

## ISOLATION AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF BETA-LACTAMASE PRODUCING GRAM-POSITIVE BACTERIA ISOLATED FROM OPEN URINATION SPOTS ACROSS OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE, NIGERIA.

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### ABSTRACT

Urine-contaminated soils are increasingly recognized as environmental reservoirs of antimicrobial resistance, contributing to the spread of resistant bacteria within human-associated ecosystems. However, data on the resistance profiles of Gram-positive bacteria inhabiting such environments within Obafemi Awolowo University remain limited. This study investigated the occurrence, antibiotic susceptibility patterns, and  $\beta$ -lactamase producing Gram-positive bacteria isolated from urine-contaminated soils across selected open urination spots on campus. Ten soil samples from visible open urination spots were aseptically collected and microbiologically analyzed within six hours. Bacterial isolates were recovered using standard microbiological techniques and identified through cultural and biochemical characterization. Susceptibility of isolates to a panel of antibiotics was determined following the standard organization (CLSI) guide. Using the nitrocefin test, the ability of the test isolates to produce  $\beta$ -lactamase was determined. The identity of  $\beta$ -lactamase-positive isolates was confirmed by partial 16S rRNA gene sequencing. A total of 29 bacterial isolates were recovered, comprising 15 Gram-positive and 14 Gram-negative bacteria. The Gram-positive isolates were identified as members of the genera *Enterococcus*, *Streptococcus*, *Listeria*, *Bacillus*, and *Staphylococcus*. High levels of resistance (>90%) to  $\beta$ -lactam antibiotics were observed across all isolates, alongside variable resistance to other antibiotic classes. Multiple antibiotic resistance indices ranged from 0.58 to 1.00, indicating substantial exposure to antibiotic selective pressure. Two isolates, identified as *Bacillus* species through molecular analysis were confirmed as  $\beta$ -lactamase producers. These findings highlight urine-contaminated soils as significant reservoirs of multidrug-resistant Gram-positive bacteria, posing potential public health risks. The study underscores the need for improved sanitation infrastructure and enforcement of policies to eliminate open urination practices within university environments.

**Keywords:** Antibiotic resistance,  $\beta$ -lactamase, Gram-positive bacteria, urine-contaminated soil.

### INTRODUCTION

Antimicrobial resistance (AMR) is a top threat to global public health, economics, and development (Jonas *et al.*, 2017; WHO, 2023). The World Health Organization (WHO) has estimated an annual 4.9 million deaths due to AMR, and this includes 860,000 deaths in Africa. The United Nations (UN) also projected that AMR could cause as many as 10 million deaths globally by the year 2050 (Anderson, 2023) if it is not addressed on time. One of the strategies for combating the AMR malady is by providing information on the resistance patterns of bacteria in ecological niches via local monitoring and national surveillance (WHO, 2023, 2024). One family of antibiotics that is of major public health concern is the  $\beta$ -Lactams (Karunarathna *et al.*, 2025; Pandey and Cascella, 2025).

$\beta$ -Lactams, due to their safety, reliable killing properties and clinical efficacy, are among the most frequently prescribed antibiotics for bacterial infections. Of all antibiotics,  $\beta$ -Lactams are of utmost importance because of their high selective toxicity in favour of humans and as such are commonly prescribed for the management of bacterial infections (Naas *et al.*, 2017). The  $\beta$ -lactam antibiotics are generally categorized into penicillins, cephalosporins, carbapenems and monobactams, while carbapenems are the  $\beta$ -Lactam drugs of last resort (Bush and Bradford, 2020; Hayer *et al.*, 2022; Tilahun *et al.*, 2021). Pathogens that exhibit resistance against  $\beta$ -Lactams often exhibit resistance against other major classes of antibiotics, such as fluoroquinolones and aminoglycosides, and at times exhibit resistance against all available drugs (Hayer *et al.*, 2022; Naas *et al.*, 2017). These

pathogens' high resistance has been linked to the presence of resistance determinants, most especially beta-lactamases producing genes such as extended-spectrum  $\beta$ -lactamases (ESBL) and Metallo- $\beta$ -lactamase (MBL) genes in their genome. These resistance determinants are of great concern because they are highly diverse (Adekanmbi *et al.*, 2020; Boyd *et al.*, 2020; Husna *et al.*, 2023a; Igbinosa *et al.*, 2023; Liu *et al.*, 2022). Also, infections caused by bacteria harbouring different beta-lactamases are complicated to treat and require specific therapeutic strategies. (Bubpamala *et al.*, 2018; Castanheira *et al.*, 2021; Hosu *et al.*, 2021; Husna *et al.*, 2023b; Sawa *et al.*, 2020; Tseng *et al.*, 2023). Therefore, regular monitoring of the human environment is crucial for developing local strategies for combating the spread of antibiotic resistance (Murray *et al.*, 2022).

Understanding the resistance pattern of bacteria circulating in a geographical area such as tertiary institutions, which is a transient population environment, is essential for developing effective strategies to prevent the spread of antibiotic resistance. This knowledge can inform targeted surveillance, guide appropriate treatment protocols, and help in the design of localized interventions. Additionally, it can aid in public health planning and policy-making by identifying high-risk areas and populations. Unfortunately, there is limited information on the resistance profile of Gram-positive bacteria of soil samples from urination spots in OAU. The University is a community of a transient population of more than 34, 000 students, 4,000 staff members and many people from the Ile-Ife community who come in and out of the University community daily (<https://oauife.edu.ng/wp-content/uploads/2023/01/busary.pdf>).

The students are from different parts of Nigeria and West African countries who travel in and out of the community on a semester basis, while the university workers, farmers and other visitors come in and out of the community daily. A transient human population such as that of OAU plays a significant role in the spreading of antibiotic-resistant bacteria and resistant genes (Comelli *et al.*, 2024). This suggests that the environmental components of a transient population, such as the OAU community soil, especially human waste-contaminated soil needs

regularly monitoring in the interest of public health.

### Urine-contaminated soil and human bacterial pathogens

Urine is a mixture of biochemicals that can create an enabling environment for bacteria to adapt, multiply, and exchange genetic material, including resistance determinants, in soil environments contaminated with it (Dabo *et al.*, 2023). In the study carried out by Dabo *et al.*, (2023), they reported the isolation of *Bacillus* sp., *Escherichia coli*, *Klebsiella* sp., *Staphylococcus* sp. and *Salmonella* sp. from urine-contaminated soil and the pathogens were found to exhibit relatively high resistance to Imipenem, Oxacillin, Ciprofloxacin, and Gentamicin. In a similar work at Ebonyi State University, Abakaliki, *Staphylococcus aureus* isolated from urine-contaminated soil was found to exhibit high resistance against cefotaxime, while all *Enterobacter* spp. isolated were 100% resistant to oxacillin, cefoxitin, clindamycin, meropenem and mupirocin (Orji *et al.*, 2022). In the same study, the majority of *Streptococcus* spp. isolated from urine-contaminated soil exhibited high resistance against meropenem, mupirocin and ceftazidime but were all susceptible to ceftazidime. This study also reported isolation of *Escherichia coli*, *Staphylococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Streptococcus* sp., and *Enterobacter* sp. with varying degrees of resistance against antibiotics, most especially first-line antibiotics. Mahdi *et al.*, (2020) reported the isolation of several bacteria that are commonly implicated in urinary tract infections (UTI). These pathogens also exhibited varying degrees of resistance against commonly recommended antibiotics, and *E. coli* was found to be the most prevalent bacterium in the urine-contaminated soil. The study also observed high resistance against  $\beta$ -lactam antibiotics. Furthermore, the findings of (Dada and Aruwa, 2014) on urine-contaminated soils around lecture theatres in the Federal University of Technology, Akure, Nigeria were similar to those of the studies mentioned earlier. The study also reported the occurrence of *Bacillus* species, *Corynebacterium* species, *Micrococcus* species *Flavobacterium rigense* and *Acinetobacter Anitratus* and *Alcaligenes entrophus* from urine-contaminated soil. Unlike most of the studies on urine-contaminated soil, Dada and Aruwa (2014) found *Bacillus* sp. as the most predominant bacterium. These studies revealed that urine-contaminated soil has the potential to serve a dual

purpose of spreading antibiotic resistance and infection, most especially UTI. Hence, the current study focuses on the Obafemi Awolowo University soil samples from open-urination spots

## MATERIALS AND METHODS

### Sampling site

The sampling sites involve open urination points within the University academic area.

### Collection of soil sample

A total of 10 samples of urine-contaminated soil were collected from different identified sites within the University. Ten grams of topsoil between 5-20 cm depth was scooped into sterile sample bottles and immediately transported to the Department of Microbiology laboratory for analysis. Uncontaminated soil samples determined as area without visible urine contamination, which is 1 meter away from the urine-contaminated area, were also collected as control samples (Paulyn *et al.*, 2022; Vince, 2018).

### Isolation and Identification of Gram-positive bacteria

Ten-fold serial dilutions of the collected soil samples were prepared up to a dilution power of 6 using sterile normal saline as the diluent. Afterwards, 100 µl aliquots from different dilution tubes were spread on sterile nutrient agar plates. Plates without overcrowded colonies were pooled together and examined for morphologically different colonies. One representative from each morphologically distinct population was selected and streaked onto another freshly prepared sterile nutrient agar plate to ensure purity. Following this, Gram-staining was carried out as described by (Tripathi *et al.*, 2025), and the purity of the colonies was ascertained based on uniformity of Gram status, shape, and arrangement of cells. A 25% glycerol stock solution of Gram-positive pure isolates was prepared and stored at -20 °C for further analysis.

To identify the isolates, biochemical tests were carried out. The set of biochemical tests carried out for Gram-positive cocci differs from those for Gram-positive bacilli based on the recommendation of a previous study (Wanger *et al.*, 2017). The identity of the isolates was afterwards determined using the ABIS online biochemical identification system (ABIS, 2025). The biochemical test carried out included spore

staining (Ahern, 2025), catalase (Iwase *et al.*, 2013; Sapkota, 2022), oxidase (Aryal, 2015a), hemolysis (Aryal, 2022, 2015b), halotolerance (Liu *et al.*, 2026), coagulase (UK Health Security Agency, 2025) and lecithinase tests (Hmani *et al.*, 2023).

### Antimicrobial susceptibility test (AST)

The Kirby-Bauer disc diffusion technique was used to determine the antibiotic susceptibility of the isolates to a panel of antibiotics (Bauer *et al.*, 1966). The preparation of Mueller-Hinton Agar (MHA) was as described by the manufacturer. It was allowed to cool and then poured into sterile Petri dishes and then allowed to solidify. An 18-24-hour-old culture of isolates was prepared on sterile nutrient agar plates, and a few colonies were picked using a sterile inoculating loop and transferred into bacteriological test tubes containing 5 ml sterile distilled water. The inoculum was standardized using a colorimeter to 0.5 McFarland standard at 580 nm. Sterile swab sticks were dipped into the test tubes containing the already standardized inoculum (one swab stick per sample). Excess fluid was removed from the swab stick by pressing it against the side of the test tube above the fluid level. The swab sticks were afterwards used to spread the inoculum evenly over the MHA plates. The discs were introduced on the MHA plate that had been seeded with test bacteria using a MAST antibiotics dispenser. The plates were inverted and incubated for 24 hours at 37 °C. After the 24-hour incubation, the plates were examined. A clear zone of inhibition around the discs was measured and interpreted as resistance (R), intermediate (I) or susceptible (S) according to interpretive criteria defined by the Clinical and Laboratory Standards Institute (CLSI, 2023; Rai *et al.*, 2023).

The panel of antibiotics used are: Tetracycline-TET (30µg), Gentamicin-GEN (10µg), Erythromycin ERY (15µg), Ciprofloxacin CIP (5µg), Cefuroxime-CRX (30µg), Meropenem-MEM (10µg), Ampicillin-AMP (10µg), Augmentin-AUG (20/10µg), Vancomycin-VAN (30µg), Cotrimoxazole COT (1.25/23.75µg), Ceftazidime-CPZ (30µg), and Chloramphenicol-CP (30µg).

### Beta-lactamase Test

The beta-lactamase test is used to determine whether a bacterial isolate produces the enzyme beta-lactamase, which has the capacity to

deactivate beta-lactam antibiotics e.g. penicillin. To carry out the test, 20 µL of 0.5 mM aseptically prepared nitrocefin solution was added to a premade dense bacterial suspension on a glass slide. A change of the natural yellow colour of the nitrocefin solution to red within 1-2 min confirms beta-lactamase production. A weak beta-lactamase production activity occurs within 10 mins while colour change above 10 mins is regarded as a false positive result. The false positive result could be as a result of secondary β-lactamase activity of penicillin-binding proteins that form unstable acyl complexes (Tankeshwar, 2015).

### DNA extraction

Using the boiling and freeze-thaw method as earlier described, DNA was extracted from the beta-lactamase-positive bacterial isolates for PCR assay (Benga *et al.*, 2014). The procedure is such that a few colonies from an 18- 24 h pure culture nutrient agar plate were aseptically transferred into sterile microfuge tubes containing exactly 300 µL of sterile distilled water. The tubes were then transferred into a heating block and heated at 98 °C for 10 min. Afterwards, the tubes were immediately transferred into a -20 °C chiller for 10 mins. This was repeated three times, after which centrifugation was carried out at 13,000 g for 10 mins. The resulting supernatant was kept at 4 °C for further analysis.

### Confirmation by PCR and 16S rRNA partial sequencing

Amplification of partial 16S rRNA was performed with the universal primers RW01 (5-

AACTGGAGGAAGGTGGGGAT-3) and DG74 (5-AGGAGGTGATCCAACCGCA-3) in a PCR assay. The cell lysate (5 µL) prepared in section 2.6.1 above was used in a 20 µl reaction containing 5 µl of 5X FIREPol® Master Mix (Solisbiodyne, Estonia), 1 µl of 10 µM of forward and reverse primer and 13 µl of nuclease-free water. The thermal cycler profile was a single enzyme activation for 15 min at 93 °C followed by 35 cycles at 92 °C for 40 s, 52 °C for 1 min and 72 °C for 1 min and a final extension at 75 °C for 7 min. The expected amplicon size is 372 bp. (Benga *et al.*, 2014; Teng *et al.*, 2004). Afterwards, the amplicon was sent to inqaba biotec Ibadan, Nigeria for Sanger sequencing. The resulting chromatogram was analysed using FinchTV, and the resulting nucleotide sequence was analysed using the BLAST tool of the National Centre for Biotechnology Information (NCBI).

## RESULTS

### Occurrence of Gram-negative and Gram-positive bacteria across sampling sites

A total of 10 urine-contaminated soil sampling sites were identified. A total of 29 bacterial isolates were recovered from the ten sampling sites, of which 14 were Gram-negative and 15 were Gram-positive. Of the Gram-positive isolates, 11 were cocci while 4 were bacilli. The distribution of the isolates across sampling site and their biochemical results are presented in Table 1.

**Table 1:** Distribution of isolates across sampling sites and their biochemical test results

| LOCATION           | ISOLATE CODE | G | S | B | Ca | Ha | Co | He       | Sp | Le | Presumptive ID                                                             |
|--------------------|--------------|---|---|---|----|----|----|----------|----|----|----------------------------------------------------------------------------|
| 1K Seaters         | A10a1        | + | C | - | -  | +  | -  | $\gamma$ | -  | -  | <i>Enterococcus faecalis</i>                                               |
|                    | A7a2         | + | C | - | -  | +  | -  | $\gamma$ | -  | -  | <i>Enterococcus faecalis</i>                                               |
| Awo Hall           | A9a2         | + | C | - | -  | -  | -  | $\alpha$ | -  | -  | <i>Streptococcus</i> spp. (possibly <i>S. mitis</i> group)                 |
| Bus Stop           | A2a          | + | C | - | -  | +  | -  | $\gamma$ | -  | -  | <i>Enterococcus faecalis</i>                                               |
|                    | A1a2         | + | C | - | -  | -  | -  | $\beta$  | -  | +  | <i>Streptococcus agalactiae</i>                                            |
|                    | V1a2         | + | B | - | +  | +  | -  | $\beta$  | +  | +  | <i>Listeria monocytogenes</i>                                              |
| Fajuyi Hall        | A1a1         | + | C | - | -  | +  | -  | $\gamma$ | -  | -  | <i>Enterococcus faecalis</i>                                               |
|                    |              |   |   |   |    |    |    |          |    |    | <i>Bacillus cereus</i> group (atypical), possibly <i>Bacillus subtilis</i> |
|                    | V3a          | + | B | + | +  | +  | -  | $\beta$  | +  | +  | <i>Listeria</i> spp.                                                       |
|                    | A3a2         | + | B |   | +  | +  | -  | $\beta$  | +  | +  | <i>Streptococcus pyogenes</i>                                              |
| Library            | A3a1         | + | C | - | -  | -  | -  | $\beta$  | -  | -  | <i>Streptococcus pyogenes</i>                                              |
|                    | A6a          | + | C | - | -  | -  | -  | B        | -  | +  | <i>Streptococcus agalactiae</i>                                            |
|                    | V6a1         | + | B | + | +  | +  | -  | $\gamma$ | +  | +  | <i>Bacillus</i> spp.                                                       |
| New Market         | V5a2         | + | C | - | +  | +  | -  | $\gamma$ | -  | -  | <i>Staphylococcus epidermidis</i>                                          |
|                    | A5a1         | + | C | - | +  | +  | -  | $\gamma$ | -  | -  | <i>Staphylococcus epidermidis</i>                                          |
| Taekwando Training | A5a2         | + | C | - | +  | +  | -  | $\gamma$ | -  | -  | <i>Staphylococcus epidermidis</i>                                          |

**Key:** G = Gram status, S = Shape, B = beta-lactamase test, Ca = catalase, Ha = halotolerant, Co = coagulase, He = hemolysis, Sp = spore, Le = lecithin

The antibiotic susceptibility test result is shown in Figure 2 below. The classes of antibiotics used were Beta-lactam (cefuroxime, ceftazidime, meropenem and ampicillin), beta-lactam-beta-lactamase inhibitor (augmentin), fluoroquinolone

(ciprofloxacin), macrolide (erythromycin), tetracycline (tetracycline), phenicol (chloramphenicol), glycopeptide (vancomycin), aminoglycoside (gentamycin) and sulfonamide (cotrimoxazole).

| Sites         | ISOLATE CODE | CIP   | ERY   | TET   | CP    | GEN   | COT   | AUG | CRX | CPZ   | MEM | AMP   | VAN   |
|---------------|--------------|-------|-------|-------|-------|-------|-------|-----|-----|-------|-----|-------|-------|
| 1K Seaters    | A10a1        |       |       |       |       |       |       |     |     |       |     |       |       |
| Awo Hall      | A7a2         |       |       |       |       |       |       |     |     |       |     |       |       |
|               | A9a2         |       |       |       |       |       |       |     |     |       |     |       |       |
| Bus Stop      | A2a          |       |       |       |       |       |       |     |     |       |     |       |       |
|               | A1a2         |       |       |       |       |       |       |     |     |       |     |       |       |
| Fajuyi Hall   | V1a2         |       |       |       |       |       |       |     |     |       |     |       |       |
|               | A1a1         |       |       |       |       |       |       |     |     |       |     |       |       |
|               | V3a          |       |       |       |       |       |       |     |     |       |     |       |       |
| Library       | A3a2         |       |       |       |       |       |       |     |     |       |     |       |       |
|               | A3a1         |       |       |       |       |       |       |     |     |       |     |       |       |
|               | A6a          |       |       |       |       |       |       |     |     |       |     |       |       |
| New Mark      | V6a1         |       |       |       |       |       |       |     |     |       |     |       |       |
|               | V5a2         |       |       |       |       |       |       |     |     |       |     |       |       |
| Taekwand      | A5a1         |       |       |       |       |       |       |     |     |       |     |       |       |
|               | A5a2         |       |       |       |       |       |       |     |     |       |     |       |       |
| %Susceptible  |              | 46.67 | 6.67  | 26.67 | 6.67  | 13.33 | 33.33 | 0   | 0   | 6.67  | 0   | 6.67  | 0     |
| %Intermediate |              | 13.33 | 6.67  | 20    | 6.67  | 0     | 13.33 | 0   | 0   | 0     | 0   | 0     | 13.33 |
| %Resistance   |              | 40    | 86.67 | 53.33 | 86.67 | 86.67 | 53.33 | 100 | 100 | 93.33 | 100 | 93.33 | 86.67 |

**Figure 2:** Antibiotic susceptibility test results

**Key:** Tetracycline – TET, Gentamicin – GEN, Erythromycin – ERY, Ciprofloxacin – CIP, Cefuroxime – CRX, Meropenem – MEM, Ampicillin – AMP, Augmentin – AUG, Vancomycin – VAN, Cotrimoxazole – COT, Ceftazidime – CPZ, Chloramphenicol – CP, Site = sampling sites, Green = susceptible, Yellow = intermediate, Red = resistant.

More than 90% of the isolate population exhibited resistance against the beta-lactam antibiotics, while population of the isolates that exhibited resistance

against other classes of antibiotics ranged between 40% for ciprofloxacin to 86.67% for erythromycin, vancomycin and chloramphenicol. The multiple

antibiotic resistance index of the Gram-positive isolates (Table 2) ranged between 0.58 and 1.

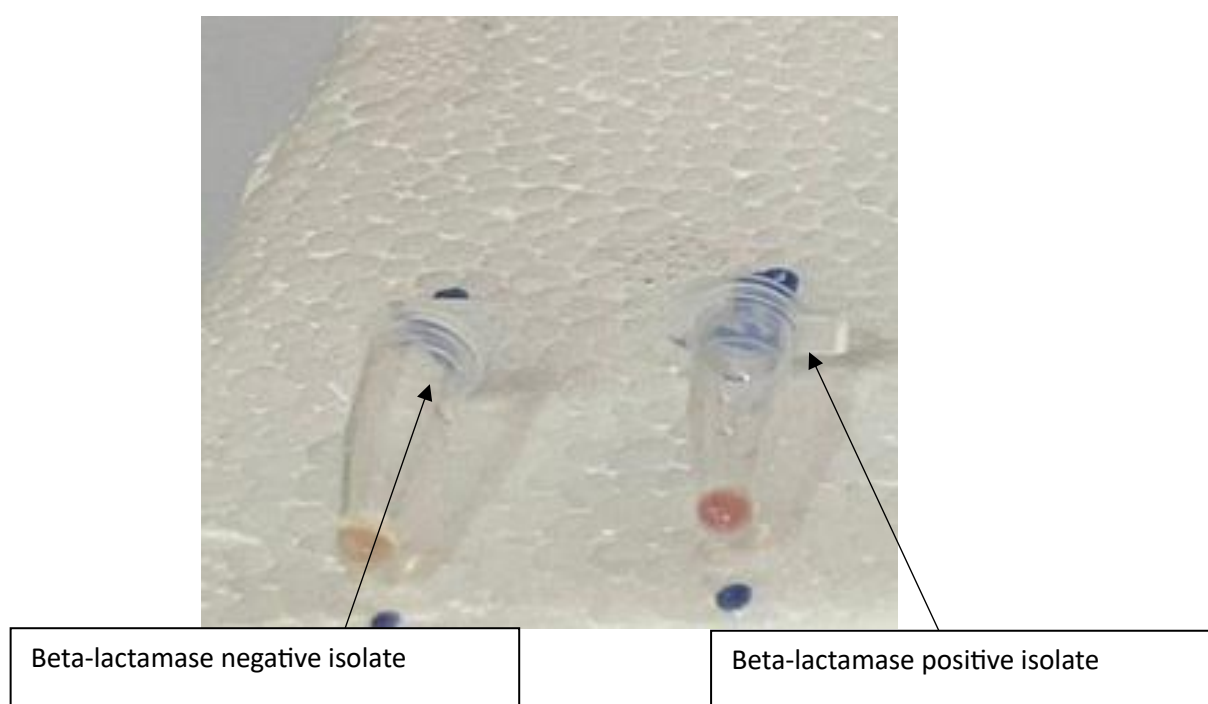
**Table 2:** Multiple antibiotic resistance index for isolates

| ISOLATE CODE | a  | b  | MARI (b/a) |
|--------------|----|----|------------|
| A10a1        | 12 | 7  | 0.58       |
| A7a2         | 12 | 12 | 1.00       |
| A9a2         | 12 | 10 | 0.83       |
| A2a          | 12 | 11 | 0.92       |
| A1a2         | 12 | 9  | 0.75       |
| V1a2         | 12 | 12 | 1.00       |
| A1a1         | 12 | 10 | 0.83       |
| V3a          | 12 | 4  | 0.33       |
| A3a2         | 12 | 7  | 0.58       |
| A3a1         | 12 | 11 | 0.92       |
| A6a          | 12 | 10 | 0.83       |
| V6a1         | 12 | 11 | 0.92       |
| V5a2         | 12 | 10 | 0.83       |
| A5a1         | 12 | 10 | 0.83       |
| A5a2         | 12 | 11 | 0.92       |

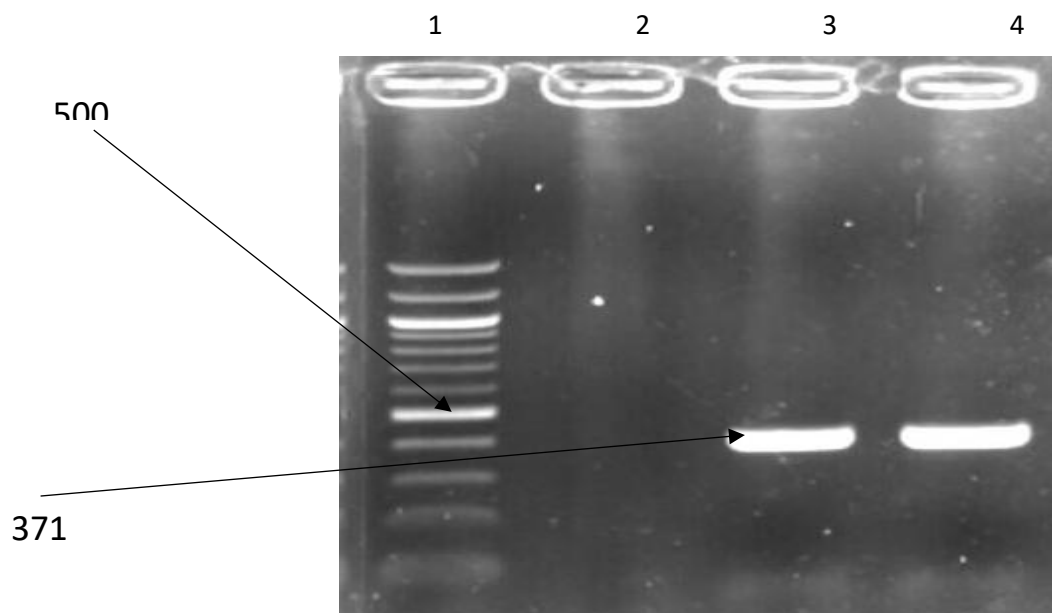
Key: a = number of antibiotics used, b = number of antibiotics with resistance, MARI = multiple antibiotic resistance index.

Of the fifteen isolates, two were positive for beta-lactamase production, and they were confirmed as *Bacillus* sp. by the partial 16S rRNA sequence result. A sample picture of the  $\beta$ -lactamase-positive and negative isolates is given in Plate 2, a gel picture confirming the amplification of the

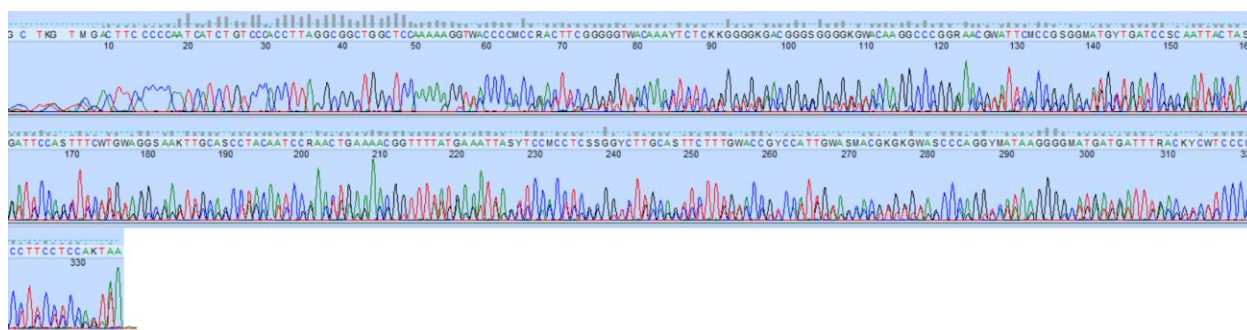
target site of the 16S rRNA with an amplicon size of 372 bp for the two  $\beta$ -lactamase-positive isolates and chromatograms of the sequenced amplicon are shown in Plates 3, 4 and 5, respectively.



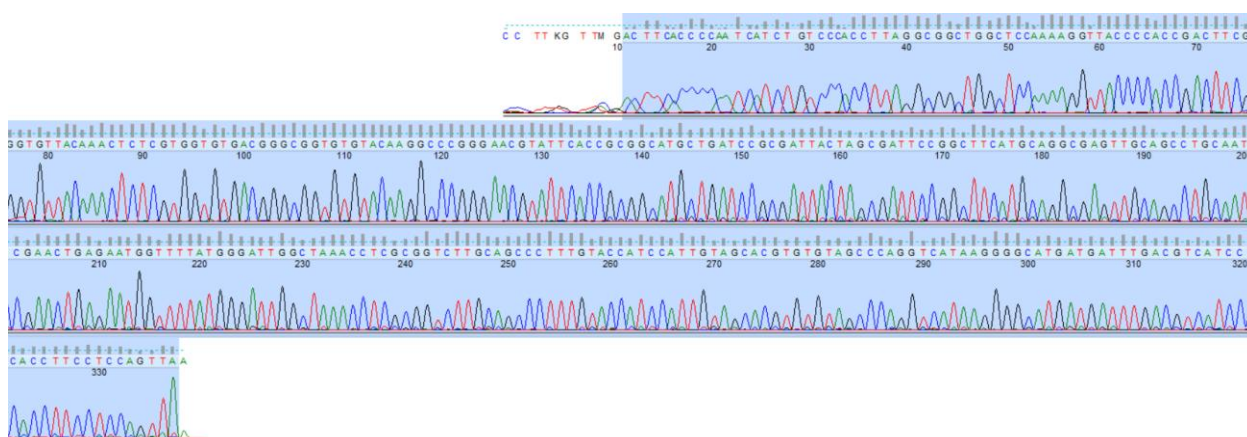
**Plate 2:** Nitrocefin-dosed nutrient broth Tubes showing beta-lactamase-positive and negative isolates



**Plate 3:** Gel picture showing the amplification (372 bp) of the V8-V9 variable regions of 16S rRNA of the two beta-lactamase-positive isolates.



**Plate 4:** A chromatogram of isolate V3a (A\_AB31F\_C10.07.ab1)



**Plate 5:** A chromatogram of isolate V6a1 (3\_AB31F\_C09\_09)

## DISCUSSION

The relatively balanced occurrence of Gram-positive ( $n = 15$ ) and Gram-negative ( $n = 14$ ) isolates suggests that urine contamination supports a broad range of bacterial taxa, consistent with previous reports that both groups can adapt to

urea-rich, nitrogenous environments (Ezeonyido, 2021; Haindongo *et al.*, 2016; Vince Anyim, 2018; Yu *et al.*, 2025).

In this study, isolated Gram-positive cocci ( $n = 11$ ) were more common than bacilli ( $n = 4$ ). The observed predominance of cocci (e.g. *Enterococcus*

*faecalis*, *Streptococcus agalactiae*, and *Staphylococcus epidermidis*, etc.) has been linked to their ability to persist in harsh environmental conditions, including desiccation and high salt concentrations, due to robust cell wall structures and stress response systems (García-Solache and Rice, 2019; Imlay, 2008; Jordan *et al.*, 2008; Murray, 1990; Onyango and Alreshidi, 2018; Ranganathan *et al.*, 2020; Tamhankar *et al.*, 2020). The occurrence of *Enterococcus faecalis* in multiple sites is notable in this study. This is a public and environmental health concern because the bacterium is a recognised faecal indicator organism (suggesting open defecation in the sampling area) and opportunistic pathogen (suggesting high risk of contracting opportunistic infections within the sampling area) (Byappanahalli *et al.*, 2012).

The Gram-positive bacilli detected in the urine-contaminated soil samples included *Listeria* spp., and members of the *Bacillus cereus* group. *Listeria* species are known to persist in soil and organic-rich environments, posing zoonotic and food safety concerns (Vivant *et al.*, 2013). Also, *Bacillus* spp., particularly *B. cereus*, are common soil saprophytes but can be opportunistic pathogens, with spores enabling long-term survival in fluctuating soil conditions (Bottone, 2010; Ehling-Schulz *et al.*, 2019; Vilain *et al.*, 2006).

The biochemical test patterns further support the identity and ecological roles of the isolates. For instance, catalase positivity in *Staphylococcus* and *Bacillus* species reflects adaptation to oxidative stress in exposed soil surfaces (Imlay, 2008), while hemolytic activity in *Streptococcus pyogenes* and *Streptococcus agalactiae* underscores their pathogenic potential (Fontaine *et al.*, 2003; Hensler *et al.*, 2005; Limbago *et al.*, 2000; Randis *et al.*, 2014; Sierig *et al.*, 2003; Whidbey *et al.*, 2013). Spore formation in *Bacillus* species confirms their resilience against environmental stressors (Petrus and Casida, 1985; Setlow, 2014; Zeigler, 2017).

Overall, these findings suggest that urine-contaminated soils act as reservoirs for both environmental and clinically significant bacteria, some of which are indicators of fecal contamination (*Enterococcus*, *Streptococcus*), while others (*Listeria*, *Bacillus*) highlight the zoonotic and opportunistic infection risks. The co-occurrence of Gram-positive and Gram-negative bacteria in such environments underlines the potential for

horizontal gene transfer, particularly of antimicrobial resistance determinants, which has been documented in similar environmental contexts (Wellington *et al.*, 2013).

Near-total resistance to penicillins and cephalosporins across isolates (augmentin, cefuroxime, cefoperazone, and ampicillin), together with 100% resistance to meropenem, was observed. Non- $\beta$ -lactam antibiotics (ciprofloxacin, cotrimoxazole, gentamicin) retain some activity but with substantial resistance rates, showing that isolates are multidrug-resistant bacteria. This is in line with earlier studies (Dada and Aruwa, 2014; Vince, 2018). The pattern ( $\beta$ -lactam resistance broadly, carbapenem non-susceptibility) is most consistent with a mix of  $\beta$ -lactamase mechanisms in the collection — plasmid-borne ESBLs (CTX-M/TEM/SHV), inducible or plasmid AmpC cephalosporinases, and/or carbapenemases (NDM/OXA/KPC/IMP/VIM) — possibly combined with permeability defects (porin loss) or efflux that can raise carbapenem MICs in the presence of ESBL/AmpC. These mechanisms are commonly reported in environmental bacteria that have been exposed to human waste streams, e.g. urine-polluted soil areas that have recurrent exposure to human excreta and therefore to antibiotic residues and resistant organisms — a setting recognised as a reservoir and selective niche for ARGs (Zhou *et al.*, 2021).

Only two isolates were positive for the beta-lactamase production assay despite high phenotypic resistance against beta-lactam antibiotics among isolated bacteria in this study. This observation suggests that the isolates with high phenotypic resistance to beta-lactam antibiotics, but negative for the beta-lactamase test, employ other antibiotic resistance mechanisms (Bush and Bradford, 2020; Nasrollahian *et al.*, 2024). This underscores the need to explore different mechanisms of antibiotic resistance before a detailed explanation of the observed phenotypic antibiotic resistance of an isolate can be exhaustively discussed. All the isolates exhibit a multiple antibiotic resistance index (MARI) > 0.2, which reflects that all the isolates are from an area of high-risk antibiotic exposure. Isolates with MARI above 0.2 have been adjudged to come from a source of high-risk exposure to antibiotics (Krumperman, 1983). This

further corroborates the high risk of antibiotic resistance spread posed by open urination.

Finally, the  $\beta$ -lactamase production in *Bacillus cereus*, which was formerly known to be intrinsic, is becoming more diverse and increasingly complex, driven by chromosomal genes (*bla*1, *BcII*), leading to emerging ESBL traits and amplified by environmental reservoirs, biofilm formation and antibiotic pressure (Bahr *et al.*, 2021; Farina *et al.*, 2024), suggesting that the organism is transitioning from being an environmental contaminant to a clinically relevant AMR pathogen.

## CONCLUSION

The study confirmed the occurrence of known pathogens in the soil samples collected at the open urination spots within the University environment. Furthermore, all the isolated bacteria are multidrug-resistant and the two *Bacillus* spp. isolated were positive for beta-lactamase production. These suggest that the open urination spots at the University are potential sources of pathogens and antibiotic resistance spread within the University community.

## RECOMMENDATION

The findings of this study align with calls in the literature to reduce or discourage anthropogenic activities such as open urination, which contribute to environmental discharge of human waste because human waste can carry residues of antibiotics and antibiotic-resistant genes that select for antimicrobial-resistant strains in soil communities. Hence, management of institutions, most especially those with transient populations, should discourage open urination by appropriate policy and provision of more public toilets in order to prevent the spread of antibiotic resistance that could occur as a result of open urination.

## AUTHORS' CONTRIBUTIONS

AOE, AOA, OFT, AFI, TFF, AOH, AAM  
Principal Investigator: AOE Writing – Original Draft Preparation: AOE and AFI Writing – Review & Editing: AOA, OFT, TFF, AOH, AAM Conceptualizations: AOE and AFI Data Curation: OEA and AAM Funding Acquisition: AOE, AOA, OFT, AOH Investigation: AFI, AOH, AAM Methodology: AOE, AOA, OFT, AFI, TFF, AOH, AAM Project Administration: AOE, AOA, AOH

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## DECLARATION OF CONFLICT OF INTEREST

All authors declare no conflict of interest

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