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Molecular epidemiology of vancomycin resistant enterococci in a tertiary care hospital in Saudi Arabia

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ABSTRACT

Aim: Vancomycin-resistant enterococci (VRE) are a major cause of nosocomial infections with high mortality and morbidity. There is limited data on the molecular characterization of VRE in Saudi Arabia. This study was carried out to investigate the premise that a shift in VRE epidemiology is occurring in our setting.

Methods: *Enterococcus* species identification and susceptibility testing plus VRE phenotypic confirmation by vancomycin and teicoplanin E-test were carried out. Vancomycin resistance genes were detected by PCR. Strain typing was conducted using PFGE.

Results: Among the strains of *Enterococcus* spp. investigated in this study, 17 (4.5%) were VRE. With the exception of one isolate from rectal swab, all others were clinical specimens with blood being the commonest source (n = 11; 64.7%), followed by urine (n = 3; 17.6%). The 17 VRE isolates were *Enterococcus faecium* (n/N = 13/17) and *Enterococcus gallinarum* (n/N = 4/17). Among *E. faecium* isolates, *vanA*$^+$/*vanB*$^+$ (n/N = 8/13; 62%) exhibiting VanB phenotype were predominant. One of the five *vanA*$^+$ *E. faecium* isolates exhibited a VanB phenotype indicative of *van*A genotype-VanB phenotype incongruence. *E. gallinarum* isolates exhibited a Van C phenotype although two were *vanA*$^+$/*vanC1*$^+$. PFGE revealed a polyclonal distribution with eight pulsotypes.

Conclusion: These findings indicate an evolving VRE epidemiology with *van*A$^+$/*van*B$^+$ isolates and *van*A genotype-VanB phenotype incongruence isolates, which were previously described as colonizers, are now causing clinical infection.

1. Introduction

Vancomycin-resistant enterococci (VRE) were first reported in 1986 and have since emerged as one of the most important pathogens causing hospital-acquired infections, especially among patients in intensive care units [1–4]. VRE infections are associated with high mortality and morbidity with *Enterococcus faecium* now being the leading cause of nosocomial infections [5,6]. In the clinical setting, VRE-associated blood stream infections are of great concern as mortality rates can be as high as 40% [7]. Glycopeptide-resistant genotypes in enterococci include *vanA, vanB, vanC1/C2/C3, vanD, vanE, vanG, vanL, vanM and vanN*. The *vanA* gene cluster is located within transposon Tn*1546* and can be transferred through acquired resistance [1,8]. Both high-level resistance to vancomycin and teicoplanin is a characteristic of a VanA phenotype whereas VanB phenotype is characterized by inducible variable levels of vancomycin resistance but susceptibility to teicoplanin [1]. The VanC resistance phenotype which has been described in *Enterococcus casseliflavus* and *Enterococcus gallinarum*, is characterized by

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intrinsic, low-level resistance to vancomycin (MICs, 4–32 mg/ml) and teicoplanin susceptibility [1].

Although there are limited studies on molecular characterization of VRE isolates in Saudi Arabia, available literature suggests an evolving pattern of vancomycin resistant *E. faecium* in our setting. This includes the recent demonstration of *van*B phenotype-*van*A genotype incongruent isolates, identification of new type F Tn1546 transposon in isolates belonging to clonal cluster 17 and detection of novel hospital-associated VanB(2)-type teicoplanin-resistant strain [9–12]. This study was carried out to investigate the indication from previous studies that a shift in VRE epidemiology is occurring in our setting. The findings from this study adds to the limited body of scientific literature on VRE from the Arabian Gulf region and provide new data indicative of a shift in VRE epidemiology in Saudi Arabia as *vanA*$^+$/*vanB*$^+$ isolates and *van*A genotype-VanB phenotype incongruence isolates which were previously described as colonizers are now causing infections.

2. Methods

2.1. Bacterial isolates

This study was carried out in the Department of Microbiology, King Khalid University Hospital, King Saud University, Riyadh, Kingdom of Saudi Arabia, from January to December 2013.

All isolates of *Enterococcus* species obtained from VRE surveillance and clinical specimens were eligible for inclusion. The medical records of patients with VRE isolates were reviewed and demographic data as well as laboratory and clinical information were obtained. Ethical approval was provided by the Institutional Review Board of King Saud University College of Medicine and King Khalid University Hospital after review of the study protocol.

2.2. Identification and susceptibility testing

The *Enterococcus* species identification and susceptibility testing were performed using MicroScan WalkAway Pos Combo 28 Panel Kit (Dade, West Sacramento, CA). The confirmation of VRE was performed and interpreted using vancomycin and teicoplanin E-test according to CLSI recommendations. Briefly, minimum inhibitory concentrations were determined by the E-test (bioMerieux, Marcy-l'Etoile). The MIC was interpreted as the value at which the inhibition zone intercepted the scale on the E-test strip. The E-test method was applied according to the manufacturer's instructions. Bacterial suspensions were first adjusted to a turbidity equivalent of 0.5 McFarland standard using a calibrated turbidometer as recommended by the CLSI [13]. Mueller–Hinton agar (Oxoid, UK) was used for susceptibility testing and all test media were incubated at 35 °C in normal atmosphere for 24 h. The susceptibility test results were interpreted according to CLSI breakpoints for vancomycin and teicoplanin. For vancomycin break points followed were $\leq$4, 8–16, and $\geq$32 mg/L for susceptible, intermediate, and resistant strains, respectively and for teicoplanin $\leq$8, 16, and $\geq$32 mg/L for susceptible, intermediate, and resistant strains, respectively [13]. *Enterococcus faecalis* ATCC 29212 was included as a quality control strain. All isolates which were confirmed as VRE by phenotypic methods were stored in skimmed milk at −80 °C for molecular confirmation and typing.

2.3. Molecular characterization of van genes

Method described by Lemcke et al. was used for oligonucleotide sequencing and amplification of the *van* genes [14]. There are many genotypes of vancomycin resistance described in enterococci, however, we have attempted to identify the commonest ones, *i.e.*, *vanA*, *vanB* and *vanC* genotypes (*vanC1*/*C2*/*C3* gene).

Molecular typing was conducted using Pulsed Field Gel Electrophoresis (PFGE) as previously described and interpreted according to Tenover et al. [9,15,16].

3. Results

3.1. Prevalence of VRE isolates

From the 378 strains of *Enterococcus* spp. isolated from clinical samples during the study period, only 17 (4.5%) were confirmed to be VRE by phenotypic and genotypic testing. With the exception of one isolate from rectal swab, all others were deemed to be associated with infection with blood being the commonest source (n = 11; 64.7%), followed by urine (n = 3; 17.6%). Table 1.

3.2. Demographic characteristics

The patient's age ranged from 3 to 83 years (mean ± SD: 54 years ± 22.4) and 53% were female. The majority of the patients (n/N = 9/17; 53%) were in the intensive care unit and most of the them had a combination of various comorbidities/risk factors including malignancies, diabetes mellitus, steroid therapy and central line insertion (Table 1). The mortality rate was 64.7%.

3.3. Phenotypic and genotypic characteristics

The majority of the 17 VRE isolates were identified as *E. faecium* (n/N = 13/18; 76.4%) while the remaining four isolates were identified as *E. gallinarum*. Of the 13 *E. faecium* isolates, genotyping revealed that majority were *van*A$^+$/*van*B$^+$ (n/N = 8/13; 62%). All *van*A$^+$/*van*B$^+$ isolates exhibited a VanB phenotype with vancomycin the break resistance (MIC range: 32–128 μg/ml) and teicoplanin susceptibility (MIC range: 0.5–0.8 μg/ml). The remaining five *E. faecium* isolates harbored only the *van*A gene and four of these exhibited the VanA phenotype. However, one *van*A$^+$ isolate was sensitive to teicoplanin (MIC 0.8 μg/ml) indicative of a VanB phenotype and therefore considered as having *van*A genotype-VanB phenotype incongruence. Of the four *E. gallinarum* isolates, two were positive for *vanC*1 gene only while the other two were *vanA*$^+$/*vanC1*$^+$ but all exhibited a Van C phenotype (Table 2).

3.4. Molecular characterization

PFGE typing of these 17 VRE revealed 8 pulsotypes (A-H) based on 80% similarity (Fig. 1). Isolates were distributed in four major pulsotypes (A, C, E & I) and majority of isolates within each pulsotype had the same *van* genotype (Table 2). The *vanA*$^+$/*vanB*$^+$ isolates were grouped into four pulsotypes namely type C (n = 2), type D (n = 1), type E (n = 3) and type F (n = 1). Figure shows the PFGE patterns of the isolates.

4. Discussion

Although molecular characterization of vancomycin genotype in enterococcal isolates and VRE prevalence has been well reported in many parts of the world, there remains a paucity of data from Saudi Arabia. Of the three reported studies describing the *van* genotype of VRE isolates from Saudi Arabia, only one study has described the clinical parameters of patients in relation to the vancomycin genotype [9–11]. Therefore the findings in this study provide much needed information on the clinical characteristics of VRE positive patients as well as genotypic and phenotypic distribution of circulating isolates.

The prevalence of VRE in our hospital is low (4.5%) when