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Research Paper

Antibiotic Resistance and ESβLs Producing in *Enterococcus* spp. Isolated From Patients Admitted to Al Women's and Children's Educational Hospital in Al-Qadisiyah, Iraq

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ABSTRACT

Introduction: *Enterococcus* spp., which produce extended-spectrum β-lactamases (ESβLs), are resistant to nearly all β-lactam antibiotics. Due to the limited number of therapeutic options available, enterococcal infections are often difficult to treat, resulting in higher antibiotic and healthcare costs and posing a threat to patients' lives. This research, therefore, aimed to determine the antibiotic resistance pattern of *Enterococcus* spp. and evaluate their potential to produce ESβLs both phenotypically and genotypically in Iraq. The epidemiological data available on β-lactam-resistant enterococci in Iraq are inadequate. This laboratory-based study was conducted from December 2020 to May 2021.

Materials & Methods: A total of 500 clinical specimens were obtained from patients with clinical cases and identified using standard microbiological procedures. The antibacterial resistance profiles of all *Enterococcus* spp. isolates were evaluated using the disk diffusion method. Enterococcal strains were examined for ESβL production utilizing the phenotypic method and PCR, respectively.

Results: All *Enterococcus* spp. isolates were completely resistant to ceftriaxone and cefotaxime. 85.45% of the isolates were resistant to gentamicin, 43.63% to chloramphenicol, 10.9% to ampicillin, and 7.2% to nitrofurantoin. The prevalence rates of multidrug-resistant (MDR) and extensively drug-resistance (XDR) isolates were 41.81% and 3.63%, respectively. Phenotypically, all 55 isolates were ESβLs-positive. Genotypically, the prevalence rates of the *bla-TEM*, *bla-SHV*, and *bla-CTX-M2* genes were 27.27%, 23.63%, and 7.27%, respectively.
Conclusion: In conclusion, a wide prevalence of ESβL among *Enterococcus* spp. isolated from patients in Hospital Al-Women's and Children's Educational Hospital in Al-Qadisiyah Province, Iraq, was observed. A high rate of resistance to β-lactam, was also observed. This highlights the need for a rational policy to reduce the use of antibiotics.

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1. Introduction

E*nterococcus* spp. are facultative anaerobic, gram-positive cocci found in both the gastrointestinal tracts of humans and animals. However, they are significant opportunistic pathogens that form biofilms on medical devices and catheters, leading to urinary tract infections (UTIs), endocarditis, bacteremia, abdominal and biliary infections, burns, and surgical site infections [1]. Due to their capacity to grow and survive in extreme environments and their intrinsic and multidrug resistance, diseases caused by enterococci pose a unique challenge and are an object of interest [2]. They include two main species: *Enterococcus faecalis* and *Enterococcus faecium*, the most common pathogenic species of enterococci, which can pose a public threat due to their antimicrobial resistance [3].

Enterococcus spp. are resistant to a wide range of antibiotics, which makes them challenging to treat. They are also known to acquire antibacterial resistance easily [4]. The level of Enterococcal intrinsic resistance varies among different beta-lactams. Penicillins generally have the highest activity against enterococci, due to their penicillin-binding proteins (PBPs) having low affinity, followed by carbapenems and cephalosporins, with the latter having the least activity. However, this does not apply to modern-generation cephalosporins. Cephalosporins such as ceftobiprole and ceftaroline [5] are effective against some resistant strains.

One of the more significant mechanisms by which enterococci resist antibiotics is the production of enzymes that hydrolyze the β-lactam ring in the antibiotic structure. ESβLs are one of these important enzymes [6]. The emergence of enterococci that produce ESβLs is a growing problem in healthcare institutions [7]. This decreases the efficiency of previously successful antibiotics, resulting in a detrimental effect. As ESβLs-production is responsible for resistance to mostly cephalosporins, carbapenems are the major antibiotics used to treat enterococci infections [8]. As there are no comprehensive studies on ESβLs-producing *Enterococcus* spp. in the Al-Qadisiyah Province of Iraq, we aimed to assess the prevalence of antibiotic resistance profiles of these bacteria in clinical samples.

2. Materials and Methods

2.1. Description of study specimens

A cross-sectional study was conducted at AL-Women›s and Children's Educational Hospital in AL-Qadisiyah, Iraq, from December 2020 to May 2021, on hospitalized patients and those visiting the hospital. A total of 500 samples were collected from urine, vaginal swabs, diarrhea, and CSF specimens. Midstream urine samples were collected in the early morning using a sterile cup, followed by vagina samples collected using sterile cotton swabs. Fresh diarrhea samples were also collected in a sterile, leak-proof container. Finally, a physician collected CSF specimens via lumbar puncture and immediately transported them to the microbiology laboratory within 30 minutes for cultivation.

2.2. Bacteriological examinations

Cultivation of all clinical specimens was performed on various agar media such as Rapid HiEnterococci agar (HiMedia, India), MacConkey agar N2, Blood agar, and Bile Esculin agar (Oxoid Company, Britain). Briefly, samples were inoculated on the media using a sterile wire loop and incubated aerobically for 24 hours at 37 ˚C. Enterococci were isolated and identified based on cultural characteristics on agar media, biochemical tests such as catalase test, salt tolerance test (growth at 6.5% NaCl), and heat tolerance test (growth at 60 ˚C for 30 minutes), and gram staining morphology under a microscope. Finally, all isolations were stored at -20 ˚C in tryptic soy broth plus 15% glycerol, following the European Manual of Clinical Microbiology [9].

2.3. Susceptibility testing for antimicrobials

Using the standard Kirby-Bauer disk diffusion method, the antibiotic susceptibility profiles of *Enterococcus* spp. Were determined [7]. After swabbing the enterococci suspension on Mueller-Hinton agar (MHA) plates, the plates were incubated for 24 hours at 37 ˚C. The inhibition zones were then measured using a metric ruler and compare with Clinical and Laboratory Standards Institute (CLSI) standards [7]. Seven antibacterials were tested: Ampicillin (AM 30 µg), Ceftriaxone (CTR 10 µg), Cefotaxime (CTX 30 µg), Gentamycin (10 µg), Vancomycin (30 µg), Nitrofurantoin (F 300 µg), and Chloramphenicol (C 30 µg). MDR was defined as any bacterium resistant to ≥3 classes of antibiotics.

Table 1. Culture-positive and culture-negative clinical specimens

Clinical Specimens	No. of Specimens	No. (%)	
		Positive Bacteria Culture	Negative Bacteria Culture
Urine	250	(74)190	(11.53)60
Vaginal	150	(83.33)125	(16.66)25
Diarrhea	50	(100)50	-
CSF	50	(20)10	(80)40
Total	500	(75)375	(25)125
χ^2	-	103.02	
P	-	0	

2.4. Phenotypic confirmation of ESβLs

The disc approximation method was used to detect the ESβLs production in selected enterococcal strains. For confirmation, an enterococcal suspension of five pure, single colonies was prepared in 5 mL of sterile broth, and turbidity was adjusted to 0.5 McFarland. The broth was spread on MHA (Hi-Media, India). An ampicillin 25 µg disc was placed in the center, flanked by cefotaxime 30 µg, ceftriaxone 10 µg, and cefepime 10 µg discs at 30 mm apart on the lawn culture. The plates were incubated for 24 hours at 37 ˚C to observe inhibition zones, resulting in a characteristic area called a "champagne cork" around the antibiotic discs.

2.5. DNA extraction and PCR assay

Genomic DNA from *Enterococcus* spp. isolates was extracted using a DNA extraction kit from Anatolia Company, Turkey. PCR was conducted in a total volume of 25 µL, consisting of 12.5 µL of master mix, 3 µL of genomic DNA, 3 µL of primers, and 6.5 µL of PCR-grade water. The primer sequences used were: bla-CTX-m2-F (5'-ACGCTACCCCTGCTATTT-3') and bla-CTX-m2-R (5'-CCTTTCCGCCTTCTGCTC-3') [8], bla-TEM-F (5'-TTTCGTGTCGCCCTTATTCC-3') and bla-TEM-R (5'-CCGGCTCCAGATTTATCAGC-3') [10], and bla-SHV-F (5'-ATTTGTCGCTTCTTTACTCGC-3') and bla-SHV-R (5'-TTTATGGCGTTACCTTTGACC-3') with amplicon sizes of 941, 1030, and 446 bp, respectively [11]. PCR conditions were as follows: an initial denaturation step at 97 ˚C for 5 minutes, followed by 35 cycles of denaturation at 94 ˚C for 30 seconds, annealing at 54 ˚C for 30 seconds, and final extension at 72 ˚C for 2 minutes. PCR was conducted using a thermal cycler (Eppendorf, Germany). Electrophoresis was performed in 1.5% agarose gel prepared in TBE buffer at 95 V for 30 minutes. Ethidium bromide was added to the gel before solidification. DNA fragments and a 100 bp DNA ladder were loaded into wells (Fermentas, Germany). PCR products were visualized under a UV transilluminator.

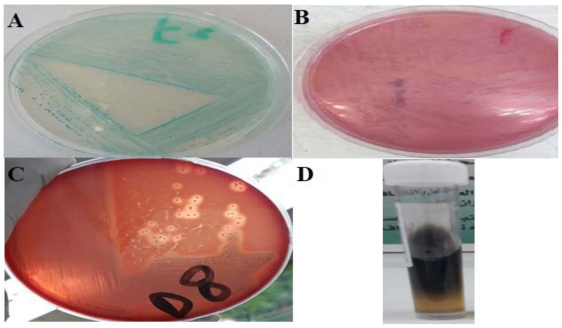

Figure 1. Phenotypic appearance of *Enterococcus* spp. on the specific media

A) Rapid HiEnterococci agar, B) MacConkey agar N2, C) Blood agar, D) Bile Esculin agar

2.6. Data analysis

Data were analyzed using SPSS software version 23 (IBM SPSS Statistics). The chi-square test was used to assessstatistical significance, and a P< 0.05 was statistically significant.

3. Results

In our study, 500 clinical specimens were collected from patients of different ages attending AL-Women's and Children's Educational Hospital in AL-Qadisiyah, Iraq, from December 2020 to May 2021, as shown in

Table 2. Distribution of *Enterococcus* spp. clinical isolates based on gender and age.

Clinical Specimens	Frequency	No. (%)			Age Range (y)
		Enterococci	Female	Male	
Urine	190	31(16.31)	19(61.29)	12(38.7)	1-87
Vaginal	125	11(8.8)	11(100)	-	25-45
Diarrhea	50	10(20)	6(60)	4(40)	1-5
CSF	10	3(30)	2(66)	1(33)	4-6
Total	375	55(14.66)	38(69.09)	17(30.09)	20-35
χ^2	-	6.86	6.2		-
P	-	0.076	0.102		-

Table 1. Out of 500 specimens processed, 375 specimens (75%) yielded positive bacterial cultures. The remaining 125 specimens (25%) were considered negative, either due to non-bacterial causative agents such as a viruses, parasites, or fungi, or because patients had received medications prior to specimen collection. The statistical results in Table 1 showed no significant difference between positive and negative cultures for clinical samples (P>0.05).

Among the 375 clinical samples, 55 isolates were identified as *Enterococcus* spp., of which 31 were derived from urine, 11 from vaginal swabs, 10 from diarrhea, and 3 from CSF specimens, with prevalence of 16.31 %, 8.8%, 20%, and 30%, respectively. Enterococci were identified by colony morphology on culture media. On Rapid HiEnterococci agar, colonies were circular, small, and greenish. On MacConkey agar, colonies were small, dry, smooth, circular, and rose-colored due to lactose fermentation. On blood agar colonies appeared white to gray, surrounded by a clear zone of beta hemolysis. On Bile Esculin agar, colonies were small, smooth, slightly convex, white to creamy and turned the agar black, as shown in Figure 1. They are gram-positive, catalase-negative, tolerant to 6.5% NaCl, and able to hydrolyze esculin. The average age of patients included in the study was between 20 to 35 years, ranging from 1 to 78 years. The percentage of *Enterococcus* spp. among the 55 isolates varied across age groups, with females 69.09% and males 30.09% (Table 2). However, the difference in the prevalence of enterococci among the four clinical sources and between females and males in various clinical sources was not significant (P>0.05), as shown in Table 2.

Table 3. Resistance rates of *Enterococcus* spp. isolates to antimicrobial agents.

Antibiotic Disks	%											
	Urine (n=31)			Vaginal (n=11)			Diarrhea (n=10)			CSF (n=3)		
	S	I	R	S	I	R	S	I	R, %	S	I	R
Ampicillin	90.3	0	9.9	100	0	0	70	0	30	100	0	0
Ceftriaxone	0	0	100	0	0	100	0	0	100	0	0	100
Cefotaxime	0	0	100	0	0	100	0	0	100	0	0	100
Gentamycin	3.2	9.6	87	0	0	100	10	30	60	0	0	100
Chloramphenicol	41.9	6.4	51.6	54.5	0	45.4	60	20	20	66.6	0	33.3
Nitrofurantoin	93.5	0	6.4	90.9	0	9	80	10	10	100	0	0
Vancomycin	100	0	0	100	0	0	100	0	0	100	0	0

Abbreviations: S: Susceptible, I: Intermediate, R: Resistant.

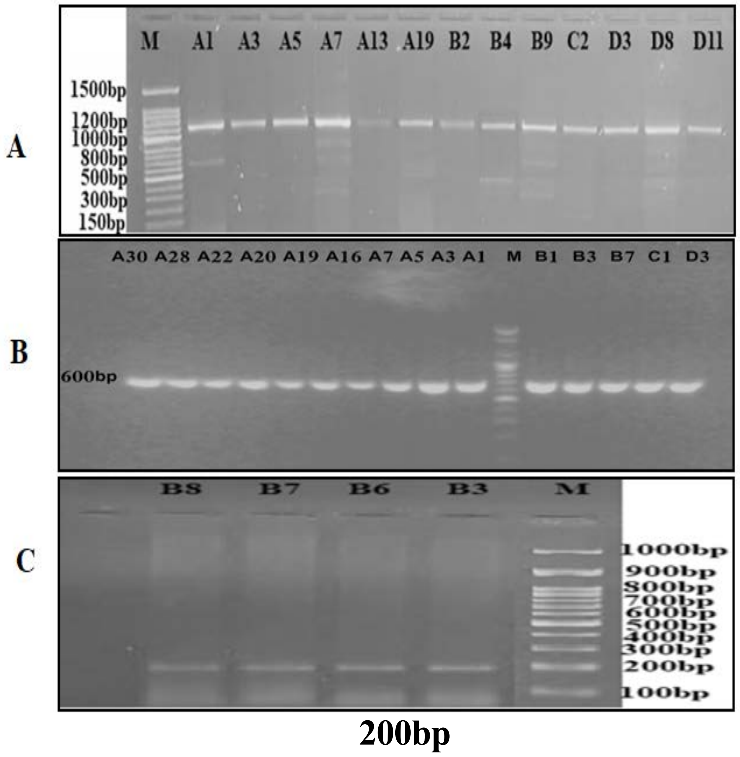

Figure 2. PCR products showing amplification of (A) *bla-SHV*, (B) *bla-TEM*, and (C) *bla-CTX-m2* genes

M: Molecular ladder (1500 bp), A: Urine samples, B: Diarrhea samples, C: CSF samples, D: Vaginal samples.

3.1. Antibacterial resistance phenotyping

β-lactam antibiotic resistance was among the most prevalent phenotypes in *Enterococcus* spp. In general, cefotaxime and ceftriaxone showed the highest resistance rates in enterococci isolated from different clinical sources: Urine: 100% (31/31); Vaginal: 100% (11/11); Diarrhea: 100% (10/10); CSF: 100% (3/3). The lowest resistance was observed for ampicillin: Urine: 9.97% (3/31); Vaginal: 0% (0/11); Diarrhea: 30% 3/10; CSF: 0% (0/3). Enterococci also showed resistance to gentamicin 47/55 (85.45%), chloramphenicol 24/55 (43.63%), and nitrofurantoin 4/55 (7.27%). In terms of antibiotic sensitivity, all *Enterococcus* spp. isolates were 100% sensitive to vancomycin across all clinical sources, as shown in Table 3.

Concerning the resistance patterns, among 55 enterococci isolates tested, 23/55 (41.81%) were MDR, 2/55 (3.63%) were XDR, and 0/55 (0%) were PDR. MDR was more common in urine 17/31 (54.83%), vaginal 3/11 (27.27%), diarrhea 3/10 (30%), and CSF 1/30 (33.33%) samples, respectively. XDR isolates were detected in urine and vaginal samples (Table 4).

When enterococci isolated from urine, vaginal, diarrhea, and CSF specimens were compared, 50 patients (90.9%) had similar resistance profiles (Table 4). Enterococcus isolates displayed a multi-drug resistance (MDR) phenotype. MDR prevalence varied among clinical sources: Urine 10/31 (32.2%), vaginal 7/11 (63.6%), diarrhea 4/10 (40%), and CSF 2/3 (66.6%). All MDR strains showed high resistance to cefotaxime, ceftriaxone, gentamicin, and chloramphenicol. There was a statistically significant difference between similar antibiotic resistance patterns among *Enterococcus* spp. strains isolated from different clinical sources, whereas no significant differences were observed in resistance to CTR, CTX, and AM (P>0.05), as shown in Table 5.

3.2. Prevalence of ESβLs among *Enterococcus* spp.

All strains were phenotypically screened for ESβLs, and it was revealed that all Enterococcus isolates produced ESβLs (100%). Molecular detection of *bla-SHV, bla-TEM,* and *bla-CTX-M2* genes in *Enterococcus* isolates was performed by PCR assays. As shown in Table 6 and Figure 2, In urine isolates, the rates of *bla-TEM* and *bla-SHV* genes were 32.2% and 19.35%, respectively. In diarrhea isolates, the rates of *bla-CTX-M2*, *bla-TEM*, and *bla-SHV* genes were 40%, 30%, and 30%, respectively.

Table 5. Antibiotic resistance profiles of *Enterococcus* spp. isolated from clinical specimens

Antibiotic Resistance Patterns	No. (%)			Same Resistance Patterns	χ^2	P
	Vaginal (n=11)	Diarrhea (n=10)	CSF (n=3)			
CTR,CTX,AM,GN,F,C	0(0)	0(0)	0(0)	0	0.789	0.852
CTR,CTX,GN,F,C	1(9)	0(0)	0(0)	0	4.07	0.254
CTR,CTX,AM,GN	0(0)	1(10)	0(0)	2	1.69	0.637
CTR,CTX,AM,C	0(0)	1(10)	0(0)	2	1.69	0.637
CTR,CTX,GN,C	3(27.2)	1(10)	1(33.3)	19	4.46	0.215
CTR,CTX,GN,F	0(0)	0(0)	0(0)	0	5.20	0.157
CTR,CTX,AM	0(0)	2(20)	0(0)	0	9.34	0.025
CTR,CTX,GN	7(63.6)	4(40)	2(66.6)	23	4.09	0.252
CTR,CTX,F	0(0)	1(10)	0(0)	0	4.58	0.205
CTR,CTX	0 (0%)	1(10%)	0 (0%)	4	1.47	0.688
No resistance	0 (0%)	0 (0%)	0 (0%)	0	-	-

In vaginal isolates, the rates of *bla-TEM* and *bla-SHV* genes were 27.27 and 9%, respectively. In CSF isolates, the rates of *bla-TEM* and *bla-SHV* genes were 33.33% and 33.33%, respectively. The results revealed that *bla-TEM* and *bla-SHV* genes were more prevalent in Enterococci isolates. However, no bla-CTX-M2-positive *Enterococcus* spp. isolates were detected in urine, vaginal, and CSF samples. There were no significant differences in ESβLs genes among *Enterococcus* spp. isolates from different clinical sources (P>0.05), As shown in Table 6.

4. Discussion

The growing and rapid development of MDR enterococci is a global concern, especially ESβLs-producing enterococci. Enterococci have become major pathogens in recent decades, not only because of their ability to cause serious infections such as endocarditis, gastrointestinal infections, bacteremia, and UTIs, but also because of their increasing antibiotic resistance. The rapid rise of β-lactams resistant enterococci, caused by β-lactamase production, has made treating major enterococcal infec-

Table 6. Prevalence of β-lactamase genes among *Enterococcus* spp.

ESβLs Genes	No. (%)				
	Urine (n=31)	Vaginal (n=11)	Diarrhea (n=10)	CSF (n=3)	Total (n=55)
bla-CTX-m2	0(0)	0(0)	4(40)	0(0)	4(7.27)
bla-TEM	10(32.2)	1(9)	3(30)	1(33.33)	15(27.27)
bla-SHV	6(19.35)	3(27.27)	3(30)	1(33.33)	13(23.63)
Total	16(51.61)	4(36.36)	10(100)	2(66.66)	32(58.18)
χ^2	0.58				
P	12.176				

Table 4. Resistance patterns of *Enterococcus* spp. isolates against different antimicrobial agents

Samples	Isolate Code	Gender	Antimicrobial Resistance	No. of Antimicrobial Classes	*ESβLs*-genes	Resistance Patterns
Urine	A1	Female	CRO^{II}, CTX^{II}, G^{NIII}, C^{IV}	3	*bla-SHV, bla-TEM*	MDR
	A3	Female	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	*bla-SHV, bla-TEM*	MDR
	A5	Male	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	*bla-SHV, bla-TEM*	MDR
	A7	Male	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	*bla-SHV, bla-TEM*	MDR
	A9	Female	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	-	MDR
	A10	Female	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	-	MDR
	A13	Female	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	*bla-SHV*	MDR
	A15	Female	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	-	MDR
	A16	Female	AM^{I}, CRO^{II}, CTX^{II}, C^{IV}	3	*bla-TEM*	MDR
	A18	Female	AM^{I}, CRO^{II}, CTX^{II}, GN^{III}	3		MDR
	A19	Female	AM^{I}, CRO^{II}, CTX^{II}, GN^{III},F^{V}, C^{IV}	5	*bla-SHV, bla-TEM*	XDR
	A20	Female	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	*bla-TEM*	MDR
	A22	Male	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	*bla-TEM*	MDR
	A25	Female	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	-	MDR
	A28	Female	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	*bla-TEM*	MDR
	A30	Female	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	*bla-TEM*	MDR
	A31	Male	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	-	MDR
Diarrhea	B1	Female	AMI, CRO^{II}, CTX^{II}, GN^{III}	3	*bla-TEM*	MDR
	B4	Female	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	*bla-SHV*	MDR
	B7	Male	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	*bla-TEM, bla-CTX-m2*	MDR
CSF	C2	Male	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	*bla-SHV*	MDR
Vaginal	D1	Female	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	-	MDR
	D3	Female	CRO^{II}, CTX^{II}, GN^{III},FV, C^{IV}	4	*bla-SHV, bla-TEM*	XDR
	D8	Female	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	*bla-SHV*	MDR
	D11	Female	CRO^{II}, CTX^{II}, GN^{III}, C^{IV}	3	*bla-SHV*	MDR

Abbreviations: AM: Amoxicillin; CRO: Ceftriaxone; CTX: Cefotaxime; GN: Gentamicin; F: Nitrofurantoin; C: Chloramphenicol, - : Non-*ESβLs*-genes.

^{I}β-lactams (penicillins), ^{II}β-lactams (cephalosporins); 3rd generation, ^{III}Aminoglycosides, ^{IV}Chloramphenicol, ^{V}Nitrofurantoin.

tions increasingly challenging, leaving physicians with very few therapeutic options.

A total of 55 (14.66%) Enterococcus strains were recovered from 375 clinical samples. The prevalence among clinical sources was as follows: UTI patients 31/55 (56.36%), vaginal swabs 11/55 (20%), diarrhea 10/55 (18.18%), and CSF 3/55 (5.45%). This finding is in agreement with the report published by Sharif et al. [12] but differs from results obtained by other researchers [13]. In this study, the UTIs infection rate was higher among females, 19/31 (61.29%), than among males, 12/31 (38.7%). Females are more significantly affected due to anatomical differences in their genitalia. They have a short urethra and its proximity to the anus, which facilitates bacterial transfer [14]. Additionally, this study found a high prevalence of *Enterococcus* spp. in vaginal samples. This high prevalence in women may be primarily due to personal hygiene practices and potentially inappropriate sexual practices between partners. The spread rate of diarrhea cases was 6/10 (60%) in males and 4/10 (40%) in females. Additionally, males had a higher prevalence of enterococci infection in CSF specimens, which may be related to weaker immune responses, making them more susceptible to infections [15]. This finding is in agreement with previous studies [16].

According to the results of our study, all Enterococcus spp. strains were resistant to cefotaxime (100%), *ceftriaxone* (100%), and gentamicin. Resistance to other antibiotics was lower, including chloramphenicol (43.63%) and ampicillin (10.90%). Nitrofurantoin showed the lowest resistance (7.27%). All the *Enterococcus* spp. isolates were completely susceptible to vancomycin. Our findings are consistent with several international studies [17, 18]. The spread of antibacterial-resistant *Enterococcus* spp. in this study may be attributed to the widespread and indiscriminate use of antibiotics for infection treatment and disease prevention. High-level resistance to β-lactams may result from a low affinity between the PBPs of *Enterococcus* strains and the antibiotic, or from the production of β-lactamase enzymes that hydrolyze the β-lactam ring in antibiotic molecule. Alternatively, resistance may involve a two-component regulatory system, including IreK, a serine/threonine kinase, and IreP, a phosphatase, which has been shown to contribute to cephalosporin resistance [19]. *Enterococcus* spp. resist gentamicin by producing the enzyme AAC(6')-Ie/APH(2"), which has acetyltransferase and phosphotransferase activities [20].

Enterococcus isolates exhibited two patterns of MDR: 41.81% of isolates were resistant to three classes of antibiotics, and 3.36% were resistant to more than three classes. Isolates A19 and D3 were resistant to four antibacterial classes, whereas other isolates showed resistance to three. This result agrees with the findings of Dadfarma et al. [21]. In contrast, other studies reported higher MDR and XDR resistance rates in enterococci [17, 22]. Multidrug or extensive drug resistance, resulting mainly from the misuse and indiscriminate use of antibiotics, can lead to severe and costly infections that are difficult to treat, consequently increasing the mortality rate [23].

Urinary *Enterococcus* isolates showed significant diversity in patterns of antibiotic resistance. They also exhibited higher rates of MDR compared to isolates from other clinical sources.

Phenotypically, we found that the prevalence of ESβLs-producing Enterococcus in clinical specimens was 100%. This is extremely high percentage for such a common bacterium, which could significantly complicate treatment management in hospitals. In contrast, the molecular study reported a lower prevalence compared to phenotypic results. PCR results showed the ability of *Enterococcus* spp. to produce extended-spectrum β-lactamases through the detection of *bla-TEM*, *bla-SHV*, and *bla-CTX-M2* genes in 51.61%, 36.36%, 100%, and 66.66% of urine, vaginal, diarrhea, and CSF isolates, respectively, which agrees with some previous studies [24] and differs from others [25]. Enterococci are resistant to cephalosporins. Although this is a well-known characteristic, the molecular basis of this phenotype is not fully understood. However, the general observation is that resistance correlates with decreased binding affinity of cephalosporins for enterococcal PBPs, particularly Pbp5. Additionally, a specific mutation in the *rpoB* gene confers enhanced cephalosporin resistance [26].

Our study concludes a high prevalence of β-lactamases produced by β-lactam-resistant *Enterococcus* isolates carrying *bla-TEM*, *bla-SHV*, and *bla-CTX-M2* genes in Al-Women's and Children's Educational Hospital in Al-Qadisiyah, Iraq. The hospital is a key facility specializing in high-risk cases, including newborns, infants, and adults. This finding is alarming, as it suggests that these plasmid-borne genes could be transferred to other plasmid-free enterococci or gram-positive bacteria, both within the gastrointestinal tract and in the hospital environment. Therefore, it is imperative to maintain careful vigilance to prevent the spread of beta-lactam-resistant *Enterococcus* within the hospital and from the hospital to the community.

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Data availability

The data supporting the findings of this study are available upon request from the corresponding author.

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Authors' contributions

Conceptualization, study design, data acquisition, analysis, data interpretation, administrative, technical, and material support, and writing: Raid Razzaq Ojaimi: Raid Razzaq Ojaimi; Supervision: Azhar Noory Hussein.

Conflict of interest

The authors declared no conflict of interest.

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