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Virulence and Antimicrobial Resistance in Enterococci Isolated from Urinary Tract Infections

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ABSTRACT

***Purpose*:** Urinary tract infection (UTI) is the most common nosocomial infection among hospitalized patients. Meanwhile, most frequent infections involving enterococci affect the urinary tract. The aims of this study were to investigate the susceptibility pattern of isolated enterococci from UTI and the prevalence of virulence genes. ***Methods*:** The study used enterococci isolated from urinary tract infections obtained from 3 university teaching hospitals in Northwest Iran. The antimicrobial susceptibility of the strains was determined using the disc diffusion method. Multiplex PCR was performed for the detection of genus- species specific targets, and potential virulence genes. ***Results*:** Of 188 enterococcal isolates, 138 (73.4%) and 50 (26.6%) were *Enterococcus faecalis* and *E. faecium*, respectively. Antibiotic susceptibility testing showed high resistance to amikacin (86.2%), rifampicin (86.2%) and erythromycin (73.9%), irrespective of species. In total, 68.1% were positive for *gelE*, and 57.4%, 53.2%, 56.4%, and 52.1% of isolates were positive for *cpd*, *asa1*, *ace*, and *esp*, respectively. ***Conclusion*:** The study revealed that most of UTI isolates were multidrug resistance against the antibiotics tested and antibiotic resistance was more common among *E. faecium* isolates than *E. faecalis*. A significant correlation was found between UTI and the presence of *gelE* among *E. faecalis* strains ($p < 0.001$).

Introduction

The enterococci are a dominant bacterial group in the intestinal flora of human and animals[1] and it is recognized that they cause serious infections such as endocarditis, septicemia and UTI.[2] The natural ability of enterococci to acquire, accumulate, and share extra chromosomal elements encoding virulence traits or antibiotic resistance genes , in part, explains their increasing importance as nosocomial pathogens.[3] Acquired resistance to various antimicrobial agents and available antibiotics currently limits the therapeutic options. It is believed that nosocomial enterococci might have virulence elements that increase their ability to colonize hospitalized patients.[4] The aims of this study were to investigate the susceptibility pattern of isolated *E. faecalis* and *E. faecium* from UTI and the prevalence of genes encoding gelatinase (*gelE*), aggregation substance (*asa1*), enterococcal Surface protein (*esp*), collagen adhesine (*ace*), and sex pheromones (*cpd*).

Materials and Methods

Bacterial isolates

One hundred and eighty eight enterococcal isolates obtained from urine specimens of patients with urinary tract infections in 3 university teaching hospitals located in Tabriz (Imam Reza and Sina Hospitals) and Orumieh (Imam Khomeini Hospital), Iran, from April 2008 to June 2010. All isolates were phenotypically identified to the species level using conventional methods[5] and their identities were later confirmed by PCR.[6]

Antibiotic susceptibility testing

The antimicrobial susceptibility of the strains was determined using the disk diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI)[7] guidelines for the following antimicrobial agents: penicillin G, imipenem, erythromycin, rifampicin, teicoplanin, ampicillin, streptomycin, ciprofloxacin, and amikacin. Considering to an

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increasing rate of vancomycin and high level gentamicin resistant enterococci in clinical isolates worldwide, the minimum inhibitory concentrations (MICs) of vancomycin and gentamicin were determined using the agar dilution method based on the CLSI (2006) guidelines. *E. faecalis* ATCC 29212 was used as a quality control strain for performing antimicrobial tests.

DNA extraction and molecular approach

The DNA of clinical isolates was extracted using a commercial kit (DNG™-Plus; CinnaGen, Iran). Multiplex PCRs were performed on enterococcal isolates for simultaneous detection of potential virulence genes (*esp*, *gelE*, and *asa1*), as described previously[8] but conditions have been optimized for detection *cpd*[9] and *ace*.[10] Briefly, the 25 µL PCR mixture contained; 2.5 µL of bacterial DNA, 10 pM of each primer for *cpd* and 4 pM of each primer for *ace*, 1.5 mM MgCl2, 0.2 mM of each dNTP, and 2.5 U of Taq DNA polymerase (CinnaGen).

Reactions were performed on thermal cycler (ASTEC-Japan) with an initial denaturation at 95 °C for 10 min, followed by 30 cycles of denaturation (94 °C for 1 min), annealing (56 °C for 1 min), extension (72 °C for 1 min), and a final extension step at 72 °C for 10 min. PCR products were analyzed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and photographed under UV light. Each PCR assay was accompanied with a negative control, containing all of the reagents without template DNA.

Statistical analysis

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.

Results

Bacterial isolates and susceptibility testing

In total, 138 (73.4%) *E. faecalis* and 50 (26.6%) *E. faecium* were collected from urine specimens. Antibiotic susceptibility testing by the disk diffusion showed high resistance to rifampicin and amikacin (86.2%) and erythromycin (73.9%), followed by penicillin G (68.6%), ciprofloxacin (65.4%), streptomycin (47.3), ampicillin (28.2%) and teicoplanin (18.6%), irrespective of species concern. The resistance patterns of two species were shown in Table 1.

The agar dilution method indicated that 35 (18.6 %) strains were vancomycin resistant (MIC ≥ 256 µg/mL excluding 1 isolate with MIC = 8 µg/mL) and 113 (60.1%) isolates were high-level gentamicin- resistant with MICs ≥ 512 µg/mL. Of vancomycin resistance strains, 33 (94.3%) were associated with high-level resistance to gentamicin. Of these, 7 (21.2%) were *E. faecalis* and 26 (78.8%) were *E. faecium* strains.

Table 1. Results of Disk Diffusion tests of Isolated Enterococci.

Antibiotics	*E. faecalis* (%)			*E. faecium* (%)			Total (%)		
	S	I	R	S	I	R	S	I	R
Ampicillin	132(95.7)	-	6(4.3)	3(6)	-	47(94)	135(71.8)	-	53(28.2)
Amikacin	15(10.9)	9(6.5)	114(82.6)	1(2)	1(2)	48(96)	16(8.5)	10(5.3)	162(86.2)
Ciprofloxacin	15(10.9)	47(34.1)	76(55.1)	0(0)	3(6)	47(94)	15(8)	50(26.6)	123(65.4)
Erythromycin	20(14.5)	26(18.8)	92(66.7)	2(4)	1(2)	47(94)	22(11.7)	27(14.4)	139(73.9)
Imipenem	132(95.7)	1(0.7)	5(3.6)	3(6)	0(0)	47(94)	135(71.8)	1(0.5)	52(27.7)
Penicillin G	58(42)	-	80(58)	1(2)	-	49(98)	59(31.4)	-	129(68.6)
Rifampicin	16(11.6)	10(7.2)	112(81.2)	0(0)	0(0)	50(100)	16(8.5)	10(5.3)	162(86.2)
Streptomycin	69(50)	2(1.4)	67(48.6)	28(56)	0(0)	22(44)	97(51.6)	2(1.1)	89(47.3)
Teicoplanin	130(94.2)	-	8(5.8)	23(46)	-	27(54)	153(81.4)	-	35(18.6)

Presence of virulence genes in E. faecalis and E. faecium

The presence of genes encoding for potential virulence factors were studied by multiplex PCR. Of 188 isolates, 68.1% were positive for *gelE*, and 57.4%, 53.2%, 56.4%, and 52.1% of isolates were positive for *cpd*, *asa1*, *ace*, and *esp*, respectively (Figure 1). The percentages of *E. faecalis* and *E. faecium* isolates harboring virulence genes were demonstrated in Table 2. All *E. faecalis* strains carried 2 or more virulence determinants, whereas, 14 *E. faecium* strains did not harbor any of the genes tested and 4 isolates positive for two or more virulence determinants.

Moreover, *esp*-positive *E. faecium* strains recovered from urine samples, exhibited high resistance to gentamicin (90.9 %), ampicillin (97%) and penicillin (100 %), ciprofloxacin (100%), rifampicin (100 %), amikacin (100%), imipenem (100 %). Nearly 64% of the isolates were also resistant to vancomycin.

Discussion

In this study, most (79.3 %) of UTI enterococcal isolates were resistant to at least three of the antibiotics tested, possibly, these reflect miss-using of antibiotics and selective pressure in our setting. While *E. faecalis* was clearly the predominant species in urine samples,

E. faecium showed a much higher incidence of resistance to antibiotics tested. *E. faecium* strains displayed resistance to ampicillin, imipenem, teicoplanin, vancomycin and high level gentamicin (p<0.001). With an exception, resistance against streptomycin was more common among *E. faecalis* than *E. faecium* strains.

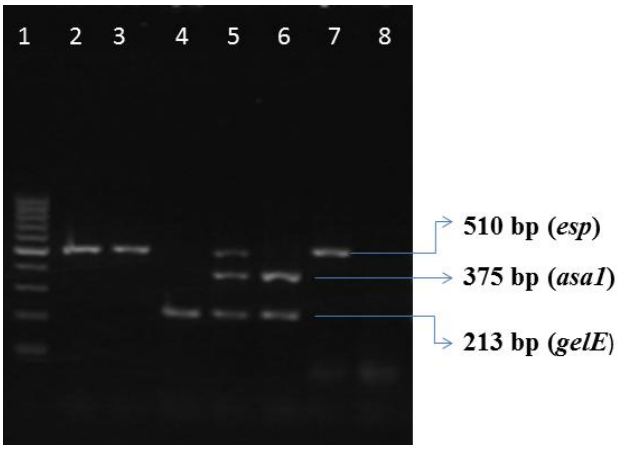

Figure 1. Agarose gel electrophoresis of amplified *asa1, esp* and *gelE* by multiplex PCR.
Lane 1: 1-kb DNA ladder
Lanes 2, 3 and 7: isolates positive for *esp* (510 bp)
Lane 4: isolate positive for *gelE* (213 bp)
Lane 5: isolate positive for *asa1* (375 bp), *esp* and *gelE*
Lane 6: isolate positive for *asa1* and *gelE*
Lane 8: negative control (without DNA)

Detection of multidrug resistance enterococci in this study, particularly VRE and HLGR is an alarming situation, since these organisms limit the number of therapeutic options available to the clinician.[11]
Antibiotic resistance alone cannot explain the virulence of enterococci. The pathogenesis of most infections follows a common sequence of events involving colonization of and adhesion to host tissues, invasion of the tissue and resistance to defense mechanisms of the host. The pathogen must produce pathological changes either directly by toxin production or indirectly by inflammation.[12] However, each of virulence traits may be associated with one or more of the stages of infection mentioned above.

Table 2. The percentages of virulence genes among isolates.

Virulence Genes	No. (%)		Total No. (%)
	E. faecalis	*E. faecium*	
gelE	124(89.9)	4(8)	128(68.1)
asa1	96(69.6)	4(8)	100(53.2)
cpd	106(76.8)	2(4)	108(57.4)
ace	104(75.4)	2(4)	106(56.4)
esp	65(47.1)	33(66)	98(52.1)

According to results from the present study, the major differences on the incidence of virulence determinants found both in *E. faecalis* and *E. faecium* isolates from the urinary tract infection, involved a remarkably higher average number of traits in *E. faecalis* isolates and a much lower average number of traits in *E. faecium* isolates. The *E. faecalis* strains tested all harbor multiple virulence determinants. The *gelE* gene (codes for gelatinase is an extracellular zinc metalloendopeptidase) was the most widespread virulence determinant. In this study, *gelE* enriched in *E. faecalis* isolates in comparison with *E. faecium* and may involve in the creation of a urinary tract infection (p<0.001). Likewise, previous studies in *E. faecalis* demonstrate the presence of this gene in high incidence among their isolates.[9,13] Consistent with our findings, *gelE*-positive *E. faecium* isolates has been found in less frequency in clinical isolates.[14] In contrast, some studies did not found *gelE* gene in any *E. faecium* isolates.[8]
The present study revealed higher frequency of the *esp* gene (coding enterococcal surface protein) among *E. faecium* isolates. In agreement with our research, other studies revealed higher incidence in *E. faecium* isolates.[4,15] In contrast, a research study showed the higher prevalence of this gene in *E. faecalis* isolates from urine samples[16] but the results of other study[17] was mostly the same as our findings.
Furthermore, *esp*-positive *E. faecium* strains, showed high resistance (> 90 %) to the most of tested antibiotics as mentioned in results. Nearly 64% of *E. faecium* strains were also resistant to vancomycin. Considering these results, it seems that the presence of *esp* may facilitate for *esp*-positive *E. faecium* isolates to obtain more antibiotic-resistance genes. The prevalence of this gene between two species indicated possible role for *esp* in *E. faecium* strains to cause urinary tract infection (p<0.05), although an earlier study on *E. faecalis*, has been demonstrated a role for *esp* gene product for *E. faecalis* isolates causing UTI.[18]
In present investigation, the *asa1* gene, (which encodes aggregation substance), was found in high frequency among *E. faecalis* strains. A high incidence of this gene in *E. faecalis* was reported in previous studies.[19] Results of studies on clinical *E. faecium* isolates are contradictory. In some studies, *asa1* was not found in *E. faecium*[4] but in contrast, in our study and some other studies[14] this gene was detected in less frequency among *E. faecium* isolates. In total, the rate of *asa1* gene in urine isolates did not indicate significant association between the presences of *asa1* and emergence of UTI.
Gene *cpd* encoding for sex pheromone peptides showed a lower incidence among *E. faecium* isolates whereas it was in higher incidence among *E. faecalis*. Other studies also reported higher frequency of this gene among clinical *E. faecalis* isolates.[20] Presence of multidrug resistance among our isolates may be related to the higher incidence of *cpd* gene, since the presence of sex-pheromone genes facilitate the acquisition of the relevant sex-pheromone plasmid and therefore the associated virulence and resistance determinants.[21]

The *ace* gene (codes for collagen-binding protein) has been detected in high frequency in *E. faecalis* strains that is in agreement with previous studies.[22] Although, Ace has been suggested as a valuable drug target against human UTI,[23] but in this investigation, the presence of *ace* gene was not statistically significant.

Conclusion

Our study demonstrated that *E. faecalis* is more common among our isolates than *E. faecium*, but *E. faecium* strains had a great ability to show drug resistance. The distribution of virulence genes were more common in *E. faecalis* than in *E. faecium* strains and the high incidence of multiple virulence factors could potentially contribute to bacterial colonization and pathogenesis of *E. faecalis* in the urinary tract.
The higher prevalence of *esp* determinant in *E. faecium*, may explain the role of this gene in emergence of resistance to the tested antibiotics.

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Conflict of interest

The authors report no conflicts of interest.

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