The Frequency of Enterococcus species and Resistance Genes (ant (3")-III, and van A) in patients on Hemodialysis with Urinary Tract Infections

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Abstract

Urinary tract infections (UTIs) are common in dialysis patients, associates with increasing rate of complications, and may be difficult to diagnose due to the subclinical presentation. Enterococcus spp. is a frequent cause of complicated UTI in patients with risk factors. The current study aimed to estimate the percentage of Enterococcus species associated with urinary tract infection in hemodialysis patients and detection the resistance genes (ant (3")-III, ant (6)-I and van A) in isolated MDR and XDR Enterococcus species. A total of (120) urine samples, 60 from hemodialysis patients with symptomatic urinary tract infections (UTIs) and 60 from UTI patients without hemodialysis was included in this study; The isolation of bacteria was performed by culturing on MacConkey agar, Blood agar, HiCrome™ UTI Agar and Brain heart infusion broth and the identification by Vitek-2 system. The detection of resistance genes (ant (3")-III, and van A(was done by conventional polymerase chain reaction (PCR). The percentage of Enterococcus faecalis in UTI patients was 16.66% (11.66% in patients with hemodialysis and 5.0% in patients without hemodialysis). Twelve bacterial isolates (85.71%) out of 14 from patients with HD possessed ant (3")-III gene compared with 4 isolates out of 6 (66.67%) from patients without HD.

1. Introduction

About 150 million individuals worldwide, both in the community and in hospitals, suffered from urinary tract infections (UTIs), UTI being one of the most prevalent bacterial illnesses [1]. Urinary tract infections (UTIs) are frequently caused by Enterococcus species, particularly in hospitalized patients [2]. It is known that the enterococci, a dominant bacterial species in the intestinal flora of both humans and animals, are accountable for serious diseases such endocarditis, septicemia, and urinary tract infections (UTI) [3].
About 15–24% of adult hospital patients with enterococcal UTIs can develop bacteremia; this risk is most frequently associated with the use of a urinary catheter, hematologic malignancies, and recent antibiotic therapy with vancomycin or third-generation cephalosporin. [4, 5]. Vancomycin resistant, resistance to aminoglycosides, lactams, particularly ampicillin are the most serious difficulties in enterococcal antibiotic resistance [6].
The bisubstrate enzymes aminoglycoside nucleotidyltransferase (ANT) and aminoglycoside antibiotic phosphotransferase (APHs) make it easier to transfer the -phosphate and nucleotide monophosphate from a nucleotide substrate to the hydroxyl groups of aminoglycoside antibiotics, respectively [7–8].
The aac(6')-Ie-aph(2")-Ia, which codes for the bifunctional enzyme Aac(6')-Ie-Aph(2")-Ia, is one of the genes found on plasmids. The second hydroxide group in the antibiotic's chemical structure is phosphorylated by the enzyme, which again renders the antibiotic's action inactive. This gene induces acquired resistance to gentamicin and other aminoglycosides with analogous structural features, with the exception of streptomycin, as well as a significant rise in MIC compared to regularly used concentrations, Presence of the enzymes Ant(6')-Ia or Ant(3")-Ia can lead to streptomycin resistance. The medicine is rendered inactive by these adenylyl transferases, which results in the loss of the drug's antibacterial effects. The formation of point mutations in the ribosome, which prevent drug from binding and, as a result, prevent the inhibition of protein synthesis, constitutes the second kind of resistance [9].
VanA, VanB, VanC, VanD, and VanE are the five identified phenotypes of vancomycin resistance. VanA and VanB, two of these, are mediated by

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recent gene cluster acquisitions. E. faecalis and E. faecium were the main sources of descriptions of VanA and VanB resistance phenotypes. Inducible, high-level resistance to teicoplanin (MICs, 16 g/ml) and vancomycin (MICs, 64 g/ml) is present in vanA-resistant strains [10].

Biofilm is one of the most essential components of microbial pathogenesis because it makes it simpler for the organism to adapt to adverse circumstances. Many diseases, particularly wound, respiratory, and urinary tract infections, are caused by enterococci which can form biofilms. These enterococci have many distinctive features, such as greater virulence and higher resistance to bactericides than in planktonic cells [11]. The aim of this study estimates the percentage of Enterococcus species associated with urinary tract infection in patients on hemodialysis. Study the antibiogram pattern of isolated Enterococcus species. Detect the resistance genes (ant (3")-III, ant (6)-I and van A) in MDR and XDR Enterococcus species isolated from patients on hemodialysis suffering from urinary tract infection. Determine the ability of isolated enterococcus to produce biofilm to cause infections in both groups of patients.

2. Methods

Subjects: A total of (120) mid-stream urine samples, 60 from hemodialysis patients with symptomatic UTI and 60 from UTI patients without hemodialysis were collected in a period from May 2022 to July 2022 from Al-Imamain Al-kadhimain Medical City, Ibn Albalady, Alkindy medical city. This study was approved by the ethical committee of the College of Medicine-Al-Nahrain University (NO.1451) and conducted in the microbiology department of this college. The mean age of patients with HD was 45.93±16.46 years. Percentage of males 54 (45%) and females 66 (55%). The isolation bacteria were performed by culturing on MacConkey agar, Blood agar, HiCrome™ UTI Agar and Brain heart infusion broth. The identification of bacteria was done by Vitek-2 system and detection of resistance genes by conventional PCR.

Antimicrobial susceptibility tests

Resistance patterns of *Enterococcus* isolates were detected by disk diffusion test (DDT) and by VITEK 2 System to 14 different antibiotics according to Clinical and Laboratory Standards Institutes (CLSI) 2016 (12).

Detection of biofilm formation

Quantitative microtiter plate assays for biofilm formation were performed as described by Fields et al. (2008) [28].

One hundred µl of Enterococcus broth turbidity equal to 0.5 McFarland and an equal volume of Luria Bertani (LB) broth supplemented with 20% glucose were added to each well in 96-well polystyrene microtiter plates.

The plates were incubated overnight at 37 °C. The cultures were softly removed. The wells were washed three times with phosphate buffered saline (PBS) to remove non-adherent cells.

The adherent cells were fixed with (200µl) absolute methanol for 10 minutes, methanol was removed, and the plate was left dried in upside down position at room temperature, then staining with (200µl) 0.4 % crystal violet for 15 min, washed three times with sterile distilled water and then allowed for air- dried. A 250 µl of glacial acetic acid was added to one well considered as negative control, and 250 µl of bacterial broth was added to another well and considered as positive control.

All other wells were filled with 250 µl of 33 % glacial acetic acid and waited for 15 min at room temperature.

The reading of optical density (OD) at 595 nm absorbance was determined using ELISA plate reader.

Molecular methods

DNA extraction

DNA was extracted according to the manufacture instructions by using Gram Positive bacteria genomic DNA purification kit protocol (Favorgen Biotech CORP. Taiwan)

DNA was extracted according to the manufacture instructions by using Gram Positive bacteria genomic DNA purification kit protocol as following:

1-One milliliter of well-grown bacteria culture was transferred to a micro-centerifuge tube.

2-Descended the cells by centrifuging at full speed for 2 minutes and discarded supernatant completely.

3- Resuspended the cell pellet in 200 µl lysozyme reaction solution (20 mg/ml ;20 mM tris-HCL ph 8.0; 2mM EDTA; 1.2% triton). Incubated at 37 °C for 30-60 minutes.

4-RNA free genomic is required therefore added 400 µl of 100 mg/ml RNase then mix thoroughly by vortexing and incubated at room temperature for 2 minutes.

5- 20 µl proteinase k added to the sample. then added 200 µl FATG2 Buffer to the sample.Mix thoroughly by pulse-vortexing incubated at 60 °C for 30 minutes and vortex occasionally during incubation.

6- Further incubation at 95 °C for 15 minutes.

7- 200 µl ethanol (96-100%) was added to the sample mixture.mix thoroughly by vortexing .

8-briefly spin the tube then removed drops from the inside.

9- a FATG Mini Column placed in a collection tube. Transferred the mixture (including any precipitate) carefully to the FATG Mini Column. Then Centrifuged at full speed (18,000 x g) for 1 min then placed the FATG Mini Column to a new collection tube.

10- 400 µl w1 buffer was added to FATG Mini Column. Then Centrifuged at full speed for 1 min. then discarded flow-through, ethanol has been added to W1 buffer when first opened.

11- 750 µl wash buffer was added to FATG Mini

Column. Centrifuge at full speed for 1 min. then discarded flow-through. make sure that ethanol has been added to wash buffer when first open.
12- Centrifuged at full speed for an additional 3 min to dry the column. This important step to ensure the residual liquid removed.
13- 100 µl of preheated Elution buffer or ddH2O (ph 7.5-9.0) added to the membrane of the FATG Mini column stand the FATG Mini Column. This important step to effective elution. then the elution solution is dispensed onto the membrane center and absorbed completely.
14- Centrifuged at full speed for 2 min to eluted DNA.

Conventional Polymerase Chain Reaction

Sequences of primers had been designed and synthesized in Solis BioDyne (Estonia) as showed in table (1) the primers were diluted with nuclease-free deionized distilled water and prepared according to manufacturer's recommendations.

Table (1): Sequences and product size of ant (3")-III, and van A resistance genes				
Genes		Nucleotide sequences (5' 3')	Products bp	Reference
ant (3")-III)	FR	CAGGAATTTATCGAAAATGGTAGAAAAGCACAATCGACTAAAGAGTACCAATC	369	Vakulenko et.al2003 [62]
van A	FR	GGGAAAACGACAATTGCGTACAATGCGGCCGTTA	732	Praharaj I et. Al2013 [63]
The mixture of PCR working solution				

Component	Concentration	Volume µl
Master mix	**1X**	**Dried pellet**
Primer F.	15pm/ µl	1.5 µl
Primer R.	15pm/ µl	1.5 µl
DNA Template	1-50 ng	4 µl
Deionized D.W.	-	13 µl
Total volume		20 µl
PCR program for amplification of ant (3")-III, Van A resistance gene by thermal cycler:		

No. of cycles	Time	Temperature	Steps	No.
1	**3-5 minutes**	**95°C**	**Initial denaturation**	**1**
25-30	15-30 s	95°C	Denaturation	**2**
	30-60 s	54-66 °C	Annealing	**3**
	40 s - 4 min	72 °C	Elongation	**4**
1	5-10 minutes	72 °C	Final elongation	**5**

Agarose gel electrophoresis for detection of PCR products

Agarose at concentrations of 1.5 % was prepared for resolution of PCR products according to Sambrook and Russell (2001) (13) as follows:
A 1.5 gm of agarose (Promega, USA) powder was dissolved in 100 ml of 1X TBE buffer (Bioneer, Korea ,)melted, and solubilized by continuous heating with stirring until clearing.
The agarose was left and waiting to cool at 55°C, then 2µl of concentration of ethidium bromide (Promega, USA) was added and poured into the taped plate with a comb located near one edge of the gel plate. Bubbles had been removed from gel by using micropipette tip.
The agarose gel was left to be solidified until it became opaque; then the comb and tape were removed gently.
TBE buffer (1X) was poured into a gel tank and the tray was situated horizontally in electrophoresis tank.
A 5µl of PCR product and negative control was inoculated to the wells by micropipette.
A volume of 5 microliters DNA molecular ladder (100 bp) were inoculated to a single well.
The power supply was adjusted at 7 v/cm for 1 hour and 45 minutes.
After the selected time was ended, the gel was displayed using UV trans- illuminator (LKB, Sweden) and then photographed by mobile device camera (iPhone) .

3. Statistical Analysis

Statistical analysis was performed using SPSS software version 25.0 (SPSS, Chicago, 2018). Data were presented as mean and standard deviation and analyzed with Student t-test and (ANOVA). Binomial variables were expressed as number and percentage and analyzed with Chi-square/Fischer exact test. A p-value less than 0.05 was considered statistically significant difference.

4. Results

The percentage of Enterococcus faecalis in all samples was 16.66% including 11.66 % in patients with hemodialysis and 5.0% in patients without hemodialysis. The mean age of patients with HD was 45.93±16.46 years which was almost similar to that of patients without HD (45.0±18.8 years) with no significant difference. Although females were more common among patients without HD than those with HD (61.67% vs. 48.33%), the difference was not

significant. However, all patients without HD had turbid urine compared with 90% of those with HD, with a significant difference. The history of UTI was reported in 93.33% and 95% in patients with and without HD, respectively, with no significant difference (Table 2).

Table 2: Demographic and clinical characteristics of the patients			
Variables	With HD (n=60)	Without HD (n=60)	p-value
Age, years:Mean±SDRange	45.93±16.4618-76	45.0±18.818-85	0.782
Gender: MaleFemale	31(51.67%)29(48.33%)	23(38.33%)37(61.67%)	0.142
Turbid urine: NoYes	6(10%)54(90%)	0(0%)60(100%)	0.027
History of UTINoYes	4(6.67%)56(93.33%)	3(5%)57(95%)	1.0
SD: Standard deviation			

Antibiogram of Enterococcus faecalis

All *Enterococcus faecalis* isolates were resistant to ciprofloxacin 100%,. In between, these bacteria showed 95% resistance to Erythromycin, 94.74% to Azithromycin, 75% to Levofloxacin and Norfloxacin while 70% of them were resistant to Amoxicillin, Ampicillin, Ampicillin/clavulanic acid, Imipenem and Meropenem. On the other hand, all isolates were sensitive to linezolid and tetracycline, and 95.24% were sensitive to Tigecycline (Table 4). Most isolates (13, 65%) were XDR, while 6 isolates (30%) were MDR. On the other hand, only one isolate (5%) was neither MDR nor XDR.

Table 4: Antibiogram of *Enterococcus faecalis* isolated from patients with renal failure (RF) with and without Hemodialysis		
Antibiotic	Resistance	Sensitive
Ciprofloxan	14(100%)	0(0%)
Levofloxacin	15(75%)	5(25%)
Norfloxacin	15(75%)	5(25%)
Azithromycin	18(94.74%)	1(5.26%)
Erythromycin	19(95%)	1(5%)
Linezold	0(0%)	20(100%)
Teicoplanin	7(36.84%)	12(63.16%)
Vancomycin	13(65%)	7(35%)
Tetracyclin	0(0%)	20(100%)
Tigecycline	1(4.76%)	20(95.24%)
Nitrofurantoin	10(50%)	10(50%)
Amikacin	13(65%)	7(35%)
Gentamycin	13(72.22%)	5(27.78%)
Amoxicillin	14(70%)	6(30%)
Ampicillin	14(70%)	6(30%)
Ampcillin/clauv	14(70%)	6(30%)
Imipenem	14(70%)	6(30%)
Meropenem	14(70%)	6(30%)
Association of *ant (3")-III* gene with Aminoglycoside Resistance of *Enterococcus faecalis*		

Generally, there was no significant association between ant ant (3")-III gene and resistance aminoglycoside antibiotics. All isolated (whether having or lacking ant (3")-III gene) were resistant to tetracycline. On the other hand, resistance to amikacin and gentamycin was less common in the presence than in the absence of the gene (75% vs. 62.5%); however, the differences were not significant (Table 5).

Table 5: Association of *ant (3")-III* gene with aminoglycoside resistance in *Enterococcus faecalis*				
Antibiotic	Pattern	With *ant (3")-III* (n=16)	Without *ant (3")-III* gene (n=4)	P-value
Tetracycline	RS	160	40	..
Amikacin	RS	10(62.5%)6(37.5%)	3(75%)1(25%)	0.561
Gentamycin	RS	10(62.5%)6(37.5%)	3(75%)1(25%)	0.561
Association of van A gene with Resistance to Vancomycin and Beta-Lactam Antibiotics of *Enterococcus faecalis*				

The presence of *van A* gene was associated with 80% of resistance to vancomycin, while the absence of the gene associated with 60% resistance to this antibiotic without significant difference. On the other hand, all *Enterococcus faecalis* isolates were resistant to imepenem, meropenem, ampicillin, amoxicillin, amoxicillin/clavulanic acid whether having or lacking *van* A gene (Table 6).

Table 6: Association of van A gene with antibiogram of Enterococcus faecalis isolated from patients with Renal failure with and without Hemodialysis				
Antibiotic	Pattern	With van A (n=5)	Without van A (n=15)	P-value
Vancomycin	RS	4(80%)1(20%)	9(60%)6(40%)	0.613
Amoxicillin	RS	44	1010	..
Ampicillin	RS	40	100	..
Ampcillin/clauv	RS	40	100	..
Imipenem	RS	40	100	..
Meropenem	RS	40	100	..
Presence of ant(3 ")-III and van A Genes in Enterococcus faecalis				

The results of PCR showed that Twelve bacterial isolates (85.71%) out of 14 of patients with HD possess *ant (3")-III* gene with 369 bp product compared with 4 (66.67%) isolates out of 6 positive for this gene from patients without HD. Statistically, there was no significant difference between the two groups (*p*= 0.329). While the *van* A gene with 732 bp product detected in 5 (35.71%) isolates from patients with HD, and none of Enterococcus isolates from patients without HD have this gene (*p*= 0.091) as shown in figures (1).

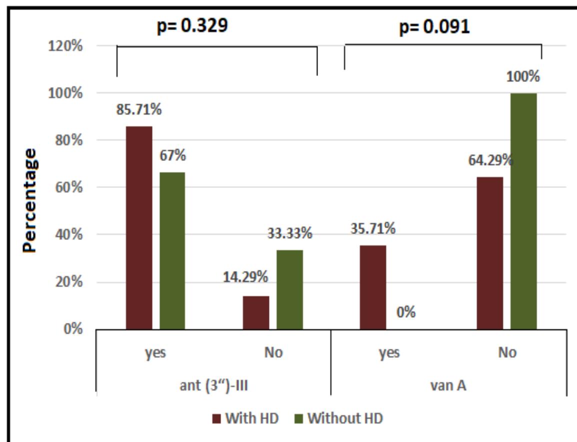

Figure 1: Percentage of ant (3 ")-III and van A genes in Enterococcus faecalis isolates.

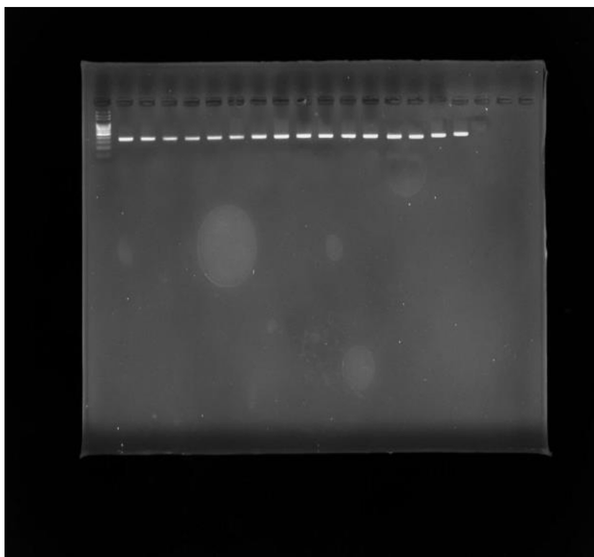

Figure 2: Gel electrophoresis of PCR products (369 bp) for ant (3")-III) resistance gene.

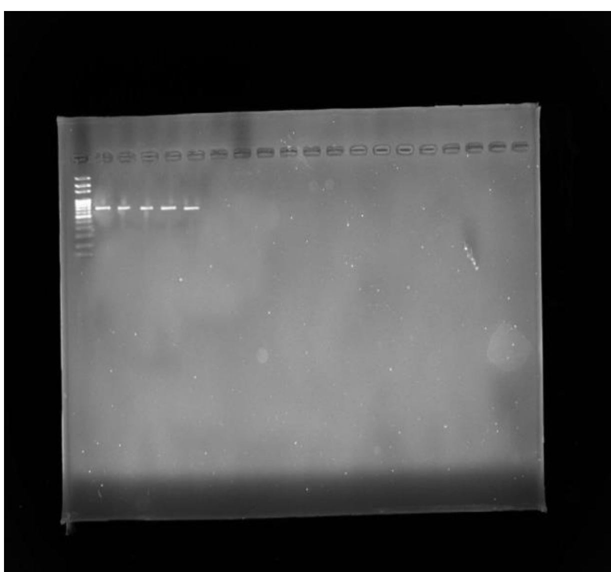

Figure 3: Gel electrophoresis of PCR products (732 bp) for VAN A resistance gene.

Biofilm Production of Enterococcus faecalis

Regarding the association between biofilm formation with MDR and XDR *Enterococcus faecalis*, the mean absorbance of biofilm for MDR *Enterococcus faecalis* isolates was 0.11±0.024 which was higher than that of non-MDR bacteria (0.09±0.032); however, the difference was not significant. In contrast, non-XDR bacteria shower higher mean absorbance of biofilm than those with XDR prosperity (0.12±0.024 vs. 0.091±0.31) with no significant difference.

Table 9: The mean absorbance of biofilm in MDR and XDR *Enterococcus faecalis*

Resistance pattern	Absorbance at 620 nm	P-value
MDR: YesNo	0.11±0.0240.09±0.032	0.186
XDR: YesNo	0.091±0.310.12±0.024	0.076

Microtiter plate assay was used to evaluate the biofilm production of *Enterococcus faecalis* isolates. The mean absorbance of biofilm for the bacteria isolated from patients with HD was 0.11±0.026 which was much higher than that of patients without HD (0.08±0.034) with a significant difference (Figure 4).

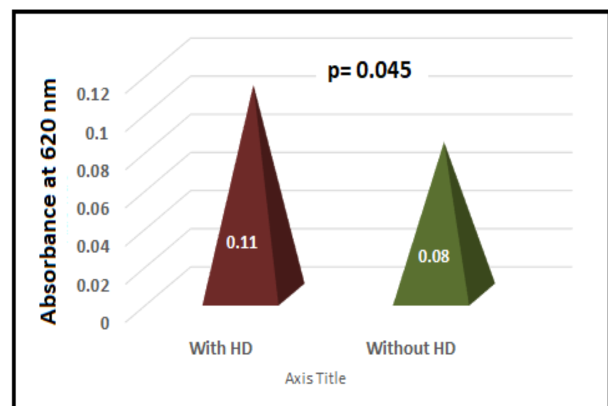

Figure 4: The mean absorbance of biofilm for Enterococcus faecalis isolated form renal failure patients with and without HD

5. Discussion

This study aimed to estimate the percentage of *Enterococcus* species associated with urinary tract infection in patients on hemodialysis.

In present study the percentage of *Enterococcus fecalis* in all samples were 16.66% (11.66% in patients with hemodialysis while is 5.0% in patients without hemodialysis). UTIs are more common in some subpopulations, such as newborns, pregnant women, the elderly, those with spinal cord injuries or catheters, people with diabetes, multiple sclerosis, AIDS/HIV, and people with underlying urologic abnormalities [14]. Due to their weakened immune systems, serious clinical conditions, and requirement

for vascular access for renal replacement therapy, patients with renal impairment are more likely to contract an infection. In study done at Zliten Teaching Hospital in Libya by Haider *et al.* 2016 [15] found that percentage of *Enterococcus faecalis* was 18.2%. the current results are similar to a study done in Iran by Jafarzadeh *et al.* 2021 [16] stated that percentage of *Enterococcus faecalis* was 16.66%. This discord in the prevalence of *Enterococcus faecalis* is may be due to the differences in sample size and included geographical areas.

Interestingly, Enteroccocus faecalis was isolated from 14 (11.66%) patients with HD versus 6 patients (5%) without HD, with a significant difference.

Regarding to gender distribution, this study revealed that, the percentage of females were 29(48.33%) with HD and 37(61.67%) without HD while the percentage of males were 31(51.67%) with HD and 23(38.33%) without HD and the highest frequency of *Enterococcus faecalis* was in age group (18-76 years) that with study published by Folliero *et al.* 2017 [18], the percentage of female were (60%) which was more than male (39.9%) within age group (46-60 years).Whereas in study done by Haider *et al.* 2016 [15] found that percentage of female were 50.5% and males were 49.5% within age group (56-65 Years).

In contrast to Haider et al. [15], who reported that the frequency of UTI among dialysis patients was 33 (32.3%), the current study indicated that the frequency of UTI among dialysis patients was 56(93.33%). Another study conducted in Taiwan by Hsiao, et al. in 2014 [20] discovered that the UTI frequency among dialysis patients was (100%) that higher than that of the current study.

In present study the most common cause of renal failure in patients under HD was hypertension accounting for more than half of the patients (56.67%) followed by T2DM (48.33%) and idiopathic (18.33%). Less common causes of RF were congenital (6.67%) and other causes. This results are different from study done in the Center of Renal Diseases and Transplantation at Baghdad Medical City by Manhal, *et al.* 2012 [21] that showed 37 (92.5%) of the patients were with hypertension and 3 (7.5%) with diabetes mellitus.

Whereas in study reported by Hsiao, *et al.* 2014 [20] found that the percentage of hypertensive patients was (35.6%) followed by T2DM (34.2%) and these results were also disagreed with the present results.

Antimicrobial susceptibility test of *Enterococcus faecalis*

Due to its ability to resist and develop different resistance mechanisms against antibacterial medications, the emergence of antibiotic resistance is a significant phenomenon. The results of this study showed that All *Enterococcus faecalis* isolates were resistant to ciprofloxacin, while 70% of them were resistant to Amoxicillin, Ampicillin, Ampcillin/clavulanic acid, Imipenem and Meropenem. In between, these bacteria showed 95% resistance to erthythromycin, 94.74% to Azithromycin 75% to levofloxacin and Norfloxacin, 65% resistance to Amikacin, 50% resistance to Nitrofurantoin while resistance to vancomycin 35%.

On the other hand, all isolates were sensitive linezolid and tetracycline, and 95.24% sensitivity to Tigecycline, this results is in agreement with study done in Poland by Kot *et al.* 2020 [25] who concluded that the susceptibility of *Enterococcus faecalis* to tigecycline remained 100%, , there were more resistant strains in males than females for gentamicin, and there was an increasing number of resistant strains to ampicillin. Therefore, disagree with study done in Tehran, Iran by Jafarzadeh *et al.*2021 [26] was shown the high resistance reported to cotrimoxazole, vancomycin and amikacin and the lowest resistance to nitrofurantoin (8.33%) was reported, Whereas In study conducted in Eastern India by Srujana *et al.* 2016 [27] was shown the resistance of *Enterococcus faecalis* to Ampicillin was (7.5%), Vancomycin (1.5%), Teicoplanin (1.5%), Ciprofloxacin (91.0%), Chloramphenicol (38.8%), Erythromycin (95.5%), Linezolid (1.5%) that agreed with study done by Mahmut , 2019 [24] shown the highest resistance rate was determined for ampicillin (87.3%).

Current study stated Twelve bacterial isolates (85.71%) out of 14 from patients with HD contain *ant (3")-III* genes compared with 4 isolates out of 6 (66.67%) from patients without HD.

Using the PCR technique, resistance genes (ant (3")-III, and van A) were found in the isolates of *Enterococcus faecalis* in this investigation. Current study stated presence *van A gene* in 5 isolates (35.71%) of patients with HD, while none of Enterococcus isolates from patients without HD have this gene (p= 0.091). the current study is in agreement with a study conducted in Greece in 2014 [29]. patients are more likely to have resistant microorganisms, such as vancomycin-resistant Enterococci, cause their urinary tract infections.

The present study agreed with study done in Iran by Moosavian *et al.* 2018 [28] was shown that, *van A* gene was detected in (91.5%) of the vancomycin-resistant or semi-susceptible isolates. While in study done in three major hospitals in Zanjan, Iran by Haghi *et al.* 2016 [30] stated that among 21 VRE isolates, 12 (57.1%) isolates were positive for the presence of *van* genes, also stated the most prevalent aminoglycoside resistance genes (ARGs) was *ant (3")-III* (78%) that in agreement with current study.

Regarding biofilm production, the present study showed the mean absorbance of biofilm for the bacteria isolated from patients with HD was 0.11±0.026 which was larger than that of patients without HD (0.08±0.034) that this result was in agreement with study conducted in Cairo by El-Atrees *et al.* 2022 [31] stated that the 35 isolates formed strong biofilm (OD range 0.24–2.66, while the other isolates were either moderate (n = 29) or weak/non-biofilm forming (n = 36), and in agreement with study done in Iran by Shahi *et al.* 2020 [32]

stated that the biofilm formation ability was observed in (63.0%) of all isolates.

In study done in India by Giridhara Upadhyaya *et al.* 2010 [33] stated that the (32.5%) clinical isolates were positive for biofilm production, out of which (11.5%) were high biofilm producers and the remaining (21%) were moderate biofilm producers. Among the commensal isolates, (16%) were biofilm producers with 4 producing high and 12 producing moderate amounts of biofilm. High formation of bacterial biofilm means that *Enterococcus faecalis* is multidrug resistance (MDR) while low formation of bacterial biofim means that *Enterococcus faecalis* used all pathogenic mechanisms lead to low expression of biofilm.

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