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Molecular Detection of *vanA* Gene among Vancomycin Resistance Clinical Isolates of *Enterococcus Faecalis* in Al-Najaf Hospitals, Iraq

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Abstract

Background: *Enterococcus faecalis* is one of the major causes of nosocomial infections, which can be problematic to treat because the bacteria can become resistant to most of the antimicrobial drugs used in hospitals. Antibiotic resistance to vancomycin is a significant threat. The aim of this study was to examine the antimicrobial resistance pattern in *E. faecalis* isolates from clinical specimens in two main hospitals in al-Najaf, Iraq, focusing on vancomycin resistance and the prevalence of the *vanA* gene in vancomycin-resistant *E. faecalis* (VREF) isolates.

Methods: This study analyzed 162 *E. faecalis* isolates from patient samples taken from the two largest hospitals in Najaf, Iraq. A conventional biochemical test and subsequent confirmation by an automated Vitek®2 system were used to identify all isolates. Ten antibiotics were tested for susceptibility using the disc diffusion method. The minimum inhibitory concentration (MIC) value of each *E. faecalis* isolates against vancomycin was determined using the E-test method. The presence of the *vanA* gene in VREF isolates was investigated by the polymerase chain reaction (PCR) method.

Results: Antimicrobial susceptibility patterns against 162 *E. faecalis* isolates collected from different clinical samples in two main hospitals in Najaf showed the highest rate of resistance to cephalosporin and aminoglycoside. The incidence of VREF was in13 (8.0%) after MIC testing. Among 13 VREF isolates, the *vanA* gene was detected in four isolates (31%). These isolates show susceptibility to linezolid antimicrobial.

Conclusion: The emergence of the VREF carrying the *vanA* gene is alarming; therefore, early detection of this gene type in *E. faecalis* isolates will help obtain the most appropriate antibiotics and control the spread of this resistance gene.

Keywords: Vancomycin-Resistant E. *Faecalis*, *vanA* Gene, *Enterococcus Faecalis*, Linezolid Antimicrobial

Introduction

Enterococcus, especially *E. faecalis* is considered to be the major organism that causes nosocomial infection [1]. In developing countries, *E. faecalis* is a significant source of bacteremia, peritonitis, urinary tract infections, and wound and soft tissue infections [2].

E. faecalis have limited treatment options for severe infections because of their propensity to acquire resistance genes readily and several specific mechanisms giving resistance to antibiotics, such as aminoglycosides and cephalosporin [3].

Glycopeptides, including vancomycin (VA), are essential antibiotics that inhibit bacterial cell wall formation. Nosocomial severe infections are caused by *E. faecalis*, which has developed resistance to multiple antibiotics; this bacterium can be effectively treated with vancomycin [4]. The increased usage of vancomycin causes the activation of genes that are resistant to this antibiotic, eventually resulting in the resistance of the antibiotic [5].

Vancomycin-resistant *E. faecalis* has acquired resistance to vancomycin through a plasmid and can spread the vancomycin resistance. This allows sensitive bacteria to become resistant to antibiotics [6]. Infections caused by VREF continue to be a major global cause of morbidity and mortality [7, 8].

There are six types of vancomycin resistance in *E. faecalis*, which are called Van-A, Van-B, Van-C, Van-D, Van-E, and Van-G. Van-A showed the most resistance, and it can pass that resistance on to methicillin-resistant *Staphylococcus aureus*(9,10).

In Iraq, vancomycin resistance in *E. faecalis* has been the subject of a few investigations, and data are rare on the genetic basis of vancomycin resistance among *E. faecalis* isolates from Iraq. Thus, the current study aimed to examine the antimicrobial resistance pattern in *E. faecalis* isolates from clinical specimens in al-Najaf hospitals, Iraq, focusing on vancomycin resistance and the prevalence of the *vanA* gene in VREF isolates.

Materials and Methods

Bacterial Strains

The current study was carried out between September /2022 and March /2023 at Al-sadder Medical City and Al-Hakem General Hospital in Al-Najaf City. There were 162 *E. faecalis* isolates studied. Standard biochemical tests, as reported by [11, 12], were used to identify all isolates to the species level, and confirmatory identification was performed with VITEK 2 system using VITEK 2 GP ID Card.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed. All *E. faecalis* isolates were subjected to antimicrobial susceptibility testing per Clinical Laboratories Standards Institute (CLSI) guidelines [13]. This testing was performed using the Kirby-Bauer disc diffusion method to identify isolates that were resistant to gentamicin (10 µg), amikacin (10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefixime (30 µg), cefepime (30 µg), erythromycin (10 µg), clindamycin (10 µg), and linezolid (10 µg).

E-Test Method for Detection of Vancomycin Resistance

Using a vancomycin E-test strip (Solna, Sweden), the MIC of vancomycin for each *E. faecalis* isolate was evaluated in triplicate. After incubating for 24 hours at 37°C, the findings were analyzed. Susceptibility was defined as a MIC of ≤ 4 (µg/mL); intermediate was MIC8 to 16; and resistance was ≥ 32 [13].

Molecular Detection of the *vanA* Gene

The ABC DNA Isolation Kit (Qiagen, Canda) was used to extract DNA from newly cultured bacterial colonies. Extracted DNA was added to the master mix and the forward and reverse primers in a PCR vial to initiate the polymerase chain reaction. The primers used were;

Forward Primer: 5'- AGCTGTACTCTCGCCGGAT A -3'
Reverse Primer: 5'- CCACCGGCCTATCATCTTT A -3'

PCR amplification condition for the *vanA* gene was 95˚C for 5min then 95˚C for 30 sec, 58˚C for 30 sec, 72˚C for 1min for 30 cycles, and the final extension was 72˚C for 1 min; the amplification produced yielded 421 bp [14]. The PCR products were submitted to an electrophoresis system (Biometra, Germany) and visualized with a gel documentation system (Biometra, Germany).

Results

A cross-sectional study was done on 162 *E. faecalis* that were randomly taken from various clinical samples in two main hospitals in al-Najaf hospitals. The Kirby-Bauer disc diffusion method was used to find antibiotics resistance patterns in all of these isolates, and the E-test method was used for determine of the MIC value for vancomycin. According to source specimens, the distribution of *E. faecalis* isolates was as follows: urine, 60% (n=97); wound, 13.5% (n=22); stool, 11% (n=18); vaginal swab, 8.5% (n=14); and blood, 7% (n=11). It is essential to note that the majority of *E. faecalis* isolates were obtained from urine samples.

The antimicrobial resistance pattern of 162 *E. faecalis* isolates is presented in Figure 1. A review of antimicrobial resistance pattern showed that the most of *E. faecalis* isolates included in the present study were majority resistant to Cephalosporin class antimicrobial (95% to cefixime, 92% to ceftriaxone, 91% to cefotaxime and 90% to ceftazidime). In aminoglycoside class antimicrobial, we can remark high resistance to erythromycin and gentamicin (82%), while amikacin (80%). Additionally, a high prevalence of clindamycin resistance (80%) was reported. There was the lowest resistance rate for *E. faecalis* isolates for linezolid (4%).

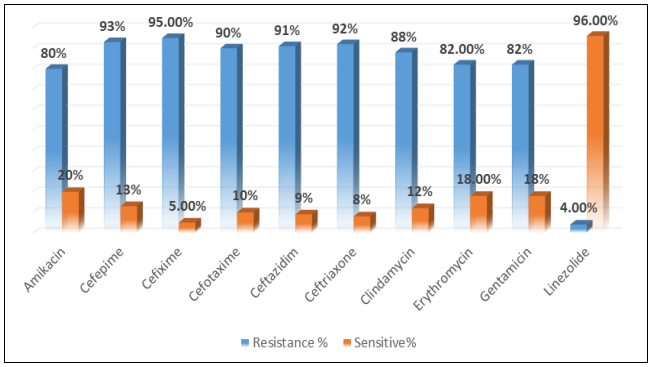

Fig 1: Antibiotic resistance patterns of162 *E. faecalis* isolates

A majority of the *E. faecalis* isolates (149 isolates) (92%) exhibited MIC of vancomycin less than 4 µg/ml. Only 13 (8%) isolates showed MIC of vancomycin ≥32 µg/ml and were identified as VREF. The distribution of VREF isolates according to source specimens, MIC valve, and antibiotics resistance profile is shown in Table 1.

Table 1: Distribution of the 13 VREF isolates according to the gender of the patients, source of infection, PCR result and antibiotic resistance profile

Isolate code	Source	MIC valve	PCR (VanA gene)	Antibiotics resistance profile
06	Urine	≥32	VanA gene	AMC,CEF,CFM,CTX,CAZ,CRO, EM
09	Urine	≥32	No detect	AMC,CEF,CFM,CTX,CAZ,CRO,CM, EM,GEN
28	Urine	≥64	No detect	AMC,CEF,CFM,CTX,CAZ,CRO,CM, EM
33	Wound	≥64	VanA gene	AMC,CEF,CFM,CTX,CAZ,CRO,CM, GEN
48	Blood	≥64	VanA gene	AMC,CEF,CFM,CTX,CAZ,CRO,CM, EM,GEN
57	Urine	≥64	VanA gene	AMC,CEF,CFM,CTX,CAZ,CRO,CM, EM,GEN
71	Wound	≥32	No detect	AMC,CEF,CFM,CTX,CAZ,CRO,CM, EM,GEN
88	Urine	≥32	No detect	AMC,CEF,CFM,CTX,CAZ,CRO, EM,GEN
106	Stool	≥64	No detect	AMC,CEF,CFM,CTX,CAZ,CRO,CM, EM
144	Urine	≥64	No detect	AMC,CEF,CFM,CTX,CAZ,CRO,CM, EM,GEN
152	Stool	≥32	No detect	AMC,CEF,CFM,CTX,CAZ,CRO,CM, GEN
158	Urine	≥64	No detect	AMC,CEF,CFM,CTX,CAZ,CRO,CM, EM,GEN
160	Urine	≥64	No detect	AMC,CEF,CFM,CTX,CAZ,CRO,CM, EM

AMK: amikacin, CEF: Cefepime, CFM: Cefixime, CTX: Cefotaxime, CAZ: ceftazidime, CRO: Ceftriaxone, CM: Clindamycin, EM: Erythromycin, GEN: gentamicin

Based on PCR results for detecting the vancomycin resistance gene (*vanA*). 4/ 13 (31%) *E. faecalis* resistant to vancomycin harbour the *vanA* gene (Figure 2) Table (1). These isolates were obtained as follows: two from urinary samples and one from both wound and blood samples.

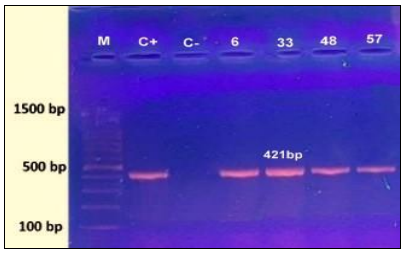

Fig 2: Agarose gel of PCR amplification products of VREF isolates amplified with *vanA* positive genes. Lane (M), molecular size marker (1500 bp), C+: control positive, C-: control negative, (6,33,48,57) display positive results with *vanA*, the agarose stained with Ethidium bromide, the electrophoresis was performed with 70 volts for 90 minutes

Discussions

Vancomycin is regarded as the last line and the most efficient antibiotic prescribed for treating of serious infections due to multiple antibiotic resistance gram-positive bacteria since the 1980s [15]. Over the past few years, vancomycin-resistant *E. faecalis* has represents a major public health issue worldwide [16, 17]. The real dissemination of vancomycin-resistant bacterial isolates remains unknown in Iraq because no hospital report rates of vancomycin susceptibility, and little published data regarding the epidemiology were readily available [18, 19]. Consequently, there is a pressing need for research into detection and infection control measures since the spread of these bacteria poses a serious threat to public health [20]. During the eight-month study period, 162 patients were diagnosed with *E. faecalis* isolates. The largest number of isolates was found in the patients with a urinary tract infection. This prevalence rate was similar to that found in other Iraqi studies in Baghdad at 46.6% [21] and in Al-Najaf at 56% [22]. Alebouyeh *et al.* [23] showed that the % of *E. faecalis* isolates from urine was 75%.

Antimicrobial susceptibility testing revealed that *E. faecalis* had a greater resistance pattern to most antibiotics tested, including cephalosporin and aminoglycoside. Nevertheless, linezolid had a significantly lower resistance level (4%).These results are similar to studies done in Iran [24] that show most *E. faecalis* samples are resistant to most cephalosporin antimicrobials. On the other hand, the antibiotic resistance rate in our study is higher than in studies done in other countries [25, 26, 27].

Inappropriate antibiotic prescribing is commonplace in Iraq, which may contribute to the widespread drug resistance problems. Extensive use of antibiotics could be another factor contributing to the prevalence of drug resistance. Additional to mutation and gene transfer among bacteria, antibiotic use in agriculture [28].

In this study, the very high sensitivity can be explained by the fact that linezolid is an effective antibiotic because it works against a wide range of gram-positive organisms and is used as a last resort to treat *enterococcal* infections that are resistant to vancomycin [29]. This result was in accordance with [30, 31].

All *E. faecalis* isolates were tested with the E-test Method, which is based on CLSI standards for detecting the rate of vancomycin resistance. Based on the results, 13(8%) of the *E. faecalis* isolates with MIC values of vancomycin (≥32 g/ml) were resistant to vancomycin. Comparatively, the results in Iraq (85.7%) [32] and Austria (80.8%) [33] are lower than this. Rather, it is a better result than the one reported in Ethiopia (29.5%) [34]. Differences in strain distribution, trends in antibiotic prescribing, and drug use patterns in the population could all contribute to the inconsistency in results.

In this study, VREF isolates were fully resistant to cephalosporin antibiotics and moderately resistant to aminoglycosides, but they had no resistance to linezolid. The emergence of VREF is attributable to inappropriate cephalosporin use and inadequate hospital infection control [35]. This resistance may be ascribable to the increasing use of vancomycin as the last resort treatment for Methicillin-resistant *Staphylococcus aureus* (MRSA) [36]. Infections caused by multi-resistant strains of *Enterococci* are more common in patients with prolonged hospital stays and taking high rates of antibiotic therapy [37].

The study used PCR experiments to observe the presence of the *vanA* gene among 13 VREF isolates. The results showed that the *vanA* gene was detected in 4/13 (31%) VREF isolates. This result is relatively lower than other previous studies in Baghdad, Iraq, performed by [38], which found that the rate of *vanA* positive was 35.3%. VanA causes the majority of human cases of VREF worldwide [39]. Moreover, the vanA operon is readily transferable via acquired resistance [40]. Our previous research [20] showed that clonal dissemination played a major role in the distribution of these isolates.

All VREFs harbour vanA genes showing susceptibility to linezolid. These strains have been reported in parts of East Asia like China, Japan and South Korea [41]. Reports of such isolates also exist in other parts of the world, like Brazil [42]. Such isolates have yet to be reported from Iraq.

Conclusion

This study revealed a high rate of *E. faecalis* against cephalosporin and aminoglycoside. The vancomycin resistance rate among *E. faecalis* isolates in our study, which was lower than other reports from Iraq. Four VREF isolates have the vanA gene. Therefore, early detection of this gene type in *E. faecalis* isolates will be useful for obtaining the most appropriate antibiotics and controlling the spread of this resistance gene.

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