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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ASTRACT

Enterococcus spp., which produce extended-spectrum β-lactamases (ESβLs), are resistant to nearly all β-lactam antibiotics. Due to the limited quantity of therapeutic options available, enterococci infections are often difficult to treat, resulting in higher antibiotic and healthcare costs and a threat to patients' lives. This research, therefore, aimed to determine the antibiotic resistance pattern of *Enterococcus* spp. and evaluate their potential to produce phenotypically and genotypically ESβLs 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. 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 approximation method and PCR, respectively. All *Enterococcus* spp. isolates were completely resistant to ceftriaxone and cefotaxime. 85.45% Isolates were resistant to gentamicin, 43.63% to chloramphenicol, 10.9% to ampicillin, and 7.2% to nitrofurantoin. The prevalence rates of Multidrug resistance (MDR) and Extensive drug resistance (XDR) isolates were 41.81% and 3.63%, respectively. Phenotypically, all 55 isolates were ESβLs-positive. Genotypically, the spread rates of the *bla-TEM*, *bla-SHV*, and *bla-CTX-$_{M2}$* genes were 27.27%, 23.63%, and 7.27%, respectively. Our conclusion demonstrated 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. Also observed was the essential rate of resistance to β-lactam. This highlights the need for a rational policy on the less use of antibiotics.

Keywords: Antibacterial-resistance ß-lactamase, Enterococci, ESβLs-genes, MDR, XDR

Introduction

Enterococcus spp. are facultative anaerobic Gram-positive cocci found in both the gastrointestinal systems of humans and animals. However, they are significant opportunistic pathogens that constitute biofilms on medical devices and catheters, leading to urinary tract infections (UTIs), endocarditis, bacteremia, abdominal and biliary infections, burns, and surgical site wound 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 defiance and an object of interest (2). They include two main species: *Enterococcus faecalis (E. faecalis) and Enterococcus faecium (E. faecium),* the most common pathogenic species of enterococci, and 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 obtain antibacterial resistance with ease (4). The level of Enterococcal intrinsic resis). tance varies among different beta-lactams. Penicillins generally have the highest activity against enterococci, due to their penicillin-binding proteins (PBPs) having a low affinity, followed by carbapenems and cephalosporins, with the final having the least activity. But this is not relevant to modern-generation cephalosporins such as ceftobiprole and ceftaroline (5).

One of the more significant mechanisms by which enterococci resist antibiotics is the production of enzymes that destroy 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 rising problem in common medical institutions (7). This decreases the efficiency of previously successful antibiotics, resulting in a bad 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 overall investigations 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 & methods

2.1. Description of study specimen.

A cross-sectional research 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. Collect 500 samples from urine, vaginal swabs, diarrhea, and CSF patients. Midstream urine samples were collected 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 with lumbar punctures and then immediately transported them to the microbiology laboratory within 30 minutes for cultivation.

2.2. Bacteriological Examinations

Cultivation of all clinical specimens on various agar media such as Rapid HiEnterococci agar (HiMedia, India), MacConkey agar N2, Blood agar, and Bile Esculin agar (Oxoid Company, Britain). In short, using a sterile wire loop to inoculate the samples on the media, then incubating aerobically for 24 hours at 37 °C. Isolated and identified the enterococci depending 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 through morphology in Gram's staining under a microscope.

Finally, all isolations were kept at -20 °C in tryptic soy broth plus 15% glycerol, as per the European Manual of Clinical Microbiology.

2.3. Susceptibility Testing for Antimicrobials

Using the standard Kirby-Bauer disk diffusion method, the antibiotic susceptibility profiles were conducted for Enterococcus spp. as mentioned in (7). After swabbing the enterococci suspension on the Mueller-Hinton agar (MHA) plates, they were incubated for 24 hours. In the end, read the results and measure the inhibition zones with a metric ruler, and compare with CLSI. 7 antibacterials were tested. These were Ampicillin (AM 30 g), Ceftriaxone (CTR 10 g), Cefotaxime (CTX 30 g), Gentamycin (10 g), Vancomycin (30 g), Nitrofurantoin (F 300 g), and Chloramphenicol (C 30 g). MDR was defined as any bacterium resistant to ≥ 3 classes of antibacterial.

2.4. Phenotypic confirmation of ESβLs

The disc approximation method was used to detect the ESβLs-producing activity of selected enterococci strains. For confirmation of ESβL production in enterococci species, prepare an enterococci suspension of 5 pure, single colonies in 5 ml of sterile broth, then compare the turbidity with 0.5 McFarland. The broth was spread by swabbing on MHA (Hi-Media, India). Ampicillin 25 mg disc placed in the middle was flanked by a disc of cefotaxime 30 mg, a disc of ceftriaxone 10 mg, and a disc of cefepime 10 mg at 30 mm apart on a lawn culture. and then the plates were incubated for 24 h at 37 °C. to observe inhibition zones. resulted in a peculiarly shaped area called a "champagne cork" around antibiotic discs.

2.5. DNA Extraction and PCR Assay

To extract genomic DNA from isolates of *Enterococcus* spp., a DNA extraction kit from Anatolia company in Turkey. The 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 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') (9), and *bla-SHV*-F (5'-ATTTGTCGCTTCTTTACTCGC-3') and *bla-SHV*-R (5'-TTTATGGCGTTACCTTTGACC-3') with amplicon sizes of 941, 1030, and 446 bp, respectively (10). Under the following conditions: a primary denaturation step for 5 minutes at 97°C, followed by a denaturation step for 30 seconds at 94°C consisting of 35 cycles, annealing for 30 seconds at 54°C, and final extension for 2 minutes at 72°C, to ensure full extension of the PCR products. PCR was conducted using a thermal cycler (Eppendorf, Germany). Then, electrophoresis was performed in a 1.5% agarose gel prepared in TBE buffer at 95 V for 30 min. After that, ethidium bromide stain was added and poured into the tray to solidify. Fragments of DNA and a 100 bp DNA ladder were placed in wells (Fermentas, Germany). The PCR products were observed under a UV transilluminator.

2.6. Data analysis

Using SPSS software version 23 (IBM SPSS Statistics) to analyze our data study. The chi-square test was used to calculate the significance test, and a P value < 0.05 was the least significant level.

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 1. Out of 500 specimens processed, 375/500 (75%) specimens gave a positive culture (caused by bacteria). The other 125/500 (25%) specimens were considered negative results (caused by another causative agent such as a viral agent, parasitic agent, or fungi agent) or resulted in patients taking drugs before the collection of specimens. The statistical results in Table 1 showed a significant difference between positive and negative cultures for clinical samples at (P>0.05).

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

Clinical specimens	No. of specimens	Positive bacteria culture (%)	Negative bacteria culture (%)
Urine	**250**	**190 (74)**	**60 (11.53)**
Vaginal	**150**	**125 (83.33)**	**25 (16.66)**
Diarrhea	**50**	**50 (100)**	**-**
CSF	**50**	**10 (20)**	**40 (80)**
Total	**500**	**375 (75)**	**125 (25)**
χ^2	-	**103.02**	
p-value	-	**0**	

Among the 375 different clinical samples, 55 isolates were identified as *Enterococcus* spp., of which 31 were derived from urine, 11 were from vaginal swabs, 10 were derived from diarrhea and 3 were isolated from CSF specimens. with the prevalence of (16.31 %), (8.8%), (20%), and (30%), respectively. Enterococci were identified by colony morphology on culture media, gave colonies grow as circles, small in size with greenish color on Rapid HiEnterococci agar on MacConkey agar shown colonies of small size dry, smooth and circular with rose color due to its lactose-ferment, and blood agar showing colonies appears as white to gray color, surrounded clear zone of beta hemolysis, While shown Enterococci on Bile Esculin agar produce small, smooth, slightly convex, white to creamy colonies. They converted the color of the agar to 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 the patients included in the study was between 20 to 35 years, ranging from 1 to 78 years old. The percentage of *Enterococcus* spp. in the 55 isolates varied across the age groups, with females 69.09% and males 30.09% (Table 2). However, the difference in the prevalence of enterococci in four clinical sources and between the numbers of females and males in various clinical sources was not significant (P>0.05). as shown in Table 2.

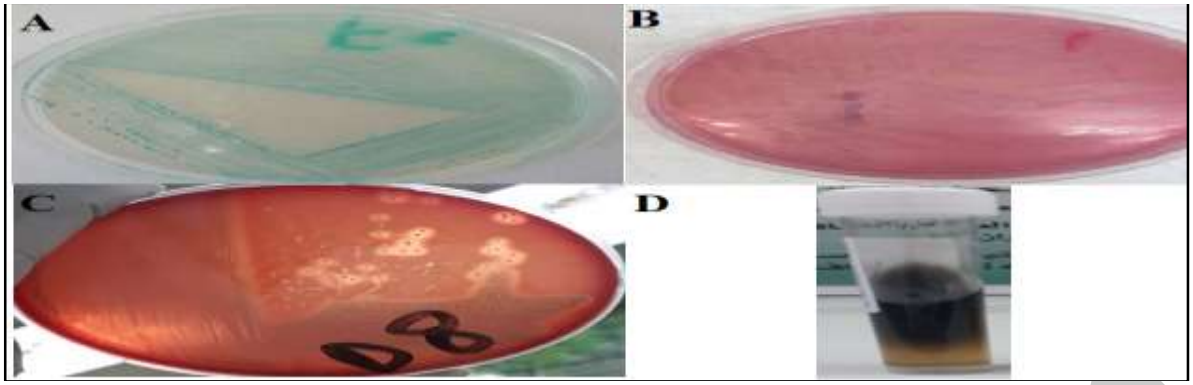

Figure 1. Phenotype photograph of *Enterococcus* spp. on the specific media**: A. Rapid HiEnterococci agar; B. MacConkey agar N2; C. Blood agar; D. Bile Esculin agar.**

Table 2. Distribution of Enterococci clinical based on gender and age.

Clinical specimens	Frequency	Enterococci (%)	Female (%)	Male (%)	age range (Years)
Urine	**190**	**31 (16.31)**	**19(61.29)**	**12 (38.70)**	**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 value	-	**0.076**	**0.102**		-

3.1. Antibacterial resistance phenotyping.

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

Table 3. Resistance rates to antimicrobials in *Enterococcus* spp. isolates.

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

S: **Susceptible**, I: **Intermediary**, R: **Resistant.**

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 the same resistance profiles Table 4. Enterococci isolates were shown to have a multi-drug resistance (MDR) phenotype. Urine specimens had a higher prevalence of this phenotype than vaginal, diarrhea, and CSF specimens, with 10 (32.2%), 7 (63.6%), 4(40%), and 2 (66.6%), respectively. All MDR strains had 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 there were no significant differences between *Enterococcus* spp. isolates resistance to CTR, CTX, and AM patterns at ($P>0.05$), as shown in Table 5.

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

Antibiotic resistance patterns	Urine N= 31	Vaginal N=11	Diarrhea N=10	CSF N= 3	Same resistance patterns	χ^2	P-value
CTR,CTX,AM,GN,F,C	1 (3.2%)	0 (0%)	0 (0%)	0(0%)	0	**0.789**	**0.852**
CTR,CTX,GN,F,C	0 (0%)	1 (9%)	0 (0%)	0(0%)	0	**4.07**	**0.254**
CTR,CTX,AM,GN	1 (3.2%)	0 (0%)	1 (10%)	0(0%)	2	**1.69**	**0.637**
CTR,CTX,AM,C	1 (3.2%)	0 (0%)	1 (10%)	0(0%)	2	**1.69**	**0.637**

CTR,CTX,GN,C	14(45.1%)	3(27.2%)	1(10%)	1(33.3%)	19	**4.46**	**0.215**
CTR,CTX,GN,F	1(3.2%)	0(0%)	0 (0%)	0 (0%)	0	**5.20**	**0.157**
CTR,CTX,AM	0 (0%)	0 (0%)	2 (20%)	0 (0%)	0	**9.34**	**0.025**
CTR,CTX,GN	10(32.2%)	7(63.6%)	4 (40%)	2(66.6%)	23	**4.09**	**0.252**
CTR,CTX,F	0 (0%)	0 (0%)	1(10%)	0 (0%)	0	**4.58**	**0.205**
CTR,CTX	3(9.6%)	0 (0%)	1(10%)	0 (0%)	4	**1.47**	**0.688**
No resistance	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0	-	-

3.2. Prevalence of ESβLs among *Enterococcus* spp.

All the strains were phenotypically screened for EsβLs, and it was revealed that all enterococci isolates produced ESβLs (100%). Then, the molecular detection of *bla-SHV*, *bla-TEM*, and *bla-CTX-M2* genes in Enterococcus isolates was by PCR assays. As shown in Table 6 and Figure 2, the rates of *Enterococcus* spp. strains isolated from urine samples harboring *bla-TEM* and *bla-SHV* genes were (32.2%) and (19.35%), respectively. Additionally, all Enterococci isolates isolated from diarrhea were carrying *bla-CTX-$_{M2}$*, *bla-TEM*, and *bla-SHV* genes rates were 40, 30, and 30%, respectively. While the rates of *Enterococcus* spp. strains isolated from vaginal samples harboring *bla-TEM* and *bla-SHV* genes were (27.27) and (9%), respectively. Finally, the rates of *Enterococcus* spp. isolates isolated from CSF samples carrying *bla-TEM* and *bla-SHV* genes were (33.33) and (33.33%), respectively. The results revealed that the prevalence of the *bla-TEM* and *bla-SHV* genes was more occurring in Enterococci isolates. However, there were no *bla-CTX-$_{M2}$*-positive *Enterococcus* spp. isolates isolated from urine, vaginal, and CSF samples. There were no significant differences between ESβLs-genes in *Enterococcus* spp. isolates isolated from different clinical sources at (P>0.05). as in Table 6.

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

ESβLs genes	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-value	12.176

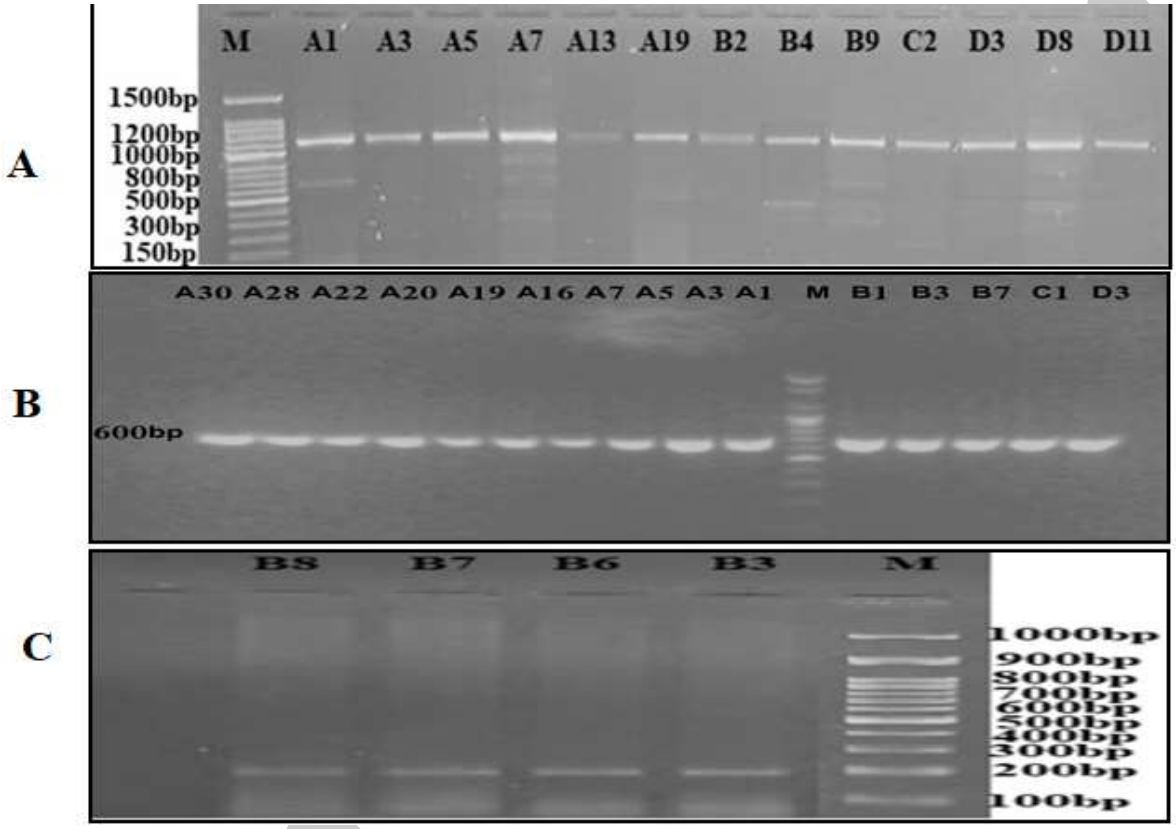

Figure 2. PCR products of the amplification of (A) *bla-SHV*,(B)*bla-TEM* and (C) *bla-CTX-m2* genes. **M** = Molecular ladder (1500 bp). (A=Urine samples, B=Diarrhea samples, C=CSF samples, D=Vaginal samples).

4. Discussion

The growing and fast development of multidrug-resistant enterococci is a universal concern, especially ESβLs-producing enterococci. Enterococci have become a major pathogen in recent decades, not only because of their capacity to cause serious infections such as endocarditis, gastrointestinal infections, bacteremia, and UTIs but also because of their rising antibiotic resistance. The fast rise of β-lactams resistant enterococci caused by generated β-lactamases has made treating major enterococcal infections increasingly challenging, leaving doctors with very few therapeutic alternatives.

A total of 55 (14.66%) enterococci strains were found from 375 clinical samples collected from patients. the prevalence of bacteria among UTI patients was 31/55 (56.36%), vaginal swabs 11/55 (20%),

diarrhea 10/55 (18.18%), and CSF 3/55 (5.45%). This study was in agreement with the report published by (11). but disagreement with the results obtained by other researchers (12). In this study, the infection rate of UTIs was higher among females, 19 (61.29%), than among males, 12 (38.70%). Females are more significantly influenced due to anatomical differences in their genitalia. They have a short urethra and its proximity to the anus, which facilitates the transfer of bacteria to it (13). Additionally, we found in a study a high spread of *Enterococcus* spp. in vaginal samples. This high spread in women can be due mainly to personal hygiene, and sexual practices between spouses may not be correct. The spread rate of diarrhea cases was 6 (60%) in males and 4 (40%) in females. Additionally, males had a more widespread of enterococci infection in CSF specimens. This may be due to their poor immune system, making them highly vulnerable to infections (14). This study is in agreement with studies (15).

According to the results of our study, all of the *Enterococcus* spp. strains were resistant to cefotaxime (100%), ceftriaxone (100%), and gentamicin. There were less resistant isolates, such as chloramphenicol (43.63%), and ampicillin (10.90%). The poor level of resistance (7.27%) was given by nitrofurantoin. All the *Enterococcus* spp. isolates were completely susceptible to vancomycin. Our findings are similar to some world studies (16,17). The spread of antibacterial-resistant *Enterococcus* spp. in this study can be demonstrated by the widespread and indiscriminate use of the treatment of infections and disease prevention. High-level resistance to β-lactams may be due to a low correlation among the PBPS of the Enterococci strains and the antibiotic, or to the production of β-lactamase enzymes that break down the β-lactam ring in the antibiotic molecule. Alternatively, it to a two-component regulatory system including IreK, a serine/threonine kinase, and IreP, a phosphatase, that has been shown to contribute to cephalosporin resistance. (18). *Enterococcus* spp. resist gentamicin by producing an enzyme AAC(60)-Ie/APH(20) that consists of 20 phosphotransferase and 60 acetyltransferase (19).

The isolates of Enterococci were characterized by having two patterns of multidrug resistance, where 41.81% of the isolates were resistant to three classes of antibiotics, and 3.36% of the isolates were resistant to more than three classes of antibiotics. A19 and D3 were resistant to four antibacterial classes, whereas other isolates showed resistance to three. This result agrees with the results of Dadfarma *et al.* (20). Contrary to our findings, higher MDR and XDR resistance rates of enterococci in other studies (16,21). The resulting multidrug or extensive drug resistance, the result of several factors, mainly the misuse and random use of antibiotics, can lead to many serious and costly diseases that are difficult to treat, and consequently increase the mortality rate (22).

Urinary enterococci isolates showed a significant diversity in patterns of antibiotic resistance. They also exhibited high rates of multidrug resistance compared to other clinical sources.

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

Samples	Isolate Code	Gender	Antimicrobial Resistance	No. of Antimicrobial Classes	ESβLs-genes	Resistance patterns
	A1	Female	CRO^{II}, CTX^{II}, GN^{III}, 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
Urine	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	AM^{I}, 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},F^{V}, 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

AM: Amoxicillin, CRO: Ceftriaxone, CTX: Cefotaxime, GN: Gentamicin, F: Nitrofurantoin, C: Chloramphenicol, ^{I}: β-lactams (penicillins), ^{II} β-lactams (cephalosporins), 3rd generation, ^{III} aminoglycosides, ^{IV} chloramphenicol, ^{V} nitrofurantoin . -: Non-ESβLs-genes.

Phenotypically, we find that the spread of ESβLs⁻ producing enterococci in clinical specimens was 100%. This is a very high level percentage for such a common bacterium, which would be tragic in the treatment management process used in hospitals. In contrast, the molecular study reported a lower prevalence compared to phenotypic results. PCR results showing Enterococcus spp. ability to produce Extended-spectrum β-lactamases through the detection of *bla-TEM*, *bla-SHV*, and *bla-CTX-M2* genes was with (51.61%), (36.36%), (100%), and (66.66%) isolates of urine, vaginal, diarrhea, and CSF specimens, respectively, which agreed with some of the previous study (23). and disagreed with others (24). Enterococci are resistant to cephalosporins. Although this is a well-known character, the molecular basis of this phenotype has not been comprehended. However, the general observation is that there is a correlation between natural resistance and decreased binding affinity of cephalosporins for enterococcal PBPs, particularly Pbp5. A specific mutation in the *rpoB* gene confers enhanced cephalosporin resistance (25).

Our conclusion finds a high prevalence of β-lactamases produced by β-lactam-resistant enterococci isolates, carrying *bla-TEM*, *bla-SHV*, and *bla-CTX-$_{M2}$* genes. The Al-Women's and Children's Educational Hospital in Al-Qadisiyah, Iraq. The hospital is an essential facility specializing in special cases, including newborns, babies, and adults. This result is alarming, as it suggests that these plasmid-borne genes could be transferred to other plasmid-free enterococcal or Gram-positive bacteria, both inside the gastrointestinal system and in a hospital environment. thence, it is imperative to maintain careful vigilance to prevent the prevalence of beta-lactam-resistant *Enterococcus* inside the hospital and from the hospital to the community

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Authors' Contribution

Study concept and design: R. R.

Acquisition of data: R. R.

Analysis and interpretation of data: R. R.

Drafting of the manuscript: R. R.

Critical revision of the manuscript for important

intellectual content: R. R.

Administrative, technical, and material support: R. R.

Study supervision: A.N.

Ethics

In this article, the authors have observed all ethical points, including those related to plagiarism, double publication, data distortion, and data manipulation.

Conflict of Interest

The author declares no known competing interests.

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Data Availability

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

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