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Antibiotic Resistance and Plasmid Profile of *Enterococcus Feacalis* Isolated from Human Anal Swab

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Abstract: *Enterococcus faecalis was isolated from human anal swab and was subjected identification procedures. The isolate was confirmed using colonial, morphological and biochemical tests. The hemolytic activity, antibiotic resistance status, Multiple antibiotic resistance index, plasmid profile and the resistance genes using primer probes were investigated using laid down procedure Enterococcus faecalis showed gamma hemolysis. Result also revealed E. faecalis to be resistant to 67% and60% of the antibiotics against Gram positive and Gram-negative strains, thereby showing Multiple antibiotic resistance index of 0.3 and 0.58 respectively.*

The plasmid profile showed E. fecalis to bear low molecular weight plasmid for antibiotic resistance. However, It bore no gene for the primers tested in this study which are ermB, blaTEM and qnrBgenes. The result of this research emphasizes the fact that intestinal bacteria are prone to developing antibiotic resistance through horizontal transfer of plasmids or genetic mutation. The need to understand how bacteria adapt to the antibiotic environment will lead to new therapeutic strategies for antibiotic-resistant infections. Interventions measures to minimize the abundance of antibiotic-resistant commensals and opportunistic pathogens may include faecal microbiota transplantation and the use of live biotherapeutics.

Keywords: *Rectal swab, Resistance genes, Primers, Plasmids, ermB, BlaTem, qnrB Hemolysis, Multiple antibiotic resistance index.*

1. Introduction

Enterococci are Gram-positive bacteria that are a part of the intestinal microbiota of warm-blooded animals. As opportunistic pathogens, they are among the major causes of nosocomial infections(Werner *et al.,* 2013**).**The treatment options for enterococcal infections are often complicated by antimicrobial resistance, especially the multiple drugs resistant strains (Arias and Murray) acquired via mobile elements (Raza *et al*., 2018) and this is a major problem for public health worldwide as it results in limited therapeutic options.

The significance of enterococci as clinically important pathogens is often associated with acquisition of AR via horizontal gene transfer using mobile genetic elements, especially plasmids in the strains (Cho *et al*., 2020). This work was designed to identify the antibiotic resistance profile, plasmids and the molecular probe of *Enterococcus faecalis* using gene primers *ermB, blaTEM* and *qnrB* genes.

2. Material and Methods

2.1. Sampling and isolation of test organisms.

Swab samples were collected from Six human (3males and 3 females) and isolation procedure was carried out on the samples using laid down procedures.

2.2. Identification of isolate

The isolate was identified using colonial, morphological and biochemical means. Biochemical tests carried out include Gram staining, sugar fermentation, Citrate utilization Test, Oxidase Test, Indole Test, Urease Test, Methyl red Test & Voges Proskauer Test (Tankeshwa, 2022).

2.3. Pathogenicity Test

Blood agar was prepared by weighing blood agar powder and dispensed into a conical flask. 100ml of distilled water was added and shaken thoroughly. The medium was then sterilized in an autoclave. Afterwards, the medium was poured into plates and allowed to gel. The plates were then inoculated with the isolate by streaking method and incubated at 37°C for 24hrs. The pathogenicity test was confirmed by determining α or β hemolytic zone of the isolates on the plate. i.e., ability of the isolate to lyse red blood cells.

2.4. Antibiotic Sensitivity test

Identified isolates were tested against standard antibiotics using the method described by Kirby – Bauer (1996) pour plate method, after which the rings of standard antibiotics were placed on the surface of gelled agar and allowed to incubate at 37^0C for 24 hrs. Antibiotics used include; Amoxicillin (30 µg) Cefotaxime (30µg), Ceftriaxone (45 µg), Cefexime (25 µg), Levofloxacin (5 µg), Ciprofloxacin (45 µg) Imipenem (10 µg), Cefuroxime (25 µg), Ofloxacin (5 µg), Erythromycin (15 µg) Gentamycin (10 µg), Azithromycin (15 µg). Augmentin (30 µg), Nitrofurantoin (300 µg),

2.5. Multiple antibiotics resistance index

The multiple antibiotics resistance index for the resistant bacteria isolates was determined according to the procedure described by Krumpman (1983). This is essentially to determine the degree of bacteria resistance to antibiotics. These indices will be determined by dividing the numbers of antibiotics to which the organisms were resistant to (a) number of antibiotics tested (b) resistance to two or more antibiotics is taken as multiple antibiotics resistance MAR greater than 0.2 shows high antibiotic resistance index.

2.6. Plasmid Profiling and molecular fingerprinting of Isolate.

The antibiotic resistant isolates were subjected to plasmid profiling to determine if the resistance factors are plasmid mediated using laid down procedure as described by (Barghouthi, 2011).

3. Result and Discussion

3.1. Identification of isolate.

The isolate was cocci in chains. Gram positive, indole negative, citrate negative, oxidase negative, methyl red negative, VP positive, urease negative and catalase negative. The organism was able to utilize glucose, sucrose, galactose and lactose with evidence of gas and acid production. The suspected organism was *Enterococcus faecalis* and this was confirmed with the aid of Bergey's manual of determinative bacteriology.

3.2. Hemolytic reaction

Enterococcus feacalis showed gamma hemolysis (Fig 1). showing that there was no hemolysis meaning the two organisms could not break down red blood cells. An α-hemolytic reaction occurs when the hemoglobin in the red blood cells is reduced to methemoglobin, causing a greenish discoloration on the agar surrounding the colonies and the absence of hemolysis or discoloration is referred to as γ-hemolysis.

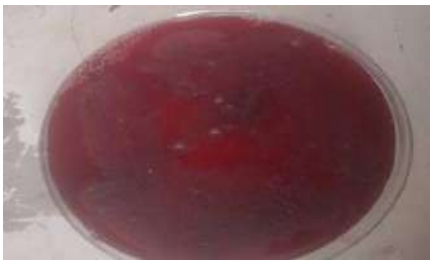

Fig 1. *Enterococcus feacalis* showing no heamolysis (ɣ)

3.3 Antibiotic susceptibility.

*Enterococcus feacalis*was sensitive to 67% of all the antibiotics used against Gram positive strains which include Amoxicillin (6mm), Levofloxacin (11mm), Ciprofloxacin (10mm), Imipenem (6mm), Cefuroxime (12mm), Ofloxacin (13mm), Gentamycin (6mm), and Azithromycin (12mm) but resistant to Cefotaxime, Ceftriaxone, Cefexime and Erythromycin. It was also resistant to 50% of the Gram negative antibiotics used. It was resistant to Amoxicillin C., Cefotaxime, Imipenem, Cefuroxime, Ceftriaxone S. Ampliclox and Cefexime.

Table1. Antibiotic susceptibility of *Enterococcus faecalis*

Antibiotics (Gram positive)	Zones of inhibition (mm)	Antibiotics (Gram negative)	Zones of inhibition (mm)
Amoxicillin .	6mm	Amoxicillin C.	R
Cefotaxime	R	Cefotaxime	R
Ceftriaxone	R	Imipenem	R
Cefexime	R	Ofloxacin	13mm
Levofloxacin	11mm	Gentamycin	11mm
Ciprofloxacin	10mm	Nalidixic Acid	10mm
Imipenem	6mm	Nitrofurantoin	6mm
Cefuroxime	12mm	Cefuroxime	R
Ofloxacin	13mm	Ceftriaxone S.	R
Erythromycin	R	Ampliclox	R
Gentamycin	6mm	Cefexime	R
Azithromycin	6mm	Levofloxacin	10mm

3.4. Multiple Antibiotic Resistance Index of isolates

Table 2 shows the multiple antibiotic resistance index of*E. feaclis.* Ithad MARI of 0.3 for Grampositive strain antibiotics0.58 for Gram negative strains antibiotics.

Table2. Multiple antibiotic resistance index of *Enterococcus faecalis.*

No of AB isolate was resistant to (G-ve)	Total number of AB tested	MARI	No of AB isolate was resistant to (G+ve)	Total number of AB tested	MARI
4	12	0.3	7	12	0.58

3.5. Plasmid profile of isolates

Fig 2 shows *Enterococcus faecalis* having plasmid borne resistance factor as shown in well four (4).Figure 3-5show the molecular fingerprints of the isolate using primers. *Enterococcus feacalis* were negative for*ermB* (macrolides), *BlaTem* (Betalactamase) and *qnrB gene*(quinolones)

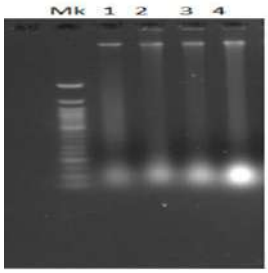

Fig 2. Plasmid profile of *Enterococcus faecalis* in well 4, showing plasmid for resistance factor.

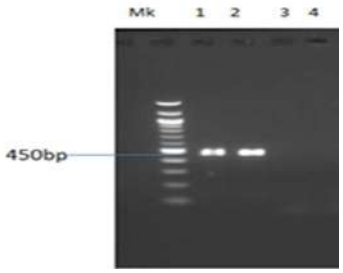

Fig 3. Gel indicates negative *ermB*gene Amplification in well 4.

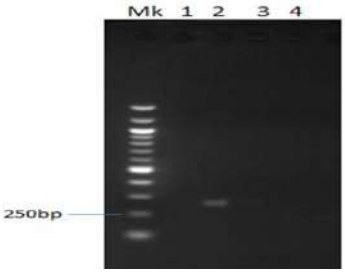

Fig 4.Gel indicates negative*BlaTem* gene Amplification in well 4.

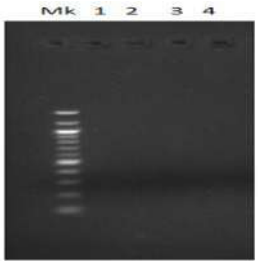

Fig 5.Gel indicates negative qnrB gene Amplification in well 4.

4. Discussion

Rampant use of antibiotics in both community and hospitals has transformed the human healthy intestinal gut flora into a reservoir of antibiotic-resistant organisms. Bacteria in the gut not only acquire ARGs (Antibiotic resistance genes), but also contribute to the transfer of ARGs to other bacteria in the gut (Ravi *et al.,* 2014). These bacteria possess great danger, have become a global issue, and it is now impossible to avoid developing strategies for the restoration of treatment options against infections caused by them. Therefore, understanding the resistance pattern is essential to fighting the battle against bacterial drug resistance.

Enterococcus faecalis is a Gram-negative bacterium that are a part of the intestinal microbiota of warm-blooded animals. However, as opportunistic pathogens, they are among the major causes of nosocomial infections (Cho *et al.,* 2020). The treatment of enterococcal infections is often complicated by antimicrobial resistant enterococci, particularly those that are resistant to multiple drugs, and this is a major problem for public health worldwide as it results in limited therapeutic options (Werner, 2013).

In this study, *E. faecalis* showed no hemolytic (gamma hemolysis) activity on blood agar. This observation is consistent with the report of Sharaf and Alshareef (2019) but contrary to the work of Furumura *et al* (2006) who observed total hemolysis on horse erythrocyte.

E. faecalis was resistant to 67% of the antibiotics used especially the cephalosporins and erythromycin. This report is corroborated by many authors (Werner, 2013; Arias and Murray, 2012; Kim*et al*., 2021) who documented resistance of *E. faecalis* to antibiotics. The plasmid profile show that the isolate has low molecular weight plasmid as shown in Fig 2. Similar observation was also documented by authors () who claim that Strains of *E. faecalis* have low molecular weight plasmids.

It is worthy of note that in this study, the resistance to cephalosporins is more pronounced in all the isolates. Cephalosporins have proven to be of immense importance in surgery and as first line therapy for a wide variety of infections, hence its continuous relevance and usage. Unfortunately, most bacteria of clinical importance have become resistant to these antibiotics, therefore, a worldwide problem (Adesoji *et al.,* 2016). Bassetti *et al.,* (2011) identified the Enterobacteriaceae as a group of microorganisms mainly involved in conferring resistance to this antibiotic.

The gene probe of the resistance factor using primers showed that *E. faecalis* had no resistance factors for the gene primers (Fig 3-5). It is possible that the resistance gene are chromosomal and not plasmid borne. Cho e*t al*., (2020) documented that although strains of *E. faecalis*was resistant to macrolide antibiotic, *ermB* genes were not detected in them. He further affirmed that not all isolates with resistance phenotypes carried AR genes.

Certain genes may be more related to the risks of the emergence or persistence of resistance than others. For example, integrons and sulfonamide resistance genes have been used to detect anthropogenic contaminants (Wang *et al*.,2014; Gillings *et al.,*2015). *Erm* genes encode resistance to macrolide antibiotics, which have long been used to treat Gram-positive and certain Gram-negative pathogens infecting humans, swine and cattle. Broadly, macrolide antibiotics act by binding to the 23S subunit of the bacterial ribosome, causing premature release of peptides during translation. The erm genes cause resistance by methylating rRNA at the active site, reducing the ability of macrolide antibiotics to bind to the ribosome (Vester and Douthwaite 2001). *Erm*-mediated resistance to macrolides has also been observed to confer resistance against other antibiotics,

including lincosamide and streptogramin B (MLSB resistance (Leclercq and Courvalin 1991).

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