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Vancomycin-Resistant Enterococci Colonization among Hospitalized Patients and Associated Risk Factors in Trinidad and Tobago

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Abstract

High-density Vancomycin-resistant Enterococci (VRE) colonization of the patient gastrointestinal tract (GIT) facilitates the transmission of multi drug resistant enterococci in hospital wards through fecal contamination. We performed a survey on hospitalized patients to determine VRE fecal colonization including the risk factors for such using univariate and multivariate analysis. We analyzed 2,066 rectal swabs from three regional tertiary hospitals in the country. Standard laboratory and molecular techniques including loop mediated amplification method (LAMP), Polymerase Chain Reaction (PCR) and pulse field gel electrophoresis (PFGE) were used to detect VRE, virulent genes (esp and hyl) and the clonal relatedness of VRE isolates. VRE fecal colonization was documented in 6.2% (129/2,066) screened patients. The VRE colonization isolates were mainly *E. faecium* and *E. faecalis* and were all (100%) resistant to ciprofloxacin, erythromycin, levofloxacin, rifampin and vancomycin but 100% susceptible to daptomycin, teicoplanin and linezolid respectively. Prior hospitalization and antibiotic use, diabetes mellitus disease, previous exposure to vancomycin, cephalosporins and quinolones use by univariate analysis were significantly associated with colonization. All vancomycin resistant E. faecium were vanA genotype while the *E. faecalis* were vanB genotype; and all possessed the enterococci surface protein (esp) gene. Molecular finger printing identified one clone of *E. faecalis* and five distinct clones for *E. faecium*.

VRE colonization in hospitalized patients, if compared with rates from some other countries is relatively low in Trinidad and Tobago. Molecular analyses suggest existence of inter and intra hospital clonal spread of *E. faecium* with predominant vanA gene and a single clonal spread of vanB *E. faecalis* strains which carry a high rate of esp genes.

Keywords: *VRE Colonization; Risk Factors; LAMP; PCR; PFGE; Trinidad & Tobago*

Abbreviations

VRE: Vancomycin Resistant Enterococci; PCR: Polymerase Chain Reactions; PFGE: Pulsed-Field Gel Electrophoresis; LAMP: Loop-Mediated Amplification Method

Introduction

Enterococci genus has been reported to consist of 54 species [1]. Although ubiquitous in nature, yet in humans, the gastrointestinal tract (GIT) is regarded as the largest reservoir of the species [2]. *Enterococcus faecalis* and *Enterococcus faecium* are the two species of Enterococci that cause the vast majority of hospital-acquired enterococci infections in humans [3].

Environmental host factors, most notably exposure to antimicrobial agents, is believed to favor an increase in colonization density of enterococci in the GIT of hospitalized patients [4,5]. This high-density colonization of the patient GIT facilitates the transmission of multi drug resistant strains among hospital ward through fecal contamination [6].

The first vancomycin resistant Enterococci (VRE) case was reported in United Kingdom in 1988 [7] and in the past three decades Enterococci species now pose great concerns to healthcare practitioners worldwide due to their increasing trend of antimicrobial resistance (notably VRE) and great adaptability in hospital environments [8]. In health care facilities, patients undergoing transplants or with underlying diseases, such as diabetes or renal failure, and patients with long-term catheter usage, are at higher risk of developing infections caused by multi-drug resistant (MDR) enterococci [6]. Global spread of VRE in several countries has been reported [9]. Also in Trinidad and Tobago, the prevalence of VRE infections have recently been reported [10], but not its colonization.

VRE colonization may not result in symptoms but could last for long periods and serve as a reservoir for its transmission to other patients. Within hospitals, widespread colonization with VRE may occur with a comparatively small number of documented infections. Geographical differences in rate of VRE colonization have been reported in several countries. For example, in Greece VRE colonization rate have ranged from 1.2% in 1999 to 34.9% in 2003 [11]. Changing trends of VRE have also occurred in French hospitals [12], while the rate is 17.5% in a tertiary hospital in Australia [13]. Colonization of VRE can lead to infections that prolong hospital stay, increase the cost of care and increase morbidity and mortality in critically ill patients [14]. Our study was designed to investigate the prevalence of VRE rectal colonization, risk factors and clonal relationships of the species in hospitalized patients in Trinidad & Tobago.

Materials and Methods

Study design and specimen collection

This cross-sectional and descriptive study was carried out at three of the five regional hospitals in Trinidad and Tobago from March 2013 to February 2014. Each hospital (designated as A, B or C) is a 400 - 500 bed facility serving about 500,000 - 600, 000 of the country's adult population. The hospitals provide general services as well as broad range of specialists care. On each chosen day, at the selected wards (Surgical, Medical, Intensive Care Unit and Burns unit – areas temporarily used for patients with burns) and hospital, rectal swab was taken from all hospitalized patients after obtaining their written or verbal consent. Designated registered nurses in each ward took the rectal swabs. This process was independent of time of patient's admission or duration of hospitalization. Patient's basic demographic and clinical information were retrieved from the medical records on a standard form. Data extracted included age, gender, risk factors such as comorbid health condition, prolonged hospitalization, type and duration of antibiotic use, and presence of invasive device.

Exclusion criteria

Patients were excluded from the study if they (a) had been confirmed to have any form of VRE infection - as previously defined [10,15]; (b) refused to participate; (c) are outpatients as well as patients on day care management; and (d) if performing rectal swab was contraindicated. Each day and during the study period, all collected rectal swabs from each study site were transported in tubes (Copan Diagnostics Inc., New York, NY, USA) to microbiology laboratory at the Dept. of Paraclinical Sciences of the University of the West Indies, St. Augustine Campus for bacterial culture and analysis.

Definitions

Colonized patient was defined as the isolation of VRE from rectal swabs in a patient who had no evidence of infection associated with enterococci – clinical or microbiological. The colonized individual is also the case patient. A control patient was an individual from whom a recovery of vancomycin sensitive enterococci (VSE) was made. For each colonized patient, two control patients were selected as controls among all individuals surveyed in a 1: 2 ratio for analysis.

Enterococci species and Vancomycin-resistance detection – Microbiology analysis

The rectal samples were processed using standard microbiological methods [16] to detect and isolate Enterococci species. In order to confirm the enterococci species within the isolates, bile esculin azide agar was used. Further testing was done in parallel through the Microscan and biochemical testing to distinguish the less common species. Vancomycin resistance screening was down by streaking the enterococci isolate on brain-heart infusion agar (DIFCO) and Bile esculin azide agar (BEAA, Oxoid) that contained 6 mg/l of vancomycin.

This was incubated in ambient air for 18 - 24 hours at 35^0C. Evidence of growth suggested that the bacterial organisms were vancomycin-resistant. Chromogenic plate - ChromID VRE (C-ID) medium (bioMerieux, France) was used to confirm vancomycin resistance and species identification. As we have previously reported [10,15], the ChromID medium is able to discriminate between VREFm and VREFs due to the production of two different colony colors after 24 hours incubation.

Antimicrobial Susceptibility testing

Antimicrobial susceptibility of all confirmed VRE isolates were determined by Microscan and disc diffusion methods, and results interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2014) guidelines [17].

All tests were validated using quality control strains. Positive controls, Vancomycin resistant *E. faecium* - VRENFM ATCC 700221 (mauve) and Vancomycin resistant *E. faecalis* - VRENFS ATCC 51299 (green) and negative controls *Escherichia coli* ATCC 25922(no growth) and *E. faecalis* ATCC 29212 (no growth) were used as previously reported [10,15].

Multiplex Loop-mediated isothermal amplification (mLAMP) method

At McMaster University Hamilton, Canada, the isolates presumptively identified as VRE on Dalynn Colorex VRE plates (n = 129) were analyzed to detect vanA and vanB genes using multiplex Loop-mediated isothermal amplification (mLAMP) methods as previously described [18]. The LAMP products were detected using 25 µm calcium (sigma) in 0.5 mM MnCl2 as described in literature [19] where vanA and vanB genotypes were identified based on the time to amplification reading on Genie® II (OptiGene, UK) as compared to the controls.

Detection of esp and hyl genes by PCR

The presence of esp and hyl genes in all VRE isolates was detected as described by Vankerckhoven V., *et al*. [20] as we previously reported [10,15].

Pulsed-field gel electrophoresis (PFGE)

Genomic DNA was prepared with some modifications as described by Murray et al and Turabelidze., et al. [21, 22]. Briefly, cell suspensions were mixed with an equal volume of low melting point agarose. Lysis of the cells by mutanolysin 50 IU/mL, lysozyme 2.5 mg/mL, followed by incubation with a proteinase K 1.5 mg/mL. The duration and temperature of lysis was 30 minutes at 37oC. DNA digestion with Sma1 (40 IU/disk) was for 6 hours at 25oC. The DNA were placed in agarose plugs after digestion and using a contour-clamped homogenous electric field apparatus (CHEF DRIII, Bio-Rad Laboratories, Hercules, CA, USA) the pulsed-field gel electrophoresis (PFGE) was performed. Gel images were captured on the Gel Doc imaging system using Quality One Software version 4.4.1 (Bio-Rad Laboratories, Hercules, CA, USA). The resulting band patterns were analyzed by visual inspection according to previously established criteria [23]. Gel analysis was performed using Bionumerics -version 3.5 (Applied Maths, Austin TX, USA) and Cluster analysis was achieved using DICE and UPGMA.

Statistical Analysis

The SPSS software (version 20) was used for data analysis. Qualitative variables were compared using Pearson's Chi-square test or Fisher's exact test and quantitative variables were compared by Mann-Whitney U-test. Univariate analysis of risk factors for colonization and acquisition was calculated. Odds ratios and the corresponding 95% confidence intervals were calculated using results obtained for the maximum likelihood estimates. Differences were statistically considered significant at $p < 0.05$.

Ethical Considerations

The Ethics Committee of The University of the West Indies, St. Augustine, Trinidad and Tobago approved the study protocol. Each hospital authority granted permissions to carry out the study at their facility.

Results

Two thousand and sixty-six (2,066) rectal swabs from eligible patients were processed and all produced Enterococci species. While 93.8% (1937/2066) of the patients produced enterococci species that were vancomycin sensitive, only 6.2% (129/2,066) were VRE colonized. Majority (90%, 116/129) of these VRE were *E. faecium* and the rest (10%, 13/129) were *E. faecalis*. The mean age of patients colonized with VRE (cases) was 59.5 years (range 20 - 90 years), 60 (40%) females. The vancomycin sensitive enterococci were distributed as follows *E. faecalis* 89% (1724/1937), *E. faecium* 8.8% (170/1937), and other enterococci species 2.2% (43/1937, including *E. gallinarum, E. casseliflavius, E. durans, E. avium*).

Distribution of VRE colonization were almost even at the three hospitals. At "A" hospital, 40 VRE isolates were recovered comprising 37 *E. faecium* and three *E. faecalis*; "B" hospital had 51 comprising 44 *E. faecium* and seven *E. faecium* and at "C" 38 consisting of 35 *E. faecium* and three *E. faecalis.*

All the VRE colonization isolates were fully (100%) resistant to ciprofloxacin, erythromycin, levofloxacin, rifampin and vancomycin but were 100% susceptible to tigecycline, daptomycin and linezolid.

Univariate analysis of risk factors for VRE colonization are clearly expressed on Table 1. The results reveal that gender (p = 0.10) and invasive devices (p = 0.63) are not significantly associated with VRE colonization. However, underlying diseases such as diabetes mellitus diseases (p = 0.01), prior administration of antibiotics (p = 0.00), use of cephalosporins (p = 0.00), use of anti-anaerobic drugs (p < 0.01) and vancomycin use (p < 0.01) showed significant differences between VRE colonized and the non-colonized patients. All the VRE isolates that were *E. faecium* possessed vanA gene while all *E. faecalis* possessed vanB gene. The virulence factor esp gene was also detected in all (100%) of the VRE isolates, *E. faecium* (n = 116) and *E. faecalis* (n = 13). Only one isolate from Hospital "B" had the hyl gene (1/129) among the VRE isolates.

Variables	FQ (%) or Mean ± SD Cases (n=129)	Univariate analysis		*p*-value
		Controls (n=258)	OR (95% CI)	
Gender				
Male	52(40.3)	127(49.2)		0.10
Female	77(59.7)	131(50.8)		
Age				
0 – 9	0	0		
10 – 19	0	0		
20 – 29	1(0.8)	18(7.0)		
30 – 39	3(2.3)	42(16.2)	0.12(0.04-0.40)	<0.01
40 – 49	4(3.1)	31(12.0)	0.23(0.08-0.68)	0.01
50 – 59	24(18.6)	43(16.7)		0.51
60 – 69	57(44.2)	50(19.4)	3.29(2.07-5.24)	<0.01
70 – 79	36(27.9)	58(22.5)		0.24
80+	4(3.1)	16(6.2)		0.20
Hospital				
A	40(31.0)	55(21.3)	1.66(1.03-2.67)	0.04
B	51(39.6)	137(53.1)	0.63(0.41-0.97)	0.04

C	38(29.4)	66(25.6)		0.33
Hospitalization				
(>10 days but<180 days)	85(66)	72(28)	4.99(3.17-7.86)	<0.01
Wards				
Surgical	62(48.1)	82(31.8)	1.93(1.25-2.97)	<0.01
Medical	51(39.5)	121(46.9)		0.22
ICU	15(11.6)	34(13.2)		0.67
Temp Burn's unit	1(0.8)	21(8.1)	0.09(0.01-0.66)	0.02
Underlying disease				
Hypertension	29(22.5)	71(27.5)		0.29
Diabetes disease	12(9.3)	8(3.1)	3.21(1.28-8.05)	0.01
Liver disease	4(3.1)	12(4.7)		0.47
Urogenital disease	35(27.1)	85(32.9)		0.24
Respiratory disease	28(21.7)	42(16.3)		0.19
Hematology	7(5.4)	15(5.8)		0.88
Malignancy	6(4.7)	12(4.7)		1.00
Invasive device				
Present	8(6.2)	13(5.0)		0.63
Antimicrobial use				
Use in <6 months	85(66)	98(38)	3.15(2.03-4.91)	<0.01
Use of one or more and type				
Vancomycin	32(24.8)	8(3.1)	10.31(4.59-23.16)	<0.01
Pip/Tazobactam	44(34.1)	23(9)	5.29(3.02-9.28)	<0.01
Metronidazole	5(3.9)	21(8)		0.12
Amox/Clavulanic acid	78(60.4)	62(24	4.83(3.07-7.61)	<0.01
Cefuroxime	58(45)	34(13)	5.38(3.26-8.88)	<0.01
Ceftriaxone	42(32.6)	18(7)	6.44(3.52-1.78)	<0.01
Carbapenems	73(56.6)	39(15.1)	4.73(2.91-7.69)	<0.01
Erythromycin	9(7)	5(2)	3.80(1.24-1.57)	0.02
Quinolones				
(Cip & Levo)	34(26.4)	15(6)	5.80(3.02-1.13)	<0.01
Gentamicin	15(11.6)	12(5)	2.70(1.22-5.95)	0.01

Table: *Comparison by univariate analysis of variables of patients with or without vancomycin-resistant enterococci (VRE) rectal colonization in hospitals in Trinidad & Tobago, March 2013 – February 2014.*

FQ = Frequency, SD = standard deviation, OR = Odds ratio, CI = Confidence interval, ICU = intensive care unit, Temp = Temporary, Pip/Tazo = piperacillin/tazobactam, Amox/Clav = amoxicillin/clavulanic acid, Carbapenems – types used include ertapenem, imipenem and meropenem, Cip and Levo = Ciprofloxacin and levofloxacin

Analysis of PFGE patterns of *E. faecium* of the VRE isolates revealed the presence of five distinct clones. Previously established criteria [23] and Bionumerics software (Applied Maths, Austin TX, USA) cluster analysis showed that all *E. faecalis* VRE isolates were identical or closely related. The DICE correlation coefficient and a dendrogram produced via the unweighted pair group method with arithmetic mean clustering (UPGMA) demonstrated excellent percentages of similarity as depicted in Figures 1 and 2.

Figure 1: *Dendrogram showing E. faecium from VRE colonized patients in Trinidad and Tobago, March 2013 – February 2014. Isolates in lane 2, 4, 6 and 30 were from Hospital "A", which revealed varied bands. Isolates in lanes 90, 12, 14, 80 were from Hospital "B", and hospital "C" had its isolates in lanes 20, 22, 24, 82 and 93. The lanes marked λ are DNA markers. Isolates from the three different hospitals revealed varied band patterns.*

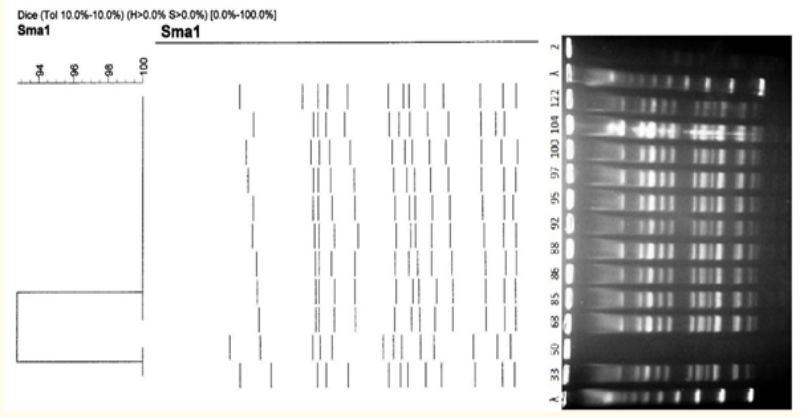

Figure 2: *Dendrogram showing E. faecalis from VRE colonized patients in Trinidad and Tobago, March 2013 – February 2014. All thirteen vancomycin-resistant E. faecalis in lanes marked 33, 63 to 100 and 122 had an identical PFGE pattern. The lanes marked λ are DNA markers. No DNA on lane 50.*

The PFGE analysis demonstrated five patterns among the vancomycin-resistant *E. faecium* isolates. Isolates from patients admitted at hospital "C" varied slightly in their banding patterns. The predominant patterns were one, four and five (PFGE-1, PFGE-4 and PFGE-5) clones. Clones one and four were present in all three hospitals "A", "B" and "C". Clone 5 consisted of isolates also recovered from patients in all the three hospitals "A", "B" and "C". All the 13 vancomycin resistant *E. faecalis* analyzed had an identical PFGE pattern indicating they belong to the same clone (Figure 2).

Discussion

Enterococci have become one of the most common causes of healthcare-associated infections with *E. faecalis* producing a greater portion of the infections while *E. faecium* is responsible for the rest [11-13]. High-density colonization of GIT in patients facilitates spread of multi drug resistant enterococci in hospital wards through fecal contamination [6]. This current survey was aimed at determining the level of colonization of VRE in some regional tertiary hospitals in Trinidad & Tobago. Our analysis revealed a prevalence (6.3%) of VRE colonization which is very much lower than what is reported in other countries (Australia, France and Greece) where rates were in the region of 17 to 34% [11-13]. The reason why VRE colonization rate is lower in Trinidad and Tobago than what was reported in other places is not fully understood. However, this low rate has to be maintained in the country. This means that the hospitals must consistently implement effective infection control measures that include active surveillance, antimicrobial stewardship, health care workers education and reliable microbiology laboratory services. These factors have been reported to adequately reduce the incidence of VRE colonization and spread of VRE healthcare associated infections [3,26].

This current analysis revealed that tigecycline and linezolid were most active agents against VRE isolates from colonized subjects but ineffective to ciprofloxacin, erythromycin, levofloxacin, rifampin and vancomycin. This is similar to the results we obtained with the VRE isolates associated with infections in the country [15]. A similar profile has also been reported elsewhere [11,26]. However, the information that teicoplanin, linezolid and daptomycin are still most active against these VRE isolates from colonized individuals is an important finding in the country because it means that there are still options available for use of these class of antimicrobial agents. The finding is also an important factor that should drive the plan or policy to institute antimicrobial stewardship, a recognized effective infection control measures, as this is lacking in the country.

Analysis of risk factors for VRE colonization in hospitalized patients are very crucial in understanding its epidemiology. In several studies and different settings VRE colonization have played key roles in impacting patients critically ill in the intensive care patients (ICU) or Burns unit etc. [6,14,11]. Prior use of antibiotics including cephalosporins and quinolones as already reported in literature, can lead to the depletion of Gram- negatives, reduce production of the antimicrobial peptide REGIIIg by Paneth cells, and this may promote the over growth of VRE [14,26,27].

Findings in our analysis were not different either since factors such as age, surgical ward and Burns unit, antimicrobial use within the last 6 months, and use of certain antimicrobial agents such as vancomycin, quinolones, cephalosporin and gentamicin or diabetes mellitus were significantly associated with VRE colonization in these hospitals. These facts have been established in our previous reports [10]. Quinolones use was also noted to be a risk factor for VRE colonization in the subjects. This should not a surprise since quinolones such as ciprofloxacin is sold over the counter without a physician's prescription. In the country, there appears to be no restriction and little or no stewardship on the other members of the group.

Predominant VRE genotype circulating in Trinidad and Tobago is *E. faecium* vanA and this is similar to vanA genotype that is predominant in the United States and Europe [15,28], but this is in contrast to vanB *E. faecium* in Australia [13]. In the current analysis of VRE isolates in colonized patients, the vanA *E. faecium* were also predominant. Therefore, from an epidemiological perspective in Trinidad and Tobago, measures should prioritize the targeting of cases of colonization due to VRE possessing vanA genes and perhaps less or little of vanB genes as the vanB genes were less or nonexistent.

In our analysis, we used a finger printing method, the pulsed-field gel electrophoresis [22,23] that may lack high reproducibility but have stood the test of time. Our molecular typing results revealed a dominant dissemination of vancomycin resistant *E. faecium* clone one and two in different wards of the same hospital, in different hospitals and in different cities or geographical areas in the country. The isolates in this analysis also were polyclonal with two major clones. These suggests a highly diverse population of hospital acquired *E. faecium* strains that have spread not only within individual hospitals but also between hospitals at various geographic locations in the country. Dissemination of these strains and clones within our hospitals may also suggest poor infection control measures and not nec-

essarily antibiotic selection pressure as elucidated elsewhere [13]. Whereas there are no effective methods to decolonize VRE-positive patients, active screening to identify additional VRE-positive patients may help in reducing cases of VRE colonization, leading to fewer VRE infections, and reduced costs or length of hospital stay.

Conclusion

VRE fecal colonization is relatively low in Trinidad and Tobago if compared with rates in other countries. Several factors were observed to significantly be associated with VRE colonization including prior hospitalization and antibiotic use, diabetes mellitus disease, previous exposure to vancomycin, cephalosporins and quinolones use. Molecular analyses suggest an inter and intra hospital clonal spread of *E. faecium* with predominant vanA gene and a single clonal spread of vanB *E. faecalis* strains which carry a high rate of esp genes.

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Conflict of interest

None to declare.

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