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Characterisation of Antibiotic Resistance Pattern of *Enterococcus Faecalis* and *Enterococcus Faecium* Isolated from Different Clinical Specimen in Al-Muthanna Province, Iraq

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Abstract

Spread of *Enterococci* has increased, causing serious infections that sometimes lead to death. Therefore, the study aimed to determine the prevalence of *Enterococci* in Al-Muthanna Governorate and their resistance patterns to antibiotics. Samples were collected from Al-Hussein Teaching Hospital, as well as Women and Children's Hospital in October 2023. Cultural diagnosis and biochemical tests confirmed sixty-three *E. faecalis*, and eighteen *E. faecium* isolates. Most infections being prevalent among females 71.6 % and young people aged from 21 to 30 years old in rural areas. Antibiotic resistance rates for *E. faecalis* were meropenem 100 %, tetracycline 65 %, ampicillin 63.4 %, azithromycin 12.6 %, ciprofloxacin 42.8 %, AMC 17.4 %, vancomycin 15.8 %, teicoplanin 7.9 %, and nitrofurantoin 7.9 %. Resistance rates for *E. faecium*, were meropenem 94.4 %, tetracycline 66.6 %, ampicillin 61.1 %, azithromycin 77.8 %, ciprofloxacin 44.4 %, AMC 22.2 %, vancomycin 33.3 %, teicoplanin 16.6 %, and nitrofurantoin 5.5 %.

Keywords: *Enterococcus faecalis*, *Enterococcus faecium*, Antibiotic Resistance.

1. Introduction

Enterococcus spp. Gram-positive, facultatively anaerobically non-spore-forming, catalase-negative enterococci are a normal component of the gastrointestinal tract's commensal *flora*. They have been identified as nosocomial pathogens despite initially being thought to have low clinical significance. Species are the reason for several fatalities because they are thought to be drug-resistant and carriers of resistance genes [1].

Enterococcus faecalis and Enterococcus faecium constitute the highest clinical isolates, although *E. durans*, *E. casseliflavus*, *E. gallinarum*, and

E. avium are isolated in a few percent [2]. *Enterococci* are the second most common cause of hospital-acquired infections. They cause urinary tract infections, endocarditis, and bacteraemia, and have causes of oral, gastrointestinal, and pelvic infections as well as intra-abdominal infections. *Enterococci* present a therapeutic challenge due to their intrinsic and acquired antibiotic resistance characteristics, virulence factors, and capacity to thrive in unfavourable environments [3].

Antibiotic resistance is one of the main risks to world health. It has led to greater medical expenses, longer hospital stays, and an increase in mortality by making common infections harder or impossible to treat. Globally, the prevalence of infections caused by multidrug-resistant organisms (MDRO) is rising. Even though new antibiotics have become more widely available recently, there are still few effective medicines against MDRO [4].

One of the most important global public health issues is the rise in antibiotic resistance in *enterococci* [5]. Antibiotic resistance in *enterococci* is due to intrinsic resistance or genetic acquisition. The most prevalent cause of intrinsic resistance is the presence of resistance genes that are directed against different types of antibiotics. DNA mutations or the acquisition of additional genes through various transfer of genes mechanisms are the causes of *enterococci*-acquired resistance [6]. Multidrug-resistant (MDR) strains are prevalent in *E. faecium* and *E. faecalis*, with vancomycin resistance in this species causing difficulties in antibiotic prescriptions. Continuous monitoring is crucial for updating local diagnostic and treatment protocols. Due to the high prevalence of *Enterococcus spp*., especially *E. faecalis* and *E. faecium*, and the failure of effective treatments, this bacterium has become a major problem in recent years.

The aim of the study is to evaluate the prevalence of *enterococci spp*.in Al-Muthanna Governorate and to identify patterns of antibiotic resistance by collecting samples from different clinical cases. Then, culturing them on a special selective medium, and for further confirmation, biochemical tests for the studied bacteria were used.

2. Material and Method

2.1 Samples Collection

The study included 170 clinical samples. Samples were collected from patients with urinary tract infection, dental caries, vaginitis, and burns, either by referring patients or lying in hospitals at Al-Hussein Teaching Hospital, and the Women’s and Children's Hospital in Al-Muthana Governorate.

These samples were subjected to activation to stimulate potential bacteria in brain-heart infusion broth (HiMedia, India) for 24 hours at 37 °C. Then cultured on Hichrome™ Enterococcus faecium agar base (HiMedia, India) for the same condition. Each sample was collected on a proper method as follows.

2.1.1 Urine Samples

Urine samples were collected in the early morning from patients who showed symptoms of urinary tract infections. After examining them microscopically to detect the presence of bacteria and leukocyte for both genders of different ages. Samples were collected also from patients, using sterile, tightly sealed collection containers and transported directly to the microbiology laboratory in hospital to activate it in brain heart infusion medium to stimulate the potential presence of bacteria.

2.1.2 Root Canal Samples

Swabs were taken from roots of inflamed teeth by the specialist doctor using a special tool known as a probe after drilling patient's teeth. Swabs later placed in amies transport media to activated after two hours.

2.1.3 Vaginal Samples

Vaginal swabs were collected from females who show symptoms of vaginitis, such as itching, discharge, and pus at the Women's and Children's Hospital in Al-Muthanna Governorate. A specialist doctor used a sterile medical probe to prevent contamination of the sample when it was passed through the lower part of the vagina using a sterile cotton swab.

The cotton swab was inserted with caution and moved gently in a rotating motion to absorb fluids, pus, and secretions. Then placed in amies transport media until activation in brain heart infusion broth within two hours.

2.1.4 Burn Samples

Burn samples were collected from patients in the Specialized Burn Centre at Al-Hussein Teaching Hospital. Burn swabs were collected using a sterile cotton swab from the most inflamed burn site and then placed in amies transport media until activation in brain heart infusion broth within two hours.

2.2 Isolates Identified

After cultivation on the HichromeTM *Enterococcus faecium* agar base (selective medium). The phenotypic characteristics were confirmed by gram stain and biochemical tests such as the catalase test, growth at 45 and 10 °C, tolerance of 6.5 % NaCl, and esculin hydrolysis.

2.3 Biofilm Formation

Phenotypic detection of biofilm formation was carried out using a 96-well microplate, according to Christensen et al [7]. *Enterococcus spp*. were grown in tryptic soy broth for 24 hours at 37 °C. Then, 20 µl of bacterial suspension equal to 0.5 McFarland Standard, was added to 180 µl of sterile tryptic soy broth fortified with glucose in wells and incubated aerobically at 37 °C for 24 hours.

After incubation period, contents of the wells were removed and gently washed three times with phosphate buffered saline to get rid of planktonic cells. Then added to 200 µl of 99 % methanol for 15 minutes to fix biofilm. Latter removed the methanol and dried it at room temperature and added to 200 µl of 1 % crystal violet for 30 minutes. a 150 µL of 33 % (v/v) glacial acetic acid was used in each well to dissolve the dye associated with the adherence cells.

The Optical Density (OD) test was OD > 4 which means strong biofilm, OD ≤ 4 consider moderate biofilm producers, OD ≤ 2 means weak biofilm formation, and OD ≤ 0.08324 means non biofilm formation [8].

2.4 Antibiotic Susceptibility Test

Vancomycin (30 µg) and other antibiotics such as Ampicillin (10 µg), Tetracycline (30 µg), Teicoplanin (30 µg), Nitrofurantoin (10 µg), Ciprofloxacin (10 µg), Amoxicillin Clavulanate AMC (30 µg), Azithromycin, Azithromycin and Meropenem antibiotic resistance were tested by the standard disc diffusion method on Muller-Hinton Agar (TM MEDIA, India) and incubated for less than 24 hours at 35 ± 2 °C.

2.5 Statistical Analysis

Statistical analysis was performed using relevant statistical methods, such as the percentage method (%), to determine the prevalence of enterococci and their resistance patterns.

3. Results

The study included 170 clinical samples, ninety females, and eighty males. Samples were collected from patients of urinary tract infection, dental caries, vaginitis, and burn in Al-Hussein Teaching Hospital, and Women's and Children Hospital. Samples were subjected to activation to stimulate potential bacteria on brain heart infusion broth. After which the turbidity of the medium was observed that indicating growth of bacteria. One hundred sixty-one samples (94.7 %) showed growth positive.

Urine samples were highest among the clinical samples with one hundred samples. Followed by dental caries samples fifty samples, vaginitis six samples, and five

burn infection samples. While turbidity was not observed in only four samples 2.35 %. That may be due to the patient antibiotics treatment or the presence of other causes of infections such as parasites or viruses or due to the nature and size of the sample. After cultivation sample on Hichrome™ *Enterococcus faecium* agar base (selective medium) as noted in (figure 1).

More tests were done to confirm the two species of *Enterococci* were stained with gram stain and subjected to many important biochemical tests in diagnosing *Enterococci* to differentiate them from the rest of the bacteria and to test their ability to grow in harsh conditions, as shown in the (table 1).

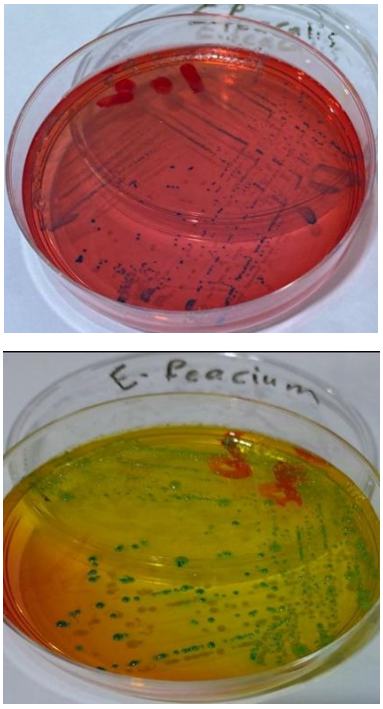


Figure 1: *Enterococcus spp*. on Hichrome™ *Enterococcus faecium* agar base. Top: Blue colonies *Enterococcus faecalis*, Bottom: Green colonies *Enterococcus faecium*.

Table 1: Phenotypic characteristics of *Enterococcus faecalis* and *Enterococcus faecium*.

Phenotypic characteristic	*Enterococcus faecalis*	*Enterococcus faecium*
Gram positive cocci /Short chain	+	+
Catalase	-	-
Growth in presence of bile esculin	+	+
Growth at 6.5% NaCl	+	+
Growth at 10-45 C	+	+
Arabinose fermentation	-	+
Lactose fermentation	+	+
Mannitol fermentation	+	+
PYR	+	+

From 170 clinical samples, the current study obtained 81 (50.3 %) *Enterococcus Spp*. distributed as follows 63 (77.7 %) and 18 (22.2 %) as in listed in (table 2).

Table 2: Number and percentage of *E. faecalis* and *E. faecium* isolated from different Clinical specimens.

Isolate Source	Specimens	*Enterococcal* isolates	*E.faecalis* (%)	*E.faecium* (%)
Urine	100	50	34 (68 %)	16 (32%)
Root Canal	50	27	26 (96.2 %)	1 (3.7 %)
Vaginal swab	6	3	2 (66.6 %)	1 (33.3 %)
Burn	5	1	1 (100 %)	0 (0 %)
Total	161	81 (50.3 %)	63 (77.7 %)	18 (22.2 %)

This study showed that *E. faecalis* spread among young groups from 21 to 30 years old with percentage of 31.7 %. While *E. faecium* common among age over 50 years old 38.8%, as listed in (table 3).

Table 3: Percentage of *Enterococcus spp*. according to age.

Age group	*E. faecalis* (%)	*E. faecium* (%)	Total
≤ 20	7 (11 %)	4 (22.2 %)	11 (13.5 %)
21 to 30	20 (31.7%)	3 (16.6 %)	23 (28.2 %)
31 to 40	12 (19.0 %)	1 (5.5 %)	13 (16 %)
41 to 50	9 (14.2 %)	3 (16.6 %)	12 (14.8 %)
50 +	15 (23.8 %)	7 (38.8 %)	22 (27.1 %)

3.1 Biofilm Formation

Most **Enterococcus spp**. formed weak biofilm, *E. faecalis* showed 70.83 % versus 44.4 % to *E. faecium*. Moderate biofilms 29.16 % and 38.8 % in *E. faecalis* and *E. faecium* respectively. All *E. faecalis* isolates were incapable to biofilm formation but *E. faecium* 16.6 % formed biofilms as showing in (figure 2).

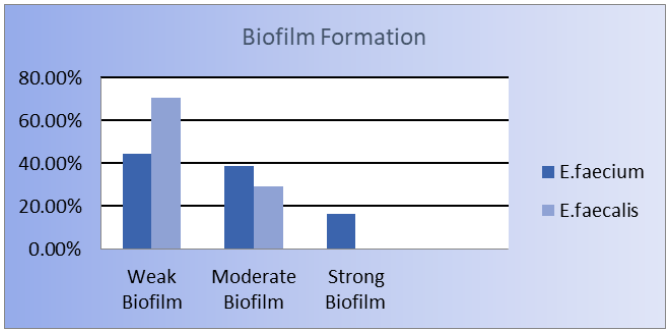


Figure 2: Biofilm Formation in *E. faecalis* and *E. faecium*.

3.2 Antibiotic Resistance Pattern

Prevalence of antibiotic resistance among *Enterococcus spp*. is shown in (figure 3). While, phenotypic antibiotic resistance of *E. faecalis* and *E. faecium* isolates from different clinical samples are shown in (figure 4).

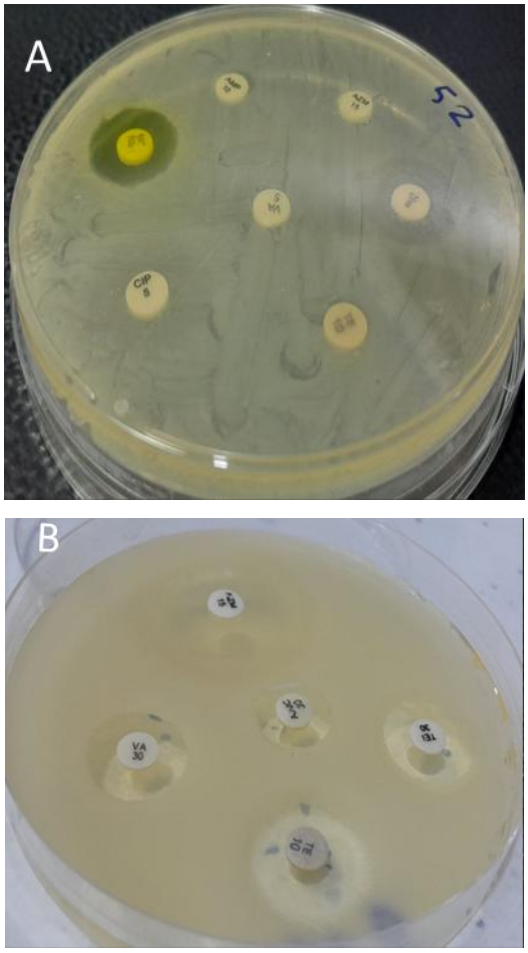
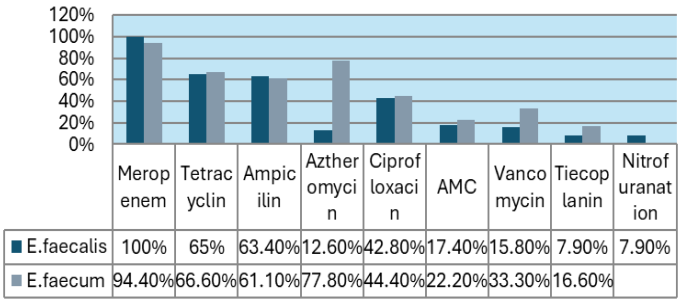


Figure 3: Phenotypic antibiotic resistance of *Enterococcus* on Muller Hinton Agar. (A) Multidrug resistance *Enterococcus*. (B) Multidrug sensitive *Enterococcus*.



	Meropenem	Tetracyclin	Ampicilin	Azthermycin	Ciprofloxacin	AMC	Vancomycin	Tiecoplanin	Nitrofuranation
E.faecalis	100%	65%	63.40%	12.60%	42.80%	17.40%	15.80%	7.90%	7.90%
E.faecum	94.40%	66.60%	61.10%	77.80%	44.40%	22.20%	33.30%	16.60%	

Figure 4: Phenotypic antibiotic resistance of *E. faecalis*, and *E. faecium* isolated from different clinical samples.

4. Discussion

Enterococcus spp. showed their inability to produce the enzyme catalase and distinguished by their ability to grow in a wide range of temperatures, from 10 to 45 °C. Also, tolerate growth in media with a pH of 9.6 and salt solutions (6.5 sodium chloride). From the 81 *enterococcal* isolates, the study obtained 63 samples (77.77 %) as *E. faecalis,* and 18 samples (22.2 %) as *E. faecium* from different clinical samples.

Results of the study were like the study of that collected *E. faecalis* at a rate of 78.8 % from urine, root canals, and burns in addition to blood [9]. Another study collected *enterococci* from urine, vaginal swabs, and other clinical samples, as they observed the presence of *E. faecalis* in 82.2 % compared to 17.8 % of *E. faecium* [10]. These results are opposite to the findings of from China, that reported 58.7 % of *E. faecium* versus 33 % of *E. faecalis*.

In the current study, the infection rate was widespread among young people aged between 21 and 30 years old at a percentage of 28.2 %, followed by those over 50 years old (27.1 %). The lowest percentage among those under 20, at 13.5 %. found that *E. faecalis* and *E. faecium* are more prevalent among ages 40 to 49, at 26.96 %.

Results of the current study showed the predominance of infection in females, with 58 isolates at a rate of 71.6 %, compared to 23 (28.3 %) in males from various sources of isolation for both species. This is compatible with Thakan [10], that reported the percentage of females was higher at 69.3 % in contrast to Rout et al. [11] that showed the opposite, as the majority of *enterococcal* infections occurred in males at 59.5 %.

According to the place of residence of the patients, there were 44 *Enterococcus* isolates (54.3 %) in the rural community compared to 37 (45.6 %) in the urban community. Another study showed that the infection in urban is 58 %, more than rural 42 % [12]. Urinary tract infections (UTI) are a prevalent type of bacterial infection that can arise in both community and hospital settings globally.

Numerous uropathogens can cause UTIs, which are among the most prevalent infectious diseases worldwide. One of these uropathogens is *Enterococcus spp*. [12]. In the current study, 50/81 enterococcal isolates from urine, representing 61.72 %, were divided into 68 % (34/50) *E. faecalis* and 32 % (16/50) *E. faecium*.

These results converge with the local study of Balata [13], that obtained *E. faecalis* 74 %, *E. faecium* 18 %, *E. faecalis* 63.3 %, and *E. faecium* 36.7% from urine. However, Salman et al. [14] reported 71.8 % for *E. faecalis* form urine, and 27.8 % for

E. faecium, and *E. faecalis* 62 % from the urine sample.

Enterococci species are regarded as transitory constituents of the oral microbiome and can cause a wide range of systemic and oral infections. A considerable reservoir of highly pathogenic and resistant to antibiotic *enterococci* may be found in the oral cavity, with adults and elderly people carrying an increasing proportion of these bacteria. *E. faecalis* and *E. faecium* in dental caries infection represent 33.3 ٪ (27/81) and are distributed as follows. A 96.29 % (26/27) for *E. faecalis*, and 3.70 % (1/27) for *E. faecium*. Abed et al. [15] reported *E. faecalis* 25 %, while Salih and Al-Quraishi [16] isolated *E. faecalis* at 65 from root canal.

Mustafa et al. [17] obtained *E. faecium* at 80 % but disagree with Fadhil Al-Taie et al. [18] in dental caries at 68.75 % versus 31.25 %. Although Enterococcus is a commensal bacterium found in the gastrointestinal system, it can turn into an opportunistic pathogen. The female genital tract may become colonized, and vaginal colonization rises in patients with aerobic vaginitis or after receiving antibiotic therapy. A wide range of infections are linked to *E. faecalis*, especially when immunocompromised or when the host microbiota is disturbed [19-20].

From the vaginal swap collected in this study, 3.7 % (3/81) of *enterococci* were isolated. Two were *E. faecalis*, 66.6 % (2/3) and *E. faecium*, 33.3 % (1/3) and *E. faecalis* in vaginitis was 6.5 %. The result of the study was like the findings of Sengupta et al. [21], with *E. faecalis* 69.23 %, *E. faecium* 30.77 %, *E. faecalis* 29.4 %, and *E. faecium* 7.7 % [21]. *Enterococcus* is becoming a more fatal infection, particularly in those with impaired immune systems who have been burned. While *enterococcal* burn infection obtained only 1.23 %, that was *E. faecalis* with 100 %.

Wang et al. [22], Latifi and Karimi [23], resulted in 3.1 % and 12.24 % of *Enterococcus spp*., respectively. Results of the current study showed a difference with the result of Heidari et al. [24], that obtained *E. faecalis* at a high percentage of 80.7 %, and Shokoohizadeh et al. [25], that showed *E. faecalis* at 62.5 %. The reason for the difference in percentages between the current study and previous studies is perhaps due to the number of samples, the difference in the isolation source, geographical location, climate, community culture, or the economic situation [13].

All *enterococci* of both species in this study formed biofilm, but the majority formed weak biofilm. *E. faecalis* formed weak biofilm at a percentage of 70.83 %, moderate biofilm at 29.16 %, and 0 % of strong biofilm. *E. faecium* formed strong biofilm at rate 16.6 %, moderate biofilm 38.8 % and 44.4 % formed weak biofilm.

A study found biofilm formation in *E. faecalis* was 91.5 % and in *E. faecium* was only 58.7 % [14]. The rising incidence of antimicrobial resistance and the consequent lack of effective antimicrobials are two major challenges facing everyone around the globe. Using broad-spectrum antibiotics for therapy increases antibiotic-resistant *Enterococci*. The current study showed that *E. faecalis* had absolute resistance to meropenem for all 63 *E. faecalis* isolates, followed by tetracycline with 41 (65 %) isolates, ampicillin 40 (63.4 %), and ciprofloxacin 27 (42. 8 %).

Percentages are lower for the rest of the antibiotics that were used in the current study, such as AMC 11 (17.4 %), Vancomycin 10 (15.8 %), and Azithromycin 8 (12.6 %). While antibiotics nitrofurantoin and teicoplanin showed the strongest effectiveness among the antibiotics used in 5 isolates, at a rate of 7.9 %. Local study showed antibiotic resistance variance with the current study by Al-Naqshbandi et al [26], *E. faecalis* resistance to ampicillin 90 %, Ciprofloxacin 100 %, Nitrofurantoin 0 %, Tetracycline 80 %, Teicoplanin 40 %, and Vancomycin 40 %.

AL-Hamadani [27] found Ampicillin *E. faecalis* resistance was 100 %. As for the second bacterial species in the study, *E. faecium*, nitrogen was also the strongest antibiotic that inhibited growth around the antibiotic disc; out of 18 isolates, only one of them was resistant to it at a rate of 5.5 %. As for teicoplanin, only 3 isolates were resistant at 16.6 %, followed by AMC, four isolates (22.2 %), vancomycin 6 (33.3 %), ciprofloxin 8 (44.2 %), ampicillin 11 (61.1 %), and tetracycline 12 (66.6 %), and the percentage of resistance to azithromycin and meropenem was 77.8 % and 94.4 %, respectively.

The current study agrees with Allami et al. [28], that found Ciprofloxacin at 47.6 % but disagree with Augmentin (AMC) at 95.2 %, Ampicillin at 90.4 %, Nitrofurantoin at 80.9 %, and Vancomycin at 0 %. A high resistance to ampicillin 89.1 %, vancomycin 68 %, teicoplanin 71.8 %, tetracycline 79.3 %, and ciprofloxacin 74.4%. An Egyptian study by Serry et al. [29], found that *E. faecalis* antibiotic resistance to Ampicillin 70 %, Vancomycin and Teicoplanin 7.5 %, Imipenem 12.9 %, Azithromycin and Ciprofloxacin were 58.6 % and 54.3 % respectively. Whereas *E. faecium* antibiotic resistance was 68.4 %, to Ampicilin, and 15.8 % Teicoplanin. Resistance to Imipenem 24.6 %, Azithromycin 47.4 %, and Ciprofloxacin resistance was 57.9 %.

33/81 (40.7 %) isolates were resistant to three categories or more of antibiotics (MDR) distributed as follows. Twenty-two isolations of *E. faecalis* at rate 34.9 % and 11/18 isolates of *E. faecium* at 61.1 %, Saeidi et al. [29], obtained MDR for *E.* was

69.6 % while 80 % of *E. faecalis* was MDR. Management of enterococcal infections is difficult due to the intrinsic and acquired resistance of *Enterococcus* to many antibiotics. In addition, previous antibiotic therapy including broad-spectrum antibiotics promotes the establishment of *enterococci* in the gut, which leads to their spread to other parts of the body and eventual infection [30].

5. Conclusion

Due to the high prevalence of *Enterococcus spp*., especially *E. faecalis* and *E. faecium*, the failure of effective treatments. This bacterium has become a major problem in recent years. The study showed a high prevalence of *Enterococcus spp*. that was isolated from different samples. Also, concern about these currently studied bacteria is due to the high levels of antibiotic resistance in addition to MDR *Enterococcus* spread in Al-Muthanna Government especially *E. faecium*.

6. References

1. Sood S., Malhotra M., Das B. K., and Kapil A., (2008). Enterococcal infections and antimicrobial resistance. Indian journal of medical research. 128, 2, 111-121.
2. Sengupta M., Sarkar R., Sarkar S., Sengupta M., Ghosh S., and Banerjee P., (2023). Vancomycin and Linezolid-Resistant Enterococcus Isolates from a Tertiary Care Center in India. Diagnostics (Basel, Switzerland). 13, 5, 945.
3. Fiore E., Van Tyne D., and Gilmore M. S., (2019). Pathogenicity of Enterococci. Microbiology spectrum. 7, 4.
4. Sy C. L., Chen P. Y., Cheng C. W., Huang L. J., Wang C. H., Chang T. H., Chang Y. C., Chang C. J., Hii I. M., Hsu Y. L., Hu Y. L., Hung P. L., Kuo C. Y., Lin P. C., Liu P. Y., Lo C. L., Lo S. H., Ting P. J., Tseng C. F., Wang H. W., and Wang F. D., (2022). Recommendations and guidelines for the treatment of infections due to multidrug resistant organisms. Journal of Microbiology, Immunology and Infection. 55, 3, 359-386.
5. Teethaisong Y., Chueakwon P., Poolpol K., Ayamuang I. O., Suknasang S., Apinundecha C., and Eumkeb G., (2023). Stephania suberosa Forman extract synergistically inhibits ampicillin- and vancomycin-resistant Enterococcus faecium. Saudi journal of biological sciences. 30, 2, 103557.
6. Esmail M. A. M., Abdulghany H. M., and Khairy R. M., (2019). Prevalence of Multidrug-Resistant Enterococcus faecalis in Hospital-Acquired Surgical Wound Infections and Bacteremia:

Concomitant Analysis of Antimicrobial Resistance Genes. Infectious diseases. 12, 1178633719882929.

7. Christensen G. D., Simpson W. A., Younger J. J., Baddour L. M., Barrett F. F., Melton D. M., and Beachey E. H., (1985). Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. Journal of clinical microbiology. 22, 6, 996-1006.
8. Novais Â., Vuotto C., Pires J., Montenegro C., Donelli G., Coque T. M., and Peixe L., (2013). Diversity and biofilm-production ability among isolates of Escherichia coli phylogroup D belonging to ST69, ST393 and ST405 clonal groups. BMC microbiology. 13, 144.
9. Boccella M., Santella B., Pagliano P., De Filippis A., Casolaro V., Galdiero M., Borrelli A., Capunzo M., Boccia G., and Franci G., (2021). Prevalence and Antimicrobial Resistance of Enterococcus Species: A Retrospective Cohort Study in Italy. Antibiotics (Basel, Switzerland). 10, 12, 1552.
10. Thakan K., and Vij A., (2022). Antibiotic susceptibility pattern in clinical isolates of Enterococci at a tertiary care hospital. Journal of The Academy of Clinical Microbiologists. 24, 1, 12-15,
11. Rout D., Bhattacharya S., Bhoi P., Sahu K., Panda N., and Otta S., (2024). Prevalence and Susceptibility Pattern of Enterococcus spp in Clinical Samples: A Retrospective Study From a Tertiary Care Teaching Hospital in an Eastern Indian State. Al- Anbar Medical Journal. 20, 1, 42-48.
12. Mancuso G., Midiri A., Gerace E., Marra M., Zummo S., and Biondo C., (2023). Urinary Tract Infections: The Current Scenario and Future Prospects. Pathogens (Basel, Switzerland). 12, 4, 623.
13. Balata R., and Abdullah B., (2023). Detection And Antibiotic Susceptibility Test of Enterococcus Spp. from Different Clinical Samples in Duhok City/ Iraq. The Journal of University of Duhok. 26, 372-384.
14. Salman H. A., Alsallameh S. M. S., Muhamad G., and Taha Z., (2022). Prevalence of multi-antibiotic resistant bacteria isolated from children with urinary tract infection from Baghdad, Iraq. Microbiology and Biotechnology Letters. 50, 1, 147-156.
15. Abed E. H., Al-Yasiri M. H., and Maktoof A. A., (2023). Isolation and diagnosis of Streptococcus mutans and Enterococcus faecalis from dental caries patients in Thi-Qar Governorate. Journal of Population Therapeutics and Clinical Pharmacology. 30, 8, 24-29.

16. Salih A. M., and Al-Quraishi G. M. S., (2024). Isolation and identification of biofilm producing enterococcus faecalis from root canal. Iraqi Journal of Agricultural Sciences. 55(Special), 314-321.
17. Mustafa E. A., Hamdoon S. M., and Shehab E. Y., (2021). Molecular detection and identification of Enterococcus faecium isolated from dental root canals. Iraqi Journal of Science. 62, 5, 1447-1551.
18. Al-Taie F. S., Al- Musawi T. M, Rasheed S. Z., (2022). Distribution and Antibiotic Resistance of Streptococci and Enterococci Isolated from Dental Caries and Healthy People. Journal of Medical Microbiology and Infectious Diseases.10, 2, 48-53.
19. Alhajjar N., Chatterjee A., Spencer B. L., Burcham L. R., Willett J. L. E., Dunny G. M., Duerkop B. A., and Doran K. S., (2020). Genome-Wide Mutagenesis Identifies Factors Involved in Enterococcus faecalis Vaginal Adherence and Persistence. Infection and immunity. 88, 10, e00270-20.
20. Alkraety I. A. A., Al-Muhanna S., Banoon S., and Ghasemian A., (2022). Bacterial vaginosis pattern and antibiotic susceptibility testing in female patients using high vaginal swabs. Biodiversitas Journal of Biological Diversity. 23, 2838-2844.
21. Sengupta M., Sarkar R., Sarkar S., Sengupta M., Ghosh S., and Banerjee P., (2023). Vancomycin and linezolid-resistant enterococcus isolates from a tertiary care center in India. Diagnostics. 13, 5, 945.
22. Wang L. F., Li J. L., Ma W. H., and Li J. Y., (2014). Drug resistance analysis of bacterial strains isolated from burn patients. Genetics and molecular research: GMR. 13, 4, 9727-9734.
23. Latifi N. A., and Karimi H., (2017). Correlation of occurrence of infection in burn patients. Annals of burns and fire disasters. 30, 3, 172-176.
24. Heidari H., Emaneini M., Dabiri H., and Jabalameli F., (2016). Virulence factors, antimicrobial resistance pattern and molecular analysis of Enterococcal strains isolated from burn patients. Microbial pathogenesis. 90, 93-97.
25. Shokoohizadeh L., Ekrami A., Labibzadeh M., Ali L., and Alavi S. M., (2018). Antimicrobial resistance patterns and virulence factors of enterococci isolates in hospitalized burn patients. BMC research notes. 11, 1.
26. Al-Naqshbandi A. A., Chawsheen M. A., and Abdulqader H. H., (2019). Prevalence and antimicrobial susceptibility of bacterial pathogens isolated from urine specimens received

in Rizgary Hospital-Erbil. Journal of infection and public health. 12, 3, 330-336.

27. Al-Hamadani Z. A. A., and Tuwaij N. S. S., (2020). Molecular Screening of Virulence Genes among Uropathogenic Enterococcus faecalis Isolated from patients in Al-Najaf City, Iraq. International Journal of Pharmaceutical Research. 12, 4.
28. Allami M., Mohammed E. J., Alazzawi F., and Bahreini M., (2021). Prevalence and Antibiotic Resistance Pattern of Pathogenic Bacteria Isolated From Urinary Tract Infections in Qal'at Saleh Hospital, Iraq. Avicenna Journal of Clinical Microbiology and Infection. 8, 4,117-122.
29. Saeidi S., Mirnejad R., Masoumi Z. S., and Rostasmzadeh S., (2018). Molecular Identification of Pathogenic Enterococci and Evaluation of Multi-drug Resistance in Enterococcus Species Isolated From Clinical samples of Some Hospitals in Tehran, Iran. Modern Medical Laboratory Journal. 1, 2, 60-67.
30. Djorić D., Atkinson S. N., and Kristich C. J., (2024). Reciprocal regulation of enterococcal cephalosporin resistance by products of the autoregulated yvcJ-glmR-yvcL operon enhances fitness during cephalosporin exposure. PLoS genetics. 20, 3, e1011215.