Short Communication

Molecular Analysis of *Enterococcus Faecalis* Isolates in a 4-year Period

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ABSTRACT

In the present research, we aimed to determine the characteristics of E. faecalis strains collected from an Iranian Children's Hospital for four years. Sixty-seven E. faecalis isolates with virulence genes detection, variable-number tandem repeat (VNTR), and multiple-locus variable-number tandem repeat analysis (MLVA) typing were investigated. A high frequency of virulence genes belonged to gelatinase (73%) and Enterococcus faecalis (62%). The MLVA of 67 E. faecalis isolates revealed 52 VNTR patterns and 38 MLVA types (MTs). Furthermore, genetic diversities with the majority of the MT1 as a major MT in different Wards of the Children's Hospital were found.

Keywords: Antimicrobial resistance, Enterococcus faecalis, MLVA, Virulence factors

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1. Introduction

Enterococcus faecalis is considered an important-acquired pathogen, which has frequently been isolated from all common infections, including urinary tract infection (UTI), bacteremia, neonatal sepsis, endocarditis, and abdominal and pelvic infection (1). In addition to the resistance to the antibiotics of their choice, especially vancomycin and gentamicin (GM), several virulence factors have contributed to the persistence of enterococci in nosocomial infections, including collagen-binding adhesin of *Enterococcus faecalis* (Ace), aggregation substance (Asa1), cytolysin (CylA), enterococcal surface protein (Esp), and gelatinase (gelE) (2, 3).

Because of the steadily increasing antibiotic resistance of *E. faecalis* strains within healthcare facilities, it is necessary to perform epidemiological investigations and identify the possible sources of contamination. Recently, multiple-locus variable-number tandem repeat analysis (MLVA) based on variations in the number of repeats at certain variable number tandem repeat (VNTR) loci has been known as a useful method for genotyping purposes of several bacterial pathogens, including *E. faecalis* (4). However, in our country, *E. faecalis* displayed a high frequency, and there are insufficient studies on molecular characteristics of enterococci obtained from pediatric infections (5, 6). In the current study, we aimed to determine the virulence genes and MLVA types (MTs) among *E. faecalis* strains isolated from an Iranian Children's Hospital over four years.

2. Material and Methods

2.1 Bacterial isolates

Sixty-seven pre-identified *E. faecalis* strains were selected from our previous study (7). These isolates were collected from clinical samples (urine (n=58), blood (n=4), cerebrospinal fluid (n=2), a wound lesion (n=1), a tracheal secretion (n=1), and a peritoneal fluid (n=1)) of children during December 2011 to July 2014. Most *E. faecalis* strains originated from outpatients (n=22), emergency (n=9), and urology hospitalized patients (n=8). Antimicrobial susceptibility testing, bacterial genomic DNA extraction, and detection of vancomycin (*vanA*) and an aminoglycoside *(aac(6')-Ie-aph(2")-Ia)* resistance genes had been performed in our previous study (7).

1.2 Virulence genes detection

The genes encoding virulence factors (*cylA, gelE, esp, ace, asa1*) were targeted by means of PCR using pre-extracted DNA by the boiling method (7, 8).

2.3 Molecular analysis

On the basis of Titze-de-Almeida study, seven repeat loci (*aceB, espA, espC, efa2, efa3, efa5, efa6*) were selected for molecular analysis of isolates (9). Briefly, the PCR protocol consisted of a pre-denaturation step at 95°C for 5 min and a final extension at 72°C for 5 min. Thirty cycles of 95°C for 45 s, 50 s at 66.3°C (for *aceB*), 56°C (for *espA* and *efa6*), 59°C (for *efa2*), 55.2°C (for *efa3*), and 49 °C (for *efa5*) were performed. Amplified amplicons were analyzed on 1% agarose gels and product bands were reflected with KBC power loading dye (GelRed Nucleic Acid Gel Stain, 10,000× in water, Kawsar Biotech Co., Tehran, Iran) under UV illumination. The number of copies in each locus was estimated based on the size of the repeats and the PCR bands. The MLVA type (MT) was given based on one or more band differences; thus, MTs were described as isolates sharing ≥ 85.7% similarity. All results were rounded down and up if they were < 0.5 and > 0.5, respectively, and were considered 0.5 itself if they were = 0.5.

3. Results

The pattern of antibiotic resistance of each strain and the genes involved in vancomycin and GM resistance are shown in Table 1. The prevalence of *gelE* and *aceB* virulence genes was high (73% and 62%), followed by *asa1*, *esp,* and *cylA* found in 58%, 31%, and 7% of isolates, respectively.

The results of MLVA typing are shown in Table 1. The MLVA typing revealed 52 variable number tandem repeat (VNTR) patterns belonging to 38 MTs

(Table 1). Eighteen isolates were assigned into two common types (13 as MT1 and 5 as MT2). The MT1 isolates were recovered from different parts of the hospital from 2011-2012. It is worth mentioning that, the most common pattern of antibiotic resistance in MT1 isolates was associated with pattern gentamicin, ciprofloxacin, erythromycin, and clindamycin (AP, GM, CIP, E, and CD). Moreover, *aac (6')-Ie-aph (2")-Ia* resistance gene and *gelE+asa1* virulence pattern in MT1 strains were frequent.

4. Discussion

Similar to previous reports, our findings showed a relatively high prevalence of *asa1*, *ace*, and *gelE* among *E. faecalis* strains (1, 10). Studies revealed that antibiotic resistance genes and *asa1* are located on a plasmid that can be transferred simultaneously. In addition, more than half of the *E. faecalis* strains isolated from nosocomial infections harbored genes encoding gelatinase and aggregation substance (11). The MLVA typing of 67 *E. faecalis* isolates revealed 52 VNTR patterns and 38 MTs. In a study conducted by Walecka *et al.*, MLVA of 56 *E. faecalis* isolates revealed 40 VNTR patterns and MTs (4). In another study in Poland, 111 VNTR patterns and MTs were determined on 153 *E. faecalis* strains (12).

This high degree of heterogenicity among isolates may indicate the persistence of enterococci strains. The MT1 strains were isolated from different parts of the Hospital during the first two years of the study. Most of these isolates have GM, CIP, E, CD, and *gelE+asa1* pattern, and an *aac(6')-Ie-aph(2")-Ia* resistance gene, which probably indicates a dominant clone compatible with the Hospital setting in our study Center and was transferred to outpatients referred to the Medical Center. Despite observing these isolates in two years of our study, more samplings from different Wards are required to identify and prevent their spread because, if the MT1 strains are not controlled, they will spread resistance and virulence factors to sensitive isolates. In conclusion, MT1, a common MT in *E. faecalis* isolates, circulated in different Wards of the Hospital in 2011-2012. Moreover, *E. faecalis* isolates with multiple resistance were common in our study Hospital.

Table 1. Phenotypic and genotypic characteristics of *E. faecalis* strains isolated from clinical samples in four years

MLVA Type	Virulence genes	Resistance genes	Resistance pattern[d]	Time of isolation (m/y)[c]	Sample[b]	isolate	Ward[a]
1	*asa1, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, CIP, E, CD	12/2011	Urine	1	
1	*asa1, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, CIP, E, CD	12/2011	Urine	2	
8	*ace, esp, gelE,*	-	E, CD	1/2012	Urine	3	
1	*asa1, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, CIP, E, CD	1/2012	Urine	4	
1	-	*aac(6')-Ie-aph(2")-Ia*	GM, CIP, E, CD	2/2012	Urine	5	
9	-	-	E, CD	3/2012	Urine	6	
9	*ace*	-	GM, CIP, E, CD	5/2012	Urine	7	
19	*ace, gelE*	-	CIP, E, CD	6/2012	Urine	8	
11	*ace*	-	GM, CIP, E, CD	6/2012	Urine	9	
2	*ace, asa1, esp, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, E, CD	6/2012	Urine	10	
4	-	-	GM, CIP, E, CD	7/2012	Urine	11	Outpatient
12	*ace, esp, gelE*	-	GM, CIP, E, CD	7/2012	Urine	12	
4	-	-	CIP, E, CD	8/2012	Urine	13	
1	*asa1, gelE*	*aac(6')-Ie-aph(2")-Ia*	CIP, E, CD	10/2012	Urine	14	
27	*ace, esp*	-	CIP, E, CD	10/2012	Wound	15	
28	*ace, esp, gelE*	-	CIP, E, CD	11/2012	Urine	16	
29	*ace, asa1, esp, gelE*	-	CIP, E, CD	2/2013	Urine	17	
32	*ace, asa1, esp, gelE*	-	CIP, E, CD	5/2013	Urine	18	
33	*ace, gelE*	-	CIP, E, CD	5/2013	Urine	19	
3	*ace, gelE*	-	CIP, E, CD	7/2012	Urine	20	
34	*ace, esp, gelE*	-	CIP, E, CD	7/2012	Urine	21	
36	*gelE*	*vanA*	AP, CIP, E, CD	1/2014	CSF	22	

Table 1 Continue

1	*asa1, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, CIP, E, CD	1/2012	Urine	23	
15	*ace, esp, gelE*	-	E, CD	1/2012	Urine	24	
16	*ace, asa1*	-	CIP, E, CD	1/2012	Urine	25	
17	*ace, asa1, cylA, gelE*	-	CIP, E, CD	3/2012	Urine	26	
10	-	-	GM, CIP, E, CD	5/2012	Urine	27	Emergency
7	*ace, asa1*	-	GM, E, CD	6/2012	Urine	28	
11	*ace, gelE,*	-	GM, CIP, E, CD	5/2013	Urine	29	
13	*gelE,*	-	GM, AP, E, CD, SYN	9/2013	Urine	30	
37	*ace, asa1, gelE,*	*aac(6')-Ie-aph(2")-Ia*	GM, CIP, E, CD	7/2014	Blood	31	
6	*asa1, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, CIP, E, CD	12/2011	Urine	32	
1	*asa1, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, CIP, E, CD	3/2012	Urine	33	
1	*asa1, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, CIP, E, CD	5/2012	Urine	34	
5	*ace, asa1, gelE*	-	CIP, E, CD	6/2012	Urine	35	Urology
22	*ace, asa1*	-	CIP, E, CD	7/2012	Urine	36	
26	*ace, gelE*	-	GM, CIP, E, CD	8/2012	Urine	37	
1	*asa1, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, CIP, E, CD	2/2013	Catheter	38	
3	*ace, gelE*	-	CIP, E, CD	7/2013	Urine	39	
1	*asa1, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, CIP, E, CD	3/2012	Urine	40	
1	*asa1, gelE*	-	GM, CIP, E, CD	3/2012	Urine	41	
2	*ace, asa1, esp, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, E, CD	3/2012	Urine	42	Dialysis Center
18	*asa1, cylA, esp, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, CIP, E, CD	5/2012	Urine	43	
2	*ace, asa1, esp, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, E, CD	6/2012	Urine	44	
1	*ace, asa1, gelE*	*vanA, aac(6')-Ie-aph(2")-Ia*	GM, AP, CIP, E, CD	5/2012	Blood	45	
5	*ace, asa1, gelE*	-	CIP, E, CD	5/2012	Urine	46	
25	*ace, esp*	-	E, CD	8/2012	Ascites	47	
31	*ace, asa1, esp, gelE*	-	CD	5/2013	CSF	48	Surgery
8	*ace, asa1, gelE*	-	CIP, E, CD	5/2013	Urine	49	
6	*asa1, gelE,*	*aac(6')-Ie-aph(2")-Ia*	GM, AP, CIP, E, CD	12/2011	Urine	50	
7	*ace, asa1, esp*	-	CIP, E, CD	10/2012	Urine	51	Neonatal
3	*ace, gelE,*	-	CIP, E, CD	5/2013	Urine	52	
30	*ace, asa1, gelE*	-	GM, CIP, E, CD	4/2013	Tracheal aspirate	53	NICU
35	*esp, gelE*	*vanA*	GM, AP, CIP, E, CD	11/2013	Blood	54	EICU
2	*ace, asa1, esp, gelE*	*aac(6')-Ie-aph(2")-Ia*	GM, E, CD	6/2012	Urine	55	Digestive
13	*asa1*	-	GM, CIP, E, CD	4/2013	Blood	56	Neurology
14	*ace, asa1, cylA, esp, gelE*	-	E, CD	1/2012	Urine	57	
20	*ace, asa1, cylA, esp, gelE*	-	CIP, E, CD	6/2012	Urine	58	
21	*ace, asa1, gelE*	-	CIP, E, CD	6/2012	Urine	59	
32	*ace*	-	GM, E, CD	7/2012	Urine	60	
35	-	-	CIP, E, CD	7/2012	Urine	61	Unknown
23	*ace, gelE*	-	GM, CIP, E, CD	8/2012	Urine	62	
24	*asa1*	-	CIP, E, CD	8/2012	Urine	63	
38	*ace, asa1, cylA, esp, gelE*	-	CIP, E, CD	9/2012	Urine	64	
10	*ace, asa1, esp*	-	GM, CIP, E, CD	12/2012	Urine	65	
1	*gelE*	*aac(6')-Ie-aph(2")-Ia*	AP, CIP, E, CD	12/2012	Urine	66	
2	*ace, asa1, esp, gelE*	-	CIP, E, CD	2/2013	Urine	67	

[a] NICU: Neonatal Intensive Care Unit, EICU: Emergency Intensive Care Unit
[b] CSF: Cerebrospinal fluid
[c] m / y month / year
[d] GM: Gentamicin, AP: Ampicillin, CIP: Ciprofloxacin, E: Erythromycin, CD: Clindamycin

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Not applicable.

Authors' Contribution

RB and ME designed the study. FJ and ASM drafted the manuscript. ASM performed data analysis. All authors provided intellectual input to the study and read and approved the final manuscript.

Ethics

The study was approved by the Ethics Committee of Tehran University of Medical Sciences and all methods were performed in accordance with the relevant guidelines and regulations. Consent to participate is not applicable for this study because the isolates included in the study were obtained from existing clinical collections routinely assembled as part of laboratory practices of university hospitals.

Conflict of Interest

The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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