Co-occurrence of Vancomycin and Multidrug Resistance in Enterococci obtained from clinical samples in a Tertiary hospital in Port Harcourt, Nigeria

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ABSTRACT

Vancomycin-resistant enterococci (VRE) are important nosocomial pathogens often regarded as "superbugs", with very limited treatment options. Continuous surveillance is essential to monitor changes in epidemiology and possible treatment options. This study was aimed to explore the co-occurrence of vancomycin and multidrug resistance in clinical enterococci isolates from Port Harcourt, Rivers State. Identities of clinical enterococci were confirmed and susceptibility was carried out using Kirby-Bauer disc diffusion method. Multidrug resistance status of the isolates was determined. Vancomycin-resistant isolates were tested for the presence of vanA and vanB vancomycin resistance determinants using PCR following DNA extraction using the boiling method. Isolates exhibited high levels of resistance to most antibiotics with 100% resistance noted to amoxicillin/clavulanate potassium, ceftazidime, cefuroxime and cloxacillin. Isolates were however generally susceptible to gentamicin and ofloxacin with a 16% resistance rate in both. A total of 7 antibiogram patterns were observed, though majority of the isolates (60%, 15/25) had the same antibiogram pattern (AUG-CAZ-CRX-CTR-CXC-ERY). Fifteen (60%) of the isolates were vancomycin-resistant, and all 15 (100%) were multidrug-resistant. The vanB gene was detected in 53.3% (8/15) of the vancomycin-resistant isolates, while no vanA gene was detected. Though reporting a high association of VRE with multidrug resistance, this study notes that majority of these isolates were sensitive to the aminoglycoside gentamicin indicating a possible treatment option. Vancomycin resistance of enterococci in this study was mediated by vanB rather than vanA genes. More wide scale surveillance is needed for understanding the epidemiology of VRE in Nigeria.

Keywords: Enterococci, Vancomycin resistance, MDR, vanA, vanB

INTRODUCTION

Vancomycin-resistant enterococci which were first reported in the late 1980s, more than 30 years after the introduction of the drug in clinical practice, have increasingly been noted as a key problematic nosocomial pathogen.[1,2] Though commensals are found in low numbers in a healthy gut, enterococci are generally opportunistic and associated with a variety of diseases like urinary tract infections, endocarditis and sepsis.[3] Their current notoriety as a superbug arises from the fact that vancomycin is a last resort drug and as such resistance to it by multidrug-resistant (MDR) strains of enterococci results in an untreatable pathogen. This organism is known for its rapid acquisition of virulence determinants, reducing effective treatment options. Due to the increasing prevalence of this group of organisms, continuous surveillance is essential to monitor changes in epidemiology and circulating clones which is region dependent.[2]

A recent review on VRE prevalence in Nigeria which focused on cross-sectional studies covering clinical, environmental and animal sources reported rates ranging from 1.1% to 88.9% though clinical had a maximum of 68.9% prevalence.[4] In a more recent review focusing only on isolates from clinical sources, reports VRE rates ranging from 3% to 34%.[5] Another 2021 study noted a 40.9% prevalence of VRE in clinical isolates in the South-West of Nigeria, all of which were said to be resistant to at least three drug classes.[6]

Vancomycin resistance is encoded by some determinants designated *vanA* to *vanE* with *vanA* and *vanB* described as the most commonly occurring.[7] The few studies in Nigeria exploring the genetics of vancomycin

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resistance mainly focused on these two though Ekuma and colleagues also assayed for *vanC*.[8-10]

The Wada review highlights the paucity of data on VRE in Nigeria.[4] This review included 19 papers and only 8 of the studies covered clinical isolates. Of the four studies from the South-South included in the Wada review, none was from Rivers State. In the 2021 review, none of the studies included was from the South-South of Nigeria.[5] This study was therefore aimed to explore the co-occurrence of vancomycin and multidrug resistance in clinical enterococci from Port Harcourt, Rivers State.

MATERIALS AND METHODS

Isolate identification

Enterococci isolates were obtained from stool and urine samples from the Medical microbiology laboratory of a tertiary hospital in Port Harcourt, Nigeria (UPTH ethical approval number: UPTH/ADM/90/S.11/VOL.XI/1110). Isolate identities were confirmed following basic protocol.[11] Briefly, isolates were inoculated onto Bile Esculin Agar (BEA) and characteristic enterococcal colonies (colonies with dark brown pigmentation) were then purified by subculturing to Nutrient agar and stored for further processing. Identification of isolates was done using standard biochemical tests which included catalase, oxidase, coagulase, sugar fermentation, citrate, starch hydrolysis, motility, methyl-red, Voges-Proskauer, growth in 6.5% NaCl, growth at 60°C.[11,12]

Antimicrobial Susceptibility Testing

The susceptibility of the isolates was determined using the Kirby-Bauer disc diffusion test.[13] This involved preparing an inoculum concentration equivalent to a 0.5 McFarland standard and inoculating the surface of a Mueller Hinton agar plate using a sterile cotton swab stick. Following a 5-minute pre-incubation at room temperature, the whole set-up was incubated at 37°C for 24 hours and zones of inhibition were measured. Isolates were then classed as resistant using the CSLI standard.[14]

A commercial standard Gram-positive test disc (Abtek, UK) was used containing augmentin (30mg), ceftazidime (30mg), cefuroxime (30mg), ceftriaxone (30mg), cloxacillin (5mg), erythromycin (5mg), gentamicin (10mg) and ofloxacin (5mg). Susceptibility against vancomycin (30mg) was also carried out with zone diameter of ≤14mm noted as resistant and ≥17 mm noted as sensitive.

Susceptibility Data Analysis

Data from the susceptibility analysis was used to determine the MAR index (calculated using the formula a/b where "a" is the total number of antibiotics to which the organism was resistant and "b" is the total number of antibiotics against which the organisms were tested), multidrug-resistant status (resistance to ≥3 drug classes) and antibiogram for each isolate.

Detection of specific Vancomycin-resistant genes

To assess the genes associated with vancomycin resistance in this study, DNA was first extracted from a random group of isolates using the boiling method.[15] The presence of *vanA* and *vanB* resistance determinants were then determined as previously described using the *vanA* (F: 5'CATGAATAGAATAAAAGTTGCAATA-3',R: 5'CCCCTTTAACGCTAATACGATCAA-3') and *vanB* primers (F: 5'GTGACAAACCGGAGGCGAGGA-3', R: 5'CCGCCATCCTCCTGCAAAAAA-3') with product sizes of 1030bp and 433bp resptectively.[16] In brief, the amplification mixture comprising of 10 × PCR buffer, 25mM $MgCl_2$, 0.2mM each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 0.5U of Taq DNA polymerase and 5pMol of both reverse and forward primers made up to a 25 l total volume using sterile ddH_2O was then amplified. The amplification protocol involved an initial denaturation at 94°C for 5 min, 30 cycles of amplification (denaturation at 94°C for 1min, annealing at 54°C for 1min, and extension at 72°C for 1min), and a final extension at 72°C for 10min. Amplification

products were then assessed by analyzing the products on a 1.5% agarose gel at a constant voltage and 1X TBE for approximately 1 hour. Products were visualized by Ethidium bromide staining and photographed under ultraviolet light using a is 1kb DNA ladder (Thermo Scientific).

RESULTS

A total of 25 isolates were confirmed as enterococci. These isolates showed high levels of resistance to most antibiotics with 100% resistance noted to 4 antibiotics (amoxicillin/clavulanate potassium, ceftazidime, cefuroxime and cloxacillin) (Figure 1). The isolates were however susceptible to gentamicin and ofloxacin (16% resistance each).

An analysis of the antibiogram patterns associated with the various isolates showed 7 patterns in total though majority of the isolates (60%, 15/25) had the same antibiogram pattern. Most of these isolates (84%, 21/25) though were multidrug-resistant, exhibiting resistance to ≥3 drug classes (Table 1).

High frequency of resistance to vancomycin (60%) was observed among the *Enterococcus* isolates (Figure 2) and all the resistant isolates were multidrug resistant (Table 2).

Table 1: Antibiogram pattern of all enterococci isolates

Resistance drug combination	Frequency (% Occurrence)	No. of antibiotic resistant classes
AUG-CAZ-CRX-CXC	1(4)	2
AUG-CAZ-CRX-CTR-CXC	3(12)	2
AUG-CAZ-CRX-CTR-CXC-ERY	15(60)	3
AUG-CAZ-CRX-CTR-CXC-ERY-GEN	2(8)	4
AUG-CAZ-CRX-CTR-CXC-ERY-OFL	2(8)	4
AUG-CAZ-CRX-CTR-CXC-GEN-OFL	1(4)	4
AUG-CAZ-CRX-CXC-ERY-GEN-OFL	1(4)	5
Total	25(100)	

Key: AUG: Augmentin, CAZ: Ceftazidime, CRX: Cefuroxime, CTR: Ceftriaxone, CXC: Cloxacillin, ERY: Erythromycin, GEN: Gentamicin, OFL: Ofloxacin

Table 2: Antibiogram pattern of vancomycin-resistant enterococci isolates

Resistance drug combination	Frequency (% Occurrence)	No. of antibiotic resistant classes
AUG-CAZ-CRX-CTR-CXC-ERY	10(66.7)	3
AUG-CAZ-CRX-CTR-CXC-ERY-GEN	2(13.3)	4
AUG-CAZ-CRX-CTR-CXC-ERY-OFL	2(13.3)	4
AUG-CAZ-CRX-CTR-CXC-GEN-OFL	1(6.7)	4
Total	15(100)	

Key: AUG: Augmentin, CAZ: Ceftazidime, CRX: Cefuroxime, CTR: Ceftriaxone, CXC: Cloxacillin, ERY: Erythromycin, GEN: Gentamicin, OFL: Ofloxacin

An assessment for the presence of both *vanA* and *vanB* genes showed a higher frequency of the *vanB* gene (433bp, Plate 1) among the *enterococcus* isolates with 53.3% (8/15), and 0% for vanA gene. No co-occurrence of both *vanA* and *vanB* in an isolate was observed.

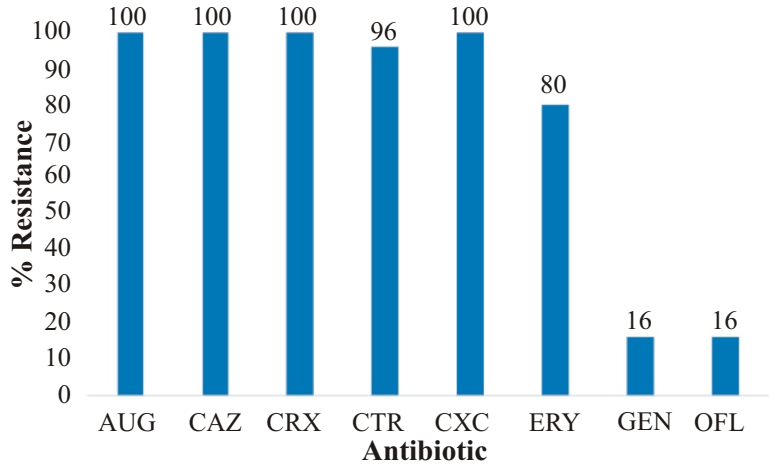

Figure 1: Drug resistance in clinical enterococci isolates

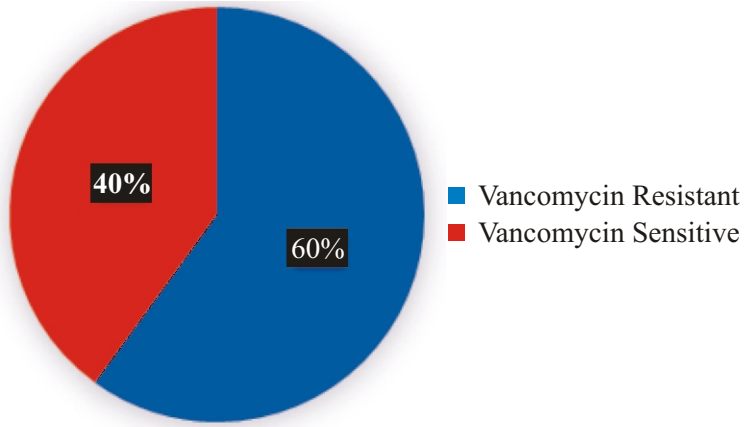

Figure 2: Rate of vancomycin resistance in enterococci isolated from clinical samples

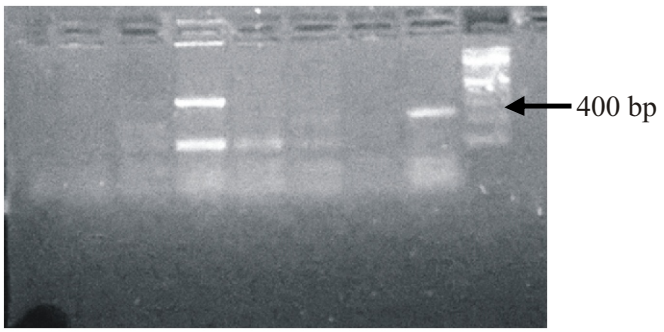

Plate 1: Representative visualization of amplified *vanB* determinant

DISCUSSION

An increasing prevalence of vancomycin-resistant enterococci (VRE) has been noted in recent years.[2,17] Considering the paucity of data from Nigeria, ongoing continuous surveillance of VRE is essential. In this small-scale study exploring the co-occurrence of vancomycin and multidrug resistance in enterococcus, a high level of resistance was noted which was similarly noted by other studies, though variations were noted in the specific antibiotic to which the organisms were resistant.[18,19] Ojo and colleagues[18] reported resistance levels of above 80% against 4 antibiotics. Three of these (cloxacillin, ceftriaxone and erythromycin) were part of the 6 antibiotics to which above 80% resistance was reported by this current study. The 4th one, gentamicin though was one of the two antibiotics to which isolates in this study were fairly sensitive (16%). This could either reflect a

variation in antibiotic use and hence development of antibiotic resistance in these areas or a general variation in circulating strains.

One significant finding of this study is the low rate of resistance to gentamicin. Gentamicin belongs to the aminoglycoside drug class, which is very useful as a combination drug with bactericidal activity against serious enterococcal infections.[20] The evolution of high-level aminoglycoside resistance (HLAR) which reduces the efficacy of aminoglycosides such as gentamicin has however threatened this application. Such strains are increasingly being reported worldwide.[9, 21-24] The low rates of resistance to gentamicin in this study are therefore encouraging as they indicate the possible continued efficacy of gentamicin as a combination drug in this region. These low rates could correlate to prescription levels of gentamicin. A 2020 study reported that gentamicin was the least prescribed (11.8%) of the most commonly prescribed antibiotics.[25]

These encouraging rates are in contrast to the data observed for vancomycin. A 60% vancomycin resistance rate of enterococci is not unusual and while it is quite high, it is lower than the 68.9% average reported by the Wada review.[4] Considering that all VRE in this current study were also MDR, is even more worrisome as it greatly reduces treatment options. The fact that 12 of the 15 VRE in this study were still susceptible to gentamicin (Table 2) is redeeming. This same phenomenon of all VRE being MDR was recently described in the Southwest of Nigeria.[10] More studies must be carried out to determine the antibiotics this MDR VREs are susceptible to.

Though *vanA* and *vanB* genes are the two most commonly occurring resistance determinants associated with vancomycin resistance, their occurrence distribution is location dependent. Initially, *vanA* was predominant in Europe but currently, the epidemiology particularly in Germany appears to be evolving with *vanB* now predominant.[1,2] Reports on vancomycin resistance determinants in Nigeria have varied,[6,8,9] ranging from the absence of *vanA* and low occurrence of *vanB* (1.1%) to 100% occurrence of *vanA*. Similar to the study by Adeyemi and colleagues,[6] no *vanA* genes were detected in this study, only *vanB*.

CONCLUSION

This study reports an association of vancomycin-resistant enterococci with multidrug resistance, in agreement with previous reports. Majority of these isolates were sensitive to the amino glycoside gentamicin indicating possible treatment options. Vancomycin resistance of enterococci in this study was however mediated by *vanB* rather than *vanA* genes. More widescale surveillance is needed for understanding the epidemiology of vancomycin-resistant enterococci in Nigeria.

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