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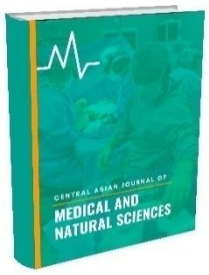

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Prevalence of Multidrug Resistance in Enterococci Isolates from Different Clinical Sources in Al-Diwaniyah City

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Abstract: Background: Enterococcus spp is one of the most important pathogens, and it has shown great resistance in recent times, and this has become a concern for health in the world.

The aim is to determine the prevalence of multidrug resistance in enterococcus spp isolates in Al-Diwaniyah city.

Methods: 150 samples were collected in this study from multiple clinical sources (urine, stool seminal fluid, vaginally swab, blood)) from patients in Al-Diwaniyah General Teaching Hospital and Maternity and Children Teaching Hospital and afaq General hospital. during the period from the first of September 2022 to January 2023, where the isolates were identified through microscopic examination, gram stain, biochemical assays, selective medium, and VITEK-2 system.

Results: A total of 50(33.33%) isolates were identified as enterococcus spp from 150) clinical samples(40 E.faecalis,10 E.faecium) that collected from different sources, that distributed(urine:13(43%)E.faecalis, 7(28%) E.faecium, Stool:12(25%) E.faecalis,3(37.5%) E.faecium,seminal fluid:2(20%) E.faecalis,vaginally swab:11(31%) E.faecalis,blood:2(10%) E.faecalis). E.faecalis was resistance to antibiotic as as (vancomycine, pencillin, chloramphenicol, ciprofloxacin, levofloxacin, Norfloxacin, Nitrofurantion, Rifampin, Erthromycin, doxycycline)

(3(7.5%), 10(25%), 16(40%), 31(77.5%) (,17(42.5%), 14(35%), 6(15%), 30(75%), 29(72.5%), 17(42.5%)) respectively while. E.faecium was resistance to antibiotic as (vancomycine, pencillin, chloramphenicol, ciprofloxacin, levofloxacin, Norfloxacin, Nitrofurantion, Rifampin, Erthromycin, doxycycline) (8(80%), 9(90%), 4(40%), 6(60%), 3(30%), 4(40%), 1(10%), 4(40%),

8(80%), 6(60%)), respectively

Conclusion: E.faecalis isolates showed high resistance to each of the-erythromycin, ciprofloxacin, rifampin, while E.faecium was highly resistant. to vankomycin, penicillin, erthromycin, as well as to ciprofloxacin, and this causes a problem of concern in this study.A high percentage of MDR among commonly isolated bacteria were found in this study would be a serious, alarming issue.

Introduction:

The genus Enterococci belongs to the lactic acid bacteria (LAB) and represents the third largest genus after Lactobacillus and Streptococcus of the Formicates phylum. Enterococcus cells are gram-positive and have an oval shape. They usually appear in the form of pairs or chains of different lengths (1). Enterococci are able to stay alive in a variety of stresses, adverse environments, with the extreme pH (4.5−10.0) and elevated concentration of Na Cl, temperature (5–65 °C),allowing them to inhabit a varied ranges of niche (2). Enterococcus species have evolved from commensal bacteria to important pathogens that cause infections in humans and animals (3). Enterococci species, especially the two types E.faecalis and E. faecium, are two common causes of urinary tract infection (4).

Sample collection

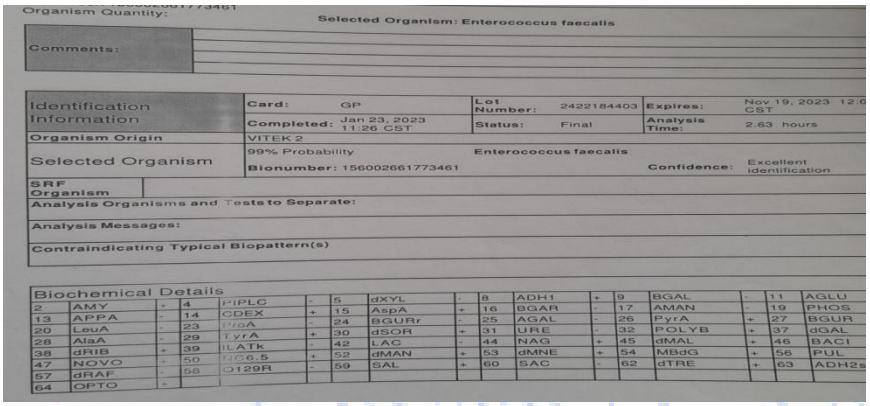
One hundred and fifty clinical samples were collected during the period from the first of September 2022 to January 2023 from different clinical cases from patients attending Al-Diwaniyah General Teaching Hospital and Materinty and Children Teaching Hospitalq and afaq General hospital. The specimens included urine, stool, vagina, seminal fluid, blood, l. urine specimen were generally collected from patients suffering from UTIs. Mid-stream urine samples were collected in sterilized screw-cap containers and stool samples were collected from patients with diarrhea, via a clean, sterile, leak-proof container. As for the vaginal specimens were generally collected from women (pregnant and non-pregnant) suffering from vaginitis, they were collected using sterile cotton swabs with Amies medium, Seminal fluid specimens were collected by the patient using sterile plastic containers from infertile men's sperm, while blood sample was collected from the suspected patients by sterile syringes delivered in blood culture bottle containing 30 ml of brain heart infusion broth, for best result insert 3-5 ml of blood and incubated at (37°C) for at least (3) days, then the sample is taken by sterile syringes and cultured on the culture medium.

All specimens were transported to the laboratory and cultured for 24 h at 37 °C on blood agar medium. The isolates were purified several times until they were pure, Microscopy and special biochemical tests were then carried out before the samples were transferred to the VITEK 2 system for confirmation of their identity

Identification of bacterial isolates

The identification of bacterial isolates were achieved by bacteriological methods including morphological characteristics of bacterial colonies on agar(blood agar medium, and Selective *Enterococcus spp* result selective media, Gram stain, and biochemical tests. Gram stain was conducted for all positive cultures, all the isolates were diagnosed by traditional biochemical tests and confirmed by automatic test (Vitek system)

Table (1) Biochemical identification of Enterococcus faecium using VITEK2 compact system



Organism Quantity:

Selected Organism: Enterococcus faecalis

Comments:

Identification Information	Card: GP	Lot Number: 2422184403	Expires: Nov 19, 2023 12:0 CST
	Completed: Jan 23, 2023 11:26 CST	Status: Final	Analysis Time: 2.63 hours
Organism Origin	VITEK 2		
Selected Organism	99% Probability	Enterococcus faecalis	
	Bionumber: 1560026617734 61		Confidence: Excellent identification
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			

Biochemical Details

2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU			
13	APPA	-	14	CDEX	+	15	AspA	+	16	BGAR	-	17	AMAN	-	19	PHOS			
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR			
28	AlaA	-	29	TyrA	+	30	dSOR	+	31	URE	-	32	POLYB	+	37	dGAL			
38	dRIB	+	39	ILATk	-	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI			
47	NOVO	-	50	IVC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL			
57	dRAF	-	58	O129R	-	59	SAL	+	60	SAC	-	62	dTRE	+	63	ADH2s			
64	OPTO	-																	

Table (2) Biochemical identification of Enterococcus faecalis infections using VITEK2 compact system

Antimicrobial Susceptibility Test

The disk diffusion method was used to test antimicrobial susceptibility using the Kirby Bauer method. After incubating for 18-24 hours at 37 ˚C, some bacteria colonies (4-5) were transferred to 5 ml of nutrient broth. The growth was compared with McFarland standard 0.5 ml solution and then 0.1 ml of bacteria suspension was transferred and spreaded on Muller Hinton agar plate, the antibiotic discs were picked up and placed on the inoculated agar by a sterile forceps on the surface of the medium and incubated for 18-24 hours at 37 ˚C. The results reported depended on measuring the diameter of the inhibition zone around the disk of antibiotic and comparing them with standard values in CLSI 2022

Results :Bacterial Isolation and Biochemical Identification The culture of different clinical samples(150) on selective media showed that the bacterial agent were detected in 50(33.33%) samples as shown in figure(1)

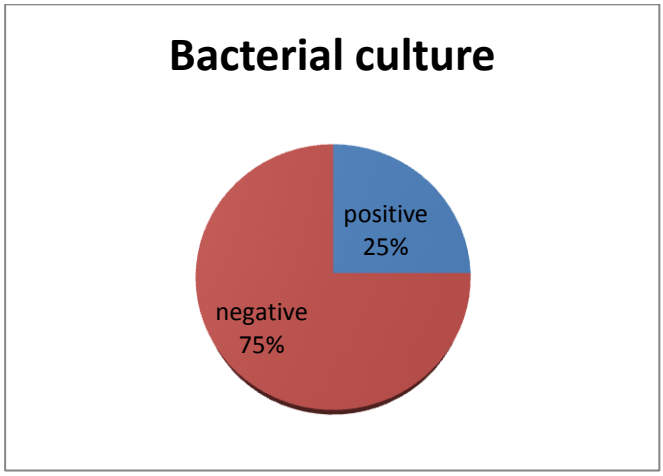


Figure (1): Percentages of Bacterial culture from clinical samples.

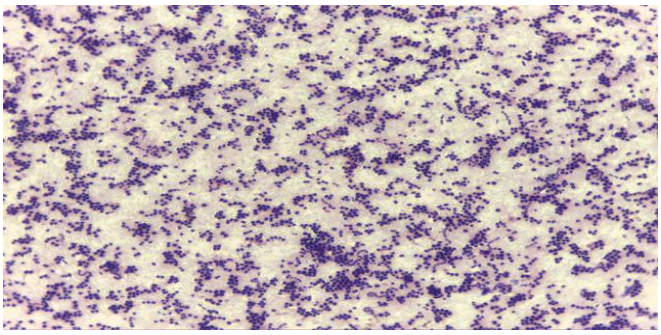


Figure (2): The shape of S.pyogenes under light microscope(100X)

A total of 50 isolates were identified as *Enterococcus. spp* from (150) clinical samples and was 10sample *E.faecium* and40 sample *E.faecalis* that collected from different sources, by Gram stain (Figure 2), traditional biochemical tests and Vitek system; that distributed by(13 (43%)*E.faecalis*,7(28%))from Urine, and (12(25%)*E.faecalis*, 3(37%) *E.faecium* from stool)(2(20)% *E.faecalis*,0 (0)%E. faecium from Seminal fluids)(11(31%)*E.faecalis*,0(0)%*E.feacium* from Vaginal swab)(2(10%) *E.faecalis*,0(0)% *E.feacium* from blood) samples as shown in table(1)

Table (3) prevalence of *Enterococcus.spp* isolated from different clinical specimens

Source	Total sample No.	Total No.	Positive specimens No.	%	X^2	P value
Urine	55	*E. faecalis*	13	43	2.2	0.138
		E. faecium	7	28		
Stool	30	*E. faecalis*	12	25	7.2	0.007*
		E. faecium	3	37.5		
Seminal fluids	10	*E. faecalis*	2	20	2.22	0.136
		E. faecium	0	0		
Vaginal swab	35	*E. faecalis*	11	31	13.05	0*
		E. faecium	0	0		
Blood	20	*E. faecalis*	2	10	2.10	0.147
		E. faecium	0	0		
Total		**150**	**50**	**33.33**		
X^2		**34.47**				
P value		**0***				

* Significant difference at P<0.05

Antibiotic susceptibility of *Entrococcus spp* isolates

The Antibiogram testing was performed with selected antibiotics that is commonly recommended by (CLSI., 2022). The rates of sensitivity and resistance to antibiotics by *Entrococcus spp* isolates are shown in table 2, table3 E.faecalis was resistance to antibiotic as as (vancomycine, pencillin, chloramphenicol, ciprofloxacin, levofloxacin, Norfloxacin, Nitrofurantion, Rifampin, Erthromycin, doxycycline) (3(7.5%), 10(25%), 16(40%), 31(77.5%) (,17(42.5%), 14(35%), 6(15%), 30(75%), 29(72.5%), 17(42.5%)) respectively as shown in table(2)

Table(4) Sensitivity test (*E. faecalis*)

Type of antibiotic	Sensitive (S)	Intermediate	Resistance
	No. (%)		
Vancomycin	26(65)	11 (27.5)	3(7.5)
Penicillin	24(60)	6 (15)	10(25)
Chloramphenicol	18(45)	6(15)	16(40)
Ciprofloxacin	9(22.5)	0(0)	31(77.5)
Levofloxacin	22(55)	1(2.5)	17(42.5)
Norfloxacin	26(65)	0(0)	14(35)
Nitrofurantoin	32(80)	2(5)	6(15)
Rifampin	6(15)	4(10)	30(75)
Erythromycin	4(10)	7(17.5)	29(72.5)
Doxycyclin	7(17.5)	16(40)	17(42.5)
X^2	148.58		
P value	0*		

* Significant difference at P<0.05

and E.faecium was resistance to antibiotic as (vancomycine, pencillin, chloramphenicol, ciprofloxacin, levofloxacin, Norfloxacin, Nitrofurantion, Rifampin, Erthromycin, doxycycline) (8(80%), 9(90%), 4(40%), 6(60%), 3(30%), 4(40%), 1(10%), 4(40%), 8(80%), 6(60%)), respectively as shown in table (3)

(Table5) Sensitivity test (*E. faecium)*

Type of antibiotic	Sensitive (S)	Intermediate	Resistance
	No. and (%)		
Vancomycin	0(0)	2(20)	8(80)
Penicillin	1(10)	0 (0)	9(90)
Chloramphenicol	4(40)	2(20)	4(40)
Ciprofloxacin	2(20)	2(20)	6(60)
Levofloxacin	6(60)	1(10)	3(30)
Norfloxacin	6(60)	0 (0)	4(40)
Nitrofurantoin	9(90)	0 (0)	1(10)
Rifampin	6(60)	0 (0)	4(40)
Erythromycin	2(20)	0 (0)	8(80)
Doxycyclin	2(20)	2(20)	6(60)
X^2	40.22		
P value	0.002*		

* Significant difference at P<0.05.

Discussion

Enterococci are considered to be a part of the normal flora of the bowel, genital tract and anterior urethra of humans (5)Enterococci cause mostly urinary tract and intra abdominal or pelvic wound infections. They can also cause bacteraemia with high mortality (6).

This study revealed that *Enterococcus spp* mostly isolated from (urine, stool, Seminal fluids, Vaginal swab, blood), Most of the isolates came from urine, which amounted to 13(43%) isolates of *E. faecalis* and 7(28%) isolates of *E.faecium*, followed by Stool, which amounted to 12(25%) isolates of *E.faecalis* and 3(37.5%) isolates of. *E.faecium).* As for the rest of the sources, only *E. faecalis* was obtained. Many studies showed the predominant species was E.faecalis but with a percentages more

than that of this study such as (Venkatesan et al., 2017) who found that E. faecalis was the predominant species isolated with percentage (77%) followed by E.faecium with percentage (20%), Among the 56 enterococci strains, (62.5%) were identified as E.faecalis and (37.5%) were E.faecium in a study by (Shokoohizadeh et al., 2018)

Emergence of antimicrobial resistant virulent enterococci is a serious problem in the treatment and control of nosocomial infections (7). Over the past two decades, enterococci have emerged as an important agent responsible for hospital acquired infection. Several virulence factors contribute to the adherence, colonization, evasion of the host immune response, and pathogenicity and severity of the infection (8) Enterococci possess efficient genetic exchange systems. The genes encoding virulence determinants can be transferred to resistant strains via these systems (9). In present study, the susceptibility test of *E.faecalis* and *E.faecium* strains showed that the resistance for vancomycin from E.faecalis was 3(7.5)% but in study in Iraq by(ojaimi, 2022) was sensitive rate 100%to both E.faecalis, E.faecium. E.faecium was resistance for vancomycine 8(80)% this is contrary to astudy in china by (Jia et al., 2014) which was reporting 0% *E.faecalis* was resistance rate to pencillin 10(25)% shown in study conducted in turkiye by (kacmaz et al., 2005) resisttance 25(12)% while E.faecium was resistance 9(90)% and this is closed to study conducted in turkiye by (kacmaz and Akosy., 2005) which was reporting25(100)% resistance this is not agree with study in italy by (carilato *et al*., 2007) which was reporting (27.5)%.

Enterococcus spp resistance to chloramphenicol is due to its effect on conserved sequences of 23S rRNA peptidyl transferase activity of the 50S subunit, thereby preventing protein synthesis by inhibiting tRNA binding to the ribosomal A site (10). Or the resistance is by acquiring this type of cat gene encoded in the plasmid, or the resistance is through the efflux mechanisms(11). *E.faecalis* was resistance16(40%) this is result nearbly from (Ojimi, 2022) resistance was 19(41%)and *E.faecium* was resistance 4(40%)(Ojimi, 2022) resistance was3(50%) while(AL duhaidhawi *et al*., 2022)was 16(45.7).

E.faecalis was resistance rate to ciprofluxacin 31(77.5) in this study, while the resistance was in study Hassoun, 2022 rate 15(40)%. astudy in china by (Jia et al., 2014) which was reporting (8.9)%, E.faecium was resistance rate 6(60)% and this closed to(AL duhaidhawi et al., 2022)was 19(54.3)% and astudy in china by (Jia et al., 2014) which was reporting 36(58.1)

E.faecalis was resistance for Levofloxacin 17(42.5)%. This study similar with results of AL-Yassary, (2011) who showed that, antibiotic susceptibility testing of the total isolates of E. faecalis from various clinical source, 55 % were sensititive to levofloxacin but, these results were not similar with results obtained by who found that strains of E. faecalis were resistant to levofloxacin with rate (4.8%). Also our study agreement with the study obtained by Lee, (2013) who found that strains of E. faecalis were resistant to levofloxacin with rate (46%) while E.faecium resistence rate 3(30%) but this not agree with shahi was resistence rate 29(90.6%)) astudy in china by (Jia *et al*., 2014) which was reporting rate 28(45).

isolates of E.faecalis were resistance to Norfloxacin 14(35)% this is agree with basher, 2022) were resistance8(21.62%) and AL-Yassary, (2011), in which the sensitivity of *E.faecalis* isolates to Norfloxacin was 80%, while *E.faecium* was resistance rate for Norfloxacin was rate 4(40%) this is agree with result study in Italy resistance rate was(47.5%)

all isolate was sensitive for Nitrofioxacin E.faecalis was resistance rate 6(15%) and study by ojaimi, 2022 resistance rate 3(6.5) and study by (Hassoun, 2022) was 0% and all isolate was sensitive E.faecium was resistance rate 1(10)% *E.faecalis, E.faecium* was sensitive rate 80%, 90% respectively

Rifampin

E.faecalis was resistance high rate 30(75%) Rifampin and this agree with study(Hassoun, 2022) resistence rate33(89.18%) but differs with the result obtained by AL-Yassary, (2011), in which the sensitivity of E.faecalis isolates to Rifampin in rate 40%. The resistance to Rifampin may be due to a genetic mutation that alters RNA polymerase enzyme and thus the antibody loses the ability to bind to this enzyme (Brooks et al., 2004).in this study *E.faecium* was resistance rate 4(40%) while(AL duhaidhawi *et al*., 2022)was resistance rate 3(30)% but astudy in china by (Jia *et al.,* 2014) reporting rate 49(79.0)% which was not agree this is study

Erthromycin

E.faecalis was resistance rate to Erthromycin was 29(72.5)% and study in iran agree with this study by (Hossein *et al.,* resistence rate was 65% while study in north of Italy not agree resistence28.9% by (cariolato *et al.*2007, *)* *E.faecium* was resistance rate 8(80)% while*(* AL duhaidhawi *et al.,* 2022)was resistance15(42.9)

E.faecalis was resistance rate to Doxycyclin 17(42.5%) but study Barbosa-Ribeiro et al., 2016) resistence rate was (5%) E.faecium was resistance rate 6(60%) but this not agree with shahi, 2019)in Egypt was resistance rate 1(3.1%)Doxycyclin Three mechanisms of resistance to tetracyclines have been described: drug inactivation, active efflux and ribosomal protection (12). Tetracycline resistance is often encoded by the ter gene in positive bacteria where it inhibits the formation of proteins by binding to the 305 ribosomal subunit and prevents the entry of tRNAs into the tetracycline. Location of the ribosome(13):

Conclusion

This study revealed that *Enterococcus spp* mostly isolated from urine, stool, seminal fluid, vaginal swab, blood. In this study, the isolates were highly resistant to mostly used antibiotics. which is considered a serious issue affecting public health. In Iraq, like in many other countries, antibiotics are readily available from the pharmacy desk. Alternatively, pharmacists prescribe medications to patients just based on their external symptoms, causing the intake of wrong antibiotic and/or over- or underdosage. Moreover, in majority of cases, patients do not complete the prescribed course of antibiotics. This causes patients to be at the hospital harboring resistant strains. These strains may cause endogenous or exogenous infections in other patients. The higher prevalence of resistance to antimicrobial agents in this environment could be due to wide- spread, indiscriminate use of antibiotics. The formulation and implementation of a national drug policy by Iraqi governments are fundamental to ensure rational drug use.

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