

Bacterial profile and antibiotics susceptibility pattern of ear swabs among students in tertiary institutions in Anambra state

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Abstract

Bacterial colonisation of the external ear canal represents an important risk factor for community-associated otitis externa, particularly among young adults with frequent exposure to shared environments. This cross-sectional study assessed the prevalence, bacterial profile, and antibiotic susceptibility patterns of ear swab isolates obtained from 107 asymptomatic undergraduate students in a tertiary institution in Anambra State, Nigeria. A total of 46 samples (42.99%) yielded significant bacterial growth, of which *Staphylococcus aureus* was the predominant isolate, accounting for 40 (86.96%) of all positive cultures, followed by *Staphylococcus epidermidis* 3 (6.52%) and *Pseudomonas aeruginosa* 3 (6.52%). No Enterobacterales species were isolated. *Staphylococcus aureus* demonstrated high resistance to β -lactam antibiotics, particularly amoxicillin/clavulanic acid (95%) and cefotaxime (87.5%), but retained notable susceptibility to azithromycin (80%) and fluoroquinolones, including ofloxacin (75%) and levofloxacin (72.5%). All *P. aeruginosa* isolates were resistant to imipenem and amoxicillin/clavulanic acid but fully susceptible to ofloxacin and levofloxacin. In conclusion, the moderate prevalence of bacterial colonisation among students, dominated by *Staphylococcus aureus* with lesser contributions from *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, alongside high resistance to commonly used β -lactam antibiotics but retained susceptibility to fluoroquinolones and azithromycin, highlights the need for continuous surveillance and rational antibiotic use to prevent the emergence and spread of resistant otic pathogens.

Keywords: Ear swabs; *Staphylococcus aureus*; *Pseudomonas aeruginosa*; Antimicrobial resistance; University students; Nigeria

1. Introduction

Bacterial infections of the ear, also known as otitis, are a common health problem that affects individuals across all age groups, with a significant prevalence in children and young adults (Smith & Jones, 2020). These infections are generally categorised into *otitis externa*, *otitis media*, and *chronic suppurative otitis media*, based on the part of the ear affected (Jamal *et al.*, 2022). *Otitis externa*, often referred to as "swimmer's ear," is an inflammation or infection of the external ear canal frequently caused by pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Wilson *et al.*, 2021). Otitis media, on the other hand, involves an infection of the middle ear and is commonly linked to respiratory

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tract infections, with *Streptococcus pneumoniae* and *Haemophilus influenzae* being the primary pathogens involved (Ngo *et al.*, 2016).

The prevalence of ear infections among students is particularly concerning due to lifestyle and environmental factors. Prolonged use of earphones, exposure to shared equipment, poor hygiene, and contact with contaminated water are known to increase the risk of developing bacterial ear infections (Kumar *et al.*, 2019). Additionally, the lack of awareness regarding proper ear care practices contributes to the persistence and recurrence of these infections in this demographic.

Untreated or poorly managed ear infections can lead to serious complications, including chronic hearing loss, speech delays, loss of balance and diminished academic performance (Smith & Jones, 2020). These outcomes underscore the importance of timely diagnosis and appropriate treatment, including the identification of bacterial pathogens and their antibiotic susceptibility patterns. Recent studies emphasise that understanding local epidemiological trends and resistance profiles is essential for the effective management and prevention of ear infections, particularly in high-risk groups like students (Garcia & Ahmed, 2021).

Ear infections are prevalent in both children and adults. When ear infections are neglected, they can result in hearing loss, deafness, potentially fatal consequences, and other serious health issues. Worldwide, between 65 and 330 million people get ear infections, and 60% of them say their hearing is impaired (Mukara *et al.*, 2017). According to reports, South Asia and Sub-Saharan Africa have the greatest rates of ear infections (Tesfa *et al.*, 2020). In Tanzania, 53.3% of people had ear infections. Ear infections are caused by Gram-positive and Gram-negative bacteria more often than by viruses and fungi. The bacteria that cause ear infections most frequently are *Streptococcus* species, *Enterococcus* species, *Klebsiella* species, *Pseudomonas* species, *Staphylococcus aureus*, *Proteus* species, and Coagulase-negative *Staphylococcus aureus* (CoNS) (Temu *et al.* 2024). One or a combination of bacteria was found in 52.1% to 98.8% of ear discharge culture investigations conducted in Africa. According to reports, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most predominant bacteria (Temu *et al.* 2024).

In resource-limited settings, most patients with ear infections delay seeking medical attention; hence, they usually present with complications. There is an increased rate of antibiotic resistance to pathogens causing ear infections due to the increased and irrational use of wide-spectrum antibiotics, which is a major global health threat. A lot of these studies have been done among hospital patients; little or nothing is documented among Asymptomatic carriers /University students. Therefore, this study is designed to assess the bacterial profile and antibiotic susceptibility pattern of ear isolates among University students.

2. Method

2.1. Study Design and Setting

This cross-sectional laboratory-based study was conducted among undergraduate students at the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Campus, Anambra State, Nigeria. The study focused on detecting bacterial pathogens colonising the external ear canal and determining their antimicrobial susceptibility profiles.

2.2. Ethical Approval and Participant Recruitment

Ethical clearance was obtained from Anambra state Ministry of Health (Ref: MH/COMM/523/VOL.124). Informed consent was obtained from all participants after explaining the purpose and procedures of the study. Voluntary recruitment targeted healthy students with no active ear infection or recent antibiotic use (within the previous two weeks).

2.3. Sample Size Determination

The minimum sample size was calculated using Cochran's formula based on a previously reported prevalence of 92.5% of culture-positive ear swabs. A total of 107 participants were enrolled to compensate for possible dropouts and laboratory exclusions.

2.4. Sample Collection

Using strict aseptic technique, sterile cotton swabs were inserted into the outer third of the external auditory canal and rotated to collect cerumen and surface microbes. Each swab was immediately transferred into individually and properly labelled sterile transport tubes and delivered to the laboratory within 1 hour of collection.

2.5. Culture and Isolation of Bacteria

The ear swabs were first inoculated into nutrient broth and incubated at 37 °C for 18–24 hours to allow enrichment. After incubation, the isolates from the enriched cultures were streaked onto selective and differential media: Mannitol Salt Agar (MSA) for the isolation of *Staphylococcus* spp., Cetrimide Agar (CA) for *Pseudomonas aeruginosa*, and MacConkey Agar (MAC) for Enterobacterales. The plates were then incubated at 37 °C for 24 hours. Afterwards, distinct colonies were sub-cultured onto nutrient agar to obtain pure isolates.

2.6. Identification of Isolates

Bacterial isolates were identified based on colony morphology (colour, size, texture, elevation, and haemolysis where applicable), Gram staining to determine Gram reaction and cellular morphology, and standard biochemical tests. Catalase test to differentiate *Staphylococcus* spp., the coagulase test to confirm *Staphylococcus aureus*, the oxidase test to identify *Pseudomonas aeruginosa*, and the citrate utilisation test to differentiate *Klebsiella* spp., were carried out.

2.7. Antimicrobial Susceptibility Testing

The antibiotic susceptibility of confirmed isolates was evaluated using the Kirby–Bauer disk diffusion method on Mueller-Hinton agar, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2020).

The commercially available antibiotic discs (Celtech diagnostic) used in this study encompassed a broad spectrum of commonly prescribed antibiotics, including.

Gram positive: Amoxicillin/Clavulanic acid (AUG) 30mcg, Cefotaxime (CTX) 25mcg, Ceftriaxone/sulbactam(CRO) 45mcg, Cefexime (ZEM) 5mcg, Levofloxacin (LBC) 5mcg, Ciprofloxacin (CIP) 5mcg, Impenem/cilastatin (IMP) 10mcg/10mcg, Cefuroxime (CXM) 30mcg, Ofloxacin (OFX) 5mcg, Erythromycin (ERY) 15mcg, Gentamicin (GN) 10mcg, Azithromycin (AZN) 15mcg.

Gram negative: Ceftriaxone/sulbactam (CRO) 45mcg, Nalidixic acid (NA) 50mcg, Cefexime (ZEM) 5mcg, Ofloxacin (OFX) 5mcg, Impenem/cilastatin (IMP) 10mcg/10mcg, Cefotaxime (CTX) 25mcg, Ampiclox (AC), Cefuroxime (CXM) 30mcg, Gentamicin (GN) 10mcg, Levofloxacin (LBC) 5mcg, Amoxicillin/Clavulanic acid (AUG) 30mcg, Nitrofurantoin (NF) 300mcg.

The turbidity of bacterial inocula was standardised to 0.5 McFarland. Antibiotic discs commonly used in otic infections were tested against Gram-positive and Gram-negative isolates, including beta-lactams, fluoroquinolones, macrolides, and aminoglycosides. Zone diameters were measured in millimetres and interpreted as Susceptible, Intermediate, or Resistant based on CLSI breakpoints.

2.8. Data Management and Statistical Analysis

All laboratory data were recorded in Microsoft Excel. Descriptive statistics (frequencies, percentages) were used to summarise bacterial distribution and resistance patterns. Results were presented in tables and charts for clarity.

3. Results and discussion

Of the 107 ear swabs collected and cultured, bacterial growth was observed in 46 samples (42.99%), while 61 samples (57.01%) showed no growth. The predominant isolates were *Staphylococcus aureus* (40 isolates, 86.96%), followed by *Staphylococcus epidermidis* (3 isolates, 6.52%) and *Pseudomonas aeruginosa* (3 isolates, 6.52%). There was no growth on MacConkey agar.

Microscopically, *Staphylococcus aureus* and *Staphylococcus epidermidis* appeared as purple coccoid clusters, reflecting their thick peptidoglycan cell walls that retain the crystal violet stain, characteristic of Gram-positive bacteria. In contrast, *Pseudomonas aeruginosa* was identified as pink, rod-shaped cells, indicative of a thin peptidoglycan layer and a Gram-negative reaction due to retention of the safranin counterstain.

Staphylococcus aureus was the predominant bacterium isolated from ear swabs of university students, consistent with previous studies from Tanzania (Temu *et al.*, 2024), Nigeria (Anie *et al.*, 2020), and Ethiopia (Worku and Bekele, 2014), which report it as a common pathogen in acute and chronic ear infections. However, Al-Tulaibawi (2023) reported *Pseudomonas aeruginosa* as the prevalent pathogen in Iraq. This contrast might be due to differences in geographic and climatic factors. The frequent isolation of *S. aureus* aligns with its status as both normal skin flora and an opportunistic pathogen, which exploits breaks in skin integrity or poor hygiene to cause infection (Ekpunobi and Adeleye, 2020).

The study also identified *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* as relevant pathogens, each comprising 6.52% of isolates. *P. aeruginosa*'s role in chronic, resistant ear infections is linked to its biofilm formation and environmental resilience, corroborating findings by Shaheda *et al.* (2024).

Globally, bacterial ear infection prevalence varies, with higher rates reported in Sub-Saharan Africa and South Asia, influenced by environmental, socioeconomic, and healthcare disparities (Temu *et al.*, 2024).

Antimicrobial susceptibility testing revealed significant resistance trends. *Staphylococcus aureus* showed high resistance to beta-lactams such as Amoxicillin/Clavulanic acid (95%) and Cefotaxime (87.5%), consistent with data from Anie *et al.* (2020) and Halilu *et al.* (2022). Resistance was also noted for Imipenem, Ceftriaxone/sulbactam, and Cefixime. However, *S. aureus* remained largely susceptible to fluoroquinolones (Ofloxacin 75%, Levofloxacin 72.5%, Ciprofloxacin 72.5%) and macrolides (Azithromycin 80%), underscoring the importance of prudent antibiotic use to preserve efficacy.

Table 1 Antibigram of *Staphylococcus aureus* isolates (n = 40) to different antibiotics

Antibiotic agent	Susceptibility (%)	Intermediate (%)	Resistant (%)
AUG	1 (2.5%)	1 (2.5%)	38 (95%)
CTX	0 (0%)	1 (2.5%)	39 (87.5%)
CRO	5 (12.5%)	5 (12.5%)	30 (75%)
ZEM	1 (2.5%)	0 (0%)	39 (87.5%)
LBC	29 (72.5%)	3 (7.5%)	8 (20%)
CIP	29 (72.5%)	7 (17.5%)	4 (10%)
IMP	0 (0%)	0 (0%)	40 (100%)
CXM	3 (7.5%)	7 (17.5%)	30 (75%)
OFX	30 (75%)	1 (2.5%)	9 (22.5%)
ERY	15 (37.5%)	3 (7.5%)	22 (55%)
GN	29 (72.5%)	0 (0%)	11 (27.5%)
AZN	32 (80%)	1 (2.5%)	7 (17.5%)

KEY: AUG- Amoxicillin/Clavulanic acid, CTX- Cefotaxime, CRO- Ceftriaxone/sulbactam, ZEM- Cefixime, LBC- Levofloxacin, CIP – Ciprofloxacin, IMP- Imipenem /cilastatin, CXM- Cefuroxime, OFX- Ofloxacin, ERY- Erythromycin, GN- Gentamicin, AZN- Azithromycin; S – Susceptible; R – Resistant; I – Intermediate

Staphylococcus epidermidis exhibited resistance to several beta-lactams and carbapenems but was fully susceptible to Levofloxacin (100%) and partially to Ofloxacin, Ciprofloxacin, Azithromycin, and Gentamicin (all 66.7%).

Table 2 Antibigram of *Staphylococcus epidermidis* isolates (n = 3) to different antibiotics

Antibiotic agent	Susceptibility (%)	Intermediate (%)	Resistant (%)
AUG	0 (0%)	0 (0%)	3 (100%)
CTX	0 (0%)	0 (0%)	3 (100%)
CRO	1 (33.3%)	0 (0%)	2 (66.7%)

ZEM	0 (0%)	0 (0%)	3 (100%)
LBC	3 (100%)	0 (0%)	0 (0%)
CIP	2 (66.7%)	0 (0%)	1 (33.3%)
IMP	0 (0%)	0 (0%)	3 (100%)
CXM	0 (0%)	0 (0%)	3 (100%)
OFX	2 (66.7%)	0 (0%)	1 (33.3%)
ERY	1 (33.3%)	1 (33.3%)	1 (33.3%)
GN	2 (66.7%)	0 (0%)	1 (33.3%)
AZN	2 (66.7%)	0 (0%)	1 (33.3%)

KEY: AUG- Amoxicillin/Clavulanic acid, CTX- Cefotaxime, CRO- Ceftriaxone/sulbactam, ZEM- Cefexime, LBC- Levofloxacin, CIP – Ciprofloxacin, IMP- Imipenem /cilastatin, CXM- Cefuroxime, OFX- Ofloxacin, ERY- Erythromycin, GN- Gentamicin, AZN- Azithromycin; S – Susceptible; R – Resistant; I – Intermediate

Pseudomonas aeruginosa showed multidrug resistance, including to Imipenem and Amoxicillin/Clavulanic acid, but remained susceptible to Ofloxacin and Levofloxacin, aligning with Shaheda *et al.* (2024). The emergence of multidrug-resistant strains such as MRSA and carbapenem-resistant *Pseudomonas aeruginosa* is alarming, reflecting WHO (2021) concerns about antibiotic misuse driving these “superbugs,” with global impacts on morbidity and mortality. This study reflects a global pattern of increasing antimicrobial resistance, especially critical in low-resource settings with limited access to advanced therapeutics (Getaneh *et al.*, 2021; Chukwunwejim *et al.*, 2025).

Table 3 Antibigram of *Pseudomonas aeruginosa* isolates (n =3) to different antibiotics

Antibiotic agent	Susceptibility (%)	Intermediate (%)	Resistant (%)
IMP	0 (0%)	0 (0%)	3 (100%)
AUG	0 (0%)	0 (0%)	3 (100%)
OFX	3 (100%)	0 (0%)	0 (0%)
GN	1 (33.3%)	0 (0%)	2 (66.7%)
ZEM	0 (0%)	0 (0%)	3 (100%)
NA	0 (0%)	0 (0%)	3 (100%)
LBC	3 (100%)	0 (0%)	0 (0%)
CRO	1 (33.3%)	2(66.7%)	0 (0%)
CXM	0 (0%)	0 (0%)	3 (100%)
ACX	0 (0%)	0 (0%)	3 (100%)
NF	0 (0%)	0 (0%)	3 (100%)
CTX	0 (0%)	0 (0%)	3 (100%)

KEY: IMP- Imipenem /cilastatin, AUG-Amoxicillin/Clavulanic acid, OFX- Ofloxacin, GN- Gentamicin, ZEM- Cefexime, NA-Nalixidic acid, LBC- Levofloxacin, CRO- Ceftriaxone/sulbactam, CXM- Cefuroxime, ACX -Ampiclox, NF- Nitrofurantoin, CTX-Cefotaxime.; S – Susceptible; R – Resistant; I – Intermediate

Behavioural and environmental risk factors identified include the use of cotton buds and sharp objects for ear cleaning, poor ear hygiene, and exposure to pathogens, consistent with findings by Massawe *et al.* (2023). The growing problem of antibiotic resistance is exacerbated by inappropriate antibiotic use, such as self-medication and incomplete treatment courses (Ekpunobi *et al.*, 2025). The WHO (2021) emphasises urgent global efforts to curb antibiotic misuse, particularly in regions with easy over-the-counter access.

Finally, this study highlights that a healthy external ear naturally harbours diverse microbes due to environmental exposure, and their presence does not necessarily indicate infection, as many are part of normal flora.

4. Conclusion

This study successfully profiled the bacterial isolates and antibiotic susceptibility patterns from ear swabs among students in a tertiary institution in Anambra State. *Staphylococcus aureus* emerged as the predominant isolate, followed by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, confirming their roles as important etiological agents of ear infections. Alarming high resistance was observed against commonly prescribed antibiotics, particularly Amoxicillin/Clavulanic acid and Cefotaxime, reflecting the growing challenge of antimicrobial resistance (AMR). Nonetheless, the observed susceptibility to Levofloxacin, Ciprofloxacin, Azithromycin, and Ofloxacin suggests viable therapeutic alternatives.

The findings highlight the urgent need for rational antibiotic use, strengthened antimicrobial stewardship, and stricter regulation of over-the-counter drug access. Preventive strategies such as promoting proper ear hygiene, discouraging harmful practices like cotton bud insertion or self-medication, and encouraging routine ear check-ups are equally critical. Overall, this study underscores both the clinical and public health importance of early detection, appropriate antibiotic therapy, and proactive AMR mitigation strategies to curb the burden of ear infections in the student population.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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