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ORIGINAL ARTICLE

Assessment of biofilm formation by enterococci isolates from urinary tract infections with different virulence profiles

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Abstract This study aimed to investigate possible associations between virulence profiles and biofilm formation in Clinical UTI isolates. Isolates were collected from five university hospitals and identified and characterized for the presence of virulence factors by PCR. Biofilm assays were conducted in 96 well microtiter plates by reading the OD570 after crystal violet staining. 75% of isolates had *esp* gene, 38.77% had *asa1*, 84.18% had *ace*, 81.63% had *efaA*, 93.36% had *ebpR*, 34.18% had *cylA*, 81.63% had *gelE* and 17.35% had *hyl*. Biofilm experiences were done and isolates having *asa1* or *efaA* genes produced more biofilms than negative ones ($P = 0.011$, $P = 0.008$), but the presence of *esp*, *ace*, *cylA* or *gelE* genes in isolates had no effect on biofilm formation. Isolates possessing *hyl* had much lower biofilm formation ($P = 0.000$). Present study showed that the *esp*, *ace*, *gelE* and *cylA* genes do not seem to be necessary nor sufficient for the production of biofilm in enterococci but the presence of *efaA* and *asa1* correlates with increased biofilm formation of urinary tract isolates. Also the low prevalence of *hyl* among enterococci isolated from UTIS and its association with poor biofilm production indicate that the absence of this gene can be an advantage in the pathogenesis of UTIs.

1. Introduction

Enterococci are Gram-positive member of the human gastrointestinal flora, and are also an important cause of opportunistic nosocomial infections (Marra et al., 2007). These organisms are capable of infecting numerous body sites, causing bacteremia, intra-abdominal infections, endocarditis, and urinary tract infections (Pillar and Gilmore, 2004). *Enterococcus faecalis* and *Enterococcus faecium* are the most common enterococci species, and they are responsible for up to 95% of human enterococcal infections (Hall et al., 1992; Jett et al., 1994; Jones et al., 2004).

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Biofilm is a population of cells attached irreversibly on various biotic and abiotic surfaces and encased in a hydrated matrix of exopolymeric substances, proteins, polysaccharides and nucleic acids (Costerton, 2001). The ability of enterococci to form biofilms may confer an ecological advantage in certain situations. For example, clinical strains of *E. faecalis* isolated from infective endocarditis patients were significantly associated with the greater biofilm formation than nonendocarditis clinical isolates (Mohamed et al., 2004); this may be attributable in part to specific virulence factors in enterococci (Mohamed and Murray, 2005). Several enterococcal virulence factors have been identified, including adhesions and secreted virulence factors. The most important adhesion factors are Asa (aggregation substance), Esp (extracellular surface protein), EfaA (*E. faecalis* antigen A), Ace (adhesin of collagen from *E. faecalis*) and Ebp (endocarditis and biofilm-associated pili) (Fisher and Phillips, 2009) and secreted pathogenic factors of enterococci with a value in pathogenesis are CylA (cytolysin), GelE (gelatinase) and Hyl (hyaluronidase) (Kayaoglu and Ørstavik, 2004). Several studies investigated the role of these virulence factors in biofilm formation by enterococci (Shankar et al., 1999, 2001; Sandoe et al., 2003; Dupre et al., 2003). *esp* and *gelE* were the main factors investigated in strains from different origins (Shankar et al., 1999; Baldassarri et al., 2006). However, some studies claimed correlation among the presence of these factors and biofilm formation (Mohamed et al., 2003; Toledo-Arana et al., 2001) but others suggest that these genes do not seem to be necessary for the production of biofilm in enterococci (Baldassarri et al., 2006; Dupre et al., 2003). The purpose of this study was to investigate biofilm production by enterococcal strains isolated from UTIs and showing different virulence genes profiles, to establish a possible relationship between virulence profile and biofilm formation.

2. Materials and methods

2.1. Strains collection

One hundred and ninety six clinical isolates of enterococci from Urinary tract infections were collected from October 2009 till August 2010 from five university hospitals, including (Tehran) Baqiatalah, (Tehran) Kodakan, (Tehran) Milad, (Mashhad) Shariati and (Shiraz) Namazi. All isolates were identified by Mass Spectrophotometer (MALDI-TOF MS microflex, Bruker, Germany) and biochemical and PCR tests (Table 1) (Facklam, 1972; Kafil and Asgharzadeh, 2014; CLSI, 2012).

2.2. Genomic PCR

DNA extraction was done by the protocol described before (Asgharzadeh et al., 2008, 2011). PCR was performed in 25 µl volumes that contained 20–200 ng DNA, 0.5 µM of specific primers for each gene (Table 1), 1.5 mM MgCl2, and 200 µM of each dNTP, 1× PCR buffer and 2 U DNA polymerase (Cinnage, Iran). DNA was amplified by general PCR. An initial 10 min denaturation at 94 °C was followed by 35 cycles of 1 min denaturation at 94 °C, annealing at 58 °C (for *ddlE*, *ddlF*, *esp*, *gelE*, *cylA*, *hyl*, *efaA* and *ace*)/52 °C (for *ebpR* and *asa1*) for 1 min and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. Positive controls for PCR were *E. faecalis* MMH594 (*gelE*, *asa1*, *esp*, *cylA*, *ebpR* positive), *E. faecalis* 29212 (*gelE*, *asa1* positive), *E. faecium* C38 and C68 and *E. faecalis* 217 (Khan et al., 2005; Vankerckhoven et al., 2004; Kafil et al., 2013) PCR products were analyzed in agarose gels and visualized under UV after staining with 0.5 µg ml-1 ethidium bromide.

Table 1 Target genes and primers used in this study.

Target gene	Primers (5′ → 3′)	Product (bp)	References
E. faecalis	*ddl*E1:ATCAAGTACAGTTAGTCTTTATTAG *ddl*E2: ACGATTCAAAGCTAACTGAATCAGT	941	Kariyama et al. (2000)
E. faecium	*ddl*F1: TTGAGGCAGACCAGATTGACG *ddl*F2: TATGACAGCGACTCCGATTCC	658	Cheng et al. (1997)
asa1	*asa*1: GCACGCTATTACGAACTATATGA *asa*2: TAAGAAAGAACATCACCACGA	375	Vankerckhoven et al. (2004)
efaA	*efa*F: TGGGACAGACCCTCACGAATA *efa*R: CGCCTGTTTCTAAGTTCAAGCC	101	Lowe et al., (1995)
gelE	*gel*F: TATGACAATGCTTTTTGGGAT *gel*R: AGATGCACCCGAAATAATATA	213	Vankerckhoven et al. (2004)
ebpR	*ebp*A: AAAAATGATTCGGCTCCAGAA *ebp*B: TGCCAGATTCGCTCTCAAAG	101	Bourgogne et al. (2007)
hyl	*hyl*F: ACAGAAGAGCTGCAGGAAATG *hyl*R: GACTGACGTCCAAGTTTCCAA	276	Bourgogne et al. (2007)
esp	*esp*A: GGAACGCCTTGGTATGCTAAC *esp*B: GCCACTTTATCAGCCTGAACC	95	Shankar et al. (1999)
ace	*ace*F: GGAGAGTCAAATCAAGTACGTTGGTT *ace*R: TGTTGACCACTTCCTTGTCGAT	101	Nallapareddy and Murray (2006)
cylA	*cyl*F: ACTCGGGGATTGATAGGC *cyl*R: GCTGCTAAAGCTGCGCTT	688	Vankerckhoven et al. (2004)

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