Multilocus Sequence Typing (MLST) of *Enterococcus faecalis* clinical isolates, Ilam, Iran

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Abstract

Introduction: The *Enterococcus faecalis (E. faecalis)* is the one of the pathogenic bacteria that become famous and considerable in the recent years. Here we tried to do typing the *E. faecalis* isolates to provide advantageous information that can help us to understand epidemiological communication between the *E. faecalis* isolates.

Materials and methods: One hundred *E. faecalis* were isolated from urine samples of Imam Khomeini Hospital, Ilam, Iran. Afterwards, all isolates were confirmed by the phenotyping method and then for more certainty, every isolates were authenticated by PCR analysis of 16sRNA gene. Eventually, all isolates were considered as *E. faecalis.* For Multilocus Sequence Typing (MLST), 7 housekeeping genes were used to gain MLST scheme for epidemiological study. In addition, to determine various type of *E. faecalis* pubmlst database was selected and the MLST analysis was done based on recommended instruction by the pubMLST.org.

Results: The disk diffusion results demonstrated that fifty-four out of one hundred isolates were resistant, four isolates were semi sensitive and forty-two isolates were sensitive to vancomycin. So, 90 isolates were MLST. Using seven structural genes and using pubMLST.org database, different types of *E. faecalis* were determined. The MLST results demonstrated that 26 different group and Sequence Types (ST) obtained. Our findings demonstrated that the isolates were from different types.

Conclusion: Accoeding to our results, we couldn't find any epidemic correlation between the isolates. Given that most of these isolates had resistance to vancomycin, they had low clonal correlation with each other and only had few similar STs pattern.

Keywords: *E. faecalis*, Multilocus Sequence Typing, Iran

Introduction

The *Enterococcus faecalis* is known as important bacterium in the recent years. Initially, *E. faecalis* was a commensal intestinal bacterium, but after the mid of 1970, it become remarkable bacteria by becoming opportunistic human pathogen. The *E. faecalis* is able to become a serious and life-threating pathogen in the appropriate circumstance. Generally, the *E. faecalis* can cause various disease including nosocomial infections, urinary tract infections (UTIs), interaabdominal infections, wound infections, endocarditis, bacteremia, sepsis. In details, the nosocomial infection caused by *E. faecalis* particularly happen in intensive care units (ICU) that emerge as UTIs most of the time. The *E. faecalis* includes 20% of all urinary infected isolates. However, the UTIs caused by *E. faecalis* is related to the

resistance against antibiotics including, gentamycin, vancomycin and *etc* (1).

As it was mentioned, the endocarditis can also cause by the *E. faecalis* that can be very drastic and intensive disease with 25-30% hospital mortality per year. It is also interesting to know that 90% of all endocarditis caused by the *E. faecalis*. Some researchers report that the endocarditis can happen by the UTIs as an ascendant infection. In addition, the bacteremia and sepsis of *E. faecalis* occur mostly in neonatal patients (2). Subsequently, the *Enterococcus spp.* regard as a resistance producer machine in the world of bacteriology, which has attracted researchers' substantial interest in working on it. Indeed, they able to obtain resistance, but even more, they also able to transfer resistance genes to the other bacterial species. The antibiotic resistant *E. faecalis* is engendered by three manner including, intrinsic resistance, acquired resistance and tolerance. The intrinsic resistance can be seen to penicillin, ampicillin and the most cephalosporins. The resistance of *E. faecalis* was also reported to quinupristin, dalfopristin, linezolid, daptomycin and tigecycline. Therefore, the multi drug resistance of *Enterococcus ssp.* become a very prominent challenge in the hospital environments. Consequently, continuous monitoring and surveillance of *E. faecalis* is necessary to prevent diseases, which can cause by it (3).

For this purpose, the genotyping method has been developed in the recent decades. So that the obtained data from genotyping method is very beneficial to increase the level of the health care and surveillance of bacteria. Indeed, the data from genotyping methods are commonly used for diagnosis, treatment and epidemiological goals. For more clarity, the genotyping method are the kind of the infection control tools, which can trace the bacteria at the strain level. It is certainly used to characterize the association between the clusters whether they are related to the outbreaks or not.

In particular, the Multilocus Sequence Typing (MLST) is an ordinary typing method that is based on the characterizing bacterial species via sequencing of internal fragments of multiple housekeeping genes. Usually, seven housekeeping gene evaluate by the MLST method via the internet (4-6). In this process, each sequence of internal fragments compares with the other alleles that they were already characterized. Then, each sequence classified at one of those seven housekeeping genes category. Ultimately, by the combination of obtained data, the allelic profile will be construct and each distinct profile consider as absolute sequence type. Here we tried to type the *E. faecalis* isolates to provide advantageous information that can help us to understand epidemiological communication between the *E. faecalis* isolates. We hope that the obtained data will be helpful to control and surveillance the nosocomial infections caused by *E. faecalis* in hospitals of Ilam, Iran.

Materials and Methods

Sample Collection and Bacterial Identification

Initially, 100 *E. faecalis* were isolated from urine samples of Imam Khomeini Hospital, Ilam, Iran. Afterwards, all isolates were confirmed by the phenotyping method and then for more certainty, every isolates were authenticated by PCR analysis of 16sRNA gene. Eventually, all isolates were considered as *E. faecalis*.

Antibiotic Susceptibility Test

In order to understand the status of resistant to vancomycin, disk diffusion method was carried out based of CLSI 2016 table. Subsequently the resistant monitored isolates were checked for existence of *vanA* gene by PCR analysis for final endorsement of vancomycin resistance.

Determination of Different Alleles of *E. faecalis* by MLST

For this purpose, 7 housekeeping genes were used to gain MLST scheme for epidemiological study. In addition, to determine various type of *E. faecalis* pubMLST.org was selected and the MLST analysis was done based on recommended instruction by the pub.MLST. Accordingly, the PCR analysis was performed for selected 7 housekeeping genes. Then, the amplicons were sequenced. Continuously, all data were analyzed by Chromas software to define alleles. In addition, every unambiguous sequence was considered as an allele number (allelic profile). Afterward, each allelic profile was specified as a sequence type. For this reason, specific primers of 7 housekeeping genes were selected from the pubMLST.org database.

Table 1. Multilocus Sequence Typing (MLST) results of *E. faecalis* isolates.

MLST groups	Allelic profile (gdh,gyd,pstS,gki, aroE,xpt,yqil)	Sequence type	Number of isolates
A	12-1-3-5-6-17-5	109	2
B	1-3-1-5-4-1-1	152	4
C	4-4-8-3-8-1-1	503	6
D	1-1-9-1-1-1-1	324	4
E	4-4-8-3-2-1-3	11	4
F	4-4-4-3-2-1-5	399	2
G	14-7-9-3-10-1-1	469	8
H	2-1-13- 11-3-2-5	206 (with 6 allel mach)	2
I	2-1-13-11-3-2-2	206	2
J	17-2-1-3-14-1-1	1022	2
K	4- 6-2-4-1-1-4	17	6
L	7-7- 47-1-10-18-1	613 (with 6 allel mach)	6
M	2- 7-11-1-3-4-2	248	2
N	2-3-13-4-3-2-2	10	8
O	20-2-14-20-6-1-5	49	2
P	2-2-12-8-1-6-2	595	8
Q	12-8-3-8-65-4-5	395	2
R	1-7-3-2-6-1-5	51	4
S	4- 4-8-3-8-1-3	28	2
T	13- 6-5-10-9-9-5	142	2
U	1-7-10-1- 1-10-1	22	1
V	5-1-1-1- 7-23-6	638	2
W	6-2-11-5-10-1-22	68	1
X	4-6-16-4-1-1-4	9	4
Y	2-6-23-11-3-4-2	426	2
Z	21- 2-1-3-7-1-6	63	2

Results

Our disk diffusion results demonstrated that fifty-four out of one hundred isolates were resistant, four isolates were semi sensitive and forty-two isolates were sensitive to vancomycin.

To approved resistance, *vanA* gene were identified by the standard PCR analysis, which were positive for forty-eight out of fifty-four isolates and also all semi-sensitive isolates were negative. Subsequently, the MLST typing of *E. faecalis* isolates that was carried out based on pubMLST.org database showed unexpected results. So, 90 isolates were mlst. Using seven structural genes and using pubMLST.org database, different types of *Enterococcus faecalis* were determined. In group C, which contained 6 isolates, ST503

was obtained, for G,N and P groups in each 8 isolates obtained and STs of 469 , 10 and 595, respectively, identified. In groups K, which consisted of 6 isolates, ST17 was considered. Group L, which consisted of 6 isolates, did not obtain 7 maches allelic profile. The same was true for group H. Groups of U and W, which consisted of one isolate, ST 22 and 68 observed (Table 1).

Discussion

The result of present study showed that 26 different STs were distributed among *E. faecalis* isolates, so we couldn't find any epidemic correlation between isolates. Given that most of these isolates have resistance to vancomycin, they had low clonal correlation with each other and only had few similar STs pattern that it was mentioned. The presence of several strains with the same genomic pattern can be due to the various reason, such as transferring an isolate from one patient to another patient via hand contact or from contaminated environment by drinking dirty water or eating foods that is contaminated by stool. We determined the degree of allelic variations in seven housekeeping genes of *E. faecalis* by using a sample of 90 isolates originating from human sources. The degree of isolate differentiation by MLST appears adequate for use in epidemiological investigations, as the number of different types obtained by MLST. Single-locus phylogenetic trees were noncongruent, suggesting that recombination plays an important role in the generation of diversity of the *E. faecalis* population. Our findings generally showed that there is no main source of isolates in Ilam hospitals. Also, our findings demonstrated a dispersion in vancomycin resistant *E.faecalis* and vancomycin sensetive *E.faecalis* isolates. In a study by Ruiz-Garbajosa *et al.,* (7) in 110 different *E. faecalis*, 55 STs were obtained that showed the diversity of samples and low possibility of common accient in *E. faecalis*. Our results also demonstrated the same happens. In a study by Zalipour and collegues (8) in Isfahan, among 53 *E. faecalis* isolates, 8 different STs obtained that ST 6 and 422 were dominant. Our data revealed too variation in STs that was opposite with results of Zalipour and et al.we got 26 different allellic profiles that when checked in database possible STs were suggested. Different allelic profile in our isolates showed a decrease of possiblity of samples to be from a clonal leneage. The most isolates with one allic profle were from group P and G that can estimated they were from the same source. Overally, if we estimated the possible dominant STs we can suggested ST10 , 469 and 595 in groups of G,N and P. In a study by Weng and collegues in Malaysia, ST17 was dominat in *E. feacium* (9). Nallapareddy and *et al.,* (10) demonstrated among 22 different *E. faecalis*, 13 STs were obtained that showed the diversity of isolates that consistent with our findings. In conclusion, there were very diversity among isoaltes. We suggested to do MLST in each hopital annually to control bacterial reponsible for nosocomial infections.

Aknowledgments

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