Vancomycin-resistant enterococci among clinical isolates from north-west Iran: identification of therapeutic surrogates

Enterococci rank among the three major pathogens isolated from the bloodstream, surgical sites and urinary tract infections. Studies from many countries, including Iran (Cetinkaya et al., 2000; Feizabadi et al., 2004), have shown that vancomycin-resistant enterococci (VRE) are formidable pathogens and a serious concern for both physicians and patients. Therefore, it is essential to explore alternative therapeutic options for treating patients with VRE infections. New antibiotics were recently licensed in the UK, Europe and the USA, including linezolid, daptomycin and quinupristin/dalfopristin (Lentino et al., 2008). However, the paucity of literature available on strains isolated from high-risk hospitalized patients in North and West Azerbaijan, Iran, prompted us to analyse vancomycin resistance in Enterococcus faecalis and Enterococcus faecium using phenotypic and molecular methods. To explore alternative treatments, the efficacies of the aforementioned antibiotics were evaluated prior to use.

This laboratory-based study was conducted using sequential isolates of enterococci obtained from different clinical specimens at university teaching hospitals in Tabriz (Imam Reza and Sina Hospitals) and Orumieh (Imam Khomeini Hospital), Iran, from April 2008 to June 2010. Multiplex PCR was performed on all isolates for the simultaneous detection of vanA, vanB, and genes encoding d-alanine-d-alanine ligases specific to E. faecalis (ddlE Faecalis) and E. faecium (ddlE Faecium), as described previously (Kariyama et al., 2000). Each PCR assay included vanA- and vanB-positive control strains (kindly provided by Dr M. Emaneini, Department of Microbiology, Tehran University of Medical Sciences) and a negative control, which contained all the reagents but not the template DNA.

The antimicrobial susceptibility of the strains was determined using the disc diffusion method, according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2006). The MICs of vancomycin and gentamicin were determined using the agar dilution method and Etest (bioMérieux) based on the CLSI (2006) guidelines and the manufacturer’s instructions, respectively. Susceptibility of the VRE strains to linezolid, daptomycin and quinupristin/dalfopristin was determined using the Etest. E. faecalis ATCC 29212 was used as the quality-control strain.

The data were analysed using the chi-squared test by the SPSS statistical software (version 18.0). A P-value <0.05 was considered statistically significant.

A total of 220 isolates were obtained from different wards. Most were isolated from intensive care units (n=43; 19.5 %), followed by nephrology (n=38; 17.3 %) and internal wards (n=27; 12.3 %). Urine was the most common source (85.5 %), followed by blood (7.7 %), body fluids (4.1 %), wounds (1.8 %) and intravenous catheters (0.9 %).

Of the isolated enterococci, 152 (69.1 %) were E. faecalis and 68 (30.9 %) were E. faecium.

Antibiotic susceptibility testing using disc diffusion revealed high resistance to fusidic acid (89.5 %), followed by rifampicin (85 %) and erythromycin (75 %), irrespective of species. Nearly 70 % of the isolates were also resistant to ciprofloxacin and penicillin. Furthermore, the isolates were resistant to teicoplanin (21.8 %), imipenem (31.4 %) and high-content (300 µg) streptomycin (45.9 %). Vancomycin resistance was observed in 20.5 % of the isolates. In vancomycin disc diffusion tests, intermediate resistance was observed in 63.8 % of E. faecalis and 7.4 % of E. faecium isolates, with 15 mm (20.6 %) or 16 mm (79.4 %) zone diameters, respectively.

The agar dilution method indicated that 45 (20.45 %) strains were vancomycin resistant. Among these, the MIC was 256 µg ml⁻¹ for 31 isolates and ≥512 µg ml⁻¹ for 13 isolates; a MIC of 8 µg ml⁻¹ was seen for only one isolate. In addition, 133 (60.45 %) strains showed high-level gentamicin resistance (MIC ≥512 µg ml⁻¹). The results of the agar dilution were confirmed by Etests.

VRE strains were tested for alternative therapeutic options using the Etest. These isolates were found to be susceptible to linezolid (MIC ≤0.05–2 µg ml⁻¹), VRE isolates also were sensitive to daptomycin, with the exception of an E. faecium strain (MIC ≥6 µg ml⁻¹).

The MIC of quinupristin/dalfopristin ranged from 0.75 to 32 µg ml⁻¹. Among the ten vancomycin-resistant E. faecalis strains, the MIC was ≤1.5 µg ml⁻¹ for one strain, whereas the remaining nine strains showed high resistance (MIC ≥16 µg ml⁻¹). Three vancomycin-resistant E. faecium strains also showed resistance to this agent (MICs ≥4, 8 and 32 µg ml⁻¹), while six strains showed intermediate resistance.

Among the 45 VRE strains with phenotypic resistance, 43 (8 E. faecalis and 35 E. faecium) carried vanA, whereas vanA and vanB were not detected in two E. faecalis strains. None of the vancomycin-susceptible isolates carried vanA. Surprisingly, vanB was detected in three vancomycin-sensitive isolates that produced 18–19 mm inhibition zones in the disc diffusion test and for which the MIC was ≤4 µg ml⁻¹ in the agar dilution test. These isolates were E. faecium from Tabriz (Fig. 1). Based on the phenotypic and genotypic analyses, we identified 48 VRE isolates.

The prevalence of VRE is obvious in developed countries, and the burden has been partially lifted; however, periodic evaluations of antibiotic susceptibility and
the early detection of VRE are a prerequisite in developing countries. In this study, the analysis of distribution of VRE species in the two cities revealed a higher frequency of VRE isolates from Tabriz (28.2%) than Orumieh (15.5%), although this difference was not statistically significant. The majority of enterococcal isolates were identified as \textit{E. faecalis}; however, most of the VRE were \textit{E. faecium} (77.8%); \(P<0.001\).

A decline in the prevalence of \textit{E. faecalis} (69.1% versus 90.5%) and an almost fivefold increase in the prevalence of \textit{E. faecium} (30.1% versus 5.84%) and VRE isolates (21.8% versus 4.38%) in the present investigation were remarkable compared with those in a previous report from Tabriz, Iran (Akhi \textit{et al.}, 2009). This is an alarming situation because the prevalence of VRE has increased almost fivefold over an 8 year time period.

In this study, antibiotic susceptibility testing by disc diffusion revealed 46.4% strains with intermediate vancomycin resistance; however, the vancomycin MICs were \(\leq 2 \mu g \text{ ml}^{-1}\) for these strains using the agar dilution and Etest methods, indicating that they were susceptible to this antibiotic. This implies that vancomycin susceptibility testing should be replaced by MIC determination, which has been shown to be more reliable (CLSI, 2006).

Most (90.4%) of our clinical isolates were resistant to at least three of the antibiotics tested, and antibiotic resistance was more common among \textit{E. faecium} isolates than \textit{E. faecalis}, which is in agreement with previous studies (Simonsen \textit{et al.}, 2003; Top \textit{et al.}, 2007). For example, ampicillin resistance was detected in 31.4% of the isolated enterococci, but nearly 93% of these were \textit{E. faecium} (\(P<0.001\)).

In this study, \textit{vanA} was the predominant vancomycin-resistance type, which is consistent with studies from Iran (Feizabadi \textit{et al.}, 2004; Emaneini \textit{et al.}, 2008) and other countries (Abele-Horn \textit{et al.}, 2006).

We detected one \textit{vanA}-positive strain (OEfm-71; isolated from a urine specimen) for which the vancomycin MIC was 8 \(\mu g \text{ ml}^{-1}\) that was susceptible to teicoplanin (MIC 1.5 \(\mu g \text{ ml}^{-1}\)) and thus showed the VanB\(^+\) phenotype but \textit{vanA} genotype. To the best of our knowledge, this is the first report of this type of clinical enterococcal isolate from north-west Iran. VanB\(^-\)\textit{vanA} enterococci were previously reported from south-east Asia (Eom \textit{et al.}, 2004).

All the VRE were found to be susceptible to linezolid and daptomycin, except one \textit{E. faecium} isolate that was resistant to daptomycin. These data are in agreement with previous reports (Hsueh \textit{et al.}, 2005).

An efflux pump that confers resistance to dalfopristin appears to be intrinsic in \textit{E. faecalis} (Eliopoulos, 2003). Accordingly, nine vancomycin-resistant \textit{E. faecalis} isolates showed resistance to quinupristin/dalfopristin. Unexpectedly, nine vancomycin-resistant \textit{E. faecium} strains also were non-susceptible to this agent. Quinupristin/dalfopristin is not yet used in our hospital settings because resistance prior to exposure remains a possibility that requires further investigation of the resistance mechanism.

A likely explanation is the application of antibiotics (for example, virginiamycin) as growth promoters in animal feed and the emergence of cross-resistance to quinupristin/dalfopristin. A study from Iran showed that more than 50% of meat samples contained detectable antibiotic residues (Tajick \& Shohreh, 2006).

In conclusion, our findings highlight the incremental emergence of VREs in our clinical setting. This necessitates mandatory testing of isolates for vancomycin resistance, by the phenotypic and genotypic methods alongside each other because some VRE strains (two \textit{vanA}- and \textit{vanB}-negative strains; three \textit{vanB}-positive strains) would have been overlooked otherwise.

Among the new therapeutic regimes, linezolid and daptomycin exhibited better \textit{in vitro} activity than quinupristin/dalfopristin against the VRE isolates. Nonetheless, careful and continuous monitoring of the effectiveness of these antibiotics will be required to detect any changes in their current status or occurrence of resistance to new agents.

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