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SURVEILLANCE FOR VANCOMYCIN RESISTANT ENTEROCOCCI IN A TERTIARY INSTITUTION IN SOUTH WESTERN NIGERIA

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Abstract

Background: Enterococci are responsible for up to 12% of cases of healthcare associated infections worldwide and cause life threatening infections among critically ill patients. They show intrinsic and acquired resistance to a wide range of antimicrobial agents. Glycopeptide resistance is due to *vanA, vanB, vanC, vanD, vanE, vanG and vanL* genes.
Objectives: To determine the carriage rate of VRE among patients on prolonged hospitalization in Lagos University Teaching Hospital, assess the antimicrobial resistance pattern of VRE, identify factors associated with VRE colonization and describe the genetic determinants of enterococcal resistance to Vancomycin.
Methods: VRE were isolated from rectal swabs collected from patients hospitalized for seven days or more in Lagos University Teaching Hospital and identified by Matrix Assisted Laser Desorption Ionization (MALDI) and Polymerase Chain Reaction (PCR). Antimicrobial susceptibility testing was performed by E-test. PCR assay for Vancomycin resistance genes was also performed. Data on demographic and risk factors collected by questionnaire was tested for significance using Chi square.
Results: Thirteen of 319 patients surveyed were colonized with VRE; one with *vanA E. faecium*, two with *vanB E. faecium*, ten with *E. gallinarum* and one with *E. casseliflavus*. Univariate analysis for risk factors associated with VRE colonization was only significant for the ward of admission. Only one VRE isolate showed full resistance to Vancomycin and Teicoplanin. Three were resistant to Ampicillin and nine to Ciprofloxacin but all were susceptible to Linezolid. High-level resistance to Gentamicin was found in four VRE isolates.
Conclusion: There is a low prevalence of VRE in Lagos University Teaching Hospital which may be spreading among patients in affected wards.

Key words: Enterococcus, Vancomycin, Resistance, VRE

Introduction

Since the late 1980s, there has been a rapid increase in glycopeptide resistance. While most of these reports have come from developed countries, it appeared that there had been a lag in development of glycopeptide resistance of enterococci in developing countries, probably due to low consumption of glycopeptide antibiotics as they are relatively expensive and the problems of Methicillin Resistant Staphylococcus aureus (MRSA) and Clostridium difficile infection (CDI) have not been so prominent in these areas.

In Nigeria, the role of enterococci in clinical infections has been poorly documented. Earlier reports had suggested that resistance to glycopeptides among enterococci had not emerged[1]. However, there have been recent reports of Vancomycin resistant enterococci (VRE) being isolated from clinical specimen and hands of health care workers in other centers in western Nigeria[2,3].

Since enterococci are part of the normal intestinal flora of humans, the gut provides a conducive environment for development and transfer of antimicrobial resistance determinants hence gastrointestinal colonization precedes infection in many cases[4]. Also, the recommendations for preventing the spread of Vancomycin resistance by the Hospital Infection Control Advisory Committee (HICPAC) of the US Centers for Disease Control include periodic culture surveys of stools or rectal swabs of patients at high risk for VRE infection or colonization[5]. Routine laboratory testing of all enterococcal isolates for Vancomycin resistance, also recommended by HICPAC, is not being practiced in most clinical laboratories in Nigeria due to the perceived absence or low incidence of Vancomycin resistance among enterococcal isolates. This practice may be masking the identification of emerging Vancomycin resistant enterococcal strains.

We report a surveillance study carried out among patients on prolonged admission (over seven days) in Lagos University Teaching Hospital, a tertiary hospital in south western Nigeria.

Methods

Study Subjects

Between February and August 2013, patients on admission for seven days and over across medical, surgical and pediatric wards were recruited. Rectal swabs were collected from patients after obtaining informed consent by the investigators, other medical personnel or the patients themselves. Information on clinical condition, antibiotic consumption, invasive procedures and other risk factors was also recorded.

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Sample Processing

The swabs were inoculated into bile esculin broth containing 6mg/ml of Vancomycin for 48 hours and then sub-cultured onto bile esculin agar. Black coloured colonies which were gram positive and catalase negative were transferred to blood agar plates for further identification.

Species Identification

Further testing of isolates was carried out at the Institute of Medical Microbiology and Infections Epidemiology, Leipzig University, Leipzig, Germany. Isolates were identified by matrix-assisted laser desorption/ionization time - off light (MALDI-TOF) Mass spectrometry using VITEK2 MS system (Biomerieux, France) and verified by specie specific enterococcal ddl PCR[6]. MALDI TOF Mass spectrometry is a mass spectrometry based technology that offers accurate, rapid, and inexpensive identification of microorganisms. Briefly, bacterial colonies are removed from agar culture plates, mixed with an excess of UV-absorbing matrix, and dried on steel target plates. The dried preparations are exposed to laser pulses, resulting in energy transfer from the matrix to the nonvolatile analyte molecules, with desorption of analyte into the gas phase. The ionized molecules are accelerated by electric potentials through a flight tube to the mass spectrometer, with separation of the biomarkers determined by their mass/charge ratio. The profile of biomarkers is then compared with profiles of a collection of well characterized organisms[7]. *vanC* genotype was determined by identification of organisms as *E. gallinarum* and *E. casseliflavus* since these species express the *vanC* genotype constitutively.

Antimicrobial Susceptibility Testing

MIC to selected antibiotics (Vancomycin, Teicoplanin, Ampicillin, Gentamicin, Linezolid and Ciprofloxacin) was determined by the E-test and interpreted according to the CLSI guidelines[8].

PCR

DNA was extracted from overnight cultures suspended in TE buffer using the MagNa Pure 96 system (Roche)[9,10]. PCR assay for Vancomycin resistance genes was carried out using standard protocols**.** Previously described primers were used[6].

Statistical Analysis

Associations between risk factors and colonization were tested with Chi square using SPSS statistics 17.0.0 (SPSS Inc., Chicago, Ill.). A *p* value of less than 0.05 was regarded as statistically significant.

Ethical Issues

This study was reviewed and approved by the Research and Ethics Committee of the Lagos University Teaching Hospital before commencement. Informed consent was obtained from all participants before specimen collection.

Results

Rectal swabs were collected from 319 patients, 165 on surgical wards, 87 from medical and 67 from pediatric wards. Of the total number of patients surveyed, 165 were males. The ages of participants ranged from 0 to 87 years with a mean age of 34.48 years. Mean duration on admission was 49.13 days. Table 1 shows the demographic and clinical characteristics of patients surveyed in this study.

Thirteen VRE strains were isolated; one *vanA E. faecium*, two *vanB E. faecium*, nine *E. gallinarum* and one *E. casseliflavus* (Table 2). Univariate analysis for risk factors associated with VRE colonization was only significant for the ward of admission (p=0.031) (Table 1).

The antimicrobial susceptibility pattern of enterococcal isolates carrying *vanA, vanB* and *vanC* genes are shown in table 3. The *vanA* isolate showed high level resistance to Vancomycin (MIC >256µg/mL) and teicoplanin (MIC = 48µg/mL) and was also resistant to ampicillin and ciprofloxacin. It was susceptible to linezolid and did not display high level resistance to gentamicin. Both *vanB* isolates showed intermediate resistance to Vancomycin (MIC = 8µg/mL) and were susceptible to teicoplanin. They were also resistant to ampicillin and ciprofloxacin and displayed high level resistance to gentamicin, but susceptible to linezolid. The *vanC* isolates showed Vancomycin MIC ranging from 1µg/mL to 8µg/mL and teicoplanin MIC <2µg/mL.

Discussion

The rapid spread of Vancomycin-resistant Enterococci (VRE) which occurred in Europe and the USA in the 1990s was driven by overuse of glycopeptides in animal farming in Europe and in clinical practice in the US[11,12]. In Africa, there have been very few reports of VRE, most being from South Africa. VRE cause a wide variety of infections involving the urinary tract, wound, bloodstream among other sites most commonly in hematological malignancy patients and transplant recipients.[13] These cases are increasing in Nigeria[14–16].

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This is the first report from Nigeria using molecular methods for differentiation of enterococci and for the determination of their resistance mechanisms. We conducted surveillance for VRE among patients admitted for 7 days and above in a tertiary hospital in South Western Nigeria. VRE were isolated from 13 (4.07%) of 319 patients screened. This is much lower than rates reported from South Africa[17]. However, this comparison may not be accurate because of differences in the characteristics of the populations studied. This finding is significant when compared to an earlier study in this center which showed no resistance to Vancomycin among enterococci[1] although only clinical isolates of *E. faecalis* where studied. Other studies from western Nigeria have reported Vancomycin resistance in 43% of hospital acquired infections due to *E. faecalis*[2] and 17.43% of 568 *E. faecalis* isolated from the hands of healthcare workers[3]. These results were obtained using phenotypic methods.

Table 1: Demographic and Clinical characteristics of patients surveyed

Variable	**VRE Absent (n= 306)**	**Present (n= 13)**	**Total (n=319)**	***P****
Mean age	33.91	47.23	34.48	0.24†
Male	161	4	165	0.106
Female	145	9	154	
Ward				
Male surgical	85	3	88	0.031
Female surgical	74	3	77	
Male medical	39	1	40	
Female medical	41	6	47	
Pediatric	36	0	36	
Pediatric surgery	31	0	31	
Mean duration on admission	50.00	27.50	49.13	0.313*
Tuberculosis	13	0	13	0.443
Malignancy	52	1	53	0.333
Diabetic	22	0	22	0.388
Renal	16	1	17	0.516
Invasive device present	110	6	116	0.318
Foley catheter	81	6	87	0.716
Chest tube	16	0	16	
CV line	8	0	8	
Ventilator	2	0	2	
Tracheostomy	3	0	3	
Anti-neoplastic therapy	19	0	19	0.443
Surgery	130	5	135	0.505
Used Antibiotics	288	12	300	0.557

* ANOVA

† Chi square/Fisher's exact test

Table 2: Distribution of VRE isolates by genotype and specie

Genotype	Specie	Number
vanA	*E. faecium*	1
vanB	*E. faecium*	2
vanC1	*E. gallinarum*	9
vanC2	*E. casseliflavus*	1
Total		13

Table 3: Antimicrobial susceptibility profile of Enterococcus isolates carrying the *vanA, vanB, vanC1* or *vanC2* genes.

ID	Specie	Genotype	Va	Te	Am	Ci	Li	HLGR
A	*E. faecium*	*vanA*	>256	48	>256	>32	1.5	12
B	*E. faecium*	*vanB*	8	1	>256	>32	0.5	>512
C	*E. faecium*	*vanB*	8	0.38	>256	>32	1.5	>512
D	*E. gallinarum*	*vanC1*	0.5	0.5	1	4	2	8
E	*E. gallinarum*	*vanC1*	6	1.5	0.75	>32	0.75	>512
F	*E. gallinarum*	*vanC1*	6	0.75	1.5	0.75	1	3
G	*E. gallinarum*	*vanC1*	6	1	0.5	1	1	2
H	*E. gallinarum*	*vanC1*	4	1	0.75	>32	0.5	>512
I	*E. gallinarum*	*vanC1*	0.38	0.5	2	2	1.5	3
J	*E. gallinarum*	*vanC1*	8	0.75	2	3	2	4
K	*E. gallinarum*	*vanC1*	4	0.75	0.75	>32	0.75	2
L	*E. gallinarum*	*vanC1*	4	1	0.5	0.75	0.75	2
M	*E. casseliflavus*	*vanC2*	8	0.75	0.125	16	0.75	1.5

Va: Vancomycin, Te: Teicoplanin, Am: Ampicillin, Ci: Ciprofloxacin, Li: Linezolid, HLGR: High Level Gentamicin Resistance

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The clinical data of patients colonized with enterococcal isolates carrying Vancomycin resistance genes are shown in table 4 while the number of patients surveyed who were receiving different classes of antibiotics is shown in table 5.

Table 4: Clinical data of the patients with enterococcus isolates carrying a gene element of Vancomycin resistance

ID	Age	Sex	Ward	Duration on admission (days)	Primary condition	Antibiotics administered	Invasive device	TWBC	Surgical intervention	Others	VRE type
A	48	M	Medical	52	Interstitial lung disease	Amoxicillin-Clavulanate, AntiTB	None	6.1	None		A
B	70	F	Medical	17	Cerebrovascular disease	Levofloxacin	None	NA	None		B
C	48	F	Medical	22	Hyperglycemic crisis, DVT and tibial fracture	Ceftriaxone, levofloxacin, metronidazole	Urethral catether	10.0	None		B
D	69	F	Medical	40	Autoimmune hemolytic anaemia	None	None	6.3	None		C1
E	25	F	Medical	21	Acute exarcebation of chronic kidney disease	Ceftriaxone, levofloxacin, metronidazole	Urethral catether	8.5	None	Dialysis	C1
F	38	F	Surgical	19	Breast cancer	Ceftriaxone, metronidazole, amoxicillin-clavulanate		13.3	None		C1
G	27	M	Surgical	28	Intestinal obstruction	Levofloxacin, metronidazole, amoxicillin-clavulanate	Urethral catether	15.2	Exploratory laparotomy		C1
H	48	F	Surgical	12	Head injury	Ceftriaxone, Cefixime	Urethral catether	5.0	Craniotomy		C1
I	25	F	Medical	17	Sepsis	Ceftriaxone, meropenem, metronidazole, amoxicillin-clavulanate	None	24.6	None		C1
J	27	M	Surgical	48	Chronic leg ulcer	Ceftriaxone, levofloxacin, ampicillin-sulbactam	None	29.9	Skin grafting		C1
K	64	F	Medical	20	Paraparesis	Levofloxacin, metronidazole, amoxicillin-clavulanate	Urethral catether	13.8	None		C1
L	59	F	Surgical	22	Leg pain	Ceftriaxone	Urethral catether	17.2	None		C1
M	66	M	Surgical	31	Tibial fracture due to gunshot	Levofloxacin, metronidazole, Ceftriaxone, Cefixime	None	5.0	Open reduction		C2

TWBC – Total White Blood Cell Count, M - Male, F - Female, NA- Not available

Table 5: Antibiotic consumption pattern of patients surveyed

ANTIMICROBIAL	COUNT	%
Cephalosporins	215	67.4
Metronidazole	185	58.0
Quinolones	162	50.8
Penicillins	87	27.3
Carbapenems	22	6.9
Anti-tuberculous	22	6.9
Glycopeptides	8	2.5
Antifungal/Antiretroviral	5	1.6
Others*	48	15
None	19	6.0

*Macrolides, Aminoglycosides, Antifolates, Nitrofurantoin, etc.

The low prevalence of VRE in this center may not be unconnected with the low consumption of Vancomycin among patients in this center. Only 2.5% of subjects studied had received Vancomycin. However, there was high consumption of cephalosporin (67.4% of subjects) and metronidazole (58.0% of subjects) which have also been implicated in the acquisition of VRE[18].

Both *vanB* isolates were recovered from patients admitted in the same ward suggesting nosocomial spread. Although *vanB* demonstrates lower level Vancomycin resistance and is more commonly susceptible to Teicoplanin, it has been shown to possess high potential for nosocomial transmission and conjugal transfer[19–21]. *VanA* VRE have been the more predominant genotype in Europe and the US, but *vanB E. faecium* may be more common in Africa as was found in South Africa[17]. Most microbiological studies of enterococci in Nigeria have only reported *E. faecalis*[1,22]. Ampicillin and Ciprofloxacin resistance were exhibited by the *vanA* and *vanB* isolates as also found in other studies[23,24], whereas high-level resistance to gentamicin occurred only in the *vanB* isolates and two *van C* isolates. This rate of high-level resistance to gentamicin is comparable to that of an earlier study in this institution[1]. All VRE isolates remained susceptible to linezolid similar to the findings of a study in South America[25]. Linezolid is a very attractive antimicrobial therapy for VRE infections due to its favorable pharmacokinetic distribution, low incidence of adverse effects, and oral bioavailability[26], however, resistance to this agent has begun to emerge[26,27].

Apart from the ward of admission, no other risk factor showed significant association with VRE colonization by Univariate analysis suggesting the possibility of nosocomial transmission. This may however be due to the relatively small number of patients who were colonized with VRE. The low prevalence of VRE will require larger studies to fully elucidate risk factors for VRE colonization in Nigeria.

The findings of this study reveals the potential for the spread of Vancomycin Resistant Enterococci among patients in this center as well as the need for continuous surveillance and laboratory testing for Vancomycin resistance in enterococcal isolates for early identification of potential outbreaks of VRE infections, and institution of control measures.

This study clearly shows that the prevalence of VRE is still low in Nigeria, giving Nigeria the opportunity to combat the emergence of VRE by measures such as antibiotic stewardship program and other infection control strategies.

Conflicts of Interest: All authors report no conflicts of interest relevant to this article.

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