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Multidrug-Resistant *Enterococcus faecalis* Clinical Isolates from a Tertiary Hospital in Gombe, Nigeria: Antibiotic Susceptibility Patterns and Implications for Therapy.

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Abstract

The emergence of multidrug-resistant (MDR) *Enterococcus faecalis* represents a growing public health concern, particularly in resource-limited healthcare settings where treatment options may be constrained. This study determined the prevalence and antibiotic susceptibility profiles of *E. faecalis* clinical isolates obtained from patients at the Federal Teaching Hospital Gombe, Nigeria. Clinical specimens were collected and subjected to standard microbiological procedures. Presumptive isolates were identified phenotypically using Gram staining, catalase testing, growth in 6.5% NaCl, and bile esculin hydrolysis, followed by molecular confirmation using polymerase chain reaction (PCR) amplification. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method against eleven antibiotics, with results interpreted according to the Clinical and Laboratory Standards Institute (CLSI) M100, 35th Edition guidelines. Four clinical isolates were recovered, of which three were confirmed as *E. faecalis* by both phenotypic and molecular methods, while one was identified as *Staphylococcus* spp. All confirmed *E. faecalis* isolates exhibited multidrug resistance, with resistance recorded against seven of the eleven antibiotics tested: amoxicillin, ceftriaxone, levofloxacin, doxycycline, cefepime, cephalothin, and chloramphenicol. Conversely, the isolates remained susceptible to erythromycin, ciprofloxacin, and clindamycin, while intermediate susceptibility to rifampicin was observed. Each *E. faecalis* isolate demonstrated resistance to antibiotics belonging to at least three different antimicrobial classes, thereby meeting the criterion for multidrug resistance. These findings demonstrate the occurrence of MDR *E. faecalis* among clinical isolates at the study hospital and underscore the importance of routine antimicrobial susceptibility testing, continuous resistance surveillance, and strengthened antimicrobial stewardship to guide effective therapy and limit further emergence and dissemination of resistant strains.

Keywords

Enterococcus Faecalis,
Multidrug-Resistant (MDR),
Antibiotic Susceptibility,
Nigeria,
Healthcare-Associated Infections,
Kirby-Bauer.



Introduction

Enterococcus faecalis is a Gram-positive, facultative anaerobic, catalase-negative coccus that forms part of the normal gastrointestinal microbiota of humans and other animals. Although it is generally considered a commensal organism, *E. faecalis* can survive under a wide range of environmental conditions and adapt to antimicrobial exposure. This ability allows it to act as an opportunistic pathogen, particularly in hospitalized patients, individuals with compromised immunity, patients with prolonged hospital stay, and those exposed to invasive medical procedures or broad-spectrum antimicrobial therapy (García-Solache & Rice, 2019; Murray et al., 2020). Enterococci are increasingly recognized as important causes of healthcare-associated infections, with *E. faecalis* commonly associated with urinary tract infections, bloodstream infections, infective endocarditis, intra-abdominal infections, wound infections, and other serious clinical conditions (Fiore et al., 2019; Sood et al., 2020).

The clinical importance of *E. faecalis* is further increased by its ability to develop and acquire resistance to several classes of antimicrobial agents. Enterococci have intrinsic resistance to a number of commonly used antibiotics and can acquire additional resistance determinants through mutation, horizontal gene transfer, and mobile genetic elements. As a result, infections caused by multidrug-resistant enterococci are becoming increasingly difficult to treat, particularly in healthcare settings where antimicrobial use and selection pressure are high (Miller et al., 2020). The emergence of resistance to glycopeptide antibiotics, particularly vancomycin, represents an important development in the evolution of antimicrobial-resistant enterococci.

Vancomycin-resistant enterococci (VRE) are enterococcal strains that have acquired mechanisms that enable them to survive exposure to vancomycin, an antimicrobial agent that has historically been important in the treatment of serious Gram-positive bacterial infections. Vancomycin resistance is mainly associated with changes in the bacterial cell-wall

peptidoglycan precursor, which reduce the ability of vancomycin to bind effectively to its target. Several vancomycin resistance genotypes have been identified, with *vanA* and *vanB* among the most clinically important determinants (Ahmed & Baptiste, 2018; Miller et al., 2020). The acquisition and spread of these resistance determinants are of particular concern because enterococci can act as reservoirs of antimicrobial-resistance genes and may facilitate their transfer to other clinically important bacterial pathogens.

The emergence and spread of VRE present major challenges for infection prevention and antimicrobial treatment. Patients infected or colonized with VRE may have limited treatment options, particularly when vancomycin resistance occurs alongside resistance to other antimicrobial classes. Agents such as linezolid, daptomycin, and tigecycline may be used to manage serious VRE infections; however, their use may be limited by factors such as toxicity, pharmacological considerations, cost, and the continued emergence of resistance (Yim et al., 2021). The increasing prevalence of antimicrobial-resistant organisms therefore highlights the need to explore new antimicrobial compounds and therapeutic approaches that could complement existing treatment options.

The global antimicrobial resistance crisis has renewed interest in medicinal plants as potential sources of biologically active compounds. Plants have been used in traditional medicine for centuries, and many contain secondary metabolites with antimicrobial, antioxidant, anti-inflammatory, and other biological properties. Plant-derived compounds may act through several mechanisms, including disruption of bacterial cell membranes, inhibition of essential enzymes, interference with nucleic acid synthesis, alteration of cellular metabolism, and inhibition of microbial communication and biofilm formation (Górniak et al., 2019; Khameneh et al., 2019). The presence of multiple bioactive compounds within a plant extract may allow it to act on different bacterial targets and may contribute to its overall antimicrobial activity.

MATERIALS AND METHODS

Study Area

This study was carried out at the Federal Teaching Hospital (FTH), Gombe, located in Gombe State, northeastern Nigeria. The hospital is situated within Gombe metropolis, the capital city of Gombe State. Gombe metropolis lies approximately between latitudes 10°N and 11°N of the Equator and falls within the Sudan Savannah ecological zone. According to the 2006 population census, Gombe State had a population of approximately 2.5 million people and covers an area of about 18,000 km² (Figure 1).

The Federal Teaching Hospital, Gombe, was established in 1996 to provide tertiary healthcare services to the people of Gombe State and neighbouring areas. The hospital also serves as a referral centre for patients requiring specialised medical care from different parts of the state and surrounding communities. The facility has an estimated bed capacity of about 500, with an average bed occupancy rate ranging from 90% to 95%. Its role as a major referral and tertiary healthcare facility makes it an appropriate setting for studies involving clinical bacterial isolates and antimicrobial resistance.

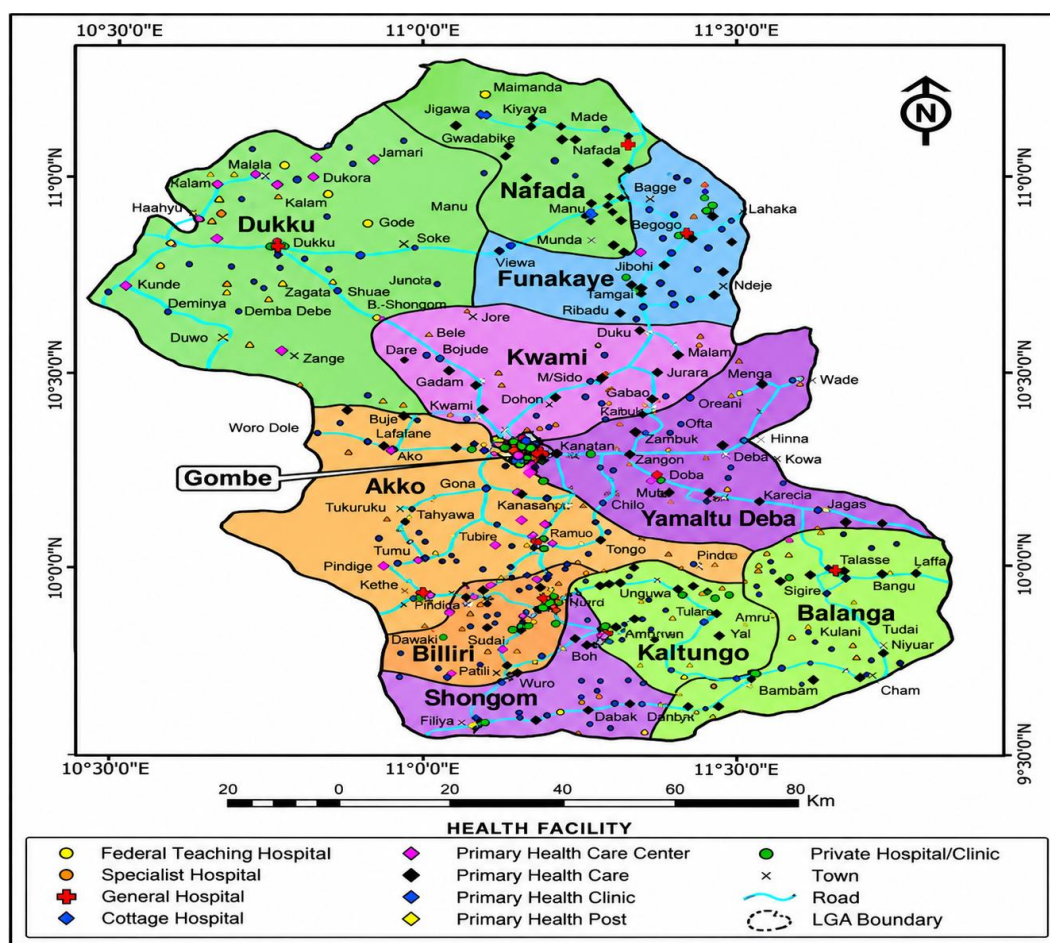


Figure 1: Map of Gombe State indicating the location of Federal Teaching Hospital, Gombe (Oghenebrume, 2021).

Study Design

This was a cross-sectional, laboratory-based study conducted at the Department of Microbiology, Gombe State University, Nigeria. Ethical approval for the study was obtained from the Research and Ethics Committee of the Federal Teaching Hospital, Gombe, before commencement of the research.

Sample Collection

Clinical samples were collected from the Federal Teaching Hospital, Gombe, using standard aseptic techniques. The collected samples were placed in appropriate sterile containers or transport media, as applicable, and transported immediately to the Microbiology Laboratory of Gombe State University for processing.

Isolation of *E. faecalis*

The clinical samples were inoculated onto Bile Esculin Azide agar plates and incubated aerobically at 37°C for 24–48 hours. Colonies showing characteristic blackening of the surrounding medium, indicating esculin hydrolysis in the presence of bile, were presumptively identified as enterococci (Murray et al., 2020). Suspected colonies were subcultured onto fresh Mueller-Hinton agar plates and incubated at 37°C for 24 hours to obtain pure cultures.

Phenotypic Identification

The presumptive enterococcal isolates were subjected to standard phenotypic tests for further identification, including Gram staining, catalase testing, salt tolerance, and bile esculin hydrolysis (Murray et al., 2020; Cappuccino & Sherman, 2011).

Gram Staining: A thin smear of each isolate was prepared on a clean glass slide and heat-fixed. The smear was stained with crystal violet for 1 minute, treated with Lugol's iodine for 1 minute, decolorized with acetone, and counterstained with safranin for 2 minutes. Gram-positive cocci occurring singly, in pairs, or in short chains were considered presumptive enterococci or streptococci.

Catalase Test: Several colonies were emulsified in 3% hydrogen peroxide on a clean glass slide. The absence of immediate effervescence or bubbling was recorded as a negative catalase reaction. This helped to differentiate enterococci and streptococci from catalase-positive staphylococci.

Salt Tolerance Test: Each isolate was inoculated into Brain Heart Infusion (BHI) broth supplemented with 6.5% sodium chloride (NaCl) and incubated at 37°C for 24–48 hours. Visible turbidity following incubation was recorded as positive growth and indicated tolerance to the high salt concentration, a characteristic commonly associated with *Enterococcus* species.

Bile Esculin Hydrolysis Test: The isolates were inoculated onto Bile Esculin Agar slants and incubated at 37°C for 24 hours. Development of a dark brown to black colour on the medium was recorded as a positive reaction, indicating esculin hydrolysis in the presence of bile and supporting identification as an *Enterococcus* species.

Molecular Confirmation by PCR

DNA Extraction: Genomic DNA was extracted from overnight cultures of the presumptive *E. faecalis* isolates using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions for Gram-positive bacteria. The concentration and purity of the extracted DNA were assessed using a NanoDrop™ spectrophotometer. DNA samples with A260/A280 ratios between 1.7 and 2.0 were considered suitable for PCR amplification.

PCR Amplification: Molecular confirmation of *E. faecalis* was performed by amplification of the species-specific *ddl* gene, which encodes D-alanine-D-alanine ligase. Previously validated primers described by Kariyama et al. (2000) were used:

- Forward primer: 5'-ATCAAGTACAGTTAGTCTTTATTAG-3'
- Reverse primer: 5'-ACGATTCAAAGCTAACTGAATCAGT-3'

The amplified PCR products were subsequently analysed by agarose gel electrophoresis to determine the presence of the expected amplification product.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI, 2025).

Preparation of Inoculum: Three to five morphologically similar colonies from an overnight pure culture were

emulsified in sterile normal saline. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 colony-forming units per millilitre (CFU/mL). The standardised suspension was used within 15 minutes of preparation.

Inoculation: A sterile cotton swab was immersed in the standardised bacterial suspension, and excess fluid was removed by pressing the swab against the inner wall of the tube. The swab was then used to inoculate the entire surface of a Mueller-Hinton agar plate by streaking in three different directions to obtain an even lawn of bacterial growth. The inoculated plates were allowed to stand for approximately 5-10 minutes to allow excess moisture to be absorbed.

Antibiotic Disk Application: The following antibiotic disks (Oxoid, UK) were aseptically placed on the surface of the inoculated Mueller-Hinton agar using sterile forceps: amoxicillin (30 µg), erythromycin (5 µg), ceftriaxone (30 µg), levofloxacin (5 µg), ciprofloxacin (10 µg), doxycycline (30 µg), rifampicin (5 µg), cefepime (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), and clindamycin (2 µg). The disks were positioned at least 24 mm apart to minimise overlapping of the zones of inhibition.

The plates were incubated aerobically at 37°C for 24 hours. Following incubation, the diameters of the zones of complete growth inhibition around each antibiotic disk were measured to the nearest millimetre using a calibrated ruler. The isolates were classified according to the applicable CLSI interpretive criteria.

Data Analysis

The data generated from the study were recorded and entered into a computer for analysis. Descriptive statistics, including frequencies and percentages, were used to summarise the findings. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 25 (IBM Corp., Armonk, NY, USA). The results were presented using tables and charts, as appropriate.

RESULTS

Identification of Clinical Isolates

Four clinical isolates were obtained from the Federal Teaching Hospital Gombe. The biochemical characteristics and identities of the isolates are summarized in **Table 1**.

Table 1: Biochemical Characteristics and Identification of Clinical Isolates

S/N	GRN	Ca	ST	BEH	ORG
1.	+	+	+	-	<i>Staphylococcus spp.</i>
2.	+	-	+	+	<i>E. faecalis</i>
3.	+	-	+	+	<i>E. faecalis</i>
4.	+	-	+	+	<i>E. faecalis</i>

KEY: Ca= Catalase, ST= Salt Tolerance, BEH = Bile Esculin Hydrolysis, GRN = Gram Reaction, ORG = Organism

Three of the four isolates (75%) were identified as *Enterococcus faecalis*, while the remaining isolate (25%) was identified as *Staphylococcus spp.* All three *E. faecalis* isolates were catalase-negative, grew in 6.5% NaCl, and showed positive bile esculin hydrolysis

Molecular Confirmation

PCR amplification of the *ddl* gene confirmed the identity of the three isolates that were phenotypically identified as *E.*

faecalis. All three isolates produced distinct bands at approximately 1500 bp following electrophoresis on a 1% agarose gel, confirming their molecular identity (Kariyama et al., 2000).

Antibiotic Susceptibility Profile

The antibiotic susceptibility profiles of the *E. faecalis* isolates are presented in **Table 2**.

Table 2. Antibiotic Susceptibility Profiles of *E. faecalis* Isolate.

S/N	Antibiotic(ug)	R	S	I
1.	Amoxicillin (30)	+	-	-
2.	Erythromycin (5)	-	+	-
3.	Ceftriaxone (30)	+	-	-
4.	Levofloxacin (5)	+	-	-
5.	Ciprofloxacin (10)	-	+	-
6.	Doxycycline (30)	+	-	-
7.	Rifampicin (5)	-	-	+
8.	Cefepime (30)	+	-	-
9.	Cephalothin (30)	+	-	-
10.	Chloramphenicol (30)	+	-	-
11.	Clindamycin (2)	-	+	-

KEY: + = Positive, - = Negative, R= Resistance, S= Susceptible, I = Intermediate.

Based on the CLSI interpretive criteria, the isolate showed a multidrug-resistant phenotype, with resistance to seven of the eleven antibiotics tested, including amoxicillin, ceftriaxone, and chloramphenicol. The isolate was susceptible to erythromycin, ciprofloxacin, and clindamycin, while an intermediate response was observed with rifampicin.

DISCUSSION

This study provides useful local information on the antimicrobial susceptibility pattern of clinical *Enterococcus faecalis* isolates obtained from a tertiary healthcare facility in Gombe, northeastern Nigeria. Four clinical isolates were recovered, of which three (75%) were confirmed as *E. faecalis* using both conventional phenotypic tests and molecular identification through amplification of the species-specific *ddl* gene. The phenotypic characteristics observed, including Gram-positive cocci, catalase negativity, growth in 6.5% NaCl, and positive bile

esculin hydrolysis, were consistent with the established characteristics of *Enterococcus* species. The molecular confirmation further supported the identification of the three isolates, providing greater confidence in the organisms used for susceptibility testing. The predominance of *E. faecalis* is consistent with previous reports identifying this species as one of the most frequently encountered enterococci in human clinical infections (Arias & Murray, 2012; Fiore et al., 2019).

The recovery of *E. faecalis* from clinical samples is clinically important because the organism has become an important opportunistic pathogen, particularly in healthcare settings. Although enterococci are normal inhabitants of the gastrointestinal tract, they can cause infections when host defences are compromised or when normal microbial flora are disrupted. These infections may include urinary tract infections, wound infections, bloodstream infections, endocarditis, and intra-abdominal infections (García-Solache & Rice,

2019; Sood et al., 2020). The detection of *E. faecalis* among the clinical isolates examined in this study therefore highlights the need for continued laboratory surveillance of enterococcal infections in tertiary healthcare facilities.

A major finding of the study was the multidrug-resistant phenotype observed among the *E. faecalis* isolates. The isolates were resistant to seven of the eleven antibiotics tested, representing several antimicrobial classes. Such a resistance pattern is concerning because it can considerably reduce the number of effective treatment options available to clinicians. Enterococci have several characteristics that contribute to antimicrobial resistance, including intrinsic resistance to certain antimicrobial agents and the ability to acquire additional resistance determinants through mutation and horizontal gene transfer. The presence of mobile genetic elements can further facilitate the acquisition and dissemination of resistance genes, particularly in healthcare environments where antimicrobial use is frequent (Miller et al., 2020).

Resistance to amoxicillin and the tested cephalosporins, including ceftriaxone, cefepime, and cephalothin, was observed among the isolates. This finding is consistent with the well-established reduced susceptibility of enterococci to many β -lactam antibiotics. Enterococci possess low-affinity penicillin-binding proteins, particularly PBP5, which reduce the effectiveness of β -lactam agents against these organisms (Kristich et al., 2014). The resistance observed in this study is therefore not unexpected and further emphasizes the importance of accurate identification of enterococcal infections before antimicrobial therapy is selected.

The resistance observed to levofloxacin, doxycycline, and chloramphenicol is also noteworthy. Unlike the β -lactam resistance described above, resistance to these agents may involve acquired mechanisms resulting from previous antimicrobial exposure and the accumulation of resistance determinants. Enterococci can acquire resistance genes through plasmids, transposons, and other mobile genetic elements,

allowing resistance traits to spread within bacterial populations (Miller et al., 2020). The resistance to several unrelated antimicrobial classes observed in this study may therefore reflect the ability of enterococci to accumulate multiple resistance traits and highlights the importance of antimicrobial stewardship in healthcare facilities.

An interesting finding was the difference in susceptibility between the two fluoroquinolones tested. While the isolates were resistant to levofloxacin, they remained susceptible to ciprofloxacin. Although both drugs belong to the same antimicrobial class, susceptibility to one fluoroquinolone does not necessarily indicate an identical response to another. Fluoroquinolone resistance can involve changes in antimicrobial targets, particularly mutations in genes such as *gyrA* and *parC*, as well as other mechanisms that may influence the activity of individual agents. The difference observed between ciprofloxacin and levofloxacin in this study therefore deserves further investigation rather than being interpreted as uniform susceptibility to fluoroquinolones.

The susceptibility of the isolates to ciprofloxacin is potentially relevant to local antimicrobial surveillance. However, this finding should be interpreted cautiously because only three *E. faecalis* isolates were confirmed in the study. The observed susceptibility cannot be generalized to all *E. faecalis* infections in the hospital or the wider Gombe population. In clinical practice, antimicrobial selection should be based on appropriate laboratory susceptibility testing and the clinical characteristics of individual patients. Continued reliance on ciprofloxacin without susceptibility confirmation could also create additional antimicrobial selection pressure and contribute to the development of resistance.

The susceptibility observed with erythromycin and clindamycin was another notable finding. Both antibiotics demonstrated activity against the isolates tested, suggesting that they retained in vitro activity against the *E. faecalis* isolates examined. Nevertheless, the small number of isolates limits the

extent to which this finding can be generalized. In addition, in vitro susceptibility does not necessarily translate directly into clinical effectiveness. Factors such as the site of infection, tissue penetration, pharmacokinetics, pharmacodynamics, and the patient's clinical condition must also be considered when selecting antimicrobial therapy. The results should therefore be viewed primarily as local susceptibility data rather than as a recommendation for empirical treatment.

The intermediate response observed with rifampicin also requires careful interpretation. An intermediate susceptibility result indicates that the clinical effectiveness of the agent may be uncertain under standard conditions. Furthermore, rifampicin is generally unsuitable as monotherapy for serious infections involving organisms that can readily develop resistance. The intermediate result obtained in this study therefore does not provide sufficient evidence to support its use as a reliable treatment option for *E. faecalis* infections.

The multidrug resistance observed in this study has important implications for antimicrobial stewardship. Effective stewardship involves ensuring that antimicrobial agents are used only when indicated and that the appropriate drug, dose, duration, and route of administration are selected. The resistance of the isolates to seven of the eleven antibiotics tested highlights the potential consequences of antimicrobial selection pressure and reinforces the need for careful antibiotic use. The adaptability and persistence of enterococci in healthcare environments further increase the importance of monitoring resistance patterns and implementing appropriate antimicrobial stewardship measures (García-Solache & Rice, 2019; Miller et al., 2020).

The findings also have implications for infection prevention and control. Resistant enterococci can persist in healthcare environments and may spread through contaminated hands, equipment, surfaces, and other healthcare-associated routes. Appropriate hand hygiene, environmental cleaning, contact precautions where indicated, and proper management of infected

or colonized patients are therefore important measures for limiting transmission. The detection of multidrug-resistant *E. faecalis* in a tertiary healthcare facility should consequently be regarded not only as a therapeutic concern but also as an infection prevention and control issue.

The findings of this study are broadly consistent with the growing concern about antimicrobial resistance among enterococci in Nigeria and other African countries. Previous studies have identified *E. faecalis* as an important cause of healthcare-associated infections and have highlighted the increasing challenge of antimicrobial resistance among enterococci (Arias & Murray, 2012; García-Solache & Rice, 2019). The present study adds local data from Gombe State, where information on the antimicrobial susceptibility of *E. faecalis* may be limited. Such local surveillance is important because resistance patterns can differ between hospitals, geographical locations, patient populations, and periods of observation.

The small number of isolates examined represents an important limitation of the study. Only four clinical isolates were recovered, of which three were confirmed as *E. faecalis*. As a result, the percentages reported are highly influenced by individual isolates and should be interpreted with caution. The findings cannot therefore be considered representative of all *E. faecalis* infections in Gombe State. Rather, they provide preliminary evidence of the presence of multidrug-resistant *E. faecalis* in the study setting and indicate the need for larger investigations involving more clinical isolates.

Another limitation is that the study focused mainly on phenotypic antimicrobial susceptibility. Although molecular testing was used to confirm the identity of the *E. faecalis* isolates, the specific genetic mechanisms responsible for the observed resistance patterns were not investigated. Molecular characterization of resistance-associated genes in future studies could provide a better understanding of the mechanisms responsible for resistance and the potential for transmission of resistance determinants within healthcare facilities.

Despite these limitations, the study provides useful preliminary evidence of multidrug-resistant *E. faecalis* in a tertiary healthcare facility in Gombe, Nigeria. Resistance to seven of the eleven antibiotics tested demonstrates the potential difficulty of treating infections caused by these organisms. At the same time, the susceptibility observed to ciprofloxacin, erythromycin, and clindamycin provides useful information for further investigation, although these findings require confirmation in studies involving larger numbers of isolates. Overall, the findings reinforce the importance of continuous antimicrobial susceptibility surveillance, rational antibiotic use, antimicrobial stewardship, and effective infection prevention and control practices to limit the emergence and spread of resistant *E. faecalis* in healthcare settings.

CONCLUSION

This study found a high level of multidrug resistance among the *Enterococcus faecalis* clinical isolates obtained from a tertiary hospital in Gombe, Nigeria, with resistance observed to seven of the eleven antibiotics tested. The isolates remained susceptible to erythromycin, ciprofloxacin, and clindamycin. These findings highlight the need for regular antimicrobial susceptibility surveillance, effective antimicrobial stewardship programmes, and improved infection prevention and control practices in healthcare facilities. Although erythromycin and ciprofloxacin showed activity against the isolates tested, their use in the treatment of *E. faecalis* infections should be guided by laboratory susceptibility testing to ensure appropriate antibiotic selection.

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