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Assessment of Antibiotics Susceptibility Pattern of Vancomycin Resistant Enterococci Isolated from Clinical Specimens in Port Harcourt

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to assess the prevalence of vancomycin resistance among *Enterococcal* species isolated from clinical specimens of patients attending two hospitals in Port Harcourt, Rivers State.
Study Design: The study employs statistical analysis of the data and interpretation.
Place and Duration of Study: Two hospitals which are Meridian hospital Port Harcourt and University of Port Harcourt Teaching Hospital, located in the city of Port-Harcourt, Rivers State were used for this study. Specimen collection lasted for 3 weeks and the analysis was carried out daily and it lasted for six months.
Methodology: A total of one hundred and eighteen (118) urine and stool specimens (60 urine and 58 stool specimens) were collected from Fifty nine (59) patients for a period of three months from Meridian hospital and University of Port Harcourt Teaching Hospital, Port-Harcourt, Rivers State. The specimens collected were grouped inpatients and outpatients and were subjected to standard microbiological procedures which include standard plate counts, identification, and sensitivity testing using Kirby-Bauer disk diffusion method, Minimum inhibitory concentration and molecular identification of the isolates.
Results: A total of 48 enterococcal isolates were isolated from the different specimens (hospitalized

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and non-hospitalized patients) of urine and stool specimens. All Enterococcal isolates showed high level of resistance to Ceftazidime and Cefuroxime (100%) followed by cloxacillin (95.8%), augumentin (85.4%) and Ceftriaxone (75.0%). The isolates showed higher sensitive rates to Ofloxacin (95.5%), followed by Gentamicin (77.1%) and Vancomycin (39.6%). All *Enterococal* isolates from this study had a MAR index > 0.2. A total of the 48 *Enterococci* were isolated, the 23 (47.9%) isolates were identified as vancomyin resistant during this study were subjected to MIC (Minimum Inhibitory Concentration) for vancomycin as a confirmatory test. Of the 23 isolates, 12 isolates were vancomycin resistant with 11 isolates showing vancomycin MIC values of 8-16µg /ml (vancomycin intermediate).

Conclusion: Conclusively, this study revealed varying Antibiotic susceptibility pattern of the isolated bacteria. Treatment guidelines for use of antibiotics should be based on the hospital formulary and the sensitivity patterns is advocated. This should be reviewed occasionally to ensure rational use of antibiotics

Keywords: Vancomycin; enterococcus; vancomycin resistant enterococci; prevalence.

1. INTRODUCTION

Enterococci were initially thought as merely harmless commensal microorganisms; enterococci have emerged as significant human pathogens and are currently the third most common nosocomial bloodstream pathogen in USA [1]. These enterococci can acquire and confer antimicrobial resistance, and ultimately lead to Vancomycin resistant enterococci [2].

Vancomycin-resistant enterococci (VRE) were first encountered in clinical isolates in England and France in 1986, and 1987 in the United States of America (USA) [3,4]. In Europe, the rise of VRE was principally in the community setting, due to transmission from animal food products to humans, whereas in the USA the predominance of VRE was in the hospital setting, probably due to the increased use of Vancomycin [5]. To date, 54 different species and two subspecies of enterococci have been described, with the most clinically relevant species being *E. faecalis* and *E. faecium* [3]. The most common clinical impact of VRE is intestinal colonization, which does not result in symptoms, and may serve as a reservoir for transmission of VRE to other patients [6,7]. In studies conducted in Europe, Ireland had the highest rate of Vancomycin resistance among enterococcal bloodstream isolates from humans, with 43.1% of *E. faecium* isolated from blood being resistant to Vancomycin in 2013 [8]. Also, in studies carried out in Australia, VRE isolated showed considerable diversity in their phenotypes, genotypes, and geographic locations [9]. All four combinations of genotype and species were found, with the commonest being *E. faecium vanB* [10]. Reports showed that the organism has caused serious nosocomial infections from all over the world hence necessitating its importance in the hospital setup. The research findings from studies carried out in different parts of the world were significant enough to prompt and support similar research in Rivers State, Nigeria about these microorganisms.

Vancomycin is a glycopeptide antimicrobial drug which was introduced in the 1950s and is produced by soil bacteria *Streptomyces orientalis* [11]. It is active against most gram-positive bacteria, whereas the majority of gram negatives are resistant [12]. It is used primarily to treat drug-resistant bacteria when other antibiotics fail. Vancomycin was first clinically used as an antimicrobial to treat enterococci infections in 1972 [13]. The rampant use of Vancomycin most often led to the promotion of colonization by VRE [8] and only 15 years later, VRE was isolated in the United Kingdom and the United States [14]. High-level Vancomycin resistance in enterococci (due to *vanA* or *vanB* genes) is associated with the acquisition of ~10 kb of DNA encoding polypeptides [15]. The use of essential drugs such as third-generation cephalosporins, clindamycin, imipenem, and metronidazole [16] which have potent activity against anaerobes, lead to VRE colonization of gastrointestinal tract (GIT) by competitive eradication of sensitive species [17]. This colonization often leads to cross-infection, dissemination, and endogenous infection [4] by VRE. VRE have caused hospital outbreaks worldwide, and these have been on the rise in recent years mainly due to widespread abuse and misuse of antibiotics [18]. VRE infections have led to an increase in clinical treatment failure and mortality when compared to Vancomycin-susceptible enterococci (VSE) infections [13]. Mortality occurs in 75% of those with VRE bacteremia infections but in only 45%

of those with VSE infections [15]. Although seven known genes (*vanA*-*vanG*) confer Vancomycin resistance, the three most prevalent genes are *van A, van B,* and *van C* [19]. These genes alter the binding target for Vancomycin in resistant enterococci through the repression and activation of certain bacterial cell wall precursors [15]. The *vanA* gene confers high-level resistance to Vancomycin and teicoplanin; however, *vanB* confers moderate to high-level resistance to only Vancomycin [20]. Both *vanA* and *vanB* are associated with acquired resistance to Vancomycin, while *vanC* is an intrinsic resistance gene that is most commonly found in *E. gallinarum, E. casseliflavus,* and *E. Flavescens* [21]. Since *vanC* is chromosomally located, this gene is non-transferable; however, *vanA* and *vanB* genes may be transferred to other gram-positive bacteria on plasmids during horizontal gene transfer [22].

Enterococci exhibit both intrinsic and acquired resistance to several of the commonly used antibiotics. Intrinsic resistance is chromosomally mediated and is found in all or most of *Enterococci* and is against *β*-lactams (cephalosporins, and penicillinase resistant penicillins), lower concentration of aminoglycosides, fluoroquinolones, trimethoprim-sulfmetoxazole and clindamycin. However, the most recommended regimen of combination of a cell wall active agent (*β*-lactams) and an aminoglycoside, for serious infections like endocarditis and in immunocompromised patients, overcomes the intrinsic resistance by exerting synergistic bactericidal killing. This is achieved by the facilitation of aminoglycoside entry into the bacteria by the damage caused by the cell wall active agent. Acquired resistance is variable and results from either mutations in existing DNA or acquisition of new genetic determinants carried on plasmids/transposans. It rather confers resistance to several classes of antibiotic agents including chloramphenicol, tetracyclines, MLS macrolides-lincosamide-streptogramins, higher concentrations of aminoglycosides and *β*-lactams, glycopeptides, rifampin and nitrofurantoin than to a single agent. Recent reports highlight the emergence of resistance to the newer agents like linezolid, daptomycin and quinupristin-dalfopristin [4].

Most infections due to VRE can be managed with antibiotics other than Vancomycin, such as cephalexin, clindamycin and metronidazole [23,24]. Some of these infections include UTIs, intra-abdominal and uncomplicated wound infections [25]. In the clinical setup, combination therapy with a cell wall active agent and a synergistic aminoglycoside should be considered when managing enterococcal infections in debilitated patients and those with evidence of sepsis, endocarditis, meningitis, or joint infections [26]. For VRE strains resistant to ampicillin because of beta-lactamase production, a combination of ampicillin and sulbactam may be employed [27,28]. Other drugs like Linezolid, daptomycin, and tigecycline including combination therapy with cell wall–active agents (e.g., ampicillin) and an aminoglycoside (eg, gentamicin) may also be used [26]. Infections due to *E. faecalis* can also be managed by prolonged therapy with high doses of a combination of ampicillin and imipenem-cilastatin, or ampicillin and ceftriaxone [29]. For *E. faecium* infection, either linezolid or daptomycin may be effective, including quinupristin- dalfopristin or tigecycline [3]. VRE infections due to isolates susceptible to penicillin or ampicillin (MICs of 0.5-2 µg/ml) may be treated with high doses of these agents [17].

Control methods for VRE include routine screening for Vancomycin resistance among clinical isolates [30], active surveillance in intensive care units [31], and contact isolation to minimize person-to-person transmission [32], rigorous decontamination of patient-contact areas [33] and judicious restriction of Vancomycin and other broad-spectrum antibiotics [31]. There is a continued need for the development of new antimicrobial agents for treating VRE infection, as well as a regimen that would eradicate VRE colonization (without selection of further antimicrobial resistance), and potentially a role for a regimen for suppressing VRE colonization during periods of high risk for enterococcal infection [3]. These measures to limit VRE spread, however, have had a few challenges [27]. Firstly not all hospitals are willing to perform active surveillance [34]. Secondly, more patients are typically colonized with VRE (3% to 47%) than are infected hence passive surveillance by routine cultures allows colonized inpatients to go unidentified and serve as point sources for continued spread of VRE [29]. It has been noted that even if all colonized inpatients were successfully identified, VRE may be spread by health-care workers through either inadequate hand washing or contact with items such as bed rails, sinks, faucets, and doorknobs [35].

A study undertaken suggested that the endemic prevalence of VRE may be reduced by

decreasing the duration of VRE colonization, limiting hospital acquisition of VRE and improving compliance with hand hygiene [36]. Further study shows increasing frequency of hand washing was associated with a decrease in nosocomial VRE infections [35]. Another study suggested that hospitals detecting their first cases of VRE colonization should particularly be aggressive in implementing appropriate infection control measures [37].

This study is aimed to assess the antibiotics susceptibility pattern of vancomycin resistant enterococci among the *Enterococcal* species isolated from clinical specimens of patients attending two hospitals in Port Harcourt, Rivers State.

2. MATERIALS AND METHODS

2.1 Study Area

The study area for this research was Port Harcourt and the sample stations were Meridian Hospital and University of Port Harcourt Teaching Hospital Rivers State, Nigeria.

2.2 Questionnaire

Patient's data were collected via a simple structured questionnaire as well as a review of patient's records after obtaining informed consent. Data collected were socio-demographic characteristics.

2.3 Sampling Method

Simple random sampling method was used.

2.4 Collection and Processing of Specimens

A total of 118 urine and stool specimens (60 urine and 58 stool specimens) were collected from 59 patients, both inpatients and outpatients and placed in sterile sample bottles. The specimens were taken to the microbiology laboratory, Rivers State University for analysis, within two hours of collection.

2.5 Microbiological Examination of Specimens

2.5.1 Enrichment

Urine and stool specimens from all subjects were enriched at 45 °C in buffered peptone water in an overnight culture. The buffered peptone water was prepared according to the manufacturer's specifications (adding 20g of buffered peptone water to 1000ml of distilled water) and 9ml was transferred aseptically into appropriate test tubes and then sterilized by autoclave at 121^{o}C for 15 minutes. After sterilization, 1g of the stool and 1ml of the urine samples were aseptically transferred into the appropriate test tubes and then incubated [38].

2.5.2 Enumeration and isolation of bacteria

Serial tenfold dilution was done on the overnight enriched culture in which 1ml of the enriched culture was transferred into 9ml of normal saline and further dilutions were done up to 10^6. Aliquot (0.1ml) of appropriate dilutions (10^4, 10^5 and 10^6) was spread plated in duplicates onto Nutrient Agar, MacConkey Agar and Bile Esculin Agar (BEA) plates. The plates were incubated at 37^{o}C for 24 hours. The colonies formed on the plates were counted and described morphologically.

2.5.3 Identification and characterization of the bacterial isolates

Pure bacterial isolates were identified by Gram-Staining technique, characterized biochemically and identified up to species level with the aid of molecular identification test. The Gram-Staining test and biochemical tests were carried out as described by Cheesbrough [39].

2.6 Antimicrobial Susceptibility Testing

Susceptibility of isolates of the following antibiotics were examined using the disk diffusion method according to Clinical and Laboratory Standards Institutes (CLSI) guidelines: Gentamicin (10µg), Ofloxacin (5µg) Augumentin (30µg) Vancomycin (30µg) Cefuroxime (30µg) Ceftazidime (30µg) Erythromycin (30µg) Ceftriaxone (10µg) Cloxacillin (5µg). Multiple antibiotics resistance (MAR) was defined as resistance to three or more different classes of antibiotics.

2.7 Determination of Vancomicin MIC

The MIC for Vancomycin were determined for all enterococcal isolates using the micro dilution method on Mueller-Hinton broth (MHB) with serial two fold dilutions range between 256 to 0.125 µg/ml, and the results were interpreted according to the standards of the CLSI [40].

2.8 Molecular Method for the Detection of VRE

For genetic detection, DNA extraction of enterococci was done and PCR was performed

to detect the glycopeptide resistance genes *vanA* and *vanB* in the Enterococci isolates using specific primers (Table 1). The PCR products were analyzed via electrophoresis in 1% agarose gels (Agarose LE, Promega) using a 100 bp DNA ladder (Gibco/BRL Life Technologies, Breda, The Netherlands). *E. durans* strain DK 004 (*vanB*+) and *E. faecalis* strain PYK 10 (*vanA*+) were used as controls in the PCR experiments.

2.9 Statistical Analysis

Data were analyzed using the statistical passage for social science (SPSS version 12). Frequencies and cross tabulations were used to summarize descriptive statistics. Statistically significance association was measured by using Analysis of Variance (ANOVA) and T-test was used to test for significance and mean separation respectively.

3. RESULTS AND DISCUSSION

3.1 Demographic Characteristics of Participants

The result of analysis for the prevalence of Vancomycin resistant enterococci in urine and stool specimens of inpatients and outpatients attending the two hospitals in Port Harcourt showed a greater number of *Enterococci* in stool specimens (31) when compared to urine (17) as seen in table 3. This could probably be as a result of the commensal nature of enterocci in the GIT [42]. Another possible explanation could be that the specimens may have been contaminated on collection by the patients prior to submission to the Microbiology Laboratory. This agreed with Gaido and Wilson, (2004), whose study ascertained samples contamination by patients [42]. This study also showed that *Enterococci* were prevalent in women (72.9%) when compared to men (27.1%) as showed in table 4. This also was in agreement with research in which females were most affected than men [43]. This study also showed *E. faecalis* was more isolated compared to *E. durans,* thus agreeing with another study by Sreeja et al., (2012) [44]. *E. faecalis* is more prevalent than *E. faecium*, as observed in other studies [34].

This study could not ascertain the sources of infection because specimens were received after being collected from the patients by health care workers. However, from other studies, it was established that for UTI, factors including catheterization and immuno-compromisation may lead to the acquisition of VRE [18]. Immunocompromisation on the other hand encourages these VRE to proliferate [45]. The immune system being compromised leads to opportunistic infections by VRE and other organisms. In this study, 118 specimens were processed and of these, 60 were urine and 58 stool. VRE was isolated from these specimens. Urine had 9 while stool specimen had 14 isolates. It was clear from the results stool specimens had more VRE isolates compared to urine. There was no patient history as to when the bacteremia was diagnosed and how long patients were hospitalized. However, according to research carried out in the last few years [28], it has been established that the source of a bacteremia due to VRE is usually the genitourinary tract, although bacteremia can also be due to indwelling central lines or soft tissue infections [46].

This study showed that age plays a role in the acquisition of VRE. Ages most affected with VRE were between 20 to 29 years. This could have probably been because patients admitted were mostly from these age groups at the time of this study as seen in Table 2. This is in correlation with other studies [34] in which it was observed that hospitalization played a major role in the acquisition of VRE. However, it was observed that *E. faecalis* was isolated from all the age groups as compared to *E. durans* (Table 5). This also agreed with the literature that *E. durans* was not as prevalent as *E. faecalis* [47]. Inpatients have a higher chance of acquiring VRE as these patients tend to stay longer in hospital and can easily be exposed to carriers of VRE [48]. Carriers for these organisms may include patient care givers and members of staff who do not adhere to strict hygiene protocols [32].

Out of 118 cultured specimens of the urine and stool, 48 Enterococci were isolated. 31 (64.6%) isolates were detected from stool specimen and 17 (35.4%) were isolated from urine specimen.

3.2 Susceptibility to Antimicrobial Agents

On studying the antibiotic susceptibility pattern, it was found that most of *Enterococci* isolates were highly resistant to Ceftazidime and Cefuroxime (100%) followed by cloxacillin (95.8%), augumentin (85.4%) and Ceftriaxone (75.0%), the enterococci are inherently resistance to cephlosporins. The isolates have shown higher sensitivity to Ofloxacin (95.5%), followed by

Gentamicin (77.1%) and Vancomycin (39.6%). The implications for this pattern of resistance are that this further narrows the drugs available to treat infections due to enterococci [7]. Studies have reported significantly higher resistance to different antibiotics among *Enterococcal* isolates similar to this study [49]. There was multi drug resistance (Tables 6). Other studies observed that VRE were multidrug-resistant opportunistic pathogens in the hospital environment. This must probably be due to selective pressure and widespread use and abuse of broad-spectrum antimicrobial drugs. Enterococci are resilient organisms that survive on the hands of health care workers and on inanimate objects [50].

The high resistant rates to the glycopeptide antibiotics is of concern as these are the last line drugs in management of infections due to gram positive organisms [30]. This has potential to complicate the already limited treatment options for UTI. This observation of drug resistance could be due to failure to follow dosage regimens by patients, and administration of drugs by health care workers. In this study, the high resistance rate observed in Vancomycin (47.9%) was probably due to the ability of Enterococci to transmit resistance amongst them. They can also transmit this resistance to other species of organisms using *VanB* genes which are found on their plasmid through Horizontal Gene Transfer [2]. Most of the antibiotics used in this study had a high resistance rate possibly due to selective pressures of antimicrobial usage in the treatment of infections due to Enterococci since these antimicrobials can readily be accessed over the country in Nigeria without need for a prescription. Gupta et al. (2015) in the study on response of Enterococci to different antimicrobials, emphasized that oral administration of antimicrobials can increase antimicrobial resistance in Enterococci.

Table 1. Primers sequences used

Gene	Primer	Sequence	Expected amplicon size (bp)
Van A	*Van A–F*	CATGAATAGAATAAAAGTTGCAATA	1000
	Van A–R	CCCCTTTAACGCTAATACGATCAA	
Van B	*Van B –F*	GTCACAAACCGGAGGCGAGGA	400
	Van B –R	CCGCCATCCTCCTGCAAAAAA	
Van C	Van C	GGGAAGATGGCAGTATCCAAGG	766

Primer sequences according to a study done by [41]

Table 2. Demographic characteristics of study participants

Variables		N=59 (%) Number of patients studied
Mean age		33.96
Age group	≤9	3 (5.1)
	10-19	7 (11.86)
	20-29	13 (22.03)
	30-39	17 (28.81)
	40-49	11 (18.64)
	≥50	8 (13.56)
Gender	Male	27 (45.76)
	Female	32 (54.24)

Table 3. Distribution of different *Enterococcal* species among specimens

Enterococcal species	Urine	Faeces	Total	Prevalence (%)
E. faecalis	15(31.25%)	21(43.75%)	36	75
E. durans	2(4.17%)	10(20.83%)	12	25
Total	17(35.42%)	31(64.58%)	48	100

Table 4. Distribution of different Enterococcal species among participants

Gender	Organism Isolated		Total	Prevalence (%)
	E. faecalis	*E. durans*		
Female	26	9	35	72.9
Male	10	3	13	27.1
Total	36	12	48	100

Table 5. Distribution of VRE among age group of participants

Age Group	VRE Isolated		Prevalence (%)
	E. faecalis	*E. durans*	
≤9	1	-	4.35
10-19	3	1	17.39
20-29	10	2	52.17
30-39	2	2	17.39
40-49	1	-	4.35
≥50	1	-	4.35
Total	18	5	100

Table 6. Antibiotic sensitivity pattern of *Enterococci* and their zones of Inhibition (mm) N=48

Antibiotics	Resistance (%)	Intermediate (%)	Susceptibility (%)
OFX	1 (2.1)	1 (2.1)	46 (95.8)
AUG	41 (85.4)	6 (12.5)	1 (2.1)
CAZ	48 (100)	0 (0.0)	0 (0.0)
CRX	48 (100)	0 (0.0)	0 (0.0)
GEN	4 (8.3)	7 (14.6)	37 (77.1)
CTR	36 (75.0)	0 (0.0)	12 (25.0)
ERY	35 (72.9)	5 (10.4)	8 (16.7)
CXL	46 (95.8)	2 (4.2)	0 (0.0)
VAN	23 (47.9)	6 (12.5)	19 (39.6)

Key: GEN (Gentamicin), OFL (Ofloxacin), AUG (Augumentin), VAN (Vancomycin), CRX (Cefuroxime), CAZ (Ceftazidime), ERY (Erythromycin), CTR (Ceftriaxone), CXL (cloxacillin)

Table 7. MAR Indices of *Enterococci* isolates (N=48)

MAR Index	Number (%)
0.1	0(0.00)
0.2	2(4.2)
0.3	0(0.00)
0.4	1(2.0)
0.5	22(45.8)
0.6	20(41.7)
0.7	3(6.25)

KEY: Multiple Antibiotic Resistance (MAR)

Table 8. MIC values of vancomycin for the VRE isolates

	Intermediate (8-16 µg/ml)		Resistant (≥32 µg/ml)				
VRE isolates n=23	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	128 µg/ml	256 µg/ml	Total
E.faecalis	2	7	1	4	2	2	18
E.durans	-	2	2	1	-	-	5
Total	2	9	3	5	2	2	23

Key: MIC- Minimum inhibitory concentration

3.4 Multiple Antibiotic Resistance (MAR) Index

A significant number of *Enterococal* isolates from this study had a multiple antibiotics resistance (MAR) index > 0.2 indicating their source to be from area where antibiotics are probably commonly used, or previous exposure of the organism to antimicrobial agents. In order words, isolates are from high risk sources of antibiotic

resistance. These isolates from both inpatients and outpatients, pointer to the fact that antibiotic resistance and development of resistant strain is not limited to hospital acquired pathogens only but can be from community acquired pathogens too (Table 7).

3.5 Determination of Minimum Inhibitory Concentration of Vancomycin

Vancomycin breakpoint was based on the CLSI cutoff for Enterococci (Resistant, MIC of ≥ 32µg/ml; Susceptible, MIC of ≤ 4; Intermediate, MIC of 8-16). Out of the 48 *Enterococcal* isolates, the 23 isolates (47.9%) which were identified presumptively as vancomyin resistant during this study were subjected to MIC (Minimum Inhibitory Concentration) for vancomycin as a confirmatory test. Of the 23 isolates, 12 isolates were vancomycin resistant with 11 isolates showing vancomycin MIC values of 8-16µg /ml (vancomycin intermediate). In this study, the MIC range of most of the VRE isolates fell within 8-256µg/ml. This was similar to the findings of Patel *et al* (1997) from mayo clinic, United States; the vancomycin MIC range of VRE isolates 8- 256µg/ml in their study. A study from South India has reported a case of vanA in *E. faecalis* with Vancomycin MIC values of 256µg/ml [51] (Table 8).

3.6 Molecular Identification and Detection of Resistant Gene

In the present study, 23 isolates were found to be vancomycin resistant by disc diffusion method. MICs of vancomycin for all isolates were determined. All the 12 isolates showed resistance to vancomycin (MIC >32 µg/ml). Five enterococcal isolates were subjected to multiplex PCR using five sets of primers as already described (Table 1). All isolates which were found to be vancomycin resistant by phenotypic methods showed the presence of *van*A and *van*B gene by multiplex PCR except one (band size – 1350 bp on gel electrophoresis). These *van* genes are responsible for the high-level resistance to vancomycin (MIC >256 µg/ml). Thus, the prevalence of vancomycin resistance among enterococcal isolates in the present study is 12 VRE isolates, which carried *van*A and *van*b genes.

Result of sequence blast on NCBI site showed 100% identity with *van*A gene. Karmarkar et al. (2004) detected 11 (2.6%) isolates of vancomycin-resistant enterococci from various clinical specimens consisting of four (12.5%) *E. faecium* and seven (1.9%) *E. faecalis* [52]. In their study of 52 enterococcal isolates, reported 12/42 (28.57%) isolates of *E. faecium* resistant to vancomycin with MIC >4 µg/ml (Plate 1-7).

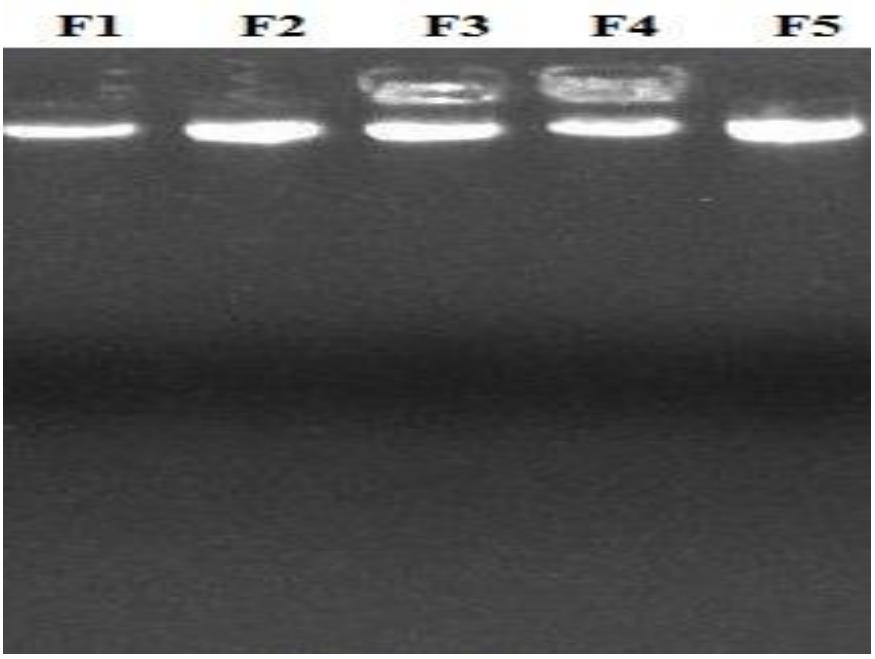

Plate 1. Genomic DNA

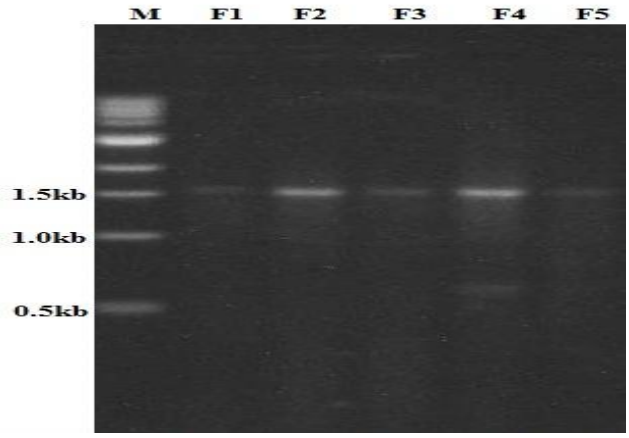

Plate 2. Agarose gel electrophoresis showing the amplified VAN genes of the *Enterococcus* spp 16S region. M is 1kb ladder

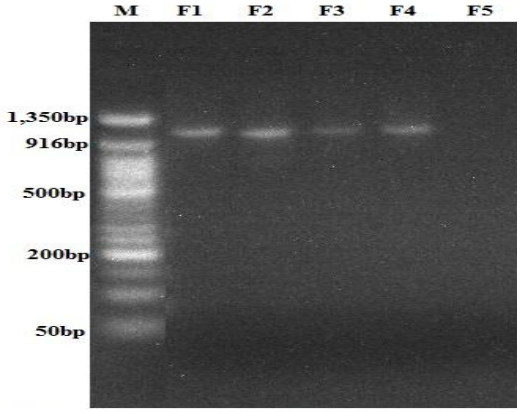

Plate 3. Gel image amplification of VAN *A* gene at about 1000bp, all lanes except lane F5 showing amplification of VAN *A* gene from the genomic DNA. Lane F5 showing no amplification signifies that VAN *A* gene is absent in the genomic DNA of the sample

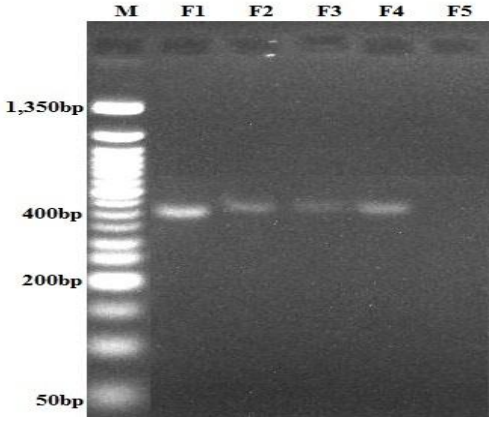

Plate 4. Gel image showing amplification of *VAN B* gene at about 400bp, all lanes except lane F5 show amplification of *VAN B* gene from the genomic DNA. Lane F5 showing no amplification signifies that *VAN B* gene is absent in the genomic DNA of the sample

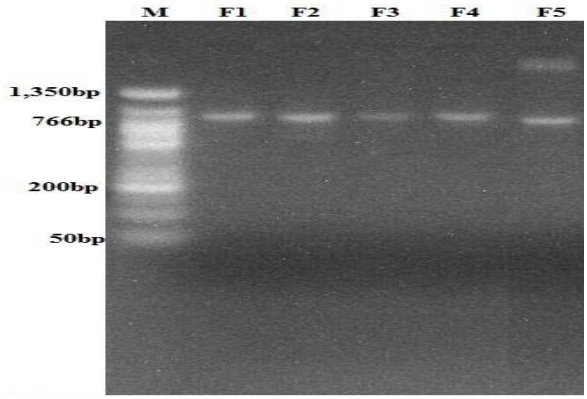

Plate 5. Gel image showing amplication of *VAN C* gene at about 766bp, all the five lanes show amplification of *VAN C* gene from the genomic DNA

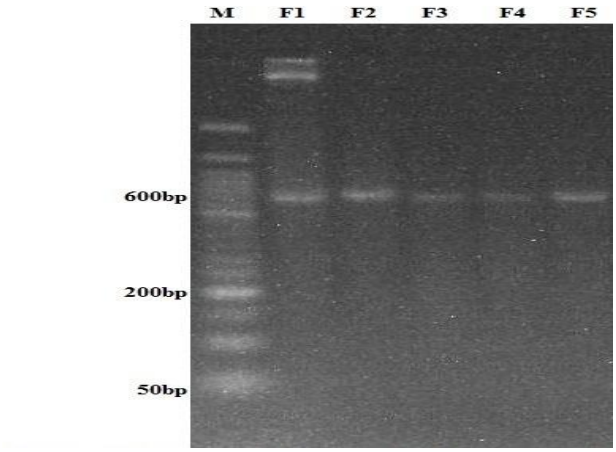

Plate 6. Gel image showing amplification of *VAN D* gene at about 600bp, all the five lanes show amplification of *VAN D* gene from the genomic DNA

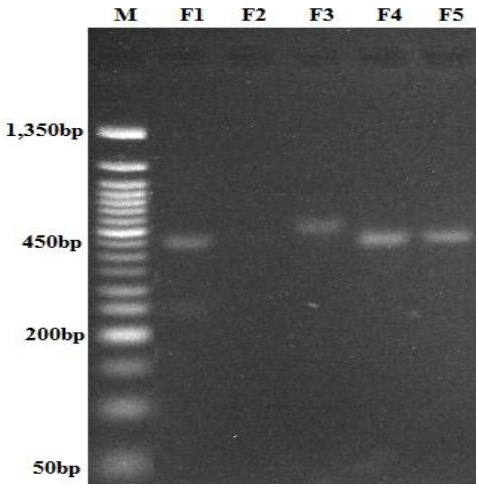

Plate 7. Gel image showing amplification of *VAN XY* gene at about 450bp, all lanes except lane F2 show amplification of *VAN XY* gene from the genomic DNA. Lane F2 showing no

5. CONCLUSION

Enterococci are emerging as an important pathogen causing variety of hospital acquired nosocomial infections as well as community acquired infections contributing significantly to patient's morbidity and mortality. The emergence of vancomycin resistant *Enterococci* worsens the problem further because of the multidrug resistance exhibited by these agents leaving fewer therapeutic options for the clinicians in treating the serious life threatening VRE infections.

In this study, of a total of 48 Enterococcal organisms isolated from various clinical samples; only two species encountered were *Enterococcus faecalis and Enterococcus duran*. Of this number, 23 isolates were identified as Vancomycin Resistant Enterococci with a prevalence rate of about 47.9%. They showed resistance to multiple antibiotics like Ceftazidime, Cefuroxime and Augumentin. The phenotyping of VRE isolates performed by detection of MIC for both vancomycin correlates well with the genotypic method of detection of vancomycin resitance gene *VanA*. Thus this method can be adopted in resource limited settings (where the genotyping may not be available) for the detection of Vancomycin resistant phenotype of *Enterococci.*

6. RECOMMENDATIONS

1. It is recommended that there is need for conducting frequent surveillance programmes for prompt identification of VRE in hospitals and community.

2. Patients being admitted for prolonged periods should be screened for VRE owing to this organism's capability of resistant gene transfer to other susceptible species within the hospital.

3. It is recommended that there is need for implementation of stringent infection control measures like rational use of antibiotics especially restricting the use of Vancomycin to minimum, proper containment and effective treatment of VRE infections.

4. Strict hand washing practices, education of the healthcare workers and other personnel involved in the patient management. These measures are to be strictly followed to bring down the mortality and morbidity associated with these nosocomial VRE infections.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

The consent of children was obtained from their family or guardian. The confidentiality of the information was maintained.

ETHICAL APPROVAL

Ethical clearance was obtained from Rivers State University, Port Harcourt and Meridian Hospital, Port Harcourt. Official permission and informed written consent were obtained from the hospitals and each study participant, respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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