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Multidrug resistance Enterococcus Faecalis isolated from patients with urinary tract infections

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***Abstract*---**Background: Globally, urinary tract infections (UTIs) constitute the most prevalent health issue. UTIs are challenging to cure due to the emergence of antibiotics resistant bacterial strains. This research aimed to determine the incidence of UTIs, identify the causative bacteria, and evaluate antibiotic resistance profile and genes in *Enterococcus faecalis.* Materials and methods: In this study, twenty *E. faecalis* isolates from patients with urinary tract infection were identified; phenotypic and genotypic characteristics were investigated in Al-Najaf City, Iraq. Results: The data revealed different rates of resistance starting with high rate (65%) of isolates which were resistant to Nalidixic acid and (60%) of isolates resistant to Ciprofloxacin and Tetracycline. then ends with lower resistance which were (20%) of isolates have resistance to Imipenem and Fosfomycine. At the same respect, all isolates of *E. faecalis* displayed multi drug resistance (MDR). Antibiotics resistance genes *bla-tem*, *emeA* and *gyrA* were found in 100%, 95% and 55% of isolates, respectively. However, this research explained that none antibiotic was entirely working against all isolates. Conclusion: All *E. faecalis* isolates exhibited multi drug resistance (MDR), and the most potent antibiotics were imipenem and fosfomycin. The ability of *E. faecalis* isolates to carry antibiotics resistance gene such as (*bla-tem, emeA and gyrA*).

***Keywords*---**Enterococcus faecalis, urinary tract infections, antibiotic resistance genes, multidrug resistance.

Introduction

Urinary tract infections (UTIs) are the most prevalent bacterial infections that often affect all components of the urinary system. It is the second most common infection following respiratory tract infections, with a greater susceptibility rate among women than men [1]. Urinary tract infection is a frequent infection of the urethra, bladder, or kidney. Bacteria may enter the urethra and migrate to the bladder, producing an infection. The infection might also spread to the kidneys. UTI occur in males as well as women, however they are most common in women [2]. *Enterococci* are gram-positive, facultative anaerobic, non-spore forming, and catalase negative bacteria. They appear as single, pairs, or short chains of cocci when seen under a microscope. *Enterococci* classified as lactic acid bacteria. An estimated thirty-five species have been named and may be found all throughout the nature [3], [4]. The most of enterococcal infections are caused by two species: *E. faecalis* and *E. faecium*. Both species have intrinsic resistance to common antibiotics such cephalosporins, aminoglycosides, clindamycin, and trimethoprim-sulfamethoxazole [5].Many infections caused by *E. faecalis*, including urinary tract infections. This pathogen has gained great resistance to many antibiotics and possesses a large number of virulence factor genes.[6], [7]. Antibiotic resistance mechanisms in multidrug-resistant *Enterococci* include efflux pump overexpression, drug target change, and antimicrobial agent inactivation prior to reaching a target site. While bacteria generally employ efflux as a means of transporting nutrients, they may also use the same mechanism to expel antibiotics [8], [9].

For effective treatment and control of UTI in a particular area, a good knowledge of the antibiotic sensitivity pattern of the causative agents in that area is of ultimate importance [10]. The aim of study include investigation the occurrence of *Enterococcus faecalis* in the urinary tract infection as well as determining of their resistance to antibiotics and Detection of antibiotics resistance genes such as (*eme*A, *gyr*A and *Bla tem*).

Materials and Methods

Bacterial isolates

During November 2021 to the end of January 2022, (20) *Enterococcus faecalis* isolates were collected from urine specimens of patients with urinary tract infection from Al-Kawthar health care center in Al-Najaf Al-Ashraf, Iraq. Specimens of urine were collected (in sterile containers, midstream urine). Identification of *Enterococcus faecalis* was performed based on a series of conventional microbiological tests include colonial morphology on blood agar, MacConkey agar, HiCrome™ UTI Agar and other biochemical tests. The 16s rRNA gene was targeted by Polymerase chain reaction (PCR) using specific primers[11] , and VITEK-2 compact system in order to confirm the identity of isolate.

Antimicrobial susceptibility test

The susceptibility of 20 *E. faecalis* strains to 11 antibiotics were measured. The following antimicrobials were tested: Amoxyclav, Fosfomycin, Naldixic acid,

Ciprofloxacin, Levofloxacin, Nitrofurantoin, Vancomycin, Rifampin, Imipenem, Tetracycline and Doxycycline.

Mueller-Hinton agar (Himedia, India) plates were inoculated using a swab that had been immersed in a bacterial suspension standardized to match the turbidity of the 0.5 McFarland standard. The surface of the plate was swabbed in three directions to ensure a complete distribution of the inoculum over the entire plate. The antimicrobial Disks were applied and the plates were inverted for incubation at 37 ºC in 18-24 h. After incubation, the plate was examined and a circle zone of growth inhibition was seen around the disk. They were translated into interpretative categories of susceptible or resistant according to the guidelines of Clinical Laboratory Standards Institute (CLSI).

DNA extraction

Total DNA was extracted from colonies grown on agar plates by boiling method according to [12] with some modifications. One bacterial colony was took using sterile wooden stick from surface of agar plates and suspended in 500µl of distilled water. The suspension heated for 15 min at 100 ºC followed by 5 minute on ice rapidly.

Gene detection

PCR assay was performed for identification of *E.faecalis,* Specific 16S rRNA gene may be used as target for identification of specific species of bacteria, such as the study that used *E. faecalis*-specific 16s rRNA gene primers were included as a control when the biochemical identification for *E. faecalis* was doubtful [13], [14]. Detection of antibiotics resistance genes include *eme A* gene (that encode multi-drug efflux pumps), *bla-TEM* gene (that encoding for extended-spectrum β-lactamase) and *gyr A* gene (quinolones resistance genes). Table (1) demositrate primers used in this study.

Table (1): primers used in this study

Primers	Sequence (5'-3')	Product size(bp)	References
16s rRNA for *E.faecalis*	F: TGGCATAAGAGTGAAAGGCGC R: GGGGACGTTCAGTTACTAACGT	290	[13], [14]
eme A	F: ACAGAAGAGCTGCAGGAAATG R: GACTGACGTCCAAGTTTCCAA	123	[15]
gyr A	F: TTGCTAATGCTAGTCCACGACC R: GCGTCAACACTTGCATTGCCGAA	575	[9]
bla TEM	F: ACCCCGTATCATTGGTTT R: ACGCATTGCTTTTCCATC	766	[16]

Results

In the present study, 412 urinary specimens were examined. UTIs are diagnosed based on patient's clinical symptoms, presence of leukocytes, and bacteria in the

urine. Of 412 specimens, 110 (26.7%) were positive for significant pyuria, while 302 (73.3%) were insignificant. gram negative bacteria isolates including *E. coli* (36), followed by (21) *Klebsiella ssp.*, (2) *Proteus ssp.*, and (1) *Pseudomonas spp.*.While gram-positive bacteria isolates were (29) *Enterococcus spp.* include (20) isolates of *Enterococcus faecalis*) followed by (21) *Staphylococcus spp.* Figure (1) show the percentage of bacteria that cause UTI in this study.

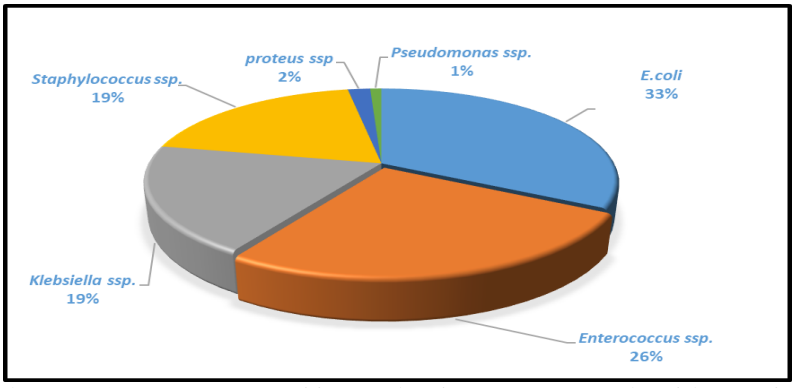

Figure (1): percentage of bacteria that cause UTI in this study.

Antimicrobial resistance pattern

In this study, a total of 20 isolates of *E. faecalis* showed a high resistance level to many antibiotics, including various resistance rate among tetracycline's antibiotics, the resistance rate in tetracycline was 12 (60%), while doxycycline was 7 (35%). Moreover, *Enterococcus faecalis* isolated in this study showed resistance to vancomycin at 10 (50%) and amoxiclave 11 (55%). At the same respect, the results displayed the resistance to nitrofurantoin was six isolates (30%), while various rates of resistance was observed with quinolones antibiotics including high resistance 13 (65%) by naldixic acid, ciprofloxacin and levofloxacin showed resistance rate 12 (60%), 11 (55%) respectively. Furthermore, the degrees of resistance for rifampin were 10 (50%). Lastly, this study declared that the most potent antibiotic was imipenem and fosfomycin that displayed a highest activity against this *E.faecalis* with low resistance rate 4 (20%) for each one of them.

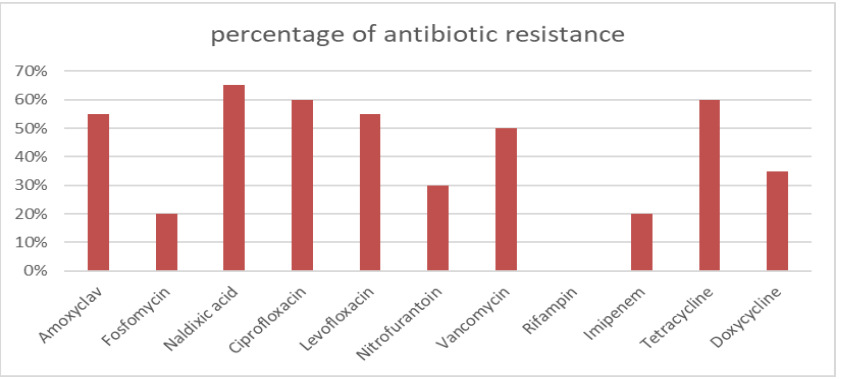

Figure (2): antibiotics resistance among *E. faecalis* isolates

E. feacalis involved in urinary tract infections isolated from patients, showed resistance for many antibiotics. In addition, the high number of MDR isolates gives rise to concern, were all isolates show resistance to three or more of antibiotics used in this study. The emergence of *enterococci* that are resistant to many antibiotics, even newer antibiotics used as a last choice, is alarming and causes widespread worry. These are increasingly associated to severe human morbidity and mortality because of their various resistance to antimicrobial agents [17].

Distribution of antibiotics resistance genes
Detection of *bla-TEM* gene

The results of PCR amplification of *bla-TEM* gene were revealed that all 20 isolates of *E. faecalis* were positive. Figure (3) demonstrated the distribution of *bla-TEM* gene among the isolates.

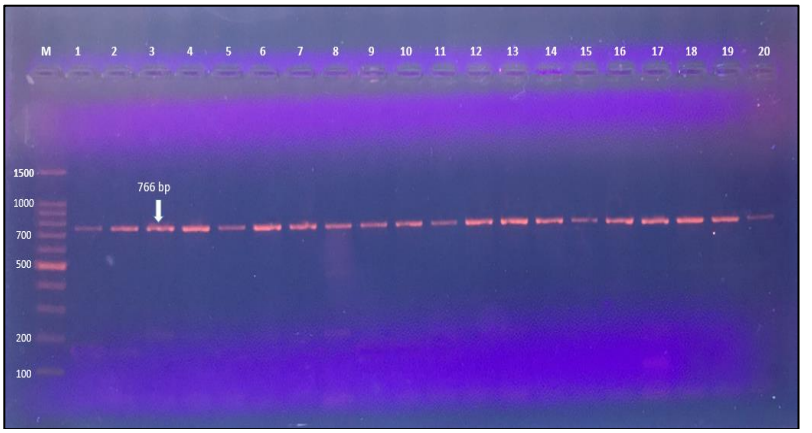

Figure (3): Electrophoresis diagram of PCR amplified products for extracted DNA of 20 isolates of *E. faecalis* using specific *bla-TEM* gene primer, lines 1 to 20 show positive products at 766 bp. Line M: molecular size DNA marker and products migrated at 75 volt for 80 minutes and stained with ethidium bromide

Detection of *eme A* gene

The results of PCR amplification of *eme A* gene (that encode multi-drug efflux pumps) revealed that most isolates of *E. faecalis* were positive. The figure (4) demonstrated that the results of PCR amplification for *eme A* gene were present in 19 (95%) of isolates.

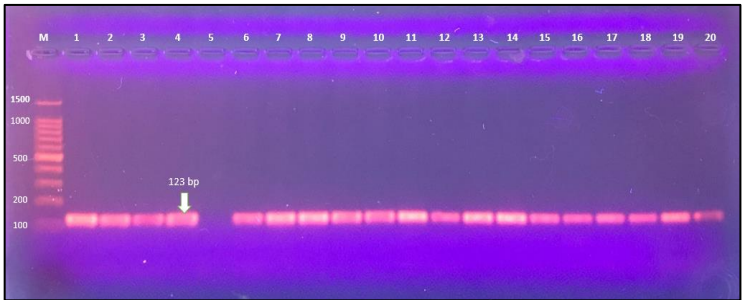

Figure (4): Electrophoresis diagram of PCR amplified products for extracted DNA of 20 isolates of *E. faecalis* using specific *eme A* gene primer show positive products at 123 bp. Line M: molecular size DNA marker and products migrated at 75 volt for 80 minutes and stained with ethidium bromide

Detection of *gyr* A gene

The results of PCR amplification of *gyr A* gene (quinolones resistance genes) were revealed that *gyr A* gene were obvious in an amount reaching 11 (55%) positive product. Figure (5) demonstrated the distribution of *gyr A* gene among the isolates.

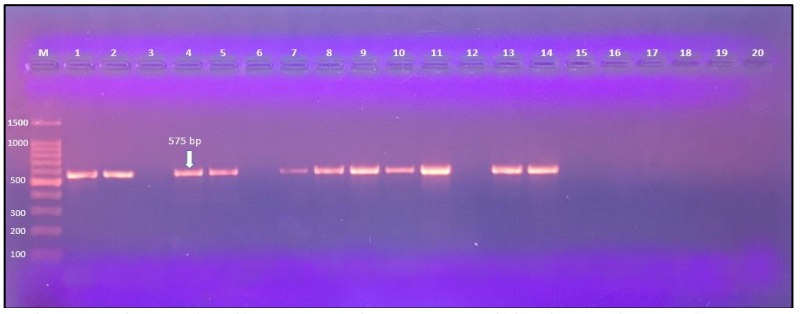

Figure (5): Electrophoresis diagram of PCR amplified products for extracted DNA of 20 isolates of *E. faecalis* using specific *gyr A* gene primer show positive products at 575 bp. Line M: molecular size DNA marker and products migrated at 75 volt for 80 minutes and stained with ethidium bromide

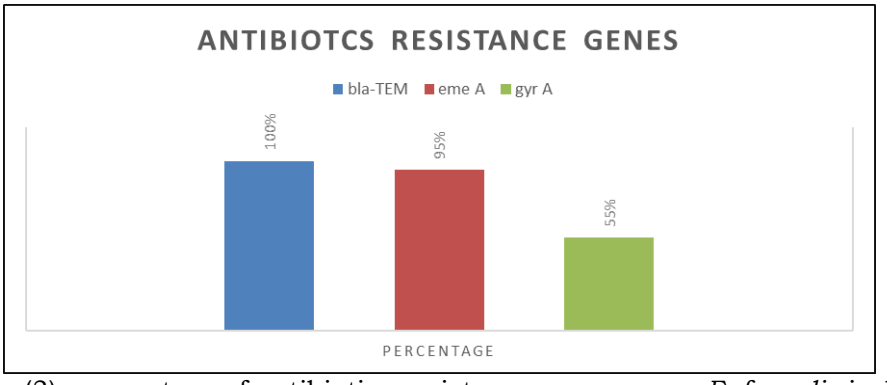

Figure (3): percentage of antibiotics resistance gene among *E. faecalis* isolates

Discussion

Enterococcus faecalis is a common cause of nosocomial infection with a significant increase in mortality rate over the past 2 decades. The failure of antibiotic therapy has led to an increased hospital stay and economic burden in patients [18]. The most potent antibiotic against *E. faecalis* in current study was imipenem with resistance rate (20%), that show increased in contrast with other studies conducted by [19], [20] that reported the resistance rate of *E. faecalis* to imipenem were (0%) and (15.9%) ,respectively. Also the result show agreement with [21] who reported that imipenem provide appropriate activity against *E. faecalis*. The same resistance result to tetracycline was also summarized with isolates of *E. faecalis* in patients with urinary tract infection (61%) [22], and [7] that report (59.5%) of *E. faecalis* was resist to tetracycline antibiotic.

A high resistance rate to vancomycin drug this may be occur because efflux pump, vancomycin-resistant associated genes or the misuse of this medicine in the country and therefore is supposed to be the last route of treatment for Enterococcal infections. This is in contrast to other studies reported by [6], [23], [24] that were 4%, 0% and 0%, respectively. This study show resistance to Amoxicillin/clavulanic acid at resistance rate (55%), more than results in other study [25] that reported the resistance rate to Amoxicillin/clavulanic acid were (33.3%) In this study, fosfomycin resembles the second most active antibiotic against *E. faecalis* with 20% resistance, more than the result in other study [26] that was 0%.

Rifampicin prevents transcription of mRNA by binding to the β-subunit of the Enterococcal DNA-dependent RNA polymerase [27]. Resistance to these drugs is common in this study, showed that (50%) of isolates were resist rifampicin, which lower than the studies [6] and [28] that reported (72%) and (85.7%) resistance rates, respectively. High resistance rate of *E. faecalis* to nalidixic acid antibiotic in this study that was (65%), that show agreement with many studies [9], [29], [30] that report high resistance rate to nalidixic acid antibiotic 100%, 96% and 61.9%, respectively.

The rate of ciprofloxacin resistance *E. faecalis* in this study was (60%). Previous researches were observed different rate of resistance to ciprofloxacin drug, in other study [23] *E. faecalis* isolates revealed that (23.6%) was resistance to ciprofloxacin while other study [28] revealed that (85.7%) was resistant. Levofloxacin antibacterial in present study approved effect against *E. faecalis* isolates more than ciprofloxacin, at resistance rate (55%), that show agreement with study [31] which reported the resistance rate to levofloxacin were (55%). And show disagreement with study [15] that reported (0%) of *E. faecalis* resist to levofloxacin, this may be occur because the isolation of *E. faecalis* from other source.

The mobile genetic elements act as reservoir for acquirement and dissemination of drug resistance factors among *E. faecalis* strains. Diverse plasmids have been described in *E. faecalis* which some of these encode important resistance gene including vancomycin, aminoglycosides, erythromycin and multi drug resistance [32]. In this study, we investigated the prevalence frequency of antibiotics

resistance genes among clinical isolates of *E. faecalis*. From this study, we report *bla-tem* that codes for extended-spectrum β-lactamase as the most prevalent antibiotics resistance genes among our isolates. The result in this study show the presence of *bla-TEM* gene in high percentage (100%) of *E. faecalis* isolates, in contrast with other study that conducted by [11] that reported the *bla-TEM* gene correlated with the origin of *E. faecalis*, were (53.3%) of the nosocomial infections isolates possessed this gene and (30%) of the endodontic isolates. Other study conducted by [33] reported (70%) of *enterococcus* have *bla-TEM* gene.

Other major antibiotics resistance gene is *emeA* was distributed (95%) among *E. faecalis* isolates. *E. faecalis* shows high levels of resistance to many antimicrobial agents, presumably due to the presence of efflux pumps [34]. this study show the presence of *eme A* gene in percentage (95%) of *E. faecalis* isolates higher than results reported by [33] that include (55%) of isolates were harboring this gene. While other studies reported that the gene *emeA* was present in all isolates [35], [36].

Fluoroquinolones frequently used to treat *E. faecalis* UTIs and the emergence of fluoroquinolone-resistant *E. faecalis* strains have recently been reported in several countries. Resistance is mediated through chromosomes and/or plasmid by various mechanisms, including mutations in the structural genes targeted by fluoroquinolones, *gyrA* and *gyrB* coding for DNA gyrase, or *parC* and *parE* coding for topoisomerase IV, *gyrA* gene mutation is more important in fluoroquinolone resistance [35]. The result in this study show the presence of *gyr A* gene in percentage (55%) of *E. faecalis* isolates less than results reported by [9] that revealed (94.1%) of isolates were harboring this gene. While other study reported that the gene *gyrA* was no present in all isolates [28].

Conclusion

All *E. faecalis* isolates exhibited multi drug resistance (MDR), and the most potent antibiotics were imipenem and fosfomycin. The ability of *E. faecalis* isolates to carry antibiotics resistance gene such as (*bla-tem, emeA and gyrA*).

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