ORIGINAL ARTICLE

Clonal spread of vancomycin resistance *Enterococcus faecalis* in an Iranian referral pediatrics center

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Key words

Enterococcus faecalis • Vancomycin-resistant • Genotyping

Summary

Vancomycin-resistant enterococci (VRE) represent as an immediate threat to public health. Since few active compounds are available for VRE infections, rapid identification of these isolates are essential. In the absence of any report on the genetic relatedness of Enterococcus faecalis especially Vancomycin-resistant E. faecalis (VREF) isolates in Iran, we undertook this study to characterize these isolates using random amplification of polymorphic DNA (RAPD–PCR) genotyping method. In this study, E. faecalis strains isolated from various samples collected from different wards of Children Medical Hospital (Tehran, Iran). These isolates were identified by standard laboratory procedures and tested for antimicrobial resistance to vancomycin and teicoplanin. The genetic similarity of the strains was investigated by amplification of the RAPD–PCR.

In our study among 91 E. faecalis isolates, 15 (16%) were identified as VREF. The similarity pattern built for E. faecalis isolates by RAPD–PCR, demonstrated the presence of four distinct clusters (A, B, C, D). It is of interest to note that 100% of VREF isolates belonged to Clusters A, indicating that there may have occurred horizontal transmission of the same strain between patients. In conclusion, rapid spread of VREF from a clonal origin calls for implementation of careful isolation and infection control measures. Therefore, environmental control by routine disinfection of patient area as well as screening of high risk patients and isolation of colonized patients should be imposed in order to diminish risk of acquiring nosocomial VRE.

Introduction

Enterococci, an important cause of clinical infections, have emerged as an increasingly important cause of nosocomial infections in the last decades, being now the third to fourth-most prevalent nosocomial pathogen worldwide [1]. The emergence and spread of resistance to vancomycin as well as other glycopeptide agents like teicoplanin among *Enterococcus* species greatly reduces the number of treatment options. In addition, spread of vancomycin-resistant enterococci (VRE) represents an immediate threat to public health [2, 3].

Since few active compounds are available for VRE infections [4], rapid identification of these isolates are essential for implementation of control measures in order to restrict the emerging trouble of these strains.

A variety of typing methods have been used to examine clonal relatedness among human VRE isolates [5] but little is known about the epidemiology of vancomycin-resistant *Enterococcus faecalis* (VREF) [6]. Comparisons with respect to the epidemiological concordance and the overlap in the data for random amplification of polymorphic DNA (RAPD) versus pulsed field gel electrophoresis (PFGE) suggested that RAPD analysis is well-suited for epidemiological typing of enterococci [7]. In our previous study, high frequency of VREF as high as 16% was reported among hospitalized patients at Children's Medical Center from August 2009 to June 2010. In the absence of any report on the genetic relatedness of *E. faecalis* especially vancomycin resistant *E. faecalis* (VREF) isolates in Iran, we undertook this study to characterize these isolates using RAPD-PCR genotyping method.

Methods

The case definition for inclusion in this study was admission to the Children's Medical Center hospital (CMC) between August 2009 and June 2010, and a culture positive for *E. faecalis* at least 48 hours after hospital admission. No attempt was made to differentiate carriage, colonization or clinical infection.

These isolates were identified using standard microbiology methods [8] . The Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility of *E. faecalis* isolates to vancomycin and teicoplanin according to the Clinical and Laboratory Standards Institute. Isolates with potential vancomycin and teicoplanin resistance by this method were confirmed by E-test method (AB Biodisk, Solna, Sweden) according to the manufacturer's specification [9].

DNA Extraction

DNA was extracted from VREF isolates using QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instruction**.**

Typing of *E. faecalis* isolates by RAPD-PCR

The genetic similarity of the strains was investigated by amplification of random amplified polymorphic DNA (RAPD). RAPD-PCR analysis was performed using primer (5'- ACG CGC CCT-3'). The reaction mixture (final volume 25 µl) consisted of 19 µl H_2O, 2.5 µl 10X reaction buffer, 0.75 µl $MgCl_2$ (100 mM), 0.6 µl dNTP mixtures (10 mM), 0.2 µl Taq-polymerase (5u/ µl), 1 µl primer (10mM), 1µl of the DNA. RAPD reactions were performed in a thermocycler by using the following temperature profile: initial denaturation at 94°C for 5 min followed by 36°C, 5 min; 72 °C, 5 min; repeat it 4 time then 94°C, 1 min; 36 °C, 1 min; 72°C, 2 min for 30 cycles [10]. Amplification products were analyzed by electrophoresis in 2% agarose gel with TBE buffer and stained with ethidium bromide 0.1%.

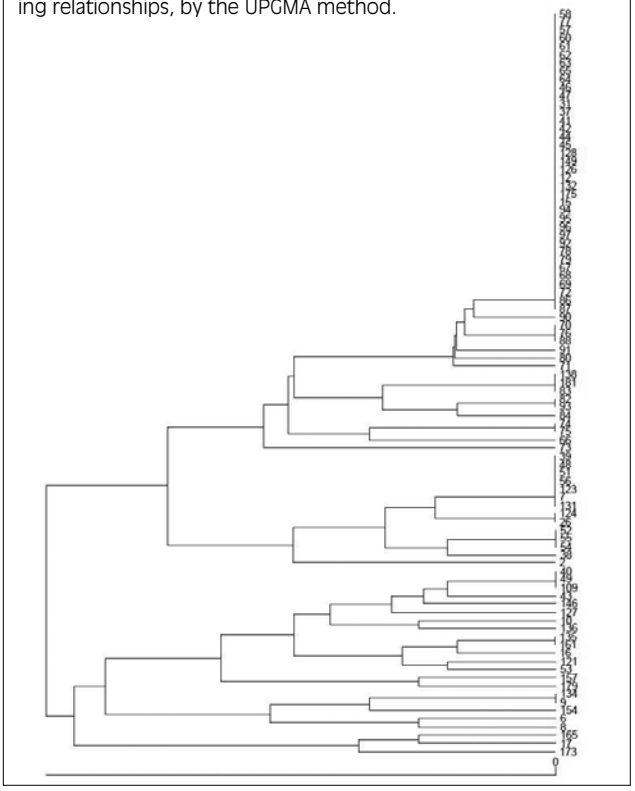
Similarity analysis

The electrophoretic profiles were scored according to the presence (1) or absence (0) of a particular band, Similarity of all pair-wise combinations of the numerical profiles was determined by Dice's coefficient and clustered by unweighted pair-group analysis using arithmetical averages (UPGMA) using the Freetree program (0.9.1.50 version). Phylogenetic trees were obtained and visualized using the TreeView program (1.30 version).

Results

During the study period, 91 children aged < 1 month to 12 years old were colonised or infected with *E. faecalis*.
Patients were hospitalized for a mean of 24 days (range 1-105 days). Twenty-one of the patients were hospitalized in urology ward, whereas the others were distributed in infectious ward (n = 14), surgical ward (n = 12), gastroenterology ward (n = 11), nephrology ward (n = 11), Neonatal Intensive Care Unit (NICU) (n = 7), Cardiovascular Intensive Care Unit (CICU) (n = 7), oncology (n = 4) and Pediatric Intensive Care Unit (PICU) (n = 4). Fifteen isolates (16%) were identified as VREF. The MICs to vancomycin and teicoplanin were ≥ 32 µg/ml in these isolates. Fingerprints of DNA fragments by RAPD-PCR were recorded. A single dendrogram of similarity was constructed for all isolates studied. Figure 1 demonstrated the similarity relationships between strains in 4 distinct clusters (A-D). Cluster A contained the majority of the isolates (n = 37, 41%), while cluster B contained 17 (19%), cluster C contained 14 (15%), and cluster D contained 23 (25%) of the isolates.
Surprisingly, all VREF isolates belonged to cluster A. Patients that were colonized/infected with VREF strains were identified in gastroenterology ward (n= 4), infectious ward (n = 3), urology ward (n = 3), CICU (n = 3) and NICU (n = 2).

Fig. 1. Phylogenetic tree among 91 *E. faecalis* isolates, constructed by Free Tree "software" and Distance Coefficient (DICE), showing relationships, by the UPGMA method.



Discussion

In the current study, we have investigated the genotypic characteristics of VREF isolates by RAPD method. Although the epidemiology of *E. faecium* is well described, little is known about the epidemiology of vancomycin-resistant isolates of *E. faecalis* [6]. Data on the prevalence of these strains are scarce in Iran, and to the best of our knowledge there is no published information concerning the genetic relatedness of VREF isolates.
The similarity phylogram built for theses strains, demonstrate the presence of four distinct clusters (A, B, C, D). In these clusters, some isolates were allocated in groups of higher or lower similarity and most strains were discriminated. Clusters A contained the majority of the isolates (n = 37, 41%) that were identical and forming one real clone. It is of interest to note that 100% of VREF isolates belongs to cluster A, indicating that there may have occurred horizontal transmission of the same strain between these patients. In our hospital, patients may be admitted briefly to a ward that does not match the medical care needed due to lack of bed on the appropriate wards. In addition, they might move to other wards when the level of care required changes. Therefore, patient movements may result in the dissemination of bacteria around the hospital, especially if proper infection control procedures are not instituted.
Predominance of one clone suggests frequent transfer of patients from one ward to another ward. In addition both person-to-person transmission and selective antibiotic

pressure can be probable mode of spread [10, 11]. Patients can remain colonized for prolonged period of time from months to years. In contrast to gram-negative bacteria, VRE broadly contaminates the environment and can remain for a long time; therefore, medical devices are commonly positive on wards with VRE patients [12] and patients might acquire nosocomial VRE from heavily contaminated environment [3].

In cluster B, 10 of 17 isolates had 100% similarity, forming four real clones. In cluster C, most of strains were similar, forming three real clones. Cluster D with 23 isolates had three real clones, which was formed by seven isolates and presented the biggest genetic distance among all. Due to the genetic distance observed especially in cluster B and D, it is probable that the non-VREF isolates in this study had diverse origins that may have been due to constant cross transmission of enterococal strains.

Our results are in agreement with those of studies of hospital-acquired VRE which indicated clonal dissemination as a major mechanism for the spread of isolates [6, 13, 14]. Typing of VREF isolates in the three studied hospitals in Spain by PFGE exhibited indistinguishable or closely related patterns [15].

Analysis of our results similar to other studies indicate *vanA* gene as common determinant for glycopeptide resistance in *Enterococcus* spp. [16-18] and clonal dissemination of VRE [19]. Identification of the source and route of dissemination of VRE is helpful for controlling outbreaks.

Clonal dissemination of *vanA* gene encoded VRE have been reported from other parts of world [19-22]. A study from Argentina have shown predominance of one epidemic clone carrying *vanA* gene [20]. Another molecular typing of VRE strains from UK revealed cross transmission of predominance VRE pulsotype with 92% containing *vanA* gene [22].

In conclusion, rapid spread of VREF from a clonal origin calls for implementation of careful isolation and infection control measures. Therefore, environmental control by routine disinfection of patient area as well as screening of high risk patients and isolation of colonized patients should be imposed in order to diminish risk of acquiring nosocomial VRE.

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■ Received on October 6, 2012. Accepted on December 6, 2012.

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