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Antibiotic Susceptibility Profile of *Enterococcus faecalis* Clinical Isolates from Federal Teaching Hospital Gombe, Nigeria.

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Abstract

The emergence of multidrug-resistant (MDR) *Enterococcus faecalis* poses a significant threat to healthcare systems worldwide, particularly in resource-limited settings. This study was conducted to determine the antibiotic susceptibility patterns of *E. faecalis* clinical isolates from Federal Teaching Hospital Gombe, Nigeria. Four isolates were confirmed using Gram's staining, catalase test, salt tolerance in 6.5% NaCl, and bile esculin hydrolysis. Antibiotic susceptibility testing was performed using Kirby-Bauer disk diffusion method against eleven antibiotics. Among the four clinical isolates tested, three were confirmed as *E. faecalis*, while one was identified as *Staphylococcus* spp. All the *E. faecalis* isolates exhibited a multidrug-resistant (MDR) phenotype, showing resistance to at least three different classes of antibiotics. The isolates were resistant to amoxicillin, ceftriaxone, levofloxacin, doxycycline, cefepime, cephalothin, chloramphenicol, rifampicin, and ciprofloxacin. Susceptibility to erythromycin, ciprofloxacin, and clindamycin varied among the isolates. These findings highlight the need for continued antimicrobial surveillance and rational antibiotic use in the study setting.

Keywords

Antibiotic Susceptibility, Clinical Isolates, Enterococcus Faecalis, Identification, Multidrug-Resistant (MDR).

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INTRODUCTION

Enterococcus faecalis is a Gram-positive coccus, facultative anaerobe, catalase-negative that forms part of the normal gastrointestinal microbiota of humans and animals (Garcia-Solache & Rice, 2019; Murray et al., 2020). 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 stays, 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 (Ahmed & Baptiste, 2018; Miller et al., 2020).

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.

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 special 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.

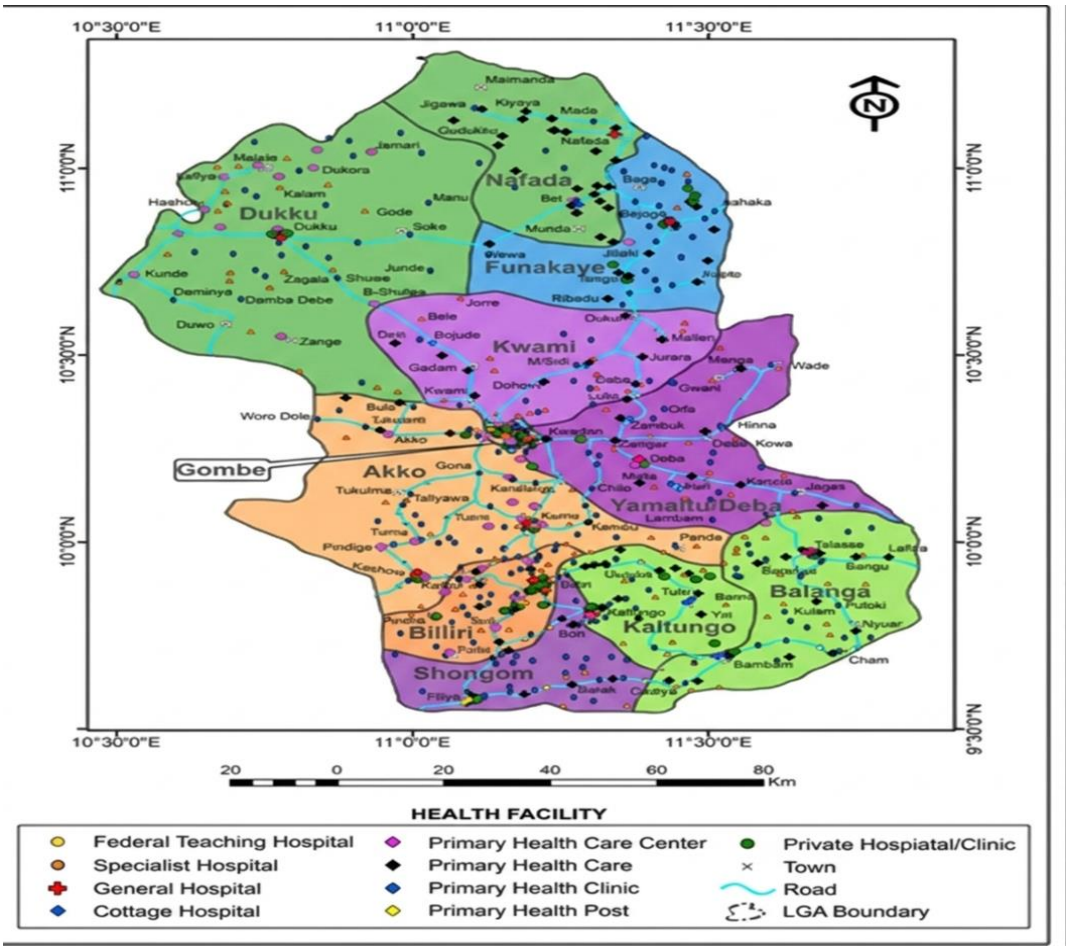


Figure 1: Map of Gombe State (Oghenebrume, 2021).

Study Design

This was a cross-sectional, laboratory-based study conducted at the Department of Microbiology, Gombe State University, Nigeria.

Sample Collection

Clinical isolates of *E. faecalis* were collected from the Department of Microbiology, Federal Teaching Hospital, Gombe and transported immediately to the Microbiology Laboratory of Gombe State University for processing.

Sub-Culturing of Isolates

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

Gram Reaction and Biochemical Characterization

The presumptive enterococcal isolates were subjected to phenotypic tests for further identification, including Gram's staining, catalase test, salt tolerance, and bile esculin hydrolysis.

Gram Reaction

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 (Cappuccino & Sherman, 2011; Michael *et al.*,2020).

Catalase Test

Several colonies were emulsified in 3% hydrogen peroxide on a clean glass slide and observed (Cappuccino & Sherman, 2011).

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 (Murray et al., 2020).

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 color on the medium was suspected as a positive reaction (Murray et al., 2020).

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).

To prepare standard inoculum, three to five morphologically similar colonies from an overnight pure culture were emulsified in a 5 mL sterile normal saline contained in a test tube. 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 milliliter (CFU/mL) (Fakuade et al., 2025).

A sterile cotton swab was immersed in the standardized bacterial suspension, and removed excess fluid 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 (Garba et al., 2019).

The following antibiotic disks 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 minimize overlapping of the zones of inhibition (CLSI,2025). 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 millimeter using a calibrated ruler. The isolates were classified according to the applicable CLSI interpretive criteria.

Data Analysis

The data generated from the study were presented in frequencies and percentages. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 23 (IBM Corp., Armonk, NY, USA). The results were presented using tables and charts, as appropriate.

RESULTS

Identification of Clinical Isolates

Three of the four isolates (75%) were confirmed as *Enterococcus faecalis*, while the remaining isolate (25%) was confirmed as *Staphylococcus* spp. The *E. faecalis* isolates were catalase-negative, tolerant to 6.5% NaCl, and showed positive bile esculin hydrolysis as shown in Table 1.

Table 1: Biochemical Characteristics and Identification of Clinical Isolates

S/N	Code	GRN	Ca	ST	BEH	ORG
1.	ISO1	+	+	+	-	*Staphylococcus spp.*
2.	ISO2	+	-	+	+	*E. faecalis*
3.	ISO3	+	-	+	+	*E. faecalis*
4.	ISO4	+	-	+	+	*E. faecalis*

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

Antibiotic Susceptibility Profile

The antibiotic susceptibility profiles of the three *E. faecalis* isolates (ISO2, ISO3, and ISO4) are presented in Table 2. Based on the CLSI interpretive criteria, all three isolates exhibited a multidrug-resistant (MDR) phenotype, showing resistance to at least three different classes of antibiotics (Table 3).

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

S/N	Antibiotic(ug)	ISO2	ISO3	ISO4
1.	Amoxicillin (30)	R	R	R
2.	Erythromycin (5)	S	I	S
3.	Ceftriaxone (30)	R	R	R
4.	Levofloxacin (5)	R	R	R
5.	Ciprofloxacin (10)	S	S	R
6.	Doxycycline (30)	R	R	R
7.	Rifampicin (5)	I	R	I
8.	Cefepime (30)	R	R	R
9.	Cephalothin (30)	R	R	R
10.	Chloramphenicol (30)	R	R	R
11.	Clindamycin (2)	S	S	S

Key: R= Resistance, S= Susceptible, I = Intermediate.

Table 3: Multidrug Resistance Status of the Isolates

S/N	Isolate	Status
1	ISO2	+
2	ISO3	+
3	ISO4	+

Key: + = Multidrug-Resistant (resistant to ≥3 different classes of antibiotics)

DISCUSSION

This study provides useful local information on the antimicrobial susceptibility pattern of clinical *Enterococcus faecalis* isolates obtained from the Federal Teaching Hospital Gombe, Nigeria. Of the four isolates collected, three (75%) were confirmed as *E. faecalis* for being Gram-positive cocci, catalase-negative, growing in 6.5% NaCl, and positive for bile esculin hydrolysis (Cappuccino & Sherman, 2011), which were consistent with the established characteristics of *Enterococcus* species (Murray et al., 2020). The predominance of *E. faecalis* among the isolates collected 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 defenses are compromised or when normal microbial flora is disrupted. These infections may include urinary tract infections, wound infections, bloodstream infections, endocarditis, and intra-abdominal infections (Garcia-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. All the isolates were resistant to at least three different classes of antibiotics, meeting the criteria for MDR (Michael et al., 2023) (Table 3). 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: Hamza et al., 2020). 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, 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 among the fluoroquinolones tested. All the isolates were resistant to levofloxacin, but susceptibility to ciprofloxacin varied: ISO2 and ISO3 were susceptible, while ISO4 was resistant. 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 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.

Susceptibility to erythromycin and clindamycin was observed in most isolates, although ISO3 showed intermediate resistance to erythromycin. These antibiotics demonstrated *in vitro* activity against the majority of 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 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 multiple 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 (Garcia-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 (Garba et al., 2020). 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 (Okeke & Aboderin, 2020; Okeke et al., 2021). 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 examined, 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, and the specific genetic mechanisms responsible for the observed resistance patterns were not investigated. Furthermore, important anti-enterococcal agents such as vancomycin, linezolid, and daptomycin were not included in the susceptibility panel due to resource constraints. Molecular characterization of resistance-associated genes and inclusion of these agents 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 the tertiary healthcare facility in Gombe, Nigeria. Resistance to multiple 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 in some isolates 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 preliminary evidence of multidrug resistance among the tested *Enterococcus faecalis* clinical isolates, with resistance observed to multiple antibiotics across different classes. All the three *E. faecalis* isolates met the criteria for multidrug resistance. The isolates generally remained susceptible to erythromycin, ciprofloxacin, and clindamycin, although susceptibility varied among isolates. 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 most of 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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