Original Article

Antibiogram of Enterococci Species and Association of *Van A/B* Genes Frequencies in Clinical Samples Isolated from Patients in Kurdistan Region Hospitals, Iraq

Haliz Saddeq Hasan, Abulrahman Towfeeq Saadi, Ahmed Mohammed Salih
Department of Microbiology, College of Medicine, University of Duhok, Duhok, Kurdistan Region, Iraq

Abstract

Background: Enterococci have over time become responsible for various infections such as endocarditis, bacteremia, and urinary tract infections. **Objectives:** This cross-sectional study aimed to determine the species, antibiogram, and *vanA*/*vanB* gene frequencies in *Enterococcus faecalis* and *Enterococcus faecium* isolated from patients at Hospitals in Kurdistan Region, Iraq. **Materials and Methods:** Samples of urine, HVS, blood, wound swab, semen, and bronchial wash were collected from the patients. Species-level confirmation and susceptibility testing were determined manually and by VITEK-2 Automated System, while *vanA*/*vanB* gene carriage was done by polymerase chain reaction. **Results:** A total of 116 *Enterococcus* isolates were identified from the patient's clinical specimens. Among these, 77 (66.4%) were *E. faecalis*, 26 (22.4%) were *E. faecium*, and 13 (11.2%) were other *Enterococcus* spp. Regardless of species identified, 100% were resistant to tetracycline and the resistant percentages for erythromycin were 86.2%, ampicillin 84.5%, gentamycin 81.9%, trimethoprim-sulfamethoxazole 68.9%, ciprofloxacin 68.1%, levofloxacin 53.4%, penicillin 41.3%, meropenem 31.9%, nitrofurantoin 30.1%, vancomycin 27.6%, teicoplanin 14.6% and a low resistant rate for fosfomycin 4.3%, for tigecycline 4.3% and for linezolid 3.4%. The percentage of multidrug resistance (MDR) in both *E. faecalis* and *E. faecium* was 100%. Out of 77 *E. faecalis* isolates, 10 (12.98%) contained *vanA* gene, but *vanB* gene was not detected. **Conclusion:** Multidrug-resistant enterococci is a potential alert in the community and healthcare facilities in the Kurdistan region and more research we need for antibiotics resistant on larger sample size.

Keywords: *Enterococcus faecalis*, *Enterococcus faecium*, multidrug resistant, *vanA* and *vanB*, vancomycin-resistant enterococci

Introduction

Enterococci are non-spore forming; facultatively anaerobic, negative to oxidase and catalase that occur as a single cell, in pairs, or short chains, they belong to the Gram-positive Enterococcaceae family.[1] Enterococcal infections may be attributed to at least 12 species, including *Enterococcus avium, E. casseliflavus, E. durans, E. faecalis, E. faecium, E. gallinarum, E. hirae, E. malodoratus, E. mundtii, E. pseudoavium, E. raffinosus, E. solitaries*, *E. faecalis, and E. faecium* are the two most common human pathogens, responsible for 85%–89% and 10%–15% of all enterococcal infections, respectively.[2] Furthermore, enterococci can persist in a variety of adverse conditions, including the presence of 6.5% NaCl, temperatures ranging from 5°C to 65°C, pH ranges from 4.5 to 10, and their ability to hydrolyze esculin in the existence of 40% bile.[1]

Enterococci is identified as super-infection in patients who take antibiotic drugs. Urinary tract infections (UTIs) are among the most prevalent *Enterococcus*-related nosocomial infections, then surgical site infections and bacteremia. Other *Enterococcus* infections, like meningitis, are found in critically unwell newborns, and in rare cases

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_406_23

Address for correspondence: Haliz Saddeq Hasan, Department of Medicine, College of Medicine, University Duhok, 1006 AJ Duhok, Kurdistan Region, Iraq.
E-mail: haliz.hasan@uod.ac

Submission: 08-Apr-2023 **Accepted:** 16-Aug-2023 **Published:** 23-Jan-2026

How to cite this article: Hasan HS, Saadi AT, Salih AM. Antibiogram of enterococci species and association of *van A*/*B* genes frequencies in clinical samples isolated from patients in Kurdistan Region Hospitals, Iraq. Med J Babylon 2025;22:1029-35.

as osteomyelitis and pulmonary infections.[3] According to the Centers for Disease Control & Prevention (CDCP), enterococci are the second most prevalent causal agent of healthcare-associated UTIs (13.9%), following *Escherichia coli*.[4] Furthermore, up to 15% of nosocomial UTIs in intensive care unit settings are caused by enterococci.[5] Additionally, the species are caused UTI in pediatric patients.[6]

Resistance to *van*comycin, one of the most powerful antibiotics, is of utmost concern as both intrinsic and acquired forms of resistance do occur in enterococci. Vancomycin-resistant enterococci (VRE) are now recognized as a critical clinical problem.[7] *Van* A, B, C, D, E, G, L, M, and N operons in enterococci have been reported to date. *VanA* and *vanB* genes are responsible for the majority of VRE outbreaks in humans.[8]

This study aimed to determine the species, antibiotic susceptibility profiles, and *vanA*/*vanB* gene of *E. faecalis* and *E. faecium* from human clinical specimens in the Kurdistan Region of Iraq.

Materials and Methods

Isolation and identification of *Enterococcus* spp.

In this cross-sectional study from October 2021 to June 2022, a total of 116 *Enterococcus* isolates were collected from specimens of patients (inpatients and outpatients) in three provinces of Kurdistan Region, Iraq: Duhok, Erbil, and Sulaimanya. The specimens included were urine, blood, HVS, pus, tissues, semen, and bronchial wash.

Identification of the enterococci was performed based on the standard microbiological tests including growth on blood agar, Gram staining, catalase reaction, and bile esculin test, and confirmed by VITEK-2 automated instrument (BioMerieux, USA).

Antimicrobial susceptibility testing

The antimicrobial sensitivity testing of enterococci isolates was evaluated against the following antibiotics (bioanalysis, Turkey): Erythromycin (E, 15 µg), Tetracycline (TE, 30 µg), Ampicillin (AMP, 10 µg), Nitrofurantoin (NIT, 100 µg), Ciprofloxacin (CIP, 5 µg), Penicillin (P, 10 µg), Vancomycin (VA, 30 µg), Teicoplanin (30 µg), Levofloxacin (5 µg) Trimethoprim-sulfamethoxazole (25 µg, 1.25/23.75), Gentamicin (10 µg), Meropenem (10 µg), Fosfomycin (200 µg), Linezolid (10 µg), and Tigecycline (15 µg) according to the Clinical and Laboratory Standards Institute (CLSI).[9] For antibiotics that were not on the CLSI list, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations were used.[10] Kirby-Bauer disk diffusion method was performed and confirmed by VITEK-2 automated identification and susceptibility testing system according to the manufacturer's recommendations (bioMerieux, USA). Multidrug resistant (MDR) resistotypes were set based on isolates' resistance to at least three antibiotic classes of the tested antibiotics.[11] The studied antibiotics fall into 11 distinct classes as follows: C1, penicillins; C2, glycopeptides; C3, fluoroquinolones; C4, macrolides; C5, folate pathway antagonists; C6, tetracyclines; C7, aminoglycosides; C8, oxazolidinones; C9, nitrofurans; C10, fosfomycins; C11, carbapenems.

Detection of vancomycin resistance genes *vanA* and *vanB*

DNA was extracted from *Enterococcus* isolates using a standard boiling procedure,[12] and DNA purity and concentration were determined using a Nanodrop Spectrophotometer (Thermo Scientific (USA). *VanA* and *vanB* genes were amplified in a thermocycler (Biosan Ltd, UAE, Dubai) by using specific targeting fragments of *vanA* and *vanB* genes, respectively.[13]

A1: 5′- GGGAAAACGACAATTGC-3

A2: 5-GTACAATGCGGCCGTTA-3′

B1: 5′- ATGGGAAGCCGATAGTC-3′

B2: 5′- GATTTCGTTCCTCGA CC-3′

Multiplex polymerase chain reaction (PCR) was used for these two primers. The PCR mix for amplification of *vanA* and *vanB* fragments contained 12.5 µL of the 2X master mix, 1 µL of each primer (10 pM), 5 µL of a DNA sample, and 6.5 µL of nuclease-free water. The amplification was performed according to the following thermal conditions: 95°C for 5 min as a pre-denaturation step, followed by (30 cycles) of 94°C for 45 s, 60°C for 45 s, and 72°C for 45 s. About 72°C for 7 min was performed as a final extension step. PCR products were visualized by using gel electrophoresis on 1% agarose gel for 45 min at 95 V. A molecular marker 100 bp (Jena Bioscience GmbH, Germany) was used to compare and determine the size of the amplicons.

Statistical analysis

The data were tabulated and analyzed by the statistical package SPSS version 25 (Chicago, IL, USA). Frequency and chi-square test was used for categorical variables to match the antibiotic-resistant rates between *E. faecalis*, *E. faecium*, and other *Enterococcus* spp. isolates and the relation between the existence of *vanA* genes and resistance to each antimicrobial in all isolates: P value < 0.05 was considered significant.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee

according to the document number 24102021-10-30 dated October 24, 2021 to get this approval.

Results

Prevalence of *Enterococcus* spp.

A total of 116 *Enterococcus* isolates were identified and included in the study, the age range was from 2 months to 75 years. About 45 (38.8%) of *Enterococcus* isolates were from Duhok followed by 44 (37.9%) from Sulaimanya and 27 (23.3%) from Erbil provinces. Among these, 77 (66.4%) were *E. faecalis*, 26 (22.4%) were *E. faecium* and 13 (11.2%) were other *Enterococcus* spp.: 5 (4.3%) were *E. gallinarum*, 1 (0.86%) was from each *E. avium* and *E. hirae* and 6 (5.2%) isolates were not being identified to species level using the VITEK-2 system.

There were 55 enterococci isolated from male patients (47.4%) and 61 (52.6%) from female patients. About 41.6% of *E. faecalis* and 61.5% of *E. faecium* were isolated from male patients, whereas 58.4% of *E. faecalis* and 38.5% of *E. faecium* were isolated from female patients; however, 53.8% of other enterococci species were from male patients and 46.2% were from female patients.

The overall frequencies of enterococci in the processed clinical samples were 68 (58.6%) from urine, 15 (12.9%) from HVS, 11 (9.4%) from blood culture, 11 (9.4%) from the wound, 10 (8.6%) from seminal fluid and 1 (0.8%) was from the bronchial wash [Table 1].

Antibiotic resistotypes

The resistant rates of *E. faecalis*, *E. faecium*, and other *Enterococci* to selected antibiotics are shown in Table 2. Regardless of species identified, 100% were resistant to tetracycline and the resistant percentages for other antibiotics were: erythromycin 86.2%, ampicillin 84.5%, gentamycin 81.9%, trimethoprim-sulfamethoxazole 68.9%, ciprofloxacin 68.1%, levofloxacin 53.4%, penicillin 41.3%, meropenem 31.9%, nitrofurantoin 30.1%, vancomycin 27.6%, teicoplanin 14.6%. The lower resistance rate and strongest effects were for fosfomycin (4.3%), tigecyclin (4.3%), and linezolid (3.4%). The resistance rates to penicillin, ampicillin, ciprofloxacin, nitrofurantoin, trimethoprim-sulfamethoxazole, fosfomycin, and meropenem antibiotics were significantly higher in *E. faecium* than in *E. faecalis* (P value < 0.05). None of the isolates were susceptible to all antimicrobials tested.

Table 1: Distribution of *Enterococcus* species isolates in clinical specimens

Source of the sample	*E. faecalis*	*E. faecium*	Other enterococci	Total source
	n (%)	*n* (%)	*n* (%)	*n* (%)
Urine	46 (59.7)	14 (53.8)	8 (61.5)	68 (58.6)
HVS	13 (16.8)	2 (7.6)	0 (0.0)	15 (12.9)
Blood	4 (5.2)	5 (19.2)	2 (15.3)	11 (9.4)
Wound swab	8 (10.3)	3 (11.5)	0 (0.0)	11 (9.4)
Semen	6 (7.7)	1 (3.8)	3 (23.07)	10 (8.6)
Bronchial wash	0 (0.)	1 (3.8)	0 (0.0)	1 (0.8)
Total	77 (66.4)	26 (22.4)	13 (11.2)	116

Table 2: Antibiotic resistance pattern of Enterococci isolates ($n = 116$)

Antibiotic	*E. faecalis* ($n = 77$)	*E. faecium* ($n = 26$)	Others enterococci ($n = 13$)	Total ($n = 116$)	*P*-value
	n (%)	*n* (%)	*n* (%)	*n* (%)	
Penicillin	23 (29.8)	19 (73.07)	6 (46.1)	48 (41.3)	0.001*
Ampicillin	65 (84.8)	25 (96.1)	8 (61.5)	98 (84.5)	0.019*
Vancomycin	20 (25.9)	8 (30.7)	4 (30.7)	32 (27.6)	0.862
Ciprofloxacin	46 (59.7)	24 (92.3)	9 (69.2)	79 (68.1)	0.009*
Erythromycin	66 (85.7)	24 (92.3)	10 (76.9)	100 (86.2)	0.412
Gentamicin	59 (76.6)	25 (96.1)	11 (84.6)	95 (81.9)	0.079
Fosfomycin	1 (1.3)	2 (7.7)	2 (15.2)	5 (4.3)	0.043*
Nitrofurantoin	13 (16.8)	15 (57.7)	7 (53.8)	35 (30.1)	0.000*
Meropenem	11 (14.2)	21 (80.7)	5 (38.4)	37 (31.9)	0.000*
Linezolid	3 (3.9)	1 (3.8)	0 (0.0)	4 (3.4)	0.770
Tigecyclin	3 (3.9)	2 (7.7)	0 (0.0)	5 (4.3)	0.512
Levofloxacin	36 (46.7)	18 (69.2)	8 (61.5)	62 (53.4)	0.115
Trimethoprim-sulfamethoxazole	54 (70.1)	21 (80.7)	5 (38.4)	80 (68.9)	0.025*
Teicoplanin	10 (12.9)	6 (23.1)	1 (33.3)	17 (14.6)	0.051
Tetracycline	77 (100)	26 (100)	13 (100)	116 (100)	–

*Significant association

Table 3: Relation between the frequency of *vanA* and antimicrobial resistance in *E. faecalis* and *E. faecium* isolates

Antibiotic	*E. faecalis* ($n = 77$)		*P* value	*E. faecium* ($n = 26$)		*P* value
	Resistance rate *n* (%)			Resistance rate *n* (%)		
	vanA positive $n = 10$	*vanA* negative $n = 67$		*vanA* positive $n = 5$	*vanA* negative $n = 21$	
Penicillin	2 (20.0)	21 (31.3)	0.465	4 (80.0)	15 (71.4)	0.698
Ampicillin	8 (80.0)	57 (85.0)	0.680	5 (100)	20 (95.2)	0.619
Vancomycin	5 (50.0)	15 (22.3)	0.063	4 (80.0)	4 (19.0)	0.007*
Ciprofloxacin	7 (70.0)	39 (58.2)	0.478	5 (100)	19 (90.4)	0.473
Erythromycin	10 (100)	56 (83.5)	0.166	5 (100)	19 (90.4)	0.473
Gentamicin	7 (70.0)	52 (77.6)	0.596	5 (100)	20 (95.2)	0.619
Fosfomycin	0 (0.0)	1 (1.4)	0.697	1 (20.0)	1 (4.7)	0.250
Nitrofurantoin	1 (10.0)	12 (17.9)	0.533	4 (80.0)	11 (52.3)	0.261
Meropenem	1 (10.0)	10 (14.9)	0.678	4 (80.0)	17 (80.9)	0.961
Linezolid	0 (0.0)	3 (4.4)	0.495	0 (0.0)	1 (4.7)	0.619
Tigecyclin	1 (10.0)	2 (2.9)	0.285	0 (0.0)	2 (9.5)	0.473
Levofloxacin	5 (50.0)	31 (46.2)	0.825	5 (100)	13 (61.9)	0.097
Trimethoprim-sulfamethoxazole	5 (50.0)	49 (73.1)	0.136	4 (80.0)	17 (80.9)	0.961
Teicoplanin	1 (10.0)	9 (13.4)	0.662	2 (40.0)	4 (19.0)	0.852
Tetracycline	10 (100)	67 (100)	–	5 (100)	21 (100)	–

*Significant association

In total, all enterococci isolates (except one isolate) (76 of *E. faecalis* and 26 of *E. faecium*) showed MDR. The antibiogram of the tested isolates categorized the 76 *E. faecalis* isolates into 44 different resistotype and the most common resistotype were 12 of Amp-CIP-LEV-ERY-CN-SXT-TET and 4 of Amp-VA-CIP-ERY-CN-SXT-TET and 4 of Amp-LEV-CN-SXT-TET. Also, a total of resistant *E. faecium* showed 12 different resistotype patterns and the most common resistotype were 10 of P-Amp-CIP-LEV-ERY-CN-F-MEM-SXT-TET; 3 of P-Amp-E-CN-MEM-SXT-TET; and 3 of P-Amp-VA-TEC-CIP-LEV-ERY-CN-F-MEM-SXT-TET.

Detection of *vanA* and *vanB* genes

VanA and *vanB* genes were amplified in all the resistant and susceptible isolates to vancomycin. The results showed that out of 77 *E. faecalis* isolates, 10 (12.98%) contained *vanA* gene, but *vanB* gene was not detected while out of 26 *E. faecium* isolates 5 (19.23%) contained *vanA* gene and no *vanB* gene. On the other hand, out of 13 other spp., only 1 (7.6%) showed *vanA* band which was *E. hirae*. The percentage of *vanA* in vancomycin-resistant *E. faecalis* (25%) compared to the percentage of *vanA* in vancomycin-sensitive *E. faecalis* (8.77%) was not statistically significant (*P* value: 0.063) [Table 3] while the percentage number of *vanA* in vancomycin-resistant *E. faecium* (50%) compared to the percentage of *vanA* in vancomycin-sensitive *E. faecium* (5.55%) was statistically significant (*P* value: 0.007).

Discussion

Enterococcus spp. has been of considerable concern in recent times because it tended to spread quickly in hospital environments among healthcare staff and critically ill patients.[14] This bacteria is also isolated from operating theaters and intensive care units.[15] The ability to develop resistance to antibiotics in *enterococcus* is another important issue associated with these bacteria.[16] So far, few studies are done on *enterococcus* in our country such as Khalid 2016[17] and Kadhim and Amin[18] Also, little is known about the enterococci resistance to antibiotics in Enterococcal hospital infections in Kurdistan region hospitals.

In the current study, there was a high frequency of *E. faecalis* (66%) compared to *E. faecium* (22%) isolated from clinical specimens. This species distribution is similar to that found by Alotaibi *et al*. who found that out of 231 *Enterococcus* spp. isolates, 168 (72.7%) were *E. faecalis* and 53 (22.8%) were *E. faecium*[19] and a study performed by Shridhar and Dhanashree, who found that of 150 *Enterococcus* isolates, 82 (58%) were *E. faecalis* and 63 (42%) were *E. faecium*.[20] In contrast, Karna *et al.* reported a high prevalence of *E. faecium*. They ascribe the results to their capacity for developing resistance against multiple antimicrobials.[21]

Other spp. (*E. gallinarum*, *E. hirae*, and *E. avium*) were also detected. The relatively acknowledged percentage (4.3%) of *E. gallinarum* among the total isolates may be alert to our clinical health facilities since no recent records are reported regarding the clinical significance of *E. gallinarum* in causing hospital-acquired infections in our locality. Despite the rarity with which *E. gallinarum* is isolated from clinical samples, many reports have linked them as sources of serious infections caused.[22]

In this study, 41.6% of *E. faecalis* and 61.5% of *E. faecium* were isolated from male patients, whereas 58.4% of

E. faecalis and 38.5% of *E. faecium* were isolated from female patients, whereas a study of Shridhar and Dhanashree revealed that males (56.3%) had higher rates of *E. faecalis* than females (43.7%).[20] In addition, enterococci species were most predominantly isolated from urine specimens (59%) followed by HVS specimens 13% then equal percentages were detected from blood and wound swabs (9%) [Table 1]. These figures are in agreement with a study done by Boccella *et al*.,[23] The high prevalence of enterococci detection from urine samples might be attributed to the proximity of the anal orifice to the urethra, as enterococci live as commensals in the gut. Furthermore, urethra catheterization played a role in some patients, resulting in a higher isolation rate of enterococci from urine samples.[24]

Based on our analysis, the majority of the strains showed low resistance to linezolid and teicoplanin, which is consistent with other findings of Boccella *et al*.,[23] and Parameswarappa *et al*. who reported high susceptibility to this antimicrobial.[25] In addition, the rate of linezolid resistance in Germany grew from 1% in 2008 to 9% in 2014 as reported by Klare *et al*.,[26]

For vancomycin, in general, 27.5% of isolates[20] *E. faecalis*, 8 *E. faecium*, and 4 isolates of other enterococci) have shown resistance to this antibiotic. Inconsistent results were recorded in Duhok, Iraq[17] also in many European countries like Norway, Denmark, France, and New Zealand.[27] Comparable results were observed in Turkey.[28] In this research, vancomycin is the choice antibiotic in terms of antibiotic sensitivity to enterococci, including gentamycin, ampicillin, erythromycin, and ciprofloxacin. There are not many treatment options for VRE, and enterococci could transfer resistance to other species including *Staphylococcus aureus* and *Listeria monocytogenes*.[29]

In the current study majority of the *Enterococcus* isolates were resistant to tetracycline 100% and for erythromycin was 86% which is comparable to a study conducted by Parameswarappa *et al*.,[25] Also, this study indicated high resistance to ampicillin 84%, gentamycin 82%, trimethoprim-sulfamethoxazole 69%, and 68% was for ciprofloxacin. The resistance to ampicillin, ciprofloxacin, and gentamicin was agreed with a study done by Rana and Sande which they showed 86% of isolates were resistant to Ampicillin, 85% to Gentamicin, and 60% to Ciprofloxacin.[30] However, studies done by Khalid[17] and Karna *et al*.,[21] indicated lower resistance to ampicillin 20%, 21.7%, and 39%, respectively.

Generally, *E. faecium* is more resistant to penicillin, ciprofloxacin, and gentamicin than *E. faecalis*. The resistance rate for penicillin in this study was 41% for all isolates, the results follow a study performed by Karna *et al*.,[21] and this proportion is much lower than a study done by Rana and Sande which was 98%.[30] In contrast, a study done by Khalid[17] has indicated a much lower level of resistance to penicillin (28%). The production of high-affinity penicillin-binding protein is a cause for the low resistant rate as attributed by Fontana *et al*.[31] and a low-affinity penicillin-binding protein may be the cause of the increased resistance to ampicillin and penicillin. Furthermore, plasmid-encoded enzymes provide resistance to β lactamase and aminoglycosides reducing the function of lactam therapy and eliminating the synergistic impact of β lactams and aminoglycosides, resulting in treatment failure.[32]

The most notable outcome of this investigation was that although linezolid, teicoplanin, fosfomycin, and tigecyclin antibiotics are not being used frequently in our healthcare facilities, however, it was low. It has been shown in the current study that enterococci show resistance to those antibiotics. This indicates that the misuse of antibiotics is not the only reason for antibiotic resistance among communities but there could be some other reasons like introducing the already resistant bacteria from a broad to our community. Whereas the high resistance rates to routinely used antibiotics [Table 2] were due to the high consumption habits, their easy accessibility, and they are relatively inexpensive might all be reasons for an increase in resistance rates. Furthermore, self-prescribing of these treatments is prevalent as also explained by Bbosa and Mwebaza,[33] which leads to the formation and dissemination of drug resistance.

Another finding in this study was a high percentage (100%) of the isolated strains were MDR. This demonstrates that *Enterococci* are a potentially growing problem and indicates a big challenge in treating infections caused by enterococci. The high level of resistance to these antibiotics is likely related to the wide use of these antibiotics for the treatment of Gram-positive infections in our provinces and not depending on the antibiotic susceptibility test in the clinical facilities. This MDR rate is comparable to that of an earlier report in Egypt[34] while it is much higher than the study done in Iraq by Alduhaidhawi *et al*.[35]

Out of 32 VRE, only 9 (28.12%) isolates contained *vanA* gene but no *vanB* gene. The phenotypic resistance of other *E. faecium* and *E. faecalis* isolates that had no *vanA* and *vanB* genes could be related to different reasons other than these two genes. Similarly, a study in Iran (2012) showed the prevalence of *vanA* gene and some strains did not have any *vanA* or *vanB* genes.[36] Another recent study in Iran (2017) recorded the predominance of *vanA* among VRE *E. faecium* species.[37] In contrast, the findings of Karki *et al*.[38] in Melbourne, Australia, were not consistent with this study. They detected only *vanB* gene among VRE, but none of them had *vanA*. The reason expressed in their study was that *vanB* gene in VRE has been endemic in the region. The cause of this variability in genes owing to the difference in antibiotics profiles used among various

countries.[39] Different from *vanA* gene, *vanB* is clustered and resides in a big area on the chromosome, and there is less chance of its transfer to other strains. Additionally, *vanB* gene is mostly linked to epidemics and food contamination, whereas *vanA* gene is linked to clinical isolates.[40] Furthermore, the fact that patients were given vancomycin for a prolonged period supports the presence of *vanA* gene in VRE.

VanA gene was also reported in 5 of vancomycin-sensitive *E. faecalis* and 1 among *E. faecium*. The difference between *E. faecalis* VRE positive and VSE positive to *vanA* gene in this study was statistically insignificant and could be related to the large difference in the number between *vanA* positive and *vanA* negative but in *E. faecium* they were significant.

In conclusion, the current study has shown that the emergence of the MDR enterococci is a potential alert in the community healthcare facilities in the Kurdistan region, which needs further future studies of antibiotic resistance in a larger sample of patients.

Acknowledgments

The authors would like to thank Duhok Medical Research Center Department and the Center staff for facilitating the task of conducting this research. Thanks to Dr Saad Younis Saeed; Assistant Professor in Community Medicine at Duhok College of Medicine for his assistance in statistical analysis. Furthermore, thanks to Dr Safeen Othman Mahmood; Lecturer at Sulaymania College of Medicine, and Dr Haval Hassan Abdulqader; Clinical Microbiologist at Erbil Rizgare Hospital for their help in the collection of samples in Sulaymania and Erbil.

Author contributions

All authors contributed to conception and design, data analysis and interpretation, article writing, and final approval of the article.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. De Filippis I, McKee ML. Molecular typing in bacterial infections. Mol Typing Bact Infect 2013;1:482.
2. Sood S, Malhotra M, Das BK, Kapil A. Enterococcal infections and antimicrobial resistance. Indian J Med Res 2008;128:111-21.
3. Murray BE. Diversity among multidrug-resistant enterococci. Emerg Infect Dis 1998;4:37-47.
4. Oberoi L, Aggarwal A. Multidrug resistant enterococci in a rural tertiary care hospital: A cause of concern. JK Sci 2010;12:157-8.
5. Murray BE. The life and times of the enterococcus. Clin Microbiol Rev 1990;3:46-65.
6. Ibrahim SA, Mohamed DA, Suleman SK. Microbial causes of urinary tract infection and its sensitivity to antibiotics at Heevi pediatric teaching hospital/Duhok City. Med J Babylon 2020;17:109.
7. Miller WR, Murray BE, Rice LB, Arias CA. Resistance in vancomycin-resistant enterococci. Infect Dis Clin North Am 2020;34:751-71.
8. Werner G, Klare I, Fleige C, Witte W. Increasing rates of vancomycin resistance among *Enterococcus faecium* isolated from German hospitals between 2004 and 2006 are due to wide clonal dissemination of vancomycin-resistant enterococci and horizontal spread of vanA clusters. Int J Med Microbiol 2008;298:515-27.
9. CLSI. CLSI M100-ED29: 2021 Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. Vol. 40, CLSI; 2020. p. 50-51.
10. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 8.1; 2018.
11. dos Santos BA, de Oliveira JS, Parmanhani-da-Silva BM, Ribeiro RL, Teixeira LM, Neves FPG. CRISPR elements and their association with antimicrobial resistance and virulence genes among vancomycin-resistant and vancomycin-susceptible enterococci recovered from human and food sources. Infect Genet Evol 2020;80:104183.
12. Dashti AA, Jadaon MM, Abdulsamad AM, Dashti HM. Heat treatment of bacteria: A simple method of DNA extraction for molecular techniques. Kuwait Med J 2009;41:117-22.
13. Praharaj I, Sujatha S, Chandra Parija S. Phenotypic & genotypic characterization of vancomycin resistant *Enterococcus* isolates from clinical specimens. Indian J Med Res 2013;138:549-56.
14. Rogers LA, Strong K, Cork SC. The role of whole genome sequencing in the surveillance of antimicrobial resistant *Enterococcus* spp.: A scoping review. Front Public Heal 2021;9:1-17.
15. Baban S, Saeed PH, Jalal DF. Microbial contamination of operating theatres and intensive care units at a surgical specialty hospital in Erbil City. Med J Babylon 2019;16:150.
16. Zaheer R, Cook SR, Barbieri R, Goji N, Cameron A, Petkau A, *et al*. Surveillance of *Enterococcus* spp. reveals distinct species and antimicrobial resistance diversity across a one-health continuum. Sci Rep 2020;10:13401.
17. Khalid H. Molecular detection of virulence factors of *Enterococcus faecalis* isolated from urine samples in Duhok City, Kurdistan Region/Iraq. Sci J Univ Zakho 2016;4:63-72.
18. Ehsan Mansoor Kadhim BKA, Amin BK. The antibacterial effect of green tea on *Enterococcus faecalis*, Iraq. Med J Babylon 2022;19:676-9.
19. Alotaibi FE, Bukhari EE. Emergence of vancomycin-resistant enterococci at a teaching hospital, Saudi Arabia. Chin Med J (Engl) 2017;130:340-6.
20. Shridhar S, Dhanashree B. Antibiotic susceptibility pattern and biofilm formation in clinical isolates of *Enterococcus* spp. Interdiscip Perspect Infect Dis 2019;2019:7854968.
21. Karna A, Baral R, Khanal B. Characterization of clinical isolates of enterococci with special reference to glycopeptide susceptibility at a tertiary care center of eastern Nepal. Int J Microbiol 2019;2019:7936156.
22. Britt NS, Potter EM. Clinical epidemiology of vancomycin-resistant *Enterococcus gallinarum* and *Enterococcus casseliflavus* bloodstream infections. J Glob Antimicrob Resist 2016;5:57-61.
23. Boccella M, Santella B, Pagliano P, De Filippis A, Casolaro V, Galdiero M, *et al*. Prevalence and antimicrobial resistance of enterococcus species: A retrospective cohort study in Italy. Antibiotics 2021;10:1552-9.
24. Abera A, Tilahun M, Tekele SG, Belete MA. Prevalence, antimicrobial susceptibility patterns, and risk factors associated with enterococci among pediatric patients at Dessie Referral Hospital, Northeastern Ethiopia. Biomed Res Int 2021;2021:5549847.
25. Parameswarappa J, Basavaraj V, Basavaraj C. Isolation, identification, and antibiogram of enterococci isolated from patients with urinary tract infection. Ann Afr Med 2013;12:176-81.
26. Klare I, Fleige C, Geringer U, Thürmer A, Bender J, Mutters NT, *et al*. Increased frequency of linezolid resistance among clinical *Enterococcus faecium* isolates from German hospital patients. J Glob Antimicrob Resist 2015;3:128-31.

27. European Antimicrobial Resistance Surveillance System (EARSS) and others. Antimicrobial resistance surveillance in Europe 2011. 2014.
28. Shah L, Mulla S, Kinjal G Patel SR. Prevalence of enterococci with higher resistance level in a tertiary care hospital: A matter of concern. Natl J Med Res 2012;2:25-7.
29. Shareef HA, Abdullah SN. Antimicrobial susceptibility of *Enterococcus* spp. isolated from different clinical sources in Kirkuk provency. Ibn AL-Haitham J Pure Appl Sci 2018;97:97.
30. Rana D, Sande S. Study of prevalence and antimicrobial susceptibility pattern of enterococci isolated from clinically relevant samples with special reference to high level aminoglycoside resistance (HLAR) in a rural tertiary care hospital. J Evol Med Dent Sci 2020;9:2472-8.
31. Fontana R, Cerini R, Longoni P, Grossato A, Canepari P. Identification of a streptococcal penicillin-binding protein that reacts very slowly with penicillin. J Bacteriol 1983;155:1343-50.
32. Thouverez M, Talon D. Microbiological and epidemiological studies of *Enterococcus faecium* resistant to amoxycillin in a university hospital in eastern France. Clin Microbiol Infect 2004;10:441-7.
33. Bbosa GS, Mwebaza N. Global irrational antibiotics/antibacterial drugs use: A current and future health and environmental consequences. Microbiology 2013;3:1645-55.
34. Abdelkareem MZ, Sayed M, Hassuna NA, Mahmoud MS, Abdelwahab SF. Multi-drug-resistant *Enterococcus faecalis* among Egyptian patients with urinary tract infection. J Chemother 2017;29:74-82.
35. Alduhaidhawi AHM, Alhuchaimi SN, Al-Mayah TA, Al-Ouqaili MTS, Alkafaas SS, Muthupandian S, *et al*. Prevalence of CRISPR-Cas systems and their possible association with antibiotic resistance in *Enterococcus faecalis* and *Enterococcus faecium* collected from hospital wastewater. Infect Drug Resist 2022;15:1143-54.
36. Moosavian M, Ghadri H, Samli Z. Molecular detection of vanA and vanB genes among vancomycin-resistant enterococci in ICU-hospitalized patients in Ahvaz in southwest of Iran. Infect Drug Resist 2018;11:2269-75.
37. Jahansepas A, Aghazadeh M, Rezaee MA, Hasani A, Sharifi Y, Aghazadeh T, *et al*. Occurrence of *Enterococcus faecalis* and *Enterococcus faecium* in various clinical infections: Detection of their drug resistance and virulence determinants. Microb Drug Resist 2018;24:76-82.
38. Karki S, Houston L, Land G, Bass P, Kehoe R, Borrell S, *et al*. Prevalence and risk factors for VRE colonisation in a tertiary hospital in Melbourne, Australia: A cross sectional study. Antimicrob Resist Infect Control 2012;1:31.
39. Ahmed MO, Baptiste KE. Vancomycin-resistant enterococci: A review of antimicrobial resistance mechanisms and perspectives of human and animal health. Microb Drug Resist 2018;24: 590-606.
40. Marothi YA, Agnihotri H, Dubey D. Enterococcal resistance—An overview. Indian J Med Microbiol 2005;23:214-9.