Identification of Enterococci Species Isolated from Clinical Specimens by Internal Transcription Spacer (ITS) and Trna Intergenic Spacer.

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Abstract
With the prevalence of enterococci that are resistant to various medicines and cause a variety of clinical illnesses in Iraqi hospitals, accurate and speedy detection and characterization of enterococci types has become critical. As a result, we used the internal transcriptional spacer region (ITS) and the *tRNA* gene to molecularly describe *Enterococcus* spp. isolated from several clinical sources (urinary, vaginal, diarrhea, spinal cord fluid). For the rapid identity of *Enterococcus* spp. show the genetic result different bands profiles, which indicated one to four DNA fragments. Using the PCR-ribotyping approach to identity 34 *Enterococcus* spp isolates according to *16S-23S rRNA* (ITS) and *tRNA* gene findings, four genotypes, and four subtypes were discovered. The DNA fragment sizes in the *16S-23S rRNA* (ITS) gene ranged from 300 to 800 bp, whereas the DNA fragment sizes in the tRNA gene ranged from 290 to 700 bp. Interspecies genetic variety was discovered through the examination of DNA band patterns. As a result, the common bands in all *Enterococcus* spp isolates are the widths 300 and 400 bp in *16S-23S rRNA* (ITS) and 290 to 700 bp in the *tRNA* gene. Genotype I was the most common, accounting for 82.35 percent (28 isolates) in *E. faecalis*, 8.82 percent (3 isolates) in *E. faecium*, 5.88 percent (2 isolates) in *E. casseliflavus*, and 2.92 percent (1 isolate) in *E. gallinarum*. Many *Enterococcus* species are cause clinically important opportunistic infections that require precise and early identification in order to get focused treatment. Currently, the PCR-ribotyping technique and method based on sequencing for the *16S–23S rRNA* (ITS) gene and the *tRNA* gene have proven to be a quick, accurate, and reliable method for identifying Enterococci species. PCR amplified of the *16S–23S rRNA* (ITS) gene and the *tRNA* gene revealed 28/34 (82.35%) *Enterococcus faecalis* isolates, with 10/10 (100%) isolates from urine, 11/11 (100%) isolates from the vagina, 10/4 (40%) isolates from diarrhea, and 3/3 (100%) isolates from the CSF. Following that are 3/34 (8.82%) *Enterococcus faecium* isolates, which comprise 3/10 (30%) isolates from diarrhea. Then there were 2/34 (5.88%) *Enterococcus casseliflavus* isolates and 1/34 (2.94%) *Enterococcus gallinarum* isolates from diarrhea. We conclude that employing PCR-ribotyping to amplify the *16S-23S rRNA* (ITS) and *tRNA* genes of pathogen enterococci provide a valid method for species identification of pathogen enterococci in clinical specimens.
Keywords: Enterococci , 16S-23S rRNA (ITS), tRNA gene

1. Introduction

Enterococci are Gram-positive bacteria that can also be found in the environment, yogurt, and other foodstuffs, as well as on vegetation, They would be most usually observed on the mucus membranes of people and animals, They can cause disease in a variety of different ways depending on circumstances. The majority of these cases occur in hospital admissions, and these bacteria are the one of the most frequent cause of nosocomial infections. [7] .

Species of enterococci in special a most prevalent enterococci species collected from diverse clinical specimens is E. faecalis, accounting for (80–90%) of isolates, followed by E. faecium, which accounting for (5–10%) [17].which are two common causes of urinary tract infection, bacteremia, wound infections, intra-abdominal infections, endocarditis, as well as infections in the pelvis Endophthalmitis, otitis, septic arthritis, and sinusitis are all possible side effects of enterococcal infections of the respiratory tract and central nervous system. [1].

the development of technologies based on nucleic acid or protein analysis has enabled exact Enterococci bacteria can now be identified and classified considerably more quickly and easily. Aside from the standard methods for identifying numerous bacterial species, there are a number of other options. such as multilocus sequence analysis (MLSA) [14] and sequencing of 16S rRNA gene [5]

In their rRNA genetic loci, prokaryotes have the among 16S, 23S, and 5S genes Spacer sections, which vary in length and sequence depending on the genus and species, PCR may be used to amplify the spacer area between the 16S and 23S rRNA genes, and the polymorphisms in the resultant product can be utilized to identify bacteria [10]. Analysis of tRNA intergenic spacer based on amplicons of spacers among tRNA genes was proposed by McClelland et al. [13], to differentiate at the species level streptococcal strains of groups A, B, and G and Streptococcus mutans. This technique has also been applied successfully for the identifying of Staphylococcus aureus. [12]. because more microbes possess multiple copies (alleles) of the rRNA operons per genetic material

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and the spacer region may vary in size inside of various operons, this corresponds to the number and type of tRNA genes (tRNA-Ile, tRNA-Ala, tRNA-Glu) found in some spacer regions, the intergenic spacer regions are subject to lower selection pressure and thus show greater genetic change. [8].

2. Materials and Methods

Bacterial strains

In this research, 34 clinical isolates of enterococci were isolated from a variety of clinical specimens, including urine, vaginal, diarrhea, and cerebrospinal fluid (CSF), were collected from inpatient and outpatient sent to the AL- Children's and Women's Educational Hospital Laboratory in AL-Qadisiyah, Iraq within (December 2020 - May 2021) [1].

HiEnterococci plate, macConkey plate, blood plate, and bile esculin plate were used to culture all isolates (Himedia, India). then incubated at 37°C for 24h. where to identify the Enterococci according to the cultural features like colony morphology, macroscopically gram staining, and biochemical assays, such as catalase, oxidase, bile esculin hydrolysis, growth at 10-45°C, tolerance at 60°C, and growth on 6.5 percent NaCl media at pH 9.6, species Enterococcus [6].

ITS-PCR and tRNA-PCR

To extract genomic DNA from all Enterococcus spp isolates, a High Pure PCR template Kit (Anatolia, Turkey) was employed[2].

The universal primer L1 (5-CAAGGCATCCACCGT-3) and G1 (5-GAAGTCGTAACAAGG-3) were used for 16s-23s rRNA ITS. and for tRNA gene use the universal primers T5A (5-AGTCCGGTGCTCTAACCAACTGAG-3) and T3B (5-AGGTCGCGGGTTCGAATCC-3) are described by [3]. A 50-liter reaction mixture containing 25 liters of master mix (Amplicon, Denmark), 16 liters of PCR buffer, 2.5 liters of each primer (F and R), and 4 liters of template DNA was used for amplification. ITS and tRNA sequences were amplified using specific primers. In a thermal cycler, an initial denaturation at 95 °C for five min was continued then 35 cycles of denaturation at 95 °C for 1 min, annealing at 56 °C for 1 min to 72 °C for 1.30 min, and a final extension at 72 °C for five min (Eppendorf, Germany). The PCR products were electrophoresed in a 2% agarose gel after electrophoresis seen using a UV transilluminator (Bioneer, South Korea)[3].

3. Results

Bacterial isolates

Thecurrent study collected 375 specimens, and 34 (9.06%) Enterococcus strains were isolated from the various clinical specimens, including; (Urine 10 strains, 29.41 percent, Vaginal 11 strains 32.35 percent, diarrhea 10 strains 29.41 percent , and CSF 3 strains 8.82 percent). Respectively 70.5 percent and 29.4 percent of the specimens were obtained from women and men.

As shown in Figure 1, out of 34 identified species, 27 (79.41 percent) were E. faecalis, 3 (8.82 percent) E. faecium, 2 (5.88 percent) E. casseliflavus, and 1 (2.94 percent) E. gallinarum identified by PCR amplification using appropriate primers (Fig.1,2).

16s-23s rRNA (ITS) gene

The 16s-23s rRNA (ITS) patterns of different enterococci species were determined based on the number and size of amplicons that are present (Fig. 1). As demonstrated in Figure 1, all enterococcal species had two or three main bands with diameters ranging from 300 to 800 bp (Fig.1,2).

All clinical isolates in specimens (urine, vaginal, and CSF) identify are E. faecalis based on had discrete 16s-23s rRNA (ITS) patterns with two primary bands of 300-500bp, whereas diarrhea specimens had distinct ITS-PCR patterns, showing a high degree of intraspecies diversity. four strains are displayed E. faecalis has two major bands varying in size from 300 to 500bp, three strains of E. faecium have two major bands varying in size from 300 to 600bp, three large bands with sizes of 300, 400, and 800bp are found in two strains of E. casseliflavus, while three significant bands with sizes of 300, 350, and 500bp are found in one strain of E. gallinarum (Fig.1).

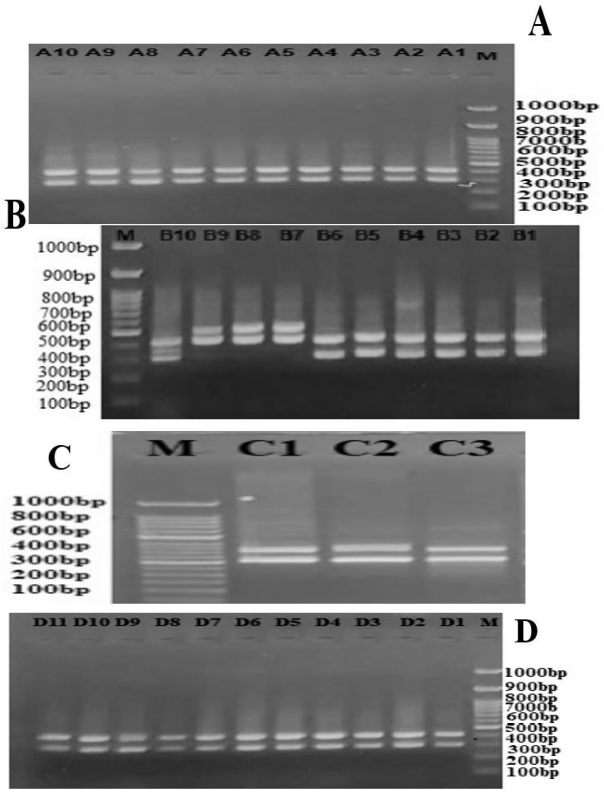

FIG. 1. 16s-23s rRNA (ITS) patterns of clinical specimens of enterococcal species (A) Urine specimens Lanes: A1 to A10 E. faecalis, (B) Diarrhea specimens Lanes: B1,B4, E. casseliflavus; B2,B3,B5,B6, E. faecalis; B7,B8,B9 E. faecium; B10 E. gallinarum, (C) CSF specimens Lanes: C1to C3 E. faecalis, (D) Vaginal specimens Lanes: D1to D11 E. faecalis. Lane M, molecular size marker (1000bp).

tRNA-gene

In Enterococcus spp. tRNA-PCR analyses revealed patterns of 1 to 4 major bands. the species was identified by a number of different main bands ranging in size from 290 to 700bp. (Fig. 2). In all clinical specimens, E. faecalis strains showed the same four bands with sizes ranging from 290 to 650 bp (Fig 2. A, B, C), E. faecium showed two bands with sizes 290 and 400 bp, E. casseliflavus possessed three bands with sizes 290, 400 and 600 bp, and E. gallinarum possessed one band with sizes of 290 bp (Fig.2,C).

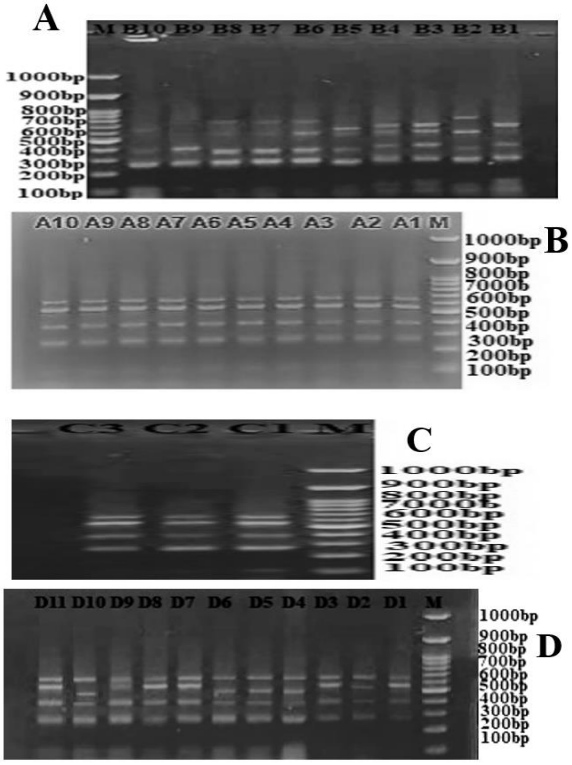

Fig. 2. Electrophoresis in a 2% agarose gel of tRNA gene patterns of enterococcal species isolated from clinical specimens. (A) Urine samples Lanes: A1 to A10 E. faecalis, (B) Diarrhea specimens Lanes: B1,B4, E. casseliflavus, ; B2,B3,B5,B6, E. faecalis; B7,B8,B9 E. faecium; B10 E. gallinarum, (C) CSF specimens Lanes : C1 to C3 E. faecalis, and Vaginal specimens Lanes: D1 to D11 E. faecalis. Lanes M, molecular size marker (1000bp).

4. Discussion

Only four species are (E. faecium, E. faecium, E. casseliflavus, and E. gallinarum) were identified based on ITS and tRNA gene obtained in this investigation for isolates of specific Enterococcus spp., which were consistent with results obtained by other researchers Nowakiewicz et al., (2015). Separating the ITS-PCR and tRNA-PCR products on a 2 percent agarose gel, as suggested by Tyrrell et al., (1997), provided higher resolution and also reported the existence of non-specific high-molecular-size band and as well as low-molecular-size bands, which showed up to be unique to each strain not to a specific Enterococci species.

The discovery of a considerable amount of variation in the ITS fingerprint of Enterococcus spp. led to this study, which was one of the more interesting outcomes. Because the ITS region consists of between one copy of 16S rRNA and one copy of 23S rRNA is most likely reflected in each major species band, these findings show both E. casseliflavus and E. gallinarum isolates have at least two or more unique ITS forms with different nucleotide compositions. The cause of this disparity is unknown. Perhaps it has something to do with the quantity or the number tRNA copies in the ITS region recent research has demonstrated that the 16S-23S rRNA ITS region of bacteria contains one or more tRNA genes. [11][4].

Because they are highly conserved among species, for bacterial identification and evolutionary studies, the rRNA genes (16S, 23S, and 5S) are appealing gene choices [19]. Between the 16S and 23S rRNA genes, the ITS1 regions have been shown to be under less evolutionary pressure [16]. As a result, they're commonly employed to distinguish and identify closely related bacteria [20-23], which have a lot change in sequence and length at the genus and species level. When there are many operons in a single cell, The spacer size varies greatly between species and even between operons within a single cell [24]. The length discrepancies are owing to the existence of numerous functional components, such as tRNA genes, inside them.; RNase III, which is involved in the splicing process to produce the mature ribosome (2), and boxA, which functions as an antiterminator during transcription, are examples of enzyme recognition sequences. [9]. Non-essential sequences that are often inserted and deleted, such as rsl in several E. coli operons, make up the majority of this area. [4].

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author's Contribution

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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