

## Detection of vancomycin resistance in multidrug-resistant *Enterococcus faecalis* isolated from burn infections

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### ABSTRACT

**Background:** *Enterococcus faecalis* is one of the main causes of nosocomial infections. The clinical importance of *E. faecalis* is related to its antibiotic resistance because it is intrinsic resistance. Vancomycin-resistant *E. faecalis* (VRE) acquired the vancomycin (VA) resistance by a plasmid that permits the sensitive bacteria to become resistant and has the ability to transfer this resistance to other unrelated bacteria. **Materials and Methods:** During the period of 3 months from 1<sup>st</sup> March to 31<sup>st</sup> May 2019, a total of 50 swabs were collected from patients with burn infections from patients in Al-Hilla General Teaching Hospital, Al-Hilla City, Babylon, Iraq. The swabs were cultured on selective and differential media and incubated aerobically at 37°C for 24–48 h. Using VITEK 2 system was used for more confirmative diagnosis. **Results:** All samples yielded bacterial growth 100%. A total of 71 bacterial isolates were identified. Morphological and biochemical characterization of bacterial cultures revealed that 14/71 (19.7%) of total isolates were *E. faecalis*. The disc diffusion method was used for antibacterial susceptibility test against 22 antibiotics. Among 14 isolates of *E. faecalis*, only 5 isolates (35.7%) have resistance against VA. **Conclusion:** The emergence of VRE became a great challenge with serious complications of the control policy for nosocomial infections in our hospitals.

**KEY WORDS:** Burn, *Enterococcus faecalis*, Iraq, Vancomycin resistance

### INTRODUCTION

*Enterococcus faecalis* is Gram-positive cocci arranged in pairs or chains, it is a part of the normal flora of human's intestines. It is a facultative anaerobe.<sup>[1]</sup> *E. faecalis* is one of the main causes of nosocomial infections which usually occur with abdominal surgery, penetrating trauma, catheterization, and intravenous therapy such as urinary tract infections, endocarditis, bacteremia, meningitis, and surgery infections.<sup>[2]</sup> Due to its characteristic cell wall carbohydrate, *E. faecalis* was first identified as Group D streptococci till 1984 that it was classified as a separate genus called *Enterococcus*. The clinical importance of *E. faecalis* is related to its antibiotic resistance because it is intrinsic resistance.<sup>[3]</sup> Despite that the first identification of vancomycin-resistant *E. faecalis* (VRE) in the 1980s at Europe and United State only, VRE have been reported worldwide at the last decades. Nowadays, VRE have been reported worldwide.<sup>[4]</sup> Vancomycin (VA) is a member of the glycopeptides group. It acts as inhibitor

of cell wall synthesis.<sup>[5]</sup> *Amycolatopsis orientalis* produces the VA. At first, the VA was used to treat methicillin-resistant *Staphylococcus aureus* (MRSA) which could not develop a significant resistance against it.<sup>[6]</sup> VA never became the first-line treatment due to its poor oral bioavailability and its toxicity (for the kidney and the ear).<sup>[7]</sup> VRE acquired the VA resistance by a plasmid and able for transferring of the VA resistance and permits the sensitive bacteria to become resistant.<sup>[8]</sup> The enterococci have six different types of VA resistance included Van-A, Van-B, Van-C, Van-D, Van-E, and Van-G. The highest resistance was shown by Van-A which has the ability to transfer the resistance to MRSA, while the lowest resistance was shown by Van-C.<sup>[9]</sup> VRE have caused many nosocomial infection outbreaks worldwide and Van A gene. The mechanism of VRE resistance to VA is alteration of synthesis pathway of the peptidoglycan.<sup>[10-12]</sup> The study of the genome sequence for *Enterococcus* was very necessary due to its many health dangers. *E. faecalis* has different metabolic strategies and has a wide range of regulatory systems.<sup>[1]</sup> The aim of the present study is the detection of VA resistance in the isolates of multidrug-resistant *E. faecalis* isolated from burn infection.

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## MATERIALS AND METHODS

### Collection of Samples

During the period of 3 months from 1<sup>st</sup> March to 31<sup>st</sup> May 2019, a total of 50 swabs were collected from patients with burn infections in Al-Hilla General Teaching Hospital, Al-Hilla City, Babylon, Iraq. The swabs were cultured on selective and differential media and incubated aerobically at 37°C for 24–48 h. According to their features, the bacterial isolates were diagnosed by the comparison with MacFaddin.<sup>[13]</sup> Using VITEK 2 system was used for more confirmative diagnosis.

### Antibacterial Susceptibility Test

The disc diffusion test was performed for 22 antibiotic discs in HiMedia/India. According to Bauer *et al.*<sup>[14]</sup> method, bacterial suspension was prepared by adding five young colonies of *E. faecalis* to 5 ml of brain heart infusion broth and incubated at 37°C for 18–24 h. After incubation, standard McFarland tube was prepared. An inoculum was obtained from the bacterial suspension and streaked on the Mueller-Hinton agar. The antibiotic disc was placed on the surface of Mueller-Hinton agar by a flamed forceps. Overnight incubation at 37°C for 18–24 h. The diameter of inhibition zone was measured by a ruler and compared to standard criteria in CLSI.<sup>[15]</sup>

## RESULTS AND DISCUSSION

During the period of 3 months from 1<sup>st</sup> March to 31<sup>st</sup> May 2019, a total of 50 swabs were collected from patients with burn infections from patients in Al-Hilla General Teaching Hospital, Al-Hilla City, Babylon, Iraq. All samples yielded bacterial growth 50 (100%). A total of 71 bacterial isolates were identified. This finding refers to the positive culture with a mixture of bacterial isolates which were considerable.<sup>[16]</sup> The laboratory diagnostic of bacterial isolates was compared with the standard features in authorized global references, for example, Collee *et al.*<sup>[17]</sup> and

MacFaddin.<sup>[13]</sup> The characteristics being investigated for diagnosis are colonial and cellular morphology on selective and differential media and biochemical tests, Table 1.

In our study, among 71 bacterial isolates, *E. faecalis* represented 14/71 (19.7%) according to the morphological and biochemical characterization and Vitek 2 system, Figures 1 and 2. The isolation rate of *E. faecalis* in the other studies was a variable Garges *et al.*,<sup>[18]</sup> 10%; Trotman and Bell,<sup>[19]</sup> 15%; Cohen-Wolkowicz *et al.*,<sup>[20]</sup> 6.6%; Alfaleh *et al.*,<sup>[21]</sup> 2.04%; and Youssef *et al.*,<sup>[22]</sup> 16.6%. In Brazil, Strabelli *et al.*<sup>[23]</sup> documented that *E. faecalis* caused an outbreak of bacteremia. The outbreaks by endemic *E. faecalis* infections are an indication of horizontal transmission due to the immunosuppression conditions.<sup>[24]</sup>

Despite *E. faecalis* is a part of normal flora in the human, in the 1980s, it was reported as an important nosocomial pathogen with high mortality due to its various survival and virulence factors.<sup>[25,26]</sup> Its ability for competition with other bacteria, represents one of the survival factors of *E. faecalis*; in addition to, it is



Figure 1: *Enterococcus faecalis* on blood agar



Figure 2: *Enterococcus faecalis* on MacConkey agar

Table 1: Diagnostic characteristics of *Enterococcus faecalis* in the present study

Number	The test	The result
1	Gram stain	Gram-positive cocci
2	Cells arrangement	Diplococci or short chains
3	Hemolysis	Non-hemolytic
4	Lancefield group	Group D
5	Motility	Non-motile
6	Spore forming	Non-spore forming
7	Oxidase	Negative
8	Catalase	Negative
9	Growth on MacConkey agar	Positive
10	Tolerance of bile salt	Positive

tolerance of hard nutritional conditions and very harsh environmental conditions such as ethanol, detergents, pH (9.6), azide, extreme concentrations of salts, bile salts tolerance, heavy metals, and desiccation tolerance,<sup>[1]</sup> in addition to the ability to survive for long periods on inanimate objects such as stethoscopes and thermometers and the growth in wide range of the temperature (10–45)°C, and in addition to, it is ability to tolerance the very high temperatures of 60°C for 30 min.<sup>[26,27]</sup> *E. faecalis* has many virulence factors including biofilm, lytic enzymes, suppression of immunity system, and cytolysin.<sup>[28,29]</sup> In addition, the high levels of antibiotic resistance contribute to *E. faecalis* pathogenicity, especially since VA-resistant *E. faecalis* is becoming most common.<sup>[30,31]</sup>

As detailed in Tables 2 and 3, full resistance 100% was shown in each ampicillin, amoxicillin/clavulanic acid, Cephalothin (CEF), cefotaxime, and ceftazidime, and high levels of resistance (85.7%) against each cost per mile and click-through rate, and 78.6% against oxacillin, Tables 2 and 3. These are  $\beta$ -lactam antibiotics that act as inhibitor of bacterial cell wall biosynthesis. These high resistance levels may ascribable to the frequently use  $\beta$ -lactam antibiotics by patients which due to randomly use of these antibiotics. The resistance against  $\beta$ -lactams usually results from  $\beta$ -lactamases, efflux pumps, and modification of penicillin-binding protein.<sup>[32,33]</sup>

In the present study, fully sensitivity (100%) was reported against each of imipenem (IPM) and meropenem (MRP) for all isolates [Tables 2 and 3]. This very high sensitivity can be attributed to the fact that carbapenems (IPM and MRP) are the effective antibiotics because they are broad-spectrum antibiotics, and it has  $\beta$ -lactam ring that has resistance to hydrolysis by most  $\beta$ -lactamases.<sup>[34]</sup> This result was in accordance with.<sup>[35,36]</sup>

On the other hand, low levels of resistance (14.3%) were shown against ciprofloxacin (CIP), norfloxacin (NX), and Clindamycin (DA). These findings are in agreement with Mitscher<sup>[37]</sup> who has stated that fluoroquinolones (NX and CIP) are effective antibiotics which can inhibit bacterial growth by effecting DNA maintenance; therefore, many types of Gram-positive bacteria were sensitive to it. On the other hand, our result about DA was in agreement with Al-Hassnawi<sup>[38]</sup> who documented low levels of resistance (11.3%) against DA, but in a variability with other studies: Hussain *et al.*,<sup>[39]</sup> 23% and Sattler *et al.*,<sup>[40]</sup> 35%. It is noteworthy that many reasons may lead to the variations in the resistance levels among the different studies such as follows: The virulence factors that found in some isolates and absent in others, the source of isolate, the conditions of test, and the type of technique (Brown *et al.*, 2005).<sup>[41]</sup>

In this study, the resistance of *E. faecalis* against aminoglycoside antibiotics such as tobramycin,

Table 2: The percentage of antibiotic resistance in *Enterococcus faecalis* isolates

Antibiotic	AK	AMC	AMP	CPM	CTX	CAZ	CTR	KF	C	CIP	DA	GEN	IPM	K	MRP	M	NX	OX	RA	TE	TOB	VA
Concentration	30	30	10	30	30	30	30	30	10	5	10	10	10	30	10	5	10	1	5	10	10	30
mcg*																						
Resistance (%)	50	100	100	85.7	100	100	85.7	100	42.9	14.3	14.3	64.3	0	64.3	0	64.3	14.3	78.6	42.9	64.3	64.3	35.7

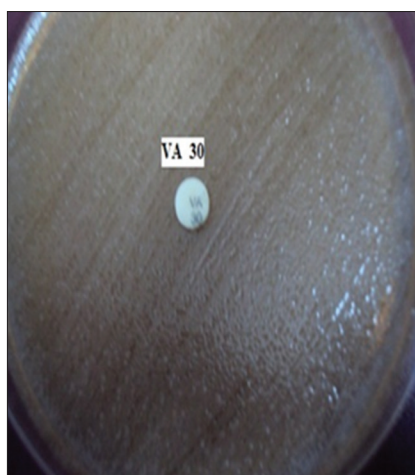
\*According to the manufacturing company HiMedia/India. AK: Amikacin, AMC: Amoxiclav, AMP: Ampicillin, CPM: Cefepime, CTX: Cefotaxime, CAZ: Ceftazidime, CTR: Ceftriaxone, CEF: Cephalothin, C: Chloramphenicol, CIP: Ciprofloxacin, DA: Clindamycin, GEN: Gentamicin, IPM: Imipenem, K: Kanamycin, MRP: Meropenem, M: Methicillin, NX: Norfloxacin, OX: Oxacillin, RA: Rifampicin, TE: Tetracycline, TOB: Tobramycin, VA: Vancomycin



**Table 3: Antibiotic resistance in 14 isolates of *Enterococcus faecalis* in the present study**

Number	Antibiotic	Number of isolates													
1	AK	S	R	R	R	S	S	S	R	S	R	S	R	S	R
2	AMC	R	R	R	R	R	R	R	R	R	R	R	R	R	R
3	AMP	R	R	R	R	R	R	R	R	R	R	R	R	R	R
4	CPM	R	R	R	R	R	R	R	R	R	R	S	R	R	S
5	CTX	R	R	R	R	R	R	R	R	R	R	R	R	R	R
6	CAZ	R	R	R	R	R	R	R	R	R	R	R	R	R	R
7	CTR	R	R	R	S	R	R	R	R	S	R	R	R	R	R
8	KF	R	R	R	R	R	R	R	R	R	R	R	R	R	R
9	C	R	S	R	R	S	S	S	R	R	R	S	S	S	S
10	CIP	S	R	R	S	S	S	S	S	S	S	S	S	S	S
11	DA	R	R	S	S	S	S	S	S	S	S	S	S	S	S
12	GEN	R	R	R	S	S	R	R	R	R	S	S	R	S	R
13	IPM	S	S	S	S	S	S	S	S	S	S	S	S	S	S
14	K	R	R	R	S	R	S	S	R	R	R	R	R	S	S
15	MRP	S	S	S	S	S	S	S	S	S	S	S	S	S	S
16	M	R	R	R	S	S	S	R	R	R	R	S	R	R	S
17	NX	S	R	R	S	S	S	S	S	S	S	S	S	S	S
18	OX	R	R	R	R	S	S	R	R	R	R	R	R	S	R
19	RIF	R	R	R	S	S	S	R	S	S	R	R	S	S	S
20	TE	R	R	R	S	S	R	R	R	R	R	S	S	R	S
21	TOB	R	R	R	R	R	S	S	R	R	R	S	S	S	R
22	VA	R	R	R	R	S	S	S	S	S	S	S	S	S	R

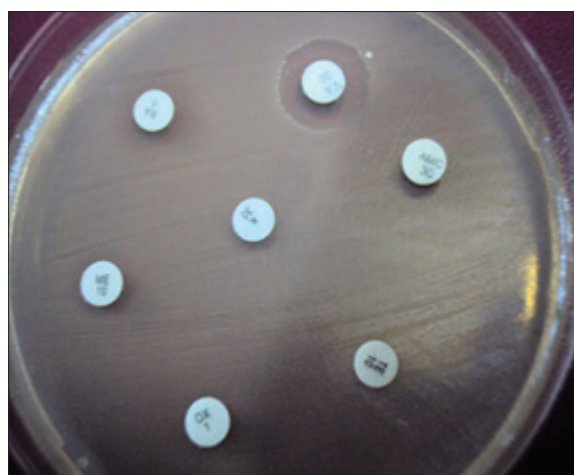
AK: Amikacin, AMC: Amoxiclav, AMP: Ampicillin, CPM: Cefepime, CTX: Cefotaxime, CAZ: Ceftazidime, CTR: Ceftriaxone, KF: Cephalothin, C: Chloramphenicol, CIP: Ciprofloxacin, DA: Clindamycin, GEN: Gentamicin, IPM: Imipenem, K: Kanamycin, MRP: Meropenem, M: Methicillin, NX: Norfloxacin, OX: Oxacillin, TE: Tetracycline, TOB: Tobramycin, VA: Vancomycin

**Figure 3:** Multidrug-resistant *Enterococcus faecalis*

gentamicin, amikacin, and kanamycin was ranged from 50% to 64.3%, Table 2. This is in agreement with the results by Panesso *et al.*<sup>[42]</sup>

As detailed in Table 2, *E. faecalis* has low resistance against rifampicin (42.9%) and chloramphenicol (42.9%). The low resistance to rifampin can be ascribable to the fact that rifampin resistance needs long period of time to develop, and rifampin could not act individually as antibacterial therapy but by the synergistic with other antibiotics to decrease the resistance.<sup>[15,43,44]</sup> It is noteworthy that *E. faecalis* has resistance against tetracycline (64.3%) and methicillin (64.3%), Table 2.

In this study, all evidences were indicating that *E. faecalis* has a multidrug resistance, Figure 3. Its intrinsic resistance and its ability to acquire the

**Figure 4:** Vancomycin-resistant *Enterococcus faecalis*

resistance genes made *E. faecalis* become a serious part of nosocomial pathogens. Recently, this bacteria developed resistance against many different groups of antibiotics.

As detailed in Tables 2 and 3, five isolates of *E. faecalis* have resistance against VA 35.7%, Figure 4. VA is a glycopeptide antibiotic. Because it is a toxic agent for the kidney as well as it is intravenously used, VA represented the last line for the treatment of the Gram-positive bacterial infections.<sup>[45]</sup> Furthermore, VA is intravenously used, so the adaptation of bacteria will be slow against it, which kept VA alive for years and had reduced the bacterial opportunities to develop the resistance.<sup>[46,47]</sup> The emergence of VRE is a dangerous nosocomial problem with serious complications of the control policy for nosocomial infections. In a study of teaching hospitals in France, glycopeptide-resistant

enterococci isolated from the hospitalized patients. This resistance may be ascribable to the increasing use of VA as the last resort treatment for MRSA. Hence, the wide use of VA made the development of resistance against this drug a significant worry.<sup>[42,48]</sup> The previous studies documented the fact that VA resistance genes can transfer horizontally from *E. faecalis* to *S. aureus* and grant it high-level VA resistance. *E. faecalis* has confirmed to be a therapeutic challenge due to it has the capacity for acquisition a broad spectrum of antibacterial resistance agents, which became a serious problem in the treatment of enterococcal infections; the VA resistance genes are carried on plasmids. Hence, *E. faecalis* can give and receive these plasmids during the conjugation.<sup>[49-52]</sup>

## CONCLUSION

The emergence of VRE became a great challenge with serious complications of the control policy for nosocomial infections in our hospitals.

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