Introduction. Enterococci have emerged as important nosocomial pathogens in the last few decades. Nowadays, few antimicrobials are active against enterococcal species and intrinsic resistance to several clinically used antimicrobials agents, making them important nosocomial pathogens [1]. Enterococcus faecalis can acquire resistance via various forms of conjugation and spread these genes through conjugative transposons, plerhone-responsive plasmids, or broad-host-range plasmids [1]. The increasing rate of vancomycin resistance Enterococcus (VRE) has emerged as the global concern [2]. The prevalence of VRE varies widely according to outbreak situations [3]. In nosocomial settings, Enterococcus faecium accounts for majority of VRE infections and E. faecalis constitutes only 2-20% of VRE isolates, depending on geographical location and healthcare facility [4].

A little is known about the epidemiology of vancomycin resistant E. faecalis (VREF) [5, 6]. PCR-based screening can rapidly detects the presence of VRE and help early prevention of VRE spread [3]. The screening of critically ill patients at high risk of VRE colonization, is recommended to prevent and control of VRE transmission [3]. Currently, eight phenotypic variants of acquired glycopeptide resistance in enterococci have been reported (VanA, VanB, VanD, VanE, VanG, VanL, VanM, and VanN), with one type of intrinsic resistance (VanC) which belongs to Enterococcus gallinarum and Enterococcus casseliflavus [7]. The vanA and vanB phenotypes confer high-level vancomycin resistance (MIC > 64 μg/mL) and is more prevalent among other phenotypes [8].

Data on the prevalence of VREF are scarce in Iran [5]. The aim of this study was to investigate the frequency of VREF and detection of two prevalent resistance genes (vanA, vanB) in pediatric population in an Iranian referral pediatric Hospital.

Original article

High frequency of vancomycin resistant Enterococcus faecalis in children: an alarming concern

F. SABOUNI1, Z. MOVAHEDI2, S. MAHMOUDI1, B. POURAKBARI1, S. KESHAVARZ VALIAN4, S. MAMISHI1 3
1 Department of Infectious Diseases, Pediatrics Center of Excellence, Children’s Medical Center, Tehran University of Medical Sciences, Tehran, Iran; 2 Department of Pediatric Infectious Diseases, School of Medicine, Qom University of Medical Sciences, Qom, Iran; 3 Pediatric Infectious Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran; 4 School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Keywords
E. faecalis • Vancomycin resistant • Children

Results. Seventy-five (42%) of patients were male and 105 (58%) were female. Mean age of patients was 34.74 months. Cephalosporin resistance was found in majority of E. faecalis isolates (98.7% to ceftazidim, 95% to cefixime, 93.3% to ceftriaxone, and 89.4% to cefotaxime). Most of the isolated were susceptible to cefepime (91.7%). In addition, high level of erythromycin and clindamycin resistance was reported (93.4% and 91.2%). There were no linezolid-resistant E. faecalis among all isolates. Teicoplanin resistance was observed in 13.8% of E. faecalis (n = 25). Minimum Inhibitory concentration (MIC) ≥ 32 μg/ml for vancomycin was found in 29 isolates (16%) and vanA gene was detected in 21 (72%) VREF strains, while vanB gene was not detected in any of these isolates. The mortality rate of all cases was 3.4%.

Conclusions. This study revealed high rate of vancomycin resistance in E. faecalis strains. Therefore, periodic surveillance of antibacterial susceptibilities is highly recommended to detect emerging resistance.
Methods

Study design
We performed a study of patients in whom E. faecalis were detected in clinical samples between January 2013, and December 2013, at Children Medical Center Hospital, tertiary care and teaching hospital in Tehran, Iran. A total of 180 E. faecalis isolates were analyzed. All isolates were identified using standard microbiology methods [9].

Microbiological methods
Antimicrobial testing was performed by Kirby-Bauer disk diffusion method to detect resistance to gentamicin, amikacin, ceftriaxone, cefotaxime, cefazidim, cefixime, piperacillin/tazobactam, cefepime, trimethoprim/sulfamethoxazole, erythromycin, clindamycin, linezolid according to Clinical Laboratories Standards Institute (CLSI) [10].

Antimicrobial resistance to vancomycin and teicoplanin was detected by measuring minimum inhibitory concentrations using E-test. Vancomycin and teicoplanin sensitivity were evaluated by the E-test (AB BIODISK, Solna, Sweden) method. The results were read after 24h incubation at 37°C. MIC of ≤ 4 (μg/mL) was considered as susceptibility, MIC 8 to 16 and ≥ 32 were considered as intermediate and resistant, respectively [10].

DNA Extraction
DNA was extracted from VREF isolates using QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer’s instruction. Polymerase chain reaction (PCR) amplification of vanA and vanB genes

The PCR assay was performed in a total volume of 25 μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), and 0.5 U of Taq DNA polymerase with the following primer F:5′-CATGAAATGAAATAAAAAGTTGCAATA-3′ and R: 5′-CCCTTTAACGCTAATACGATCAA-3′ for amplification vanA gene [11]. DNA amplification was carried out with the following thermal cycling profile: initial denaturation at 94°C for 5 min, 30 cycles of amplification (denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min), and a final extension at 72°C for 10 min. E. faecium BM4147 (vanA-positive) and E. faecalis V583 (vanB-positive) were used as positive controls. PCR products were analyzed on a 1% agarose gel with 0.5 × Tris-borate-EDTA buffer. A 100-bp DNA ladder (New England Biolabs, Beverly, Mass.) was used as the molecular size marker. The gels were stained with gel red and photographed under UV light.

Results
In this study 180 samples of E. faecalis were obtained from children aged 1 month to 12 years old. Seventy-five (42%) of patients were male and 105 (58%) were female. Mean age of patients was 34.74 months. Thirty eight of the patients were hospitalized in urology ward, whereas the others were distributed in gastroenterology ward (n = 19), nephrology ward (n = 20), infectious ward (n = 18), emergency ward (n = 40), hematology ward (n = 4), NICU (n = 17), PICU (n = 8), surgery ward (n = 7), cardiology ward (n = 6) and rheumatology ward (n = 3). The mortality rate of all cases was 3.4% and 10% of patients with VREF infection died.

Antimicrobial susceptibility was determined for a variety of antibiotics (Tab. 1). Cephalosporin resistance was found in majority of E. faecalis isolates (98.7% to cefazidim, 95% to ceftriaxone, 93.3% to cefotaxime, and 89.4% to cefotaxime). Most of the isolated were susceptible to cefepime (91.7%). In addition, high level of erythromycin and clindamycin resistance was reported (93.4% and 91.2%). More than 90% of isolated were resistant to ceftriaxone, cefotaxime, cefixime, cefazidim and clindamycin. There was no linezolid-resistant E. faecalis among all isolates. Teicoplanin resistance was observed in 13.8% of E. faecalis (n = 25). MIC ≥ 32 μg/ml for vancomycin was found in 29 isolates (16%). Among resistant group, 12(41.4%) were male and 17 (58.6%) cases were female with a mean age of 27.9 months. Ten patients with VREF were hospitalized in urology ward, the others were distributed in infectious ward (n = 3), CICU (n = 8), gastroenterology ward (n = 6) and emergency (n = 2). There were no significant differences between the age, sex and wards of the patients with VREF or vancomycin susceptible isolates (P value ≥ 0.05).

Antimicrobial susceptibility of VREF isolates was shown in Table 2. Among all patients with VREF isolates, 117 (65%) and 20 (69%) cases had underlying disease, respectively.

Tab. 1. Antibiotic susceptibility in all samples by disk diffusion method.

| Antibiotics                  | Resistant (N, %) | Sensitive (N, %) |
|-----------------------------|-----------------|-----------------|
| Gentamycin                  | 154 (74.4)      | 46 (25.6)       |
| Amikacin                    | 110 (61.1)      | 70 (38.9)       |
| Cefotaxime                  | 161 (89.4)      | 19 (10.6)       |
| Ceftriaxone                 | 171 (95)        | 9 (5)           |
| Cefazidim                   | 177 (98.7)      | 13 (1.3)        |
| Piperacillin/ tazobactam    | 100 (55)        | 80 (45)         |
| Cefepime                    | 15 (8.2)        | 165 (91.7)      |
| Trimethoprim- sulfamethoxazole | 159 (77.5)     | 41 (22.5)       |
| Erythromycin                | 168 (93.4)      | 12 (6.6)        |
| Clindamycin                 | 164 (91.2)      | 16 (8.8)        |
| Linezolid                   | 0 (0)           | 180 (100)       |
Amplification of vanA, vanB targets produced distinct bands corresponding to their respective molecular sizes (1,030 bp for vanA and 433 bp for vanB). Among VREF, vanA gene was detected in 21 (72%) isolates, while vanB gene was not detected in any of these isolates. vanA gene was found in 13 girls (62%) and 8 boys (38%) (p value ≥ 0.05).

Discussion

The emergence of VRE as an important nosocomial pathogen is due to its propensity for colonization of the gastrointestinal (GI) tract, persistence in hospital environments, genome plasticity, mobile genetic elements, and increased mortality [12]. The epidemiology of VRE varies from one hospital to another, which depends on several factors including the hospital size, patient population, antibiotic usage patterns and geographic location. According to earlier reports, risk factors that increase the likelihood of VRE infection or colonization can be due to host factors, hospital-specific factors and antibiotic usage [4].

The antimicrobial susceptibility of Enterococcus spp. showed higher resistant pattern to a majority of antibiotics compare to our previous hospital report in 1996-2000 [13]. Analysis of our results similar to other studies indicate vanA gene as common determinant for glycopeptide resistance in Enterococcus spp. [14-17]. VanA is responsible for most of the human cases of VRE around the world [7]. In addition, the vanA operon can easily be transferred through acquired resistance [18]. Our previous study demonstrated that clonal dissemination was a major mechanism of the spread of these isolates [5]. The majority of E. faecalis colonization occurs in the gastrointestinal tract infection (GI) and to a lesser extent on the skin, in the genitourinary tract, and in the oral cavity [7, 8, 19]. When GI colonization with VRE occurs, it can persist for months to years. In addition, and efforts for decolonization are typically transitory and recurrence of VRE may occur days or weeks later [7, 19]. The common pathway of nosocomial VRE acquisition might be via person-to-person contact or exposure to contaminated objects. Health care workers’ hands are the most consistent source of transmission and it has been reported that VRE can persist for up to 60 minutes on hands and as long as 4 months on surfaces [7, 20]. Therefore, healthcare facilities need a comprehensive infection control program in order to decrease the transmission of VRE among patients.

The emergence of VRE is also due to the inappropriate use of cephalosporin as well as poor hospital infection control measures [21]. Long duration of hospital stay and high rate of antibiotics treatment are the most frequently reported risk factor for multi-resistance Enterococci colonization and infection.

Another concern about VREF is the possible transfer of vanA from E. faecalis to S. aureus [22]. E. faecalis vanA-carrying plasmid was found to encode a response to sex pheromone and it raises concern about the potential uptake of vanA from Enterococci by a pheromone-related process in S. aureus [23].

Our study highlights further intervention for controlling the spread of VRE. Active periodic surveillance cultures (or molecular testing) of patients at highest risk for carriage, decontaminating the hands of healthcare workers using an antiseptic-containing preparation before and after all patient contact, adherence to barrier precautions (i.e., gloves and gowns) and cohorting colonized and/or infected patients; and cleaning of occupied rooms by patient with VRE are highly recommended [24, 25].

In conclusion, in this study high frequency of vancomycin resistance in E. faecalis strains was found. Therefore, periodic surveillance of antibacterial susceptibilities is highly recommended to detect emerging resistance.

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Authors’ contributions

FS and SM conceived designed and coordinated the research. ZM, SM, BP and SKV collected data. ZM and SM performed the statistical analyses. ZM, BP, SM and DA evaluated the results. ZM and SM wrote the manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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