Conclusion

In conclusion, the study revealed that students in this study harbor potentially pathogenic multidrug-resistant bacteria that can lead to serious urinary tract infections. The infections were recorded only in subjects who resided within the hostels, and used public toilets and water for cleaning up after defecation Antibiotic susceptibility surveillance is still needed for these silent pathogens that could cause life-threatening infections in the future. Students should be educated on healthy toilet habits and indiscriminate use of antibiotics.

Author Contributions
All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement
All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted following the Declaration of Helsinki, and the protocol was approved by the Ethical Review Committee of the LAUTECH Teaching Hospital, Ogbomosho, Oyo State, Nigeria. (LTH/OGB/EC/2022/336).

Informed Consent Statement
Informed consent was obtained from all subjects involved in the study.

Data Availability Statement
All relevant data are within the manuscript. The datasets used and/or during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest
The authors declare no conflict of interest.

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