Multidrug resistant and most virulent Enterococcus faecium (strain 2653), isolated from hospitalized patient wound in Iran

Hossein Samadi Kafil1, Ashraf Mohabati Mobarez1*, Mehdi Fourouzandeh Moghadam2

1Department of Medical Bacteriology, School of Medicine, Tarbiat Modares University, P.O.Box: 14115-111, Tehran, Iran.
2Department of Biotechnology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

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Enterococci are parts of human normal flora and the microbial association of a variety of fermented foods and probiotics but also cause a wide variety of diseases in human including urinary tract infection, bacteremia, endocarditis, peritonitis and wound infections. Different factors causes pathogenesis of Enterococci. In this study we aimed to describe pathogenic properties of a multidrug resistance strain of Enterococci isolated from wound of auricle and upper lip of a hospitalized female patient. Antibiotic resistant properties of this strain described by kerby-bauer and E. test method. The presence of 23s rRNA, ddIE, ddIF, VanA, VanB, Esp, As, Efa, Gel, Ebp, Hyl, Ace, Cyl gene were studied by PCR. This strain had extreme resistance to all common antibiotics which are using in hospital and community acquired infections and it was completely resistance to P, AMX, A, VA, TEC, E, TE, TGC, CIP, NI, RI, C, Imp, GM antibiotics and it was just sensitive to LZD. Our isolates Enterococcus faecium (2653) also was resistant to nitrofurantoin and tigecycline. This strain harbored vanA gene and interestingly had all virulence factors including colonization and secretory factors. Our study showed this strain is a suitable model for mechanism of pathogenesis and drug resistance studies of Enterococci.

Key words: Enterococcus faecium, Antibiotic resistance, Virulence factors, Colonization gene, secretory gene,

INTRODUCTION

Enterococci are facultative anaerobic gram-positive cocci that occur either singly or arranged in pairs or as short chains. They colonize the gastrointestinal tract, the oral cavity, and the vagina in humans as normal commensals (Domann et al, 2007). Theses bacteria also are present in the microbial association of a variety of fermented foods such as cheese, fermented sausages and vegetables (Mannu et al, 2002, Müller et al, 2001), some Enterococcal strains have been also used as human probiotics, because they can survive and compete in the gastrointestinal tract (Franz et al, 1999). Now, Enterococci cause a wide variety of diseases in human including urinary tract infection, bacteremia, endocarditis, peritonitis and wound infections (Jett et al, 1994). Also their increasing antibiotic resistance is narrowing options for treatment of serious infections (Landman et al, 1997). Two of the species most frequently found in infections and flora are Enterococcus faecalis and Enterococcus faecium. E. faecalis is dominant isolates from nosocomial infections, but recently, with growing antibiotic resistance especially to vancomycin, rate of isolation of E. faecium becomes significant. Several virulence factors have been described in Enterococci including as (aggregation substance), esp (extracellular surface protein), efaA (E. faecalis antigen A), ace (adhesin of collagen from E. faecalis), ebp (endocarditis and biofilm-associated pilii) as colonization factors and cyl (cytolysin), gelE (gelatinase) and hyl (hyaluronidase) as secretory factors (Fisher et al, 2009, Kayaoglu et al, 2004). The aim of present study was to describe antibiotic resistance and...
pathogenic properties of previously isolated E. faecium (2653) which have been isolated from a wound and was multi drug resistance properties and clinically interest.

**MATERIAL AND METHODS**

In national antibiogram surveillance in Iran educational hospitals we identified an multidrug resistant Enterococcus faecium (2653) which was isolated from wound of auricle and upper lip of a hospitalized female patient in Mehr hospital in Tehran who have been received ampicillin and vancomycin before isolation and was treated with prescription of Linozolide. This isolate were identified as Enterococcus faecium and specified by biochemical tests (Facklam et al, 1972) and PCR method (Vankerckhoven et al, 2004). Antibiotic resistance properties of strains were examined by Kirby-buer method according to NCCLS-CLSI guideline (CLSI M02-A10). Antimicrobial resistance examined for pericillin (P 10U), ampicillin (AMX 10 µg), amoxicillin (A 10 µg), glycopeptides (VA 30 µg), teicoplanin (TEC 30 µg), macrolides (E 15 µg), tetracycline (TE 30 µg), tigecycline (TGC 30 µg)), fluoroquinolones (CIP 5 µg), nitrofurantoin (NI 300 µg), ansamycins (RI 5 µg), phenicols (C 30 µg), Oxazolidinones (LZD 30 µg), imipenem (Imp 10 µg), gentamicin (GM 120 µg). All discs provided from Mast Diagosis disc (United Kingdom). MIC (Minimum Inhibitory Concentration) of isolate for ampicillin, oxacillin, vancomycin, teicoplanin and amikacin and ceftizoxim was examined by E. test (bioMérieux Clinical Diagnostics, France), for dilutions up to 256 we used agar dilution method. Enterococcus faecalis strain ATCC 29212 was used as a control for susceptibility testing.

DNA extraction was done by Cinnapure TM DNA extraction kit (Cinnagen, Iran). Briefly, bacterial pellet was re suspended in 100µl G+ pre lysis buffer and added 20µl lysosome and incubated at 37°C for at least 30 min. After adding lysis buffer and precipitation solution, the solution was transferred to a spin column and after washing the spin, DNA was eluted by elution buffer in 65°C. PCR was performed in 25 µl volumes that contained 20-200ng DNA, 0.5 µM of specific primers for 23srRNA (F: CCTATCGGCTCCGGCTTAG, R: AGCGAAAGACGAGTGAGAATCC) (Shepard et al, 2002), E. faecalis ddiE1: ATCAAGTCAGTTAGCTTTTATTAG, ddiE2: ACGATTTACAGCTAACATCAAATCC (Kariyama et al, 2000), E. faecalis (ddiF1: TTAGGGCCAGCCAGTGGAC, ddiF2: TTAGACAGCCACTTGGTATCAG, TGCAGTTTCCTAGTCC) (Cheng et al, 1997) and for Esp (espA: GGAACGGCTTATTGATGCTAAC, espB: GCCACCTTATACGGAC) with 95bp product length (Shankar et al, 1999), for As(Asa1:GCACTTATTAGCAGAATATG, Asa2: TAAAGAAAACATCAGCAAs) with 375bp product length (Vankerckhoven et al, 2004), efa (efaF: TGGGACAGACCCCTCACGAATA, efaR: CGCCTGTGGTTCTAAGATCAAGC) with 101bp product length (Lowe et al, 1995), Gel (gelF: TATGAAATGCTTTTGGGAT, gelR: AGATGGCAGCGAAATAATATA) with 213bp product length (Vankerckhoven et al, 2004), ebpEbpA: AAAATGATTGGCGGCTCCAGA, EbpB: TGCCAGATTGCCTGCTCAAG) with 101bp product length (Vankerckhoven et al, 2007), hyl/HylF: ACGAAAGAGTCGAGGAAATG, HylR: GACTGAGCTCAAGTTTCCAA) with 276bp length (Vankerckhoven et al, 2004), aceAceF: GGAAGTCAATCAAGTAGTTGGT, aceR: TGTTGACACTTCCCTTTCG) with 101bp length (Nallapareddy et al, 2016), cyl (CylF: ACTCGGCGGATTGATCCG, cylR: GCTGTCAGATTCCGT, cylR: 101bp length (Coque et al, 1995), VanA (VanA F: 5- AAATGCTTGGGGTTGTCGA-3, VanA R: 5- CTTTTGCGGCTGACTTCTCT-3 ) with 734bp length and vanB (VanB F: 5- GCGGGAGGATTGCGGACAG, VanB R: 5- GGAAGTAGGATGTCGAGTACAG, VanB: GGAAGATCGCTGGCTCAAAC-3) with 420bp length (Khan et al, 2005), with 1.5 mM MgCl2, 200 µM of each dNTP, 1X PCR buffer and 2 U DNA polymerase (Cinnagen, Iran). DNA was amplified by general PCR. An initial denaturation of 10 min at 94°C was followed by 35 cycles of denaturation at 94°C (1 min), annealing at 58°C (for 23s, dille, dillF, VanA, esp, gelE, cyl, hyl, efaA and ace) and 52°C (for ebp and as) for 1 min and extension at 72°C for 1 min, followed by a final extension at 72°C for 10min. product length were 941bp for E. faecalis, 658bp for E. faecalis and 95bp for esp. Positive control for PCR were E. faecalis MMH594 (also esp positive), E. faecium C38 and C68 (gift from Dr Vankerckhoven). Negative controls consisted of the PCR components on reaction mixtures lacking Enterococci DNA. PCR products were electrophoreses in 1.5% agarose gels and after staining with 0.5µg/ml ethidium bromide visualized under UV light. The size of fragments was determined in comparing with 100bp plus DNA ladder plus size marker (Fermentas, Germany).

**RESULTS AND DISCUSSION**

This strain has been chosen because of its drug resistance and pathogenesis properties. It has been confirmed as Enterococcus faecium by biochemical and PCR examinations (23s rRNA and dll genes screening). Results of antibiogram showed that this strain had extreme resistance to all common antibiotics which are using in hospital and community acquired infections. This strain had complete resistance to P, AMX, A, VA, TEC, E, TE, TGC, CIP, NI, RI, C, Imp, GM antibiotics and it was just sensitive to LZD. This result showed that however, resistance to antibiotic, especially in hospital acquired
infections is growing, but linezolid remind as the last drug choice for treatment of resistant Enterococci. This strain had high level of resistance to nitrofurantoin. Nitrofurantoin is one of the important selected antibiotics against urinary infections of Enterococci and there is not any report on resistant to it and it’s so active against vancomycin resistant Enterococci (Zhanel et al, 2001). Enterococcus faecium 2653 had complete resistance to nitrofurantoin while have been isolated from wound sample. Also, this strain had interesting resistance to tigecycline, the most recent generation of tetracycline that is not commonly used in society and is not commercially available in Iran. Resistance to this antibiotic can indicates importance of efflux pumps in drug resistance in this isolates. MIC measurement of our isolate for ampicillin, oxacillin, vancomycin, teicoplanin, amikacin and ceftizoxim showed that this isolates resistance was higher than 1024 in all above mentioned antibiotics. In genetic studies for resistance to vancomycin, this isolates identified as vanA positive (Figure 1), which is most prevalent, consists of a cluster of seven genes present on Tn1546, a 10.8-kb transposon (Arthur et al, 1993). In This study we investigated pathogenesis of E. faecium 2653 by tracking presence of main virulence factors of Enterococci. We investigated presence of colonization factors including ebp, ace, asa, efa and esp. E. faecium 2653 had all virulence factors related to colonization and biofilm construction of bacteria (Figure 1). Ebp which is an operon encodes pilus components for Enterococci (Singh et al, 2007), is important for pathogenesis of Enterococci. The Esp gene positive isolates were considered as expressing the Esp protein (Waar et al, 2002, Shankar et al, 1999), and this protein has been considered as an essential factors for blood and wound infections of Enterococci and is associated with resistance to ampicillin (Billström et al, 2008). Efa is a common pathogenic factors in isolates of Enterococci from blood (Eaton et al, 2001) and was present in E. faecium 2653. As (aggregation substance) is a virulence factor that has shown to form large aggregates and serve as a putative factor in favor of bacterium against the host defense (Kayaoglu et al, 2004), it has lower prevalence among clinical isolates and its presence consider as advantage for pathogenesis of bacterium (Bittencourt de Marques et al, 2004). Ace as another colonization factor is a collagen binding protein belonging to the microbial surface components recognizing adhesive molecules (Koch et al, 2004), and has role in biofilm formation of bacteria. Also we found cly, gel and hyl in E. faecium 2653, these factors have role in distribution of bacteria and spread of infection in body (Kayaoglu et al, 2004). According to above result this strain had all common
virulence factors of Enterococci and extreme drug resistance to most commercially available antibiotics. Most of previously described strains have some of virulence factors like E. faecalis MMH594 (gelE, asa, esp, cyl, ebp positive), E. faecalis 29212 (gelE, asa positive), E. faecium C38 (esp positive), E. faecium C68 (hyl and esp positive) (Vankerkhoven et al, 2004). More studies are needed for describing drug resistance reasons and pathogenic properties of E. faecium 2653, but our study showed this strain is a suitable model for mechanisms of pathogenesis and drug resistance studies in Enterococci.

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REFERENCE


