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Molecular screening the emergence of Vancomycin and Gentamycin resistant *Enterococcus* species in burn' patients in Basrah government, Iraq

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***Abstract*---**Emergence of multidrug resistance bacteria (MDR) in burn infections including *Enterococcus* yet to be managed and highlighted in most of hospitals in our region. This study included 200 clinical samples collected from wound, urine, stool and blood of 75 burn patients in Al-Faiha Teaching Hospital in Basrah, Iraq, between 2020 -2021. Based on Enterococci chromo agar, biochemical test and PCR for *16S rRNA* gene, 50 isolates were identified as *Enterococcus* spp., involving 20 (40%) *E. faecium*, 14 (28%) *E. faecalis*, 7 (14%) *E. gallinarum*, 6 (12%) *E. gilvus*, 2 (4%) *E. casseliflavus* and 1 (2%) *E. avium*. All detected Enterococci were reported worldly as pathogenic bacteria to human. Six new local strains of *Enterococcus* were recorded in NCBI and the Gene bank as follow; *Enterococcus gallinarum* IraqYaHa5 and IraqYaHa19, *Enterococcus faecium* IraqYaHa23, IraqYaHa48 and IraqYaHa60, and *Enterococcus gilvus* strain IraqYaHa50. Phylogenetic tree was constructed to all isolated Enterococci. The clinical isolates showed resistance up to nine antibiotics. However, enterococci isolated from healthy people during this study were 100% sensitive to six of those antibiotics. Duplex PCR was applied to detect vancomycin and gentamicin resistance genes. All resistant *Enterococcus* to gentamicin had harboured *aph (3')*. While vancomycin resistant not consistant with the presence of *vanA*. Based on outcome here (out of 50, 44 clinical enterococci were MDR), the present study have suggested the emergences of enterococci as the third common cause of acquired infections in burn after *Staphylococcus* and *Pseudomonas*, causing difficulty in therapeutic choice and need more attention.

***Keywords*---**Burn infection, *Enterococcus* spp., vancomycin and gentamycin resistance genes.

Introduction

Burn bacterial infections are a serious hindrance to patient recovery. The most common infections in burn patients included urinary tract infection, pulmonary infections, bacteremia and sepsis. Worldwide, infections have been estimated to cause about 73% of burn patient deaths as a result of septic process (Tancheva and Hadjiiski 2005). Microorganisms colonizing burn wound originate from the patient's endogenous flora. However, they may also be transferred to the patient through contaminated hospital environmental surfaces, water, fomites, air, and the soiled hands of health care workers (Varshochi et al., 2020). In most cases, the bacterial infection of burn wounds is an unquestionable phenomenon because of the skin destruction, which plays a role as the major barrier to bacterial access to the internal tissues (Labibzadeh et al., 2018). The burn skin injury provides a rich environment of avascular necrotic tissue that supplies microorganism with a rich medium of nutrient that cause suppuration. Sometimes, the formation of scars leads to reduce migration of immune cells into the burned area and impair local host immune responses, which lead to limit distribution of antimicrobial agents, produced by the host to the burned area (Chaudhary et al., 2019; Shariati et al., 2021). Gram-positive bacteria are more predominant at first in hospitalized burn cases, but in prolonged hospitalization, gram-negative bacteria become more prevalent (Pironyeh et al., 2017). There are predominant five species of bacteria, *Staphylococcus aureus, Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Enterococcus faecalis* (Jasem et al., 2018; Li et al., 2018). According to some researchers (Hashem et al., 2017; Labibzadeh et al., 2018), enterococci have become one of the most common nosocomial pathogens. The US National Nosocomial infection Surveillance system (NISS), has recorded Enterococci among the top three common pathogens of nosocomial infections and the leading cause of nosocomial infections in burn patients (Norbury et al., 2016; Labibzadeh et al., 2018). As a genus, enterococci have a relatively short history, most of which intertwined with that of other Gram-positive cocci, especially streptococci. Presently, there are 55 enterococci species reported so far based on 16s rDNA sequences (Kim et al., 2021), only eleven species including (*E. faecalis, E. faecium, E. avium, E. casseliflavus, E. gallinarum* and *E. gilvus*) have been associated with human infection and pathogenesis (Byappanahalli et al., 2012; Lebreton et al., 2014).

The treatment of these infections has been clinically challenging because of the increasing resistance to different types of antibiotics including prevalence of multidrug resistance (MDR) enterococci. Resistance genes only does not point to pathogenicity of a bacteria, however combined with the virulence determinant it can cause bacteria to become dangerous (Mustafa et al., 2021). Strong biofilm producer exhibits multidrug resistance in many bacterial species, the ability of enterococci to form biofilms contribute to bacterial virulence in several ways including the resistance to antibiotics (Hashem et al., 2017; Shridhar and Dhanashree 2019). This work aims to detect the emergence and prevalence of highly Vancomycin and gentamycin resistant nosocomial *Enterococcus* species in burn' patients in Basrah province, Basrah, Iraq.

Method

Materials and methods

1- Sample collection and bacterial identification

Total of 200 samples collected from 75 burn patients in the burn unit of Al-Faiha Teaching Hospital Basrah, Iraq. Samples were divided into (75wound swab and 75, 38 and 12 blood, urine and stool samples respectively. All samples transplanted in Brain Heart Infusion broth (Himedia, India) and incubated in 37°C for 24 hours. The samples were then cultured on Azide blood agar (Himedia, India), with adding sodium azide a concentration 0.04% (Merck, Germany)) and incubated for 24-48 hour at 37°C. The growing bacteria were cultured then on HiCrome *E. faecium* Agar Base (Himedia, Labs) incubated for 24 - 48 hour at 37°C. Nutrient agar was used to keep isolates and to preform the biochemical tests such as Gram stain (Biotech, India), Oxidase and Catalase. In addition, hydrolytic of aesculin was tested on bile-esculin agar (Himedia, India), grow in different range of temperature included (10-45-60) °C for 24 hr. as well as growing in 6.5% NaCl, was investigated following (Facklam and Sham, 1995; MacFaddin, 2000 and Josephine et al, 2006), followed by identification using genetic approach.

2- Molecular diagnosis

A- DNA extraction

DNA extraction of *Enterococcus spp.* was applied on each sample by picking a single colony with a sterile loop, which then inoculated into sterile tubes of 5ml brain heart broth. and incubated at 37° C for 24 hr. DNA of the *Enterococcus* isolates, extracted by using of DNA purification kit, according to the instruction of the manufacture (Geneaid Company).The extracted DNA was visualized using agarose gel 1% and then it was stored at -20°C until further use.

B- Polymerase chain reaction

Polymerase chain reaction (PCR) amplification was performed using universal *16S rDNA* gene primers (table. 1). The reagents to amplify the *16S rDNA* gene were 50 µl, including (Go taq Promega green master mix (Promega USA) 25 µl , DNA template 2µl (50 ng/µl), forward primer 2 µl, reverse primer 2 µl (100 picomol\ml). the reagent one completed to 50 µl by NFW 19 µl). The mixture was vortexed and centrifuged for short time. The reaction conditions for PCR was as follow, Initial denaturation 95°C 5 min for 1 cycle. 35 cycles of denaturation 95°C for 30 sec, annealing 55°C for 30 sec, extension 72° C for 30 sec. then final extension 72°C for 5 min (1 cycle) and cooling 4°C (Miyoshi et al.,2005). The product size of *16SrDNA* gene approximately 1500bp that was visualized by 2% agarose gel electrophoresis (Niveditha et al., 2012).

C-Sample preparation for sequencing and Identification of bacterial species

The PCR product were sending for sequencing in Macrogen Company, following the sequencing instructions. After receiving the sequence results, bacterial species were identified using chromes https://chromaspro.software.informer.com/program with Basic Local Alignment search tool (BLAST) followed by National Center for Biotechnology Information (NCBI) http://www.ncbi.nlm.nih.gov (Kerbauy et al., 2011). The receiving sequences was open in Applied Bio systems (chromas program), FASTA and plain text formats was used also. High quality data was applied to automatically removing the low quality sequence to improve sequence assembly. BLAST searches through the NCBI web site and multiple alignments were the tools to identify the bacteria on genus and species level was applied in this study. The high similarity percentage between the corrected sequences under investigation with the reference one in NCBI was the first choice.

D. Phylogenetic Tree

Sixty *16S rDNA* concatenated sequences for six *Enterococcus* species from clinical isolates detected in the present study were aligned with *16S rDNA* sequences of their reference strains using multiple sequence alignment. The phylogenetic tree was constructed using (MEGAX) Molecular Evolutionary Genetics Analysis http://www.megasoftware.net (Kumar et al., 2018). This program achieves multiple alignment on nucleotide sequences and uses the neighbor joining (NJ) method for phylogenetic tree construction.

3- Antibiotic susceptibility test

A. Antibiotic discs assay

Ten antibiotics included vancomycin (VA30mg), meropenem (MRP10mg), erythromycin (E15mg), ciprofloxacin (CIP10mg), levofloxacin (LEV 5mg), tetracycline (TE 30mg), ceftriaxone (CRO 30mg), gentamicin (GEN10mg), chloramphenicol (C30mg) and piperacillin (PRL 100mg). Were selected to use in the present study based on the Clinical and Laboratory Standards Institute (CLSI 2020) and the antibiotic use in the hospital to treat burns infections. The diffusion method was used in Antibiotic susceptibility test for each isolate (Nichollas, 2000). Then the inhibition diameters were measured the sensitive, intermediate and resistant bacteria were recorded depending on the inhibition doses recommended by CLSI 2020 for each antibiotic.

B. Detection the resistance genes for vancomycin and gentamicin in Enterococci

Conventional Polymerase chain reaction (PCR) with specific primers for the vancomycin *vanA* gene and gentamicin *aph* (3') gene as shown table (1) was used in the present study. The annealing temperature for the primers was determined by gradient PCR, the reaction reagents to amplify the genes were run in 50 µl. (Go taq Promega green master mix 25µl, DNA template 5µl (50 ng/µl), 1.5µl (100 picomol\ml) from forward and reverse primer for each gene) the mixture was completed to 50 by N .F .W. (14 µl). The mixture was vortexed and centrifuged for short time, then the reaction conditions for PCR were; initial denaturation 94°C 4 min for 1 cycle, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing 57°C for 35 sec and extension 72°C for 35 sec. The final extension was 72°C for

5min and then cooled at 4°C. After optimizing the PCR conditions, duplex PCR for *vanA* and *aph* (3') were used. The product size of genes that were 732bp and 523bp respectively, were visualized by 2% agarose gel electrophoresis.

Table 1
Primer sequence for detection *16S rRNA* gene and antibiotic resistance genes (vancomycin *van A* and gentamicin *aph (3')*) using PCR.

Primer name	DNA Sequences (5'-3')	Product size bp	Reference
16S rRNA	F-AGAGTTTGATCCTGGCTCAG R-GGTTACCTTGTTACGACTT	1500	Miyoshi *et al.*,2005
van A	F-GGGAAAACGACAATTGC R-GTACAATGCGGCCGTTA	732	Dutka et al.,1995
aph(3')	F-GGCTAAAATGAGAATATCACCGG R-CTTTAAAAAATCATACAGCTCGCG	523	Vakulenko et al.,2003

Statistical analysis

Results of the current study were statistically analyzed using SPSS software (version 26). One-way ANOVA was performed to evaluate the differences among *Enterococcus* species resistance of antibiotic. $P < 0.05$ were considered as statistically significant.

Results

Bacterial identification

Based on MacFaddin (2000) and Collee et al., (1996), the microbiological and biochemical characteristics were determined for the bacterial isolates. 122(61%) clinical samples were showed turbidity in BHIb media from total samples 200 included; 75/200 (37.5%) swab, 31/200 (15.5%) urine, 12/200 (6%) stool and 4/200 (2%) blood. Out of 122, 110 samples were grow on azide blood agar, divided as 70/122(57.37%) swab, 26/122(21.31%) urine, 12/122(9.84%) stool and 2/122 (1.64%) blood. The colonies of these bacteria appeared on the medium as a slightly convex circular shape with a smooth white or creamy mounds edge. The 110 isolates grown on Azide blood agar, were then cultured on HiCrome Agar plates, 100 from these isolates showed three types of colored colonies, 46/100(46%) isolates gave white colonies, 30/100 (30%) isolates were appeared as green colonies surrounded by yellowish coloring of the ambient and 24/100(24%) isolates forms blue colonies. *E. faecalis* forms blue colonies. *E. faecium* gives green colonies, surrounded by yellowish coloring of the ambient as they were in this study, other species of *Enterococcus* gave green and blue colonies, as shown in figure(1). While, white colonies was signs for *Staphylococcus* sp. A Gram stain test was indicated that the ten isolates that did not develop on HiCrome agar plates were Candida. Testing these isolates on bile-esculin agar showed grown black or brown colonies. There are definitive biochemical tests to differentiate *Enterococcus*, which appeared as Gram-positive single, double and short chains cocci. Oxidase and catalase negative. Salt tolerant as high as 6.5% of NaCl. All strains were able to grow in temperatures of 10°C and 45°C as well as their ability to withstand at 60°C for 30 minutes. All

isolates of this bacteria was catalase-negative except five isolates include (5-*E. gallinarum*, 11-*E. casseliflavus*, 12, 39-*E. gilvus*, and 41-E. *avium*) are weakly positive-catalase.

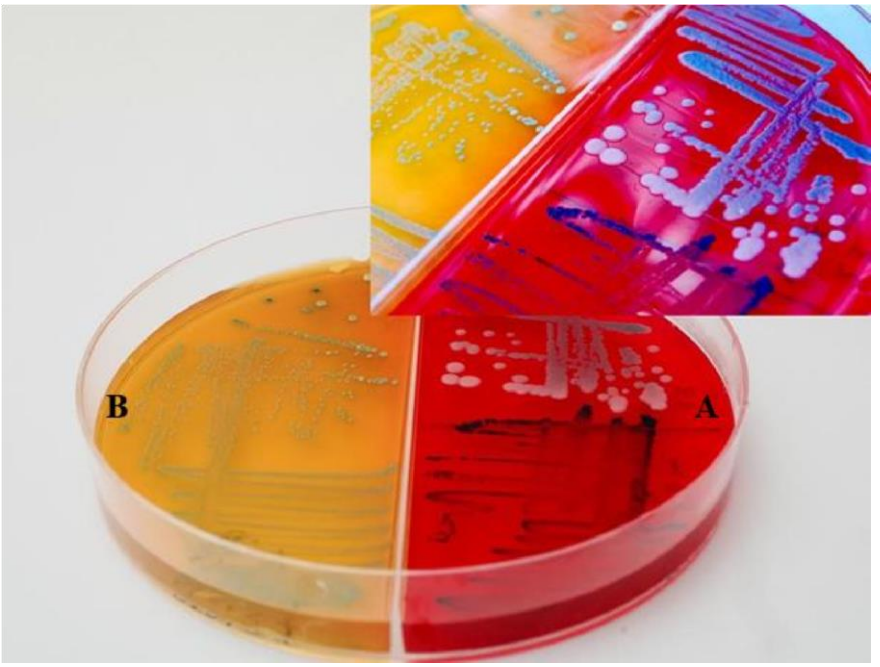

Figure (1): *Enterococcus* spp. isolates on HiCrome Agar medium, A: Blue colonies is signs to *E. faecalis*, B: green colonies surrounded by yellowish coloring is signs to *E. faecium*

Molecular work

Out of 200 clinical samples, 100 isolates showed all the tests resemble to *Enterococcus* sp., were Gram-positive cocci and grown on selective media. These Enterococci were subjected to confirmation using molecular approach to the 16s rDNA gene detection and sequence subjecting. Out of 100 clinical isolates 50, were identified as *Enterococcus* sp. the obtained 50 of *Enterococcus* were divided as follow; 20 (40%) *E. faecium*, 14 (28%) *E. faecalis*, 7 (14%) *E. gallinarum*, 6 (12%) *E. gilvus*, 2 (4%) *E. casseliflavus* and 1 (2%) was *E. avium*. The percentage of appearance of *Enterococcus* in the samples of wounds, urine, stool and blood was 24 (48%), 16 (32%), 9 (18%) and 1 (2%), respectively.

During the treatment and proofreading for incoming sequence result, six new sequences of bacterial isolates were identified by comparing nucleotides with their type strains. The sequence of new isolates were deposited in the National Center for Biotechnology Information (NCBI) and the Gene bank databases. The first and second stains were *E. gallinarum* IraqYaHa5, *E. gallinarum* IraqYaHa19 were similar to *E. gallinarum* FDAARGOS, *E. gallinarum* FUA3375 in the NCBI database respectively except a deletion mutation in T base for both of them as shown in figure (2). The third, fourth and fifth were new isolates of *E. faecium* IraqYaHa23, *E. faecium* IraqYaHa48 and *E. faecium* IraqYaHa60 were similar to *E. faecium* TEM 1, *E. faecium*

1042 and *E. faecium* M17_J8 in the NCBI database respectively. A transition mutation G base instead of A base, a deletion mutation in A base and a deletion mutation in T base respectively of them as shown in figure (3). The sixth isolate was *E. gilvus* IraqYaHa50 was similar to *E. gilvus* 1_SR_D15_70 in the NCBI database with a deletion mutation in T base, as shown in figure (4).

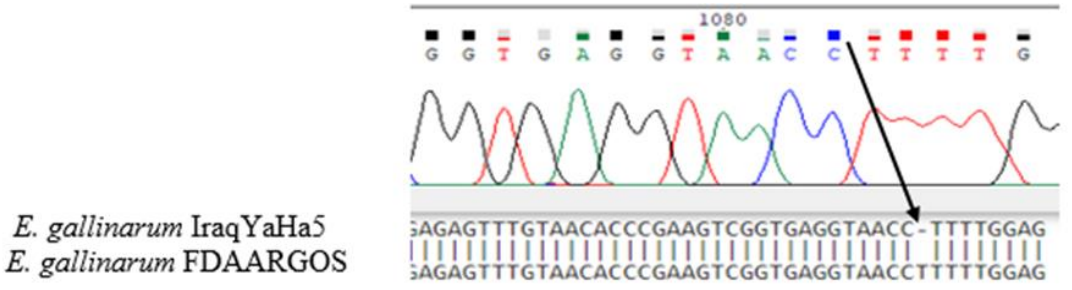

Figure (2): Comparison of *16s rDNA* nucleotide sequence for isolate 5 from present study with their type strain FDAARGOS, deletion mutation (deletion T) at the position 1084.

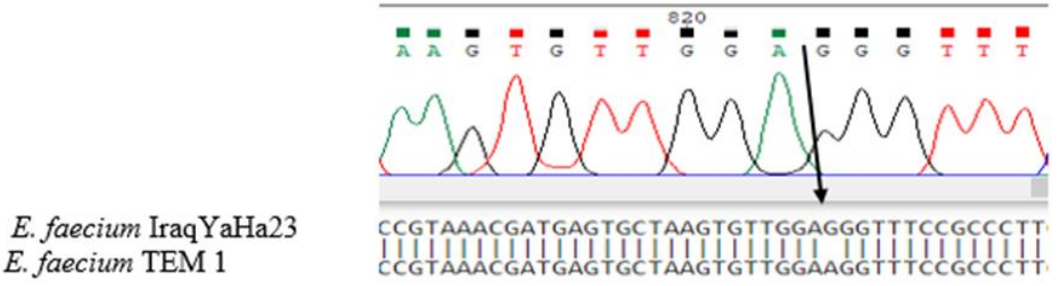

Figure (3): Comparison of *16s rDNA* nucleotide sequence for isolate 23 from present study with their type strain TEM 1, transition mutation (G instead A) at the position 823.

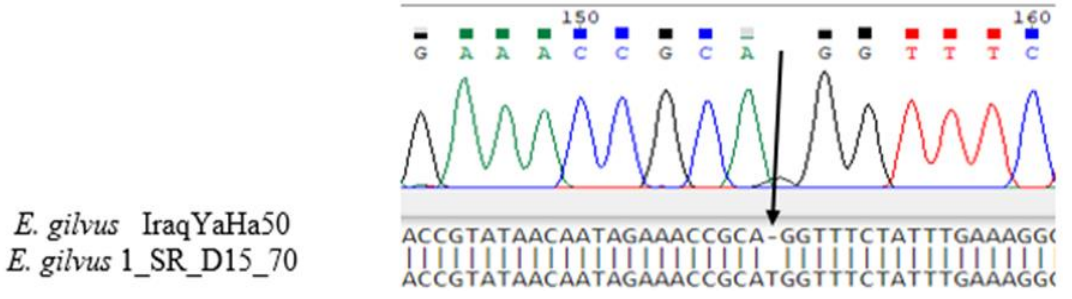

Figure (4): Comparison of *16s rDNA* nucleotide sequence for isolate 50 from present study with their type strain 1_SR_D15_70, deletion mutation (deletion T) at the position 155.

The rooted phylogenetic tree for sixty *16S rDNA* sequences for studied enterococci species was constructed, as shown in figure (5). The tree showing the distribution and phylogenetic relationships of six different *Enterococcus* spp. isolated from clinical samples of burn patients with their reference strains (ATCC).

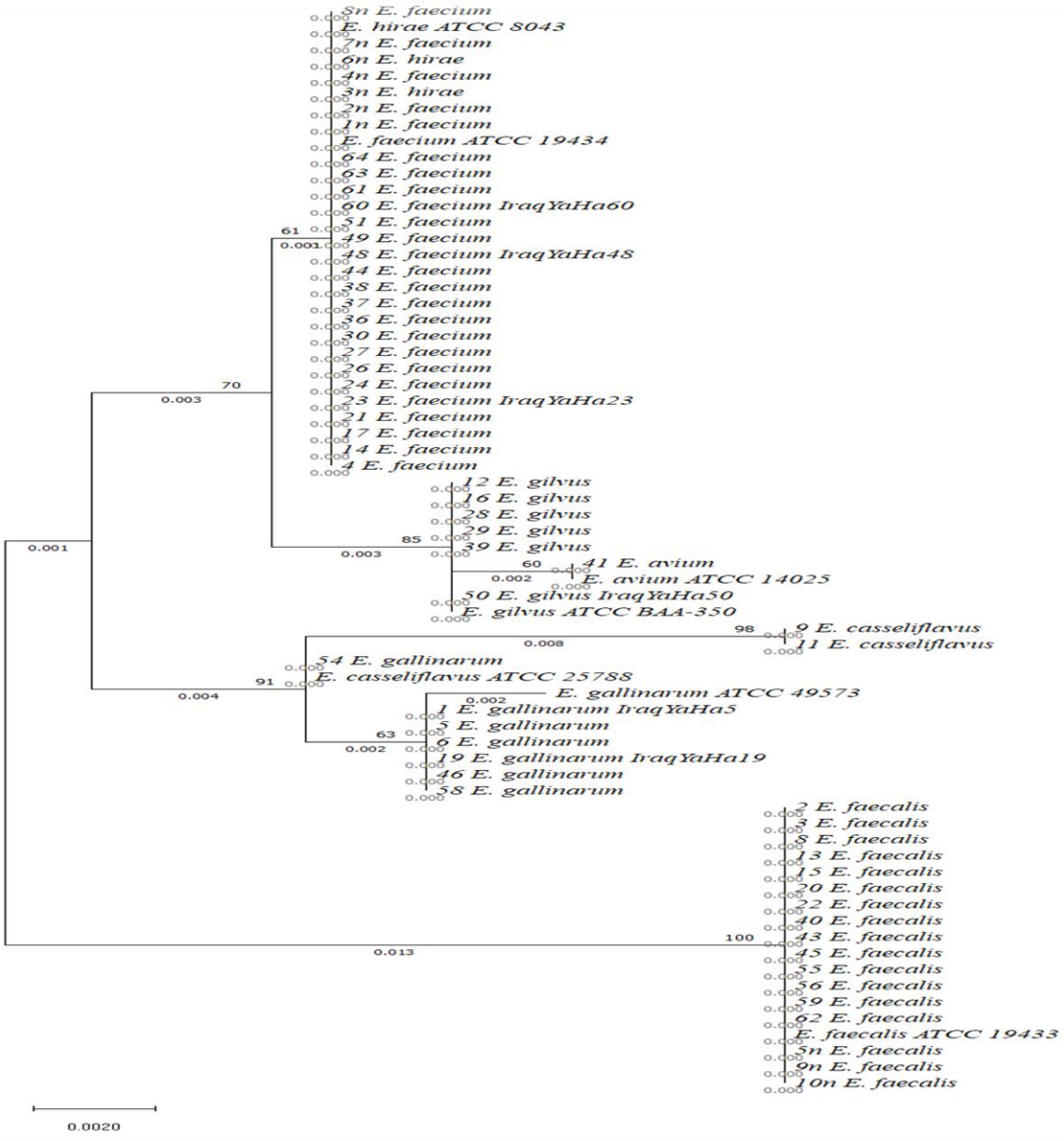

Figure (5): Neighbor-joining tree showing the distribution and Phylogenetic relationships of 60 *Enterococcus* spp. with their reference strains (ATCC). All horizontal branch lengths were drawn to scale. Bootstrap values after 1000 repetitions are indicated.

Antibiotic Susceptibility

Antibiotic susceptibility to determine the resistance and phenotypic variations of isolated strains using disk diffusion was used (Maugeri et al., 2019), as illustrated in table (2) shown the isolates resistance to antibiotics, *E. faecium* isolates was the most resistance to all used antibiotics, except chloramphenicol (C) were *E. gallinarum* isolates more resistance (10%). Emergence of multidrug resistant (MDR) strains. Out of 50 isolates, 44 were multidrug resistance, 20 (40%), 12 (24%), 6 (12%), 4 (8%) and 2 (4%) from *E. faecium, E. faecalis*, *E. gallinarum*, *E. gilvus* and *E. casseliflavus* respectively. However, *E. casseliflavus* isolates were susceptible for most antibiotics including (E, TE, C, CIP, LEV, PRL and MRP) followed as *E. avium* isolate that was susceptible for each of VA, CIP, LEV, MRP and CRO. The results of the statistical analysis showed $P<0.05$, significant differences of resistance antibiotic between *E. faecium* and *E. faecalis*. In contrast, no significant differences of other species ($P>0.05$).

Table 2
Antibiotic susceptibility of *Enterococcus* spp. in clinical isolates

Clinical isolates	Percentage of resistance antibiotics									
	VA 30mg	GEN 10mg	E 15mg	TE 30mg	C 30mg	CIP 10mg	LEV 5mg	PRL 100mg	MRP 10mg	CRO 30mg
*E. faecium**	22%	36%	30%	14%	8%	24%	36%	30%	30%	36%
*E. faecalis**	12%	24%	18%	22%	8%	6%	10%	0%	0%	12%
E. gallinarum	10%	6%	14%	8%	10%	4%	4%	10%	6%	14%
E. gilvus	4%	4%	10%	8%	6%	2%	2%	8%	2%	4%
E. casseliflavus	4%	4%	0%	0%	0%	0%	0%	0%	0%	4%
E. avium	0%	0%	2%	2%	0%	0%	0%	0%	0%	0%
Total	52%	74%	74%	54%	32%	36%	52%	48%	38%	70%

*P -value was $P<0.05$ (significant differences of resistance antibiotic between *E. faecium* and *E. faecalis*)

Detection of vancomycin and gentamicin resistance genes in Enterococci

Genes encoding proteins conferring resistance to gentamycin *aph* (3') and vancomycin *vanA* antibiotics were detected in clinical isolates and absence in healthy infants stool isolates (under investigation). The results showed the *aph* (3') gene was presence in all clinical isolates of *Enterococcus* 50 (100%) (approximately 523bp). However, 30 out 50 isolates showed to harbour the *vanA* gene (approximately 732bp), which is one of the most important antibiotics against *Enterococcus*. 16 (32%) *E. faecium*, 6 (12%) *E. faecalis*, 3 (6%) *E.gallinarum*, 3 (6%) *E. gilvus*, 1 (2%) *E. casseliflavus* and 1 (2%) *E. avium*, as shown in figure (6).

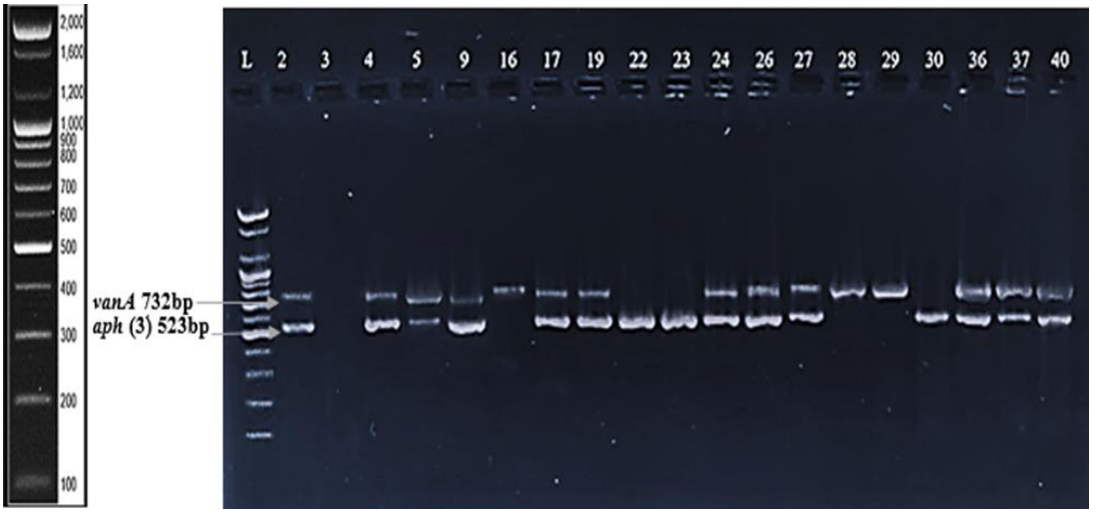

Figure (6): Agarose gel electrophoresis of duplex PCR product for *vanA* (732bp) and *aph* (3') (523bp). Lane L: 100 bp DNA ladder, 2-40 represent of isolates.

There were correlation between gentamicin resistance and presence of *aph (3')* gene in the clinical isolates. All isolates resistance of gentamicin had harboured *aph (3')* gene. While less Correlation was between vancomycin resistance and presence of *vanA* gene. Among the *E. faecium* isolates, 16 (80%) isolates harboured van gene and 9 (65.25%) isolates resistant of vancomycin. There are 6 (42.85%) isolates of *E. faecalis* resistance to vancomycin, the *vanA* gene was absent. 5 (71.42%) out of (*E. gallinarum* resistance vancomycin) 1 (20) isolate was harboured *vanA* gene. Also *E. gilvus* and *E. casseliflavus* 2 (33.33%) and 1 (50%) resistance to vancomycin respectively, only one isolate of each them contain *vanA* gene.

Discussion

Compatibility of samples collection through winter period, associated with the most severe burns, many of those who were have burn injuries are children and old patients (Ribeiro et al., 2019). Different selective media have tested for the isolation and identification of enterococci; Culture-based techniques are also used in regulatory activities to estimate the densities of enterococci in environmental sample. Since none of the enterococcal media available can be used to discriminate between the different species (Ryu et al., 2013). HiCrome *Enterococcus faecium* Agar recommended for the chromogenic detection of *Enterococcus faecium* from urine, faeces, soil, food, water, plants and animals. *E. faecium* ferment arabinose and cleaves the chromogenic substrate present in the media to produce green colored colonies along with yellow coloration to the medium. While *E. faecalis* does not ferment arabinose and therefore retains the blue color (Willinger and Manafi, 1995), not all enterococcus species are able to grow in these selective media, but the most clinically relevant species grow well (Garcia-Solache and Rice 2019). Since traditional phenotypic methods are not sufficient, it recommended to use polymerase chain reaction technique PCR with universal primers for 16S rDNA gene that provides a rapid, accurate and more sensitive for detection the species of *Enterococcus* (Mustafa et al., 2021). There are numbers of worldwide studies were showed frequency bacterial infected burn patients including study of Naqvi et al., 2014 was observed that more infections of burn wound caused by *Staphylococcus*

aureus (23%) then followed *Pseudomonas aeruginosa* (21%) and less prevalence of Enterococci (1.2%). Locally, most studies including (Al-Hamdy, 2015; Aljanaby and Aljanaby, 2018; Jasem et al., 2018; Rahim and Hateet, 2021) were shown *P. aeruginosa* was the most common pathogen associated with burn wounds followed by *S. aureus* whereas *Enterococcus* sp. recording lesser of prevalence (2-3%). Study of Alhamdani and Al-Luaibi, 2020 out of 112 samples 41 isolates were diagnosed as *Pseudomonas aeruginosa*, 26 isolates from burn patients and15 isolates from the surrounding environment. In addition, Chmagh 2016, in her study out of 65 isolates were 53 (81.5%) isolates of *S. aureus* were isolated from a burn wound. However, the current study showed that prevalence of *Enterococcus* sp. were 50%, while *Staphylococcus* sp. were 38%. Almost, all studies in our country were not awared that enterococcal infection is a third major cause in burn patients, lack of monitoring the appearance of enterococci in hospitals led to its prevalence and emergence in this high percentage of the current study. On the other hand, the spread of enterococci in recent years may be due to the excessive use of antibiotics

Enterococci have been suggested as the third most common nosocomial pathogens and frequently are the causative pathogens surgical wound and potentially life-threatening infections including sepsis. It is emerged as important agent of human disease largely because of their resistance to antimicrobial agents. They are important nosocomial pathogens capable of causing serious and the prevalence of enterococcal infections, mainly hospital-acquired isolates have with mechanism of resistance to antimicrobial agents are more frequent. Furthermore, they have great capacity for transmitting these resistances to other species and even to other genera (Iwuafor et al., 2021). In addition to the costs imposed to health systems of burn cares , the importance of the vancomycin resistance enterococci strains emergence, these were could serve as a van genes reservoir for other organisms, especially *S. aureus* and this could be a real problem because vancomycin is the therapeutic agent of choice for methicillin resistant *S. aureus* (Gardete and Tomasz 2014). The results of sequencing *16S rRNA* gene were shown 50 isolates identified as *Enterococcus* isolates, involving 20 (40%) *E. faecium*, 14 (28%) *E. faecalis*, 7 (14%) *E. gallinarum*, 6 (12%) *E. gilvus*, 2 (4%) *E. casseliflavus* and 1 (2%) *E. avium*. The incidence of *E. faecium* was higher than *E. faecalis* and other species in clinical isolates, these results was consistent with the researcher Sattari-Maraji *et al.*, (2019) and Shahi et al., (2020). This increase in the prevalence of *E. faecium* species compared to other species may be due to a serious problem is antibiotic overuse, which promotes the emergence of antimicrobial-resistant pathogens. Common resistance of these bacteria to anti-enterococcal drugs, such as ampicillin, aminoglycosides and glycopeptides (Gawryszewska et al., 2016; Sattari-Maraji et al., 2019). The current study similar to with most studies, where drug sensitivity results showed that enterococci are multidrug-resistant bacteria, this due to being Enterococci are intrinsically resistant to multiple antibiotic agents, cephalosporins, penicillins, and low concentrations of aminoglycosides (Hollenbeck and Rice 2012). Phylogenetic tree of *Enterococcus* sp., were including four groups: 1) *E. faecium* and *E. hirae*, 2) *E. avium* and *E. gilvus*, 3) *E. gallinarum* and *E. casseliflavus* and 4) *E. faecalis*. This a result shown similarity to Byappanahalli et al., 2012, *Enterococcus* sp. were divided into five group, including (*E. faecalis*, *E. faecium*, *E. avium*, *E. gallinarum* and *E. cecorum*).

The clinical importance of *Enterococcus* spp. is directly related to their antibiotic resistance that contributes to the risk of colonization and infection. The species of the greatest clinical importance are *E. faecalis* and *E. faecium* (Kajihara et al. 2015). In this study, antibiotic resistance rate in *E. faecium* isolates was significantly higher

than *E. faecalis*, the p-value was 0.001($p<0.05$). While other species no significant differences ($p>0.05$). The bacteria transferred from the environment of hospitals, tend to be more resistant to antimicrobial agents than those originating from the patient's normal flora (Elmanama et al., 2013; Jasem *et al.*, 2018). The *aph (3')* gene was presence in all clinical isolates of *Enterococcus*. While, 30 out 50 isolates showed to harbour the *vanA* gene. Among hospitalized patients, *E.faecium* has the highest resistance rate to vancomycin and gentamycin, due to the expression of *vanA* and *aph* (3') genes (Miller et al., 2014). The high prevalence of *Enterococcus* resistance to these antibiotics has been previously reported (Labibzadeh et al., 2018; Aun et al., 2021). All isolates obtained from healthy infants have not shown the presence of any resistance genes, and 100% sensitive to antibiotics Vancomycin, Meropenem, ciprofloxacin, levofloxacin, Chloramphenicol and tetracycline, as shown in study perform by Zhang et al., (2016), none of the enterococcus isolates they isolated from infants were resistant to ampicillin or chloramphenicol. Enterococci are among the first LAB to colonize the neonatal GIT and could be associated with infant health and development of the human microbiome (Dominguez-Bello et al., 2010). The threat of the multi-antibiotic resistance of enterococci to public health has attracted considerable attention from the scientific community and being a major reason of treatment failure. The current study was shown 44 of 50 isolates were multidrug resistance; 20 (40%), 12 (24%), 6 (12%), 4 (8%) and 2 (4%) from *E. faecium, E. faecalis*, *E. gallinarum*, *E. gilvus* and *E. casseliflavus* respectively.

Conclusion

In the current study, *Enterococcus* spp. has been emerged in clinical samples of burn patients as a major causative agent. *E. faecium* and *E. faecalis* were the two most abundant *Enterococcus* as pathogens of the isolates, *E. faecium* was more than *E. faecalis* in prevalence, antibiotic resistance and appearance of the *vanA* and *aph (3')* genes. These results explain that environment of hospitals and prolonged hospitalized are reason for emergence of enterococci from commensals to pathogenesis bacteria. The hospitals management was not aware of enterococci infection and emergence as main pathogens in burn patients of burn halls, and became more prevalent from last years in clinical samples due to have resistance as multidrug, also ability to acquire responsible genes for antibiotics resistance.

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