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STUDY GENETIC RELATIONSHIP AMONG *ENTEROCOCCUS FAECALIS* STRAINS COLLECTED FROM URINE HARBORED DIFFERENT VIRULENCE PROFILES USING ERIC-PCR ASSAY

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ABSTRACT

Twenty five isolates of *Enterococcus faecalis* have been previously identified and characterized were subjected to ERIC-PCR analysis in order to study the genetic relationship with regarding to their virulence profile. Nine virulence profiles were identified according to the presence/ absence of five virulence factors; *asa1,gelE, esp, cpd and ace*. Most isolates (28%) were belonged to Virprofile1 *asa1+,gelE+, esp+, cpd+,ace+* followed by Virprofile3*asa1+,gelE-, esp+, cpd+,ace+* (20%), Virprofile4 *asa1+,gelE+, esp-, cpd+,ace-* accounting 16%, while virprofiles5 *asa1+,gelE-,esp-,cpd+,ace+*, virprofile6 *asa1-,gelE+,esp+,cpd+,ace+* and virprofile8 *asa1+,gelE-, esp+, cpd+,ace-* representing 8%, virprofile2 *asa1-,gelE-,esp-, cpd-, ace+*, virprofile7 *asa1+,gelE+, esp+, cpd+,ace-* and virprofile9 *asa1+,gelE+, esp-, cpd+,ace+* were 4%. ERIC-PCR analysis divided isolates into two main clusters named; cluster A accounting 28% which further classified into groups; 8% isolates were belonged to A1 and 20% were belonged to A2. Most isolates were belonged to cluster B accounting 72%. This cluster was involved two groups; six isolates (33.3%) were belonged to B1 while 66.6% of isolates were assigned as B2. However, no relationship was found between virulence profiles with phylogentic groups of the isolates.

KEYWORDS: ERIC, ***Enterococcus, faecalis***, PCR, Virulence Genes
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INTRODUCTION

Enterococcus faecali is a gram positive bacterium that inhabits the elementary system of humankind and some other mammals. It used to be classified as part of the group D *Streptococcu* system (Scudeller *et al.,* 2009). *E. faecalis* is seen in healthy persons, resembling other species in the genus *Enterococcus*, and could cause life-threatening infections. This is particularly true in hospitals units and wards, where the levels of antibiotic resistance in *E. faecalis* is soaring that participate in its pathogenicity (Murray, 1990). About 85-90% of enterococcal infection are caused by *Enterococcus faecalis*, and it takes the third place in causing nosocomial infections, specially bacteremia, sepsis in children, endocarditis, urinary tract infection (UTI), and wound infections (Facklam *et al.,* 1995; Reimer *et al.,* 1997). Although some Enterococcal species are considered relevant for their technological properties such as ripening, aroma development and inhibition of pathogens, they are not, unlike other lactic acid bacteria, recognized as probiotics (Kuriyama *et al.,* 2003; Emaneini *et al.,* 2008; Jamet *et al.,* 2012). The enterococci have the ability to obtain, gather and share extra chromosomal elements encoding virulence traits or antibiotic resistance genes; these abilities are considered to enlighten their significance as nosocomial pathogens (Klibi *et al.,* 2006). It is assumed that some virulence elements owned by the nosocomial enterococci would enhance their capability to colonize inpatients (Hällgren *et al.,* 2009). Social and economic factors, and the customs for antimicrobial prescription in each country are among different causes that lead to surge in the antimicrobial resistance rate of enterococci (Goossens *et al.,* 2005; Huang *et al.,* 2012). Subsequently, the antimicrobial resistance rates is clearly diverge among countries, in Asia-Pacific regions for example the rates are high, in contrast to the westernized

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countries where the rates are low. Moreover, over the last few decades there was alteration in the microorganisms responsible of urinary tract infection and their susceptibility to the antimicrobials (Goossens *et al.,* 2005; Hoban *et al.,* 2012). Hence, early recognition of the causative organisms of UTI and the pattern of their sensitivity to the antibiotics will ensure effective UTI management. There are various techniques to determine the enterococci type including protein analysis, biochemical profiles and antibiotic susceptibility. Nevertheless, these methods were missing the discriminatory power, which necessitated the development of molecular-based techniques as alternatives. Furthermore, numerous nucleic acid–based techniques, including pulsed-field gel electrophoresis (PFGE) (Weng *et al.,* 2013), plasmid profiling, and polymerase chain reaction (PCR)-based methods such as; randomly amplified polymorphic DNA based PCR (RAPD-PCR), repetitive extragenic palindromic sequence PCR (Rep-PCR), and enterobacterial repetitive intergenic consensus PCR (ERIC-PCR). These techniques have been extensively utilized to determine the enterococci typing and characterize their genetic variability. Enterobacterial Repetitive Intergenic Consensus (ERIC) sequence is the sequence length is about 124-127bp, which contain about 44bp highly conservative core sequences in its center, primarily presents with multiple copies in genomes of Enterobacteracae and vibrios (Wilson and Sharp, 2006). This technique has been applied for genotyping of *E. faecalis* isolates (Martin-Platero *et al.,* 2009). Among these molecular typing approaches, PFGE is the most effective technology or typing of *E. faecalis* isolates due to its high reproducibility and discriminatory ability. However, PFGE is labor intensive and time-consuming (Weng *et al.,* 2013). In contrast, ERIC-PCR is a relatively simple and cost-effective method, which has been successfully used for genotyping of different bacterial pathogens and for tracking the bacterial source of contaminated water products (Martin-Platero *et al.,* 2009). This study aims to provide new insights and view of the diversity of the species *E. faecalis* by studying the differences among *E. faecalis* strains with different virulence profile have been collected from urine using ERIC-PCR assay.

MATERIAL AND METHOD

Sample collection and determination the virulence profile of *E. faecalis* isolates:

Twenty-five samples of *E. faecalis* have been involved in this study, they were isolated from 788 urine samples. Genomic DNA was extracted from purified and identified colonies using the genomic DNA purification kit supplied by Jena Bioscience (GmbH, Germany). The prevalence of five virulence genes *asa1 ,gelE, esp, cpd and ace* encode to Aggregation substance, Gelatinase, Enterococcal surface protein, sex pheromones and collagen binding protein respectively were also studied previously by Khalid *et al.* (2016). These genes were used to determine the virulence profile of each isolate depending on the presence/absence of studying genes.

ERIC-PCR DNA Fingerprint Analysis:

All isolates were subjected to PCR amplifications in a final volume of 25µL containing: 4µl of genomic DNA, 50 pmol of each primer, and 12.5 µl of master mix (Master Mix (Go Taq)). The primers used for ERIC-PCR typing were 5'CAGCCATGAACAACTGGTGGCG-3' and R-5'TGCTTTGCGCAGGGAAGATTCC-3' (Versalovic *et al.,* 1991). Conditions were performed as following; initial denaturation at 95°C for 7min. Then 30 cycles of a denaturation step at 90°C for 30s; annealing at 52°C for 1min; extension at 68°C for 8 min; Later, a final extension step at 65°C for 16 min and final storage at 4°C (Versalovic *et al.,* 1991). ERIC-PCR fingerprints of amplified DNA fragments obtained by 1.2% agarose gel electrophoresis were recorded. The positions of the bands on each lane and each gel were normalized using molecular marker (1500-100bp.). The molecular weight of each amplicon has been determined by Vilber Lourmat-CAPT software. The zero-one manual method was used to count the bands. Then, the data were entered on the following site: http://insilico.ehu.es/dice_upgma/, and the dendrograms were drawn (Merza *et al.,* 2018).

RESULTS

Twenty five of *E. faecalis* isolates which have been collected from urine, identified and characterized previously by (Khalid, 2016) were involved in this study. Depending on the

presence or absence of five virulence genes among these isolates, nine virulence profiles have been identified as shown in Table (1).

Table(1): Representing the virulence profile of twenty five of *E. faecalis* isolates

	Name	Virulence profile	No. isolates (%)
1.	**Viroprofile1**	***asa1+,gelE+, esp+, cpd+,ace+***	**7(28%)**
2.	**Viroprofile2**	***asa1-,gelE-,esp-, cpd-, ace+***	**1(4%)**
3.	**Viroprofile3**	***asa1+,gelE-, esp+, cpd+,ace+***	**5(20%)**
4.	**Viroprofile4**	***asa1+,gelE+, esp-, cpd+,ace-***	**4(16%)**
5.	**Viroprofile5**	***asa1+,gelE-,esp-,cpd+,ace+***	**2(8%)**
6.	**Viroprofile6**	***asa1-,gelE+,esp+,cpd+,ace+***	**2(8%)**
7.	**Viroprofile7**	***asa1+,gelE+, esp+, cpd+,ace-***	**1(4%)**
8.	**Viroprofile8**	***asa1+,gelE-, esp+, cpd+,ace-***	**2(8%)**
9.	**Viroprofile9**	***asa1+,gelE+, esp-, cpd+,ace+***	**1(4%)**

These isolates were subjected to ERIC- PCR analysis to study the genetic relationship among these isolates using ERIC primer. All strains were successfully amplified multiple bands with different molecular weight, with excluding of the amplicon with molecular weigh more than 1.5Kb, these isolates were produced 173 band, each isolates produced varied bands between 2 to 10 bands, the lowest molecular weight was 0.16Kb. (Figure 1). The most frequent band was 1.28kbp. that produced by 23/25 (92%) of these isolates. While the least band was 0.4kbp., produced by only three strains. According to the amplification profile of these isolates, 20 ERIC-types were produced. Five isolates (EF1, EF2, EF3, EF12, and EF15) shared the same profile, they have bands with 1.28, 0.72, 0.61, 0.48, 0.42, 0.35, 0.29, 0.19 and 0.16kbp., While the other isolates have different patterns

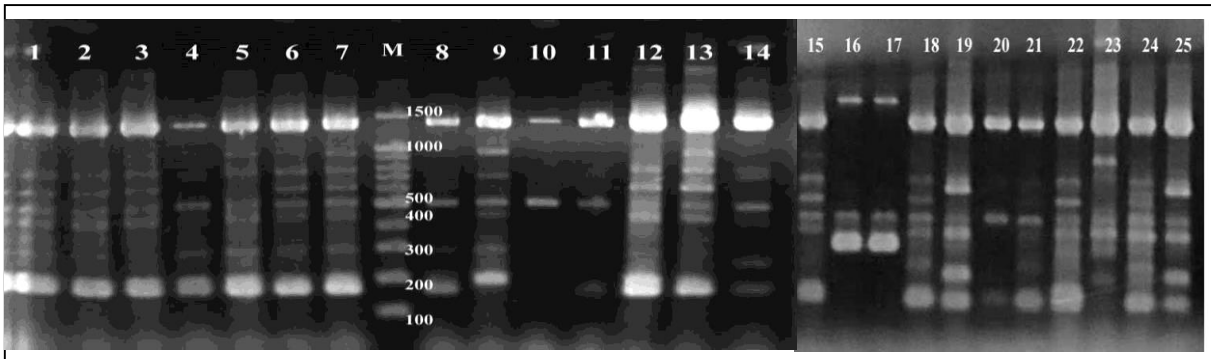
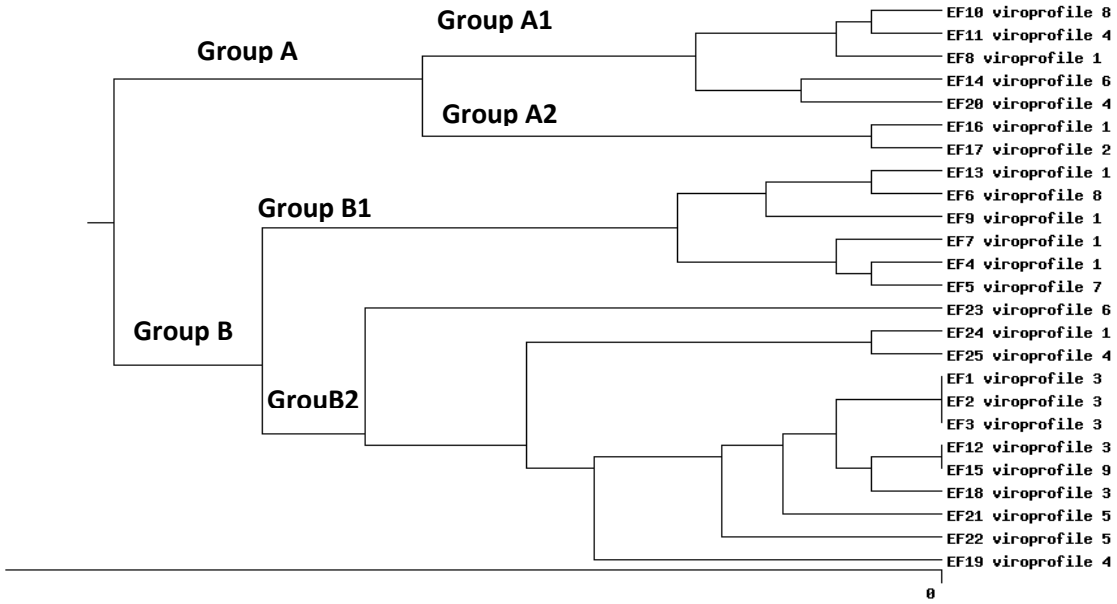


Fig. (1): Shows the ERIC -PCR amplification pattrens of 25 *E. faecalis isolates* collected from urine using 1.2% Agarose gel electrophoresis run on 8V/cm, M lane is Molecular Marker (1500-100Bp.)

Data of ERIC-PCR products of *E. faecalis* strains were analyzed by UPGMA to determined phylogenetic tree. *E. faecalis* strains have been classified into two main clusters; Group A and Group B. GroupA accounting 28% involving two subgroups; Five strains (20%) were belonged to A1 (EF8, EF11, EF18); (EF14, EF20) and subgroup A2 (8%) (EF16, EF17), while Group B was found the prominent group involving most isolates accounting 72%, Six isolates were representing 24% within groupB1 (EF6, EF9; EF13); (EF4, EF5, EF7), while group B2 was involved two subgroups; only one strain (4%) belonged to B21 (EF22) and (40%) of these strains were belonged to B22 (EF23, EF24, EF 25) (EF1, EF2, EF3, EF12, EF15, EF18, EF18, EF21, EF 22, EF 19) as shown in diagram (1).

Diagram (1): The Phylogenetic analysis of 25 *E. faecalis* strains produce different virulence profile constructed **with the use of UPGMA**



DISCUSSION

In fact, the infectious agent that causes an outbreak often result from clones that are genetically identical or related to the source of infection. In other word, it may have the same biochemical traits, virulence factors and genomic characteristics (Olive and Bean, 1999). DNA-based typing methods have been widely used to demonstrate the epidemiology and molecular characterization of most pathogens (Ranjbar *et al.,* 2014). Recently, ERIC-PCR analysis has been successfully applied to study the genetic relatedness of different microorganisms such as; *Staphylococcus aureus* (Ye, *et al.,* 2012), *Pseudomonas aueroginosa* (Han *et al.,* 2014), Uropathogenic *E. coli* (Ardakani *et al.*,2016; Merza *et al.,* 2018), *Enterococcus cecorum* (Wijetunge *et al.,* 2012). This technique provides rapid with highly discriminatory value for *E. faecalis* typing(Wei *et al.,* 2017). In this study, the genetic diversity among twenty five *E. faecalis* strains collected from urinary tract infects were determined via ERIC-PCR analysis. Although these isolates were belonged to different virulence profiles, the results of this study showed high relatedness among isolates collected from the same source, they were generally assigned into two main clusters, 72% of them were belonged to cluster B, while 28% of them were represented cluster A. However, Jurkoviç *et al,* 2007 and Bachtiar *et al.,* 2015 demonstrated considerable genetic diversity among *Enterococcus* spp. Furthermore, in this study it has been found that the isolates within each cluster were belonged to different virulence profiles, therefore, non correlation was found between virulence genes with phylogenetic analysis of these isolates. This may due to high relationship among these isolates in addition to the high virulence content for most isolates, only one isolate expressed single studied virulence gene, while most isolates have considerable content of virulence ability as shown in Table (1). This finding may agree to study by Blanco *et al.,* 2018 who found ERIC-PCR didn't cluster stains according their virulence. Besides, Eaton & Gasson, 2001 and Borst *et al.,* 2014 suggested that the molecular taxonomy of *Enterococcus spp.* might not be able to distinguish between avirulent and virulent strains.

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