Vancomycin-resistant Enterococcus faecium in Algeria: phenotypic and genotypic characterization of clinical isolates

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Abstract
Introduction: vancomycin-resistant Enterococcus faecium (VREfm) is a major public health problem worldwide. The aim of our study was to determine the microbiological, epidemiological and molecular characteristics of VREfm isolated in north-central, eastern and western Algeria.

Methodology: a collection of 48 VREfm isolated from September 2010 to April 2017 in several Algerian hospitals were studied. Minimum inhibitory concentrations (MICs) were determined by E-test method according to CLSI guidelines. the detection of van genotype of all strains was performed by PCR. Clonal relationship of five VREfm targeted by region were characterized using multilocus sequence typing (MLST).

Results: All isolates have multidrug-resistance (MDR) and were resistant to at least five classes of antibiotics; however, all were susceptible to tigecycline and daptomycin with MIC₅₀ at 0.094 µg/mL and 2 µg/mL respectively. All strains belonged to vanA genotype and have high level of resistance to vancomycin and teicoplanin. MLST revealed two sequence types (STs): ST80 (from the four regions of Algeria) and ST789, both belonging to the former hospital-adapted clonal complex CC17.

Conclusion: the alarming dissemination of MDR E. faecium vanA and the ST80 in several regions of Algeria suggest a clonal spread of VREfm strains, which urgently require implementation of adequate infection control measures.

Key words: Vancomycin-resistant E. faecium; antimicrobial susceptibility; vanA; MLST; Algeria.


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Introduction
Enterococcus faecium has become an important nosocomial pathogen, involved in healthcare-associated infections (HAIs), especially among severely ill and/or immunocompromised patients and causes multiples infections (e.g. urinary tract infections, surgical site infections, bacteremia and endocarditis) [1-3]. E. faecium characterized by its remarkable survival abilities in harsh conditions and by its capacity to colonize and persist in gastrointestinal tract of healthy carriers and patients, leading to rapid spread and outbreaks [2-4]. E. faecium, naturally resistant to many antimicrobial agents, has acquired resistance to almost all drugs, including the glycopeptides [2-4]. Therefore, multidrug-resistant (MDR) E. faecium has significantly limited therapeutic options for treating serious and sometimes deadly infections due to these pathogens. In this context, the World Health Organization recognized VREfm as a high priority in its list of 12 resistant-bacteria that pose the greatest threat to human health [5]. Moreover, VREfm can serve as a reservoir of resistance genes and can also transfer to other strains of bacteria such as Staphylococcus aureus [2,4,6]. Over last 30 years, VREfm has increased worldwide and becomes a cause of concern [2-4, 7]. The European Antimicrobial hold Surveillance Network (EARS-Net) showed that the detection rate of VREfm from 2015 to 2018 increased from 10.5 to 17.3% [8]. In 2014, the detection rate of VRE in the United States was 8.1% [9]. Furthermore, according to the Korean Antimicrobial hold state Monitoring System (KARMS) from 2013 to 2015, the detection rate of VREfm grew from 29 to 31% [10]. The Algerian Antimicrobial Resistance Network (AARN) indicated that the VREfm rate from 2014 to 2017 similarly rose from 7.1 to 16% [11,12]. Vancomycin-resistant
**Enterococci** (VRE) strains have emerged during the 1980s and have gradually increasingly reported worldwide as an important cause of nosocomial infection, due mainly to *E. faecium* [2-4]. The glycopeptide resistance genotypes include *vanA/vanE, vanG* and *vanL-vanN*) [3, 4]. The *vanA* genotype is the most prevalent in VRE and confers high level resistance to both vancomycin and teicoplanin [3, 4]. This species is characterized by its genome plasticity, which allows it to generate subpopulations highly adapted to the hospital environment such as the clonal complex CC17, which is highly virulent and has epidemic potential. In addition, the strains belonging to this clonal complex are highly resistant to ampicillin and fluoroquinolones [2,4,13]. In Algeria, the first VREfm was isolated in 2010. Since then, these MDR strains have spread in Algerian hospitals. The main objectives of this study was to determine the microbiological, epidemiological and molecular characteristics of VREfm isolated in north-central, eastern and western regions of Algeria.

**Methodology**

**Patients and bacterial strains**

Between September 2010 and April 2017, 48 non-repetitive VREfm clinical isolates from several Algerian hospitals were collected at the Medical Bacteriology Laboratory, Institut Pasteur of Algeria. VREfm strains, recovered from ill and colonized patients, were from north-central (Algiers, Boumerdes, Blida and Tipaza), eastern (Constantine, Setif and Batna) and western (Oran) Algeria. Patients’ demographic characteristics, medical conditions and clinical outcome were recorded. All the VREfm isolates collected were stored at – 80 °C until analysis.

**Species identification and antimicrobial susceptibility**

This study was carried out between January 2012 and December 2017. Clinical isolates were identified by the strep API32 system (bioMérieux, Marcy l’Etoile, France). A PCR assay based on the amplification of specific gene encoding D-alanine-D-alanine ligase (*ddl*) was used to confirm the identification of *E. faecium* as previously described [14].

The minimum inhibitory concentrations (MICs) to ampicillin, high-level gentamicin, high-level streptomycin, erythromycin, quinupristin-dalfopristin, levofloxacin, rifampicin, tetracycline, nitrofurantoin, fosfomycin, chloramphenicol, vancomycin, teicoplanin, tigecycline and daptomycin were determined by the E-test (bioMérieux, Marcy l’Etoile, France) method on Mueller-Hinton agar following the CLSI recommendations [15]. CLSI interpretive criteria were used for all antibiotics [15], excluding tigecycline for which EUCAST breakpoints were used for interpretative criteria [16]. MIC$_{50}$ and MIC$_{90}$ of all antibiotics studied were also determined. Cefinase test (bioMérieux, Marcy l’Etoile, France) was used to detect β-lactamase. Multidrug resistance (MDR) definition was adopted as reported [17]. *E. faecalis* ATCC 29212 strain was used for quality control.

**Genotyping of vancomycin-resistance genes**

Genotyping for the detection of *vanA, vanB* and *vanC-1/2* resistance genes was performed for 21 strains by PCR reverse hybridization using the GenoType Enterococcus assay (Hain Lifescience, Nehren, Bade-Wurtemberg, Germany) as recommended by the manufacturer. For the remaining 27 strains, this research was carried out by the van multiplex PCR assay as previously described [14]. Quality control strains used were: *E. faecium* BM4107 (susceptible), *E. faecium* BM4147 (vanA), *E. faecalis* V583 (vanB) and *E. gallinarum* BM4174 (vanC1).

**Multilocus sequence typing**

Molecular typing of five clinical isolates targeted on the basis of one strain by region (north-central: Algiers and Tipaza, eastern: Constantine and Batna, and western: Oran was determined by multilocus sequence typing (MLST) using internal fragments from seven housekeeping genes (*atpA, ddl, gdh, purK, gyd, pstS* and *adk*) [18].

The allelic profiles and sequence type (ST) designations were identified in the online PubMLST database (http://pubmlst.org/efaecium/). Global eBURST implemented by PHYLOViZ was used to analyze the genetic relationship of the six studied strains and those of all the CC17 referenced strains deposited in the *E. faecium* PubMLST database on December 31, 2019.

**Results**

**Description of patients’ characteristics**

From September 2010 to April 2017, 48 non-repetitive VREfm strains were isolated from inpatients in several Algerian hospitals. Thirty seven (77.1%) patients were infected and 11 (22.9%) were colonized with VREfm strains. The median age was 40 years and the gender ratio (male/female) was 1.2.

VREfm strains were more frequently recovered from intensive care units (ICUs) (n = 24; 50.0%) and haematology ward (n = 15; 31.2%), and were isolated mostly from blood (n = 15; 33.3%) and urinary tract (n = 10; 20.8%) and abscesses (n = 7; 14.6%).

Analysis of underlying diseases showed serious illnesses, mostly haematological malignancy (n = 15; 31.2%), digestive diseases, immunosuppressive chemotherapy and polytrauma (n = 5; 10.4% for each one of these illnesses).

Prior antibiotic therapy was mainly glycopeptides (n = 16; 33.3%), third generation cephalosporins (n = 14; 29.2%), aminoglycosides and carbapenems (n = 13; 27.1% for each one of these antibiotic families). Deaths were recorded among 14 (29.2%) patients. Detailed characteristics of patients infected with VREfm strains are presented in Table 1.

Antimicrobial susceptibility and genotyping of vancomycin-resistance genes

All the 48 strains belonged to the vanA genotype and showed high level of resistance to vancomycin (MICs 32-256 μg/ml) and teicoplanin (MICs 16-256 μg/ml). The vanB and vanC1/2 genotypes have not been detected in any strain. In addition, all the strains were resistant to ampicillin without production of β-lactamase. All the strains were MDR and were resistant to at least five classes of antimicrobial agents, in addition to glycopeptides. High levels of resistance (rates higher than 80%) was recorded to erythromycin, quinupristin-dalfopristin, rifampicin, levofloxacin, high-level gentamicin and high-level streptomycin. Resistance to tetracycline and fosfomycin was 66.6% and 77.1% respectively. However, tigecycline, daptomycin and chloramphenicol exhibited good activity against VREfm strains with MICs ≤ 0.25 μg/ml, ≤ 3 μg/ml and ≤ 8 μg/ml respectively. Nitrofurantoin showed moderate activity with MIC50 at 32 μg/mL. The antimicrobial resistance rates with MIC50 and MIC90 are shown in Table 2.

Multilocus sequence typing

Of the five isolates studied, four isolates (from Algiers, Tipaza, Constantine and Oran) were assigned to ST80 and one isolate, from Batna, belonged to ST789. Moreover, the comparative eBURST analysis with the all CC17 referenced strains deposited in the E. faecium PubMLST database showed that ST80 and ST789 derived from ST117 and ST17 respectively, both originated from the former hospital-adapted clonal complex CC17 (Table 3).

Discussion

The widespread of VRE is a serious issue in healthcare settings of various countries around the world. In Algeria, the first VRE strain was isolated since 2010. To date, only two papers have reported the emergence of this highly resistant pathogen, one case from Algiers and four cases from Batna [19, 20]. To the
best of our knowledge, we describe for the first time the microbiological, epidemiological and molecular characteristics of VREfm involved in HAIs from several regions of Algeria.

**Description of patients’ characteristics**

In total, 48 VREfm clinical isolates, collected from September 2010 to April 2017, were confirmed as VRE. Virtually most of them were from adults hospitalized in ICUs and haematology ward, and were isolated from blood and urinary tract specimens as also noted globally [3, 21-23]. Similarly to several reports [3, 21-24], most VREfm strains were isolated in patients with co-morbidities or immunosupression, including haematological malignancy, digestive diseases, polytrauma and immunosuppressive chemotherapy.

Nearly 23% (n = 11) of patients were colonized with VREfm strains. The most common clinical impact of VRE strains is intestinal colonization that can persist for long periods. Colonized patients are potential reservoirs for transmission of VRE strains [1]. Epidemic potential recognized for VRE strains explains their rapid diffusion [1, 2]. The lack of rapid and accurate screening of patients infected or colonized with VRE strains in admission to the hospital in our country contributes to this dissemination.

Colonization or infection due to VRE strains is recognized to be associated with therapeutic use of glycopeptides, broad-spectrum cephalosporins, anaerobes and quinolones [2,4,13]. In Algeria, vancomycin was introduced for therapeutic use since 1998; a study on the antibiotics consumption carried out in several hospitals of the Central and Eastern Algeria in 2009 showed a high vancomycin use in ICUs (66.9%) and haematology ward (28.1%) [25]. Likewise, our results showed that glycopeptides and third generation cephalosporins were the main prior antimicrobial agents used. The mortality rate was high (29.2%) as reported by other studies [3,13].

**Antimicrobial susceptibility and genotyping of vancomycin-resistance genes**

Similarly to numerous studies, that reported the prevalence of vanA genotype [2-4,13], all strains in our study harbored the vanA gene and showed high-level resistance to vancomycin (MICs 256 µg/mL) and teicoplanin (MICs 16-256 µg/mL) which are characteristic of VanA phenotype [4,10,13].

All strains showed high level of resistance to ampicillin (CMI50 at 128µg/mL) with no production of β-lactamase. It has been shown in several reports that an increase in the detection of ampicillin-resistant *E. faecium* precedes the emergence of VREfm and is most frequently due to the over production of penicillin binding protein 5 [2,4,13]. In fact, high resistance rate to ampicillin (95%) among vancomycin-susceptible *E. faecium* contributes to this dissemination.

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**Table 2. MIC50, MIC90, MIC range and antimicrobial resistance of VREfm isolates.**

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC50</th>
<th>MIC90</th>
<th>MIC range</th>
<th>Resistant strains Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>256</td>
<td>256</td>
<td>32-256</td>
<td>48 (100)</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>32</td>
<td>256</td>
<td>12-256</td>
<td>48 (100)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>128</td>
<td>256</td>
<td>24-256</td>
<td>48 (100)</td>
</tr>
<tr>
<td>High-level gentamicin</td>
<td>1024</td>
<td>1024</td>
<td>1.5-1024</td>
<td>41 (85.4)</td>
</tr>
<tr>
<td>High-level streptomycin</td>
<td>1024</td>
<td>1024</td>
<td>0.19-1024</td>
<td>45 (93.7)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>256</td>
<td>256</td>
<td>0.25-256</td>
<td>47 (97.9)</td>
</tr>
<tr>
<td>Quinupristin-dalfopristin</td>
<td>6</td>
<td>16</td>
<td>1-32</td>
<td>40 (83.3)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>32</td>
<td>32</td>
<td>2-32</td>
<td>45 (93.7)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>32</td>
<td>96</td>
<td>0.016-256</td>
<td>32 (66.6)</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>32</td>
<td>32</td>
<td>0.006-32</td>
<td>46 (95.8)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>32</td>
<td>128</td>
<td>8-512</td>
<td>23 (47.9)</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>128</td>
<td>1024</td>
<td>24-1024</td>
<td>37 (77.1)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>3</td>
<td>8</td>
<td>1-12</td>
<td>4 (8.3)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.094</td>
<td>0.125</td>
<td>0.016-0.25</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>2</td>
<td>3</td>
<td>1-3</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

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**Table 3. Sequence type and clonal complex of the five VREfm isolates.**

<table>
<thead>
<tr>
<th>Number of isolates</th>
<th>Clonal complex</th>
<th>Sequence type</th>
<th>Allelic profile*</th>
<th>Van type</th>
<th>Ward</th>
<th>Source</th>
<th>Geographical origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>CC17</td>
<td>ST80</td>
<td>9-1-1-1-1-12-1-1</td>
<td>vanA</td>
<td>ICU, surgery</td>
<td>Blood, urine, wound</td>
<td>Algiers, Tipaza, Constantine, Oran Batna</td>
</tr>
<tr>
<td>1</td>
<td>CC17</td>
<td>ST789</td>
<td>1-1-1-1-1-12-1-1</td>
<td>vanA</td>
<td>Haematology</td>
<td>Blood</td>
<td></td>
</tr>
</tbody>
</table>

*In the order: atpA, ddl, gdh, purK, gyd, pstS, adk; ICU: intensive care unit.
faecium strains isolated in 2010 have reported in a hospital of eastern Algeria [24].

Almost all strains were resistant to erythromycin, quinupristin-dalfopristin, high-level gentamicin, high-level streptomycin, levofloxacin, tetracycline, rifampicin and fosfomycin. In addition to their natural resistance to cephalosporins and low concentrations of aminoglycosides, the ability of enterococci to acquire resistance to other antibiotics is recognized, which is often supported by the same mobile genetic element harboring multiple resistance genes [2, 4, 13]. The multilocus-resistance of VREfm strains is prevalent as reported in several parts of the world [2-4, 7-10, 13] and recently in North Africa (including Tunisia and Egypt) [26-29].

The MDR E. faecium is a major concern, as it substantially limited the therapeutic options and poses a great challenges for treatment. Treatment options for VRE include tigecycline, linezolid, daptomycin, quinupristin-dalfopristin, nitrofurantoin and fosfomycin [2, 3, 10, 21, 30, 31]. Interestingly, our results showed good antibacterial activity of tigecycline and daptomycin against VREfm strains. A good activity against VREfm was also noted for chloramphenicol, which can represent a potential therapeutic option [1, 32]. However, high resistance rates to quinupristin/dalfopristin (83%) and fosfomycin (77%) were recorded among VREfm strains. In addition, linezolid and daptomycin are not yet approved in Algeria, leading to more difficulties in the management of patients infected with VREfm.

Multilocus sequence typing

The two STs (ST80 and ST789) found in the five VREfm strains belonged to the widespread adapted-hospital clonal complex CC17. Furthermore, this clonal complex was mostly reported among vancomycin-susceptible E. faecium strains isolated in 2012 and 2016 in north-eastern regions of Algeria [24,33].

The four VREfm strains from several regions (Algiers, Tipaza, Constantine and Oran) belonged to ST80, suggesting the spread of this subgroup in Algerian hospitals. The ST80, previously reported in some European countries (including Germany and Danemark) [34, 35] and in Tunisia [27, 28] was also reported in 2012 among vancomycin-susceptible E. faecium strains from Annaba hospital [24] and recently in 2018 among three VREfm isolated in Batna hospital [20].

One strain from Batna belonged to ST789, which first identified in South Korea (https://pubmlst.org/efaecium/). Interestingly, this sequence type was also reported in 2018 in Batna [20].

Conclusions

In conclusion, on the basis of our findings, the dissemination of MDR E. faecium vanA and the presence of ST80/CC17 in several regions of Algeria is alarming and suggest a clonal spread of the strains. Rational use of vancomycin and broad-spectrum antibiotics, systematically rapid and accurate screening of patients at the admission to hospital and implementation of early adequate infection control measures are urgently required to restrict the spread of this highly resistant species. Further molecular investigations are needed to better understand the epidemiology of circulating VREfm strains in Algeria and to improve infection control policies.

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**Conflict of interests:** No conflict of interests is declared.