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Vancomycin Resistant Enterococci Infections in Trinidad and Tobago

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SK and PEA designed the study and wrote the protocol. Author SK collected and processed the clinical data and samples. Authors PEA and WHS coordinated the study. Authors SK and PEA wrote the first draft of the manuscript and managed literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To document Vancomycin Resistant Enterococci (VRE) infections prevalence, risk factors, antimicrobial susceptibility patterns and evaluation of chromogenic plates in identifying VRE isolates in Trinidad and Tobago.
Study Design: This was a cross sectional prospective observational and descriptive study.
Place and Duration of Study: Study was carried out in all major regional hospitals in Trinidad & Tobago over a four year period, 2009 to 2013.
Methodology: All cases of Enterococcus infections from major hospitals in the country were reviewed. Standardized questionnaire was used to analyze epidemiological and clinical data of VRE infected patients. Enterococcal speciation, minimum inhibitory concentrations (MIC) were evaluated using Microscan Walk Away 96SI (Siemens, USA). Isolates were further identified using 6.5% NaCl and pyrrolidonyl arylamidase activity according to CLSI guidelines. Brain Heart Infusion agar, Bile esculin azide agar containing 6 mg/L of vancomycin and chromogenic agar plates - Chromagar VRE and chrom ID VRE were used to screen and confirm VRE isolates
Results: A low (3.9%) VRE infections were encountered from 1,141 enterococcal infections

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reviewed. Majority of these infections occurred in the surgical facilities (42%) and least from the burns unit (2%) respectively. No significant association was observed between VRE infections and patients' gender, age, wards or hospital facilities. However underlying diseases such as diabetes mellitus and hypertension; and prior use of antibiotics on or before admission were noted to be associated with the VRE infections.
All the VRE infections were mainly caused by *E. faecium* and *E. faecalis* that were completely (100%) resistant to ciprofloxacin, erythromycin, levofloxacin, rifampin and vancomycin, but were 95% and 100% susceptible to gentamycin and linezolid respectively. Overall, performance of the chromogenic agar plates were 100% sensitive and specific for VRE
Conclusion: In Trinidad & Tobago VRE infections prevalence although low are strongly associated with high antibiotic consumption, prolonged hospitalization and diabetes mellitus disease. Linezolid is highly susceptible to multidrug resistant VRE isolates in the country. Chromogenic VRE media produced rapid and reliable identification of VRE organism. Further use of molecular studies to monitor the epidemiology of VRE infections in hospitals in the country is highly recommended.

Keywords: Chromogenic agar; diabetes mellitus; Enterococcus faecium; Enterococcus faecalis; VRE infections; Trinidad & Tobago.

1. INTRODUCTION

The emergence and spread of glycopeptide resistance in enterococci has become a substantial clinical and epidemiological concern, and vancomycin-resistant enterococci (VRE) are now an increasingly important problem in hospitals worldwide, since it was first isolated in the UK and France in 1986 [1,2]. Prevalence of VRE has been associated with higher treatment costs, prolonged morbidity and greater mortality rates [3]. The prevalence of VRE differs widely among different countries including from one hospital to another in the same country [4]. In Latin America, less than 10% prevalence of VRE has been reported in Brazil, Colombia and Argentina [5]. In the United States (US) and Canada varied VRE prevalence has been reported; for example, vancomycin resistant *E. faecium* (VRE.*fm*) is 10-20% and vancomycin resistant *E. faecalis* (VRE.*fs*) is 5% [6].

Various risk factors such as advanced age, severe underlying disease, inter-hospital transfer, extended hospitalization, central venous catheterization, tumors, hemodialysis, antibiotic exposure to vancomycin, third generation cephalosporins antibiotics with anti-anaerobic activity and prolonged duration of antibiotic therapy may contribute to VRE colonization and infection [7]. Prompt and accurate identification of patients with VRE infections is imperative. Therefore rapid and reliable identification of these organisms is crucial for patient management and infection control measures. Chromogenic plates had been used not only to screen for VRE but also to differentiate between vancomycin resistant *E. faecium* and vancomycin resistant *E. faecalis* [8]. The tool is a very reliable method to use in VRE studies.

There has never been any report on the cases or outbreaks of Vancomycin resistant Enterococci infections in Trinidad & Tobago. In fact, nothing or little is known about VRE infections prevalence in the country partly because it does not make headline news or because it is not properly and adequately investigated. This study therefore sought to determine the prevalence of VRE infections and associated risk factors, antimicrobial susceptibility patterns of VRE isolates. It also evaluated the usefulness of chromogenic plates in identifying VRE isolates from Trinidad and Tobago. Such information should be useful in establishing management guidelines for VRE infections in the country

2. MATERIALS AND METHODS

This prospective study was carried out over a period of 4 years (August 2009 to July 2013) on all patients admitted and treated at all the regional hospitals in Trinidad & Tobago. Once an Enterococcus species is isolated from routine clinical specimen sent to the microbiology laboratory, a standardized questionnaire was used to further collect basic demographic and clinical information on the patient including, site of infection, age, ward and gender, risk factors (such as transfer from another health facility or ward, co-morbid health condition, prolonged hospitalization, previous antibiotic consumption or antibiotic treatment) and patient's outcome. Basically the questionnaire was used to collect data that would establish if this case was due to Enterococcal infection or colonizer. Parameters

used to determine infection included presence of fever (≥ 38ºC), elevated WBC and C-reactive protein and if wound infection is involved, there must be evidence of swelling, redness and tenderness, pus and other systemic symptoms. Other laboratory data findings recorded for Enterococcal bacteremia included platelet count, serum creatinine and leucopenia (which was defined as a white blood cell count < 4000/ µL and thrombocytopenia as a platelet count < 150,000/ µL). These endpoints may all cause morbidities and mortalities within 14 and 28 days in cases of Enterococcal infections.

2.1 Source of Bacterial Isolates and Identification

The enterococcal isolates from the clinical specimens were routinely identified at the collection institutions using standard laboratory procedures [9] including Gram stain (Quelab Laboratories Inc., Montreal Canada), catalase test (Regal Pharmaceuticals, Canada), 6.5% NaCl and pyrrolidonyl arylamidase activity (PYR: Oxoid Ltd., Basingstoke, Hampshire, UK) bile esculin azide agar (Oxoid Ltd., Basingstoke, Hampshire, UK) and Microscan (Siemens, USA). No duplicate isolates from a single patient were included and there was no history of VRE outbreak during the 4 year period.

2.2 Vancomycin-resistance Detection

2.2.1 Microbiological methods

Screening for vancomycin resistance was performed by plating the enterococcal isolate on brain-heart infusion agar (DIFCO) and Bile esculin azide agar (BEAA, Oxoid) that contained 6 mg/l of vancomycin. The plates were incubated in ambient air atmosphere for 18-24 hours at 35ºC. Evidence of growth suggested that the isolates were vancomycin-resistant.

Two chromogenic plates [CHROMagar VRE (CHR) medium (CHROMagar, Paris, France) and ChromID VRE (C-ID) medium (bioMerieux, France)] were used to determine vancomycin resistance. The CHR medium can detect VRE, but unable to discriminate between Vancomycin resistant *E. faecium* (VREFm) and Vancomycin resistant *E. faecalis* (VREFs), whereas ChromID medium is able to discriminate between VREFm and VREFs due to the production of two different colony colors after 24 hours incubation [8]. All tests were validated using quality control strains. Positive controls, VRENFM ATCC 700221 (mauve) and VRENFS ATCC 51299 (green) and negative controls *Escherichia coli* ATCC 25922 (no growth) and *E. faecalis* ATCC 29212 (no growth) were used as described in literature [9,10].

2.3 Antimicrobial Susceptibility and MIC Testing

In vitro susceptibilities of the isolates to several antibiotics including Ampicillin, Amoxicillin, linezolid and vancomycin were determined by the disc diffusion method as recommended by Clinical and Laboratory Standard Institute (CLSI) [11]. The antibiotics discs used were from Oxoid. The Minimum Inhibitory Concentrations (MIC) was examined by the Microscan Walk Away 96 SI (Siemens, USA). MICs were interpreted according to approved CLSI breakpoints [11].

2.4 Statistical Analysis

Data were analyzed using SPSS software (version 18). Pearson's Chi-square, Fisher's Exact test, Cramer's V and Independent Samples t-tests were statistical tests performed. Differences were statistically considered significant at $p < 0.05$.

2.5 Ethical Approval

Approval for this study was given by the Ethics Committee of The University of the West Indies, St. Augustine, Trinidad and Tobago. Permission to conduct this study at the various hospital institutions were also obtained.

2.6 Consent

No consent was obtained from any patients since data were only extracted from their case notes using codes and there was no way any information obtained would have been related to the patient in this study.

3. RESULTS AND DISCUSSION

The performance of the test methods used to detect infections and vancomycin resistance in the Enterococcus species isolates revealed very significant results. A total of 1,141 enterococcal isolates from patients fulfilling infections criteria were included in the analysis. Only 3.9% (45/1141) of these cases had vancomycin resistance enterococcal infections. Results of the infection site and distribution of the VRE isolates

in the different facility of the major hospitals in Trinidad and Tobago are depicted in Tables 1 and 2. Interestingly, Hospital "E" had no enterococcal infections while 40% of the cases were reported from Hospital "A". Among the Vancomycin-Sensitive Enterococcus infections, female patients accounted for 58% (631/1096) while the rest were males; whereas for VRE infections, males were 51% (23/45), and 49% (22/45) for females.

The Vancomycin agar plate method, the BHI and BEAA 6mg/ml of vancomycin compared excellently well with the CHROMagar VRE and ChromID VRE method, which provided a rapid method for detecting and differentiating Enterococcus species, i.e. *E. faecium* and *E. faecalis.* The antibiotic susceptibility data for all the Vancomycin-Sensitive Enterococcus and VRE isolates are depicted in Table 3 and shows that large proportions of Vancomycin-Sensitive Enterococcus (VSE) isolates were still over 87% susceptible to several antimicrobial agents available and readily used in the country. The in vitro susceptibility of VRE shows that *E. faecium* isolates were 82% resistant to ampicillin, 100% resistant to ciprofloxacin, erythromycin and vancomycin, but were 93% susceptible to gentamycin and 100% to linezolid. In contrast, VRE *faecalis* shows 100% resistance to erythromycin, chloroamphenicol, streptomycin, synercid and 100% susceptibility to levofloxacin, linezolid, gentamycin, ampicillin, tetracycline, ciprofloxacin, penicillin and rifampin, respectively.

All patients with VRE infections had a median age of 50 (range 9-91) years. Analyzed risk factors associated with VRE infection revealed a high percentage (42%) of the patients were diabetic (19/45, P=0.001), 18% hypertensive (8/45, P=0.004) and 36% with cardiovascular diseases (16/45, P=0.6); and the least 4.4% had tumors (2/45, P=0.2).

Data review of antimicrobial agents consumption from the Trinidad & Tobago National Drug Procurement Agency (also known as C40) revealed that the amount of vancomycin consumed at various regional hospitals from 2009 to 2013 were as follows, Eric Williams Medical Sciences Complex (EWMSC) 6,829 g; San Fernando General Hospital (SFGH) 6,992 g; Port of Spain General Hospital (POSGH) 6,785 g; Eastern Regional Health Authority (ERHA) 780 g; Scarborough Regional Hospital, Tobago (SRHT) 1,080 g; Others (several health centers including Mount Hope Women's Hospital) was 1,576 g. Data for cephalosporins also revealed the followings: EWMSC 85,742 g; ERHA 13,703 g; SFGH 91,775 g; POSGH 83,854 g; SRHT 12,721 g; others, 63,286 g. In terms of antibiotic use, more patients on the surgical wards and Intensive Care Unit (ICU) received carbapenems, fluoroquinolones or vancomycin.

In this study, the prevalence of VRE from several major regional hospitals in Trinidad and Tobago was found to be low (3.9%). This is lower than 5 – 10% rates noted in other countries such as Canada, USA, Argentina, and Columbia [5]. The low prevalence found in this study is similar to rates found in Central Europe, Germany, Switzerland and Austria [12]. However, in other countries such as Portugal, the prevalence was over 50%; England, Ireland and Greece over 19%, while the Netherlands and Switzerland had the lowest prevalent rate (2%) [13]. Among Western Pacific countries, percentages of VRE strains ranged from 6% in Western Australia to over 23% in Taiwan and Singapore and more than 50% in Japan and Hong Kong [14]. In South America, the rates are less than 9.6% in Colombia and 5% in Argentina, Paraguay and Brazil respectively [5].

The reason why VRE infection rates are lower in Trinidad & Tobago than in many hospitals in developing countries such as Colombia or Argentina is not clear. Perhaps we can infer that the substantial infection control measures such as hand washing, antibiotic stewardship, barrier nursing and other efforts instituted at the hospitals in controlling methicillin-resistant *S. aureus* is having an impact on other common infections that easily spread in the hospital. These measures play major roles in preventing such infection from reaching an epidemic tipping point or significant proportion as reported to date [15]. Also the different patient population we had in this study may have lacked the critical mass of susceptible patients for VRE infections to occur as reported in other places [12].

From our data analysis, the highest source of VRE infections was found in urogenital tract (UGT) and the least from blood. This trend was also seen with VSE infections and this is consistent with reports by Dupont et al. [16]. Results from this study suggests prior antimicrobial use, especially use of vancomycin, cephalosporins may have played a role with VRE infections spread at the various institutions in the country. This may be so based on the amount of antibiotics consumed in the country, as

evidenced by what was observed at hospital "E" that consumed the least amount of vancomycin and had no VRE infections. Exposure to previous antibiotics usage is strongly associated with VRE infection. Previous studies elsewhere have reported that exposure to antibiotic regimens are potent risk factors in the development of VRE [17,18].

This study showed a high percentage of VRE patients with diabetes mellitus (40%). This is no surprise giving the high prevalence of diabetes in the country. Other studies have revealed associations between VRE infections and patients with co-morbid conditions being at a high risk of being infected [19]. There must be therefore a concerted efforts to combat or control diabetes in the country so as to stem down or nib at its bud any possibilities of outbreak or increase of VRE infections or even colonization among individuals in the country. In our experience the most common site of infection was the urogenital system. This is similar to findings from studies by researchers from Korea and Portugal [20,21]. Blood and pleural samples were the least for the recovery of VRE isolates in this study. A similar finding was reported in studies conducted in France by Bourdon et al. [22].

Vancomycin resistant *E. faecium* (VREFm) isolates encountered in this study were completely (100%) resistant to ciprofloxacin, erythromycin, levofloxacin, rifampin and 90% resistance to tetracycline. A similar result was noted in Turkey (Kidar) [23]. Although the patients in that study were mainly those with haematological malignancy unlike ours that were varied.

The use of the chromogenic agar plates enabled identification of the VRE isolates within a very short turn-around time (TAT). The results obtained was not only accurate, the method was also very simple and reliable. As reported elsewhere, accurate detection of vancomycin-resistant enterococci (VRE) is essential in preventing transmission in health care settings. Chromogenic media are widely used for screening VRE because of fast turn-around times (TAT) and high sensitivity [24].

Table 1. Distribution of VRE infections at body sites in major hospitals in Trinidad & Tobago, 2009-2013 (%)

Hospital	N	*E. faecium*	*E. faecalis*	Body sites			
				Blood	SSTI	UGT	GIT
A*	19(42)	18	1	0	11	8	0
B	8(18)	6	2	1	2	5	0
C	15(33)	13	2	0	6	8	1
D	3(7)	1	2	0	0	3	0
E	0	0	0	0	0	0	0
Total	**45**	**38(84)**	**7(16)**	**1(2)**	**19(42)**	**24(53)**	**1(2)**

*N = total number of isolates; SSTI = skin and soft tissue infections; UGT= urogenital tract; GIT = gastrointestinal tract- the single infection from this site was a case of peritonitis and the isolate was recovered from a peritoneal fluid; There was a significant relation between the frequency of persons with or without VRE and the hospitals in which they were isolated as explained above. Hospital "E" had none while hospital "A" had the highest frequency of VRE infections. The difference in frequency of the total number of VRE isolates, the specific VRE species and the particular body sites these VRE isolates were recovered when compared with other parameters reveals a statistically significant result: * Hospital A, P < 0.05; ** E. faecium, P < 0.05; *** UGT, P < 0.05*

Table 2. Distribution of VRE infections among facilities of major hospitals in Trinidad & Tobago, 2009-2013

Hospital	N	*E. faecium*	*E. faecalis*	Hospital facility			
				Surgery	Medicine	ICU	Burns unit
A	19	8	1	6	7	6	0
B	8	6	2	3	3	2	0
C	15	13	2	8	6	0	1
D	3	1	2	2	0	1	0
E	0	0	0	0	0	0	0
Total	**45(100)**	**38(84)**	**7(16)**	**19(42)**	**16(36)**	**9(20)**	**1(2)**

N = total number of isolates; ICU = Intensive Care Unit

Table 3. Antibiotic susceptibility pattern of 1,141 clinical Enterococcus isolates recovered at major regional hospitals in Trinidad and Tobago, 2009-2013 (%)

Antibiotics	VSE; N=1096		VRE; N=45		p-value
	Sensitive	Resistant	Sensitive	Resistant	
Ampicillin	1085(99)	11(1)	8(18)	37(82)	0.000
Chloramphenicol	1085(99)	11(1)	25(56)	20(44)	0.000
Ciprofloxacin	1041(95)	55(5)	0	45(100)	0.000
Erythromycin	1085(99)	11(1)	0	45(100)	0.000
Gentamycin	1085(99)	11(1)	42(93)	3(7)	0.000
Levofloxacin	1085(99)	11(1)	4(9)	41(91)	0.000
Linezolid	1096(100)	0	45(100)	0	-
Penicillin	1074(98)	22(2)	8(18)	37(82)	0.000
Rifampin	932(85)	164(15)	0	45(100)	0.000
Streptomycin	1019(83)	77(17)	25(56)	20(44)	0.000
Tetracycline	701(64)	395(36)	28(62)	17(38)	.812
Vancomycin	1096(100)	0	0	45(100)	0.000

VSE = vancomycin susceptible enterococci isolates; N = number of isolates tested; VRE = vancomycin resistant enterococci isolates; A significant association between resistance to antibiotics and VRE status of the patients (p = 0.000) was found using Chi-square test in these antibiotics tested and the differences were statistically significant as indicated by the p value in the Table

4. CONCLUSION

There is low VRE infection in the country and this may be due to high infection control practices in the country despite the huge amount of broad spectrum antibiotics consumption. The few VRE infection is associated with underlying diseases such as diabetes and hypertension. With the excellent results of using the chromogenic agar, this media can be used in VRE surveillance and should facilitate in VRE infection control practices. Linezolid still appears to be the drug of choice for treating multidrug resistant VRE infections because of its perfect susceptibility.

To combat any progressive VRE prevalence trend in the hospitals in Trinidad & Tobago, a regular surveillance of hospital associated infections, effective screening monitoring of antibiotic susceptibility pattern and formulation of definite antibiotic policy will continue to be helpful. However, use of molecular studies to monitor the epidemiology of VRE in these hospitals in the country is also highly recommended.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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