Investigation of the Epidemiology and Antibiotic Resistance of Vancomycin-Resistant Enterococcus (VRE) Strains Isolated from Clinical Samples in Algeria

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Abstract

The vancomycin resistance continues to spread in Enterococcal infections throughout the world. In Algeria, these infections caused by of Vancomycin Resistant Enterococci (VRE) keep gaining ground, causing exponential morbidity and mortality every day. The greatest threat these bacteria represent is their ability to developed multi-resistance to virtually all antimicrobials currently used and they can cause serious invasive diseases. Our Aim is study and to investigate the isolation, frequency and the level of antibiotic resistance of Vancomycin Resistant Enterococci (VRE).The present study was a prospective study in which *Enterococcus* was isolated from five different departments. Enterococcal species were identified and confirmed by using VITEK® 2 systems. Antibiotic sensitivity was done by the agar diffusion and Minimum Inhibitory Concentration (MIC) method. SPSS software version 23 (IBM Statistical Package for the Social Sciences (SPSS) statistics 23) was used to analyze the data obtained from the study. A total of 135 *Enterococcus* strains were isolated during the study period, in which we revealed two predominant species *E. faecalis* 60% (81/135) and *E. faecium* (54/135). All isolates had a multidrug resistance, thirty seven of VRE isolates including *E. faecium* (23/37) and *E. faecalis* (14/37) were distinguished by their resistance to vancomycin (MICs 32-256 µg/ml) and teicoplanin (MICs 16-256 µg/ml). The most enterococcal strains were isolated from the Internal medicine (47.4%) followed by the intensive care unit ICU (31.9%). These bacterial isolates were obtained mainly from urine (45.9%), and were observed in female patients (62%). The age group between 25 and 45 was the most affected.This work revealed alarming levels of antibiotic resistance in Enterococci, and their ability to transfer resistance genes to highly pathogenic bacteria such as *Methicillin resistant Staphylococcus aureus.* We should controlling measures to reduce the transmission of these multidrug-resistant organisms.

Keywords: Multidrug resistance; Vancomycin, Enterococcal infections, VRE.

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1. Introduction

The genus *Enterococcus* belongs to the group of Gram-positive bacteria that are regarded normal colonizers of the human gastrointestinal tract, but of great relevance to human health for their role as major causative agents of health care-associated human's infections, and the selection pressure exerted by antibiotics, a dysfunction in therapeutic procedures, or an imbalance in hospital hygiene causing exponential morbidity and mortality [1],[2].The greatest threat these bacteria represent is their ability to developed multi-resistance to virtually all antimicrobials currently used and to transfer resistance genes to more pathogenic Gram-positive bacteria such as *Staphylococcus aureus*. They can cause serious invasive diseases, including endocarditis, bacteremia, urinary tract infection, and pelvic infection [3]; in additional to their intrinsic resistance, the acquired of antibiotic resistance limits the number of treatment approach [4]. This intrinsic antimicrobial resistance played a role in providing opportunities for enterococci to interact with other drug-resistant bacteria, by acquiring or transferring additional resistances on mobile elements [5].

Over the last two decades the high mortality rate caused by these dangerous strains is alarming because of their increasing of multiple antibiotic resistances, which is being a serious threat to current healthcare practices [6]. In that context, many surveys investigated the epidemiology and antimicrobial susceptibility of Vancomycin Resistant *Enterococcus*(VRE) isolated from the different human specimens. Despite its importance, few publications have been made concerning this study in Algeria and our objective of this study is to identify risk factors associated with antibiotic resistance in patients infected with *Enterococcus*.

Material and methods

Bacteria isolation and species identification

Our study was carried out in the PHI (public hospital institution) in south-eastern Algeria. A total of 135 Enterococcal isolates were obtained from hospitalized patient's specimens submitted to the microbiology laboratory, over a period of 12 months from August 2019 to August 2020. These strains were collected from a variety of sources, including urine, blood, respiratory secretions, pus swabs, vaginal/ urethra swabs and pleural fluids.

Enterococcus isolates were selected by using *Enterococcus* selective media (Bile-esculin-azide agar (BEA), Columbia blood agar and CHROMA agar for 24 h at 35 °C in 5 % CO 2; salt tolerance test (6.5% NaCl),in order to differentiate enterococci. The identification of Enterococci isolates was based on standard laboratory criteria which the strains were identified by usual and biochemical tests allowing for discrimination among species: colony morphology, microscopic observation in the fresh state and after Gram staining, screening for catalase, and

confirmation by using microbiological methods including API20 Strep system (Bio Merieux, France) .The automate identification of species was performed with the VITEK® 2 system (BioMerieux, Marcy l'Etoile, France), using Gram-positive identification (GP ID) cards.

Antibiotic susceptibility test:

Antibiotic susceptibility testing was performed by applying the method of discs diffusion in Mueller-Hinton agar using BioMerieux discs as recommended in guidelines established by the Antibiogram Committee of the French Microbiology Society (CA-SFM 2022) [7], and the results of some other discs were interpreted according to Clinical and Laboratory Standards Institute (CLSI) recommendations (2022) [8]. Confirmation of antimicrobial susceptibility was achieved by testing the minimum inhibitory concentration (MIC) and using the VITEK® 2 susceptibility system (BioMerieux, Inc., Durham, NC) AST-GP2 cards according to the manufacturer's instructions [9]; [10].

The bacterial suspension was prepared by emulsifying bacterial isolates 1.5 x 107 CFU/ml in 0.45% saline to the equivalent of a 0.5 McFarland turbidity standard.

The disk diffusion method on Mueller–Hinton agar was performed with the following antibiotic disks: Penicillin G (PG-1U), Oxacillin (Ox-5µg), Cefoxitin (FOX-30 µg), Gentamicin (GM-10µg), , Amikacin (AK-30 µg), Tetracycline (TE-30µg), Erythromycin (E-15µg), Ofloxacin (OFX-5µg),Fusidic acid (FA-10 µg), Vancomycin (VA-30µg), Teicoplanin (TEC-30 µg), Clindamycin (CC-2 µg), Rifampicin (RA-5µg),and Trimethoprim-sulfamethoxazole (cotrimoxazole) (SXT-1.25 / 23.7 5µg). The minimum inhibitory concentrations (MICs) to vancomycin, teicoplanin, were determined by the E-test (bioMérieux, Marcy l'Etoile,France) method on Mueller-Hinton agar following the CLSI recommendations [8].

Microdilution susceptibility testing of isolates was performed using the Vitek ® 2 system (bioMérieux® , Marcy L'Etoile, France). The following antibiotics were used: high-level gentamicin (HLG),erythromycin (ERY), clindamycin (CLI), high-level streptomycin (HLS),levofloxacin (LVX), linezolid (LZD), moxifloxacin (MXF), quinupristin/dalfopristin (QD), tigecycline (TGC), vancomycin (VAN),teicoplanin (TEC), tetracycline (TET), trimethroprime+ sulfamethoxazol (SXT) ,and nitrofurantoin (NIT).

The study of Antimicrobial susceptibility to glycopeptides (vancomycin, teicoplanin) was determined for each suspect strain (low sensitivity diameter) by evaluation of the Etest® quantitative minimum inhibitory concentration (MICs).

The reference strains: Staphylococcus aureus ATCC 25923 and E. faecalis ATCC 29212. were used as quality control before every antibiogram [8]

Statistical Analyses

The data were analyzed using Statistical Package for Social Sciences (SPSS) software version 23.0 (International Business Machines Inc., Armonk, NY, USA). Demographic and clinical characteristics were summarized using absolute and percentage frequencies for the qualitative variables, and calculate the mean (or median) and standard deviation for the quantitative variables. Categorical variables were compared and identify the difference between the two ratios based on the Chi-square test. The t-test was used to compare the mean/median of the 2 groups (normally distributed), and the multi-group mean/median comparisons were based on the ANOVA test. Multivariate logistic regression analysis was used to identify risk factors associated with intestinal colonization or infection with VRE. This data analysis technique adopted to achieve the evaluation of the association between the Vancomycin resistance (qualitative explained variable) and the factors likely to influence it (the qualitative explanatory variables: sex, age, risk factor and medical wards). A value of $p < 0.05$ was determined to be statistically significant. The OR was used to determine the level of risk attributable to an F factor in the exposure of the population to an antibiotic-resistant germ. This association was qualified as significant when the confidence interval did not contain 1.

Ethical considerations

The study protocol was approved by the local ethics committee (Ethics Committee of Dr SAADANE Hospital). Ethical considerations were taken into account during all steps of the survey .The study results were entered and maintained in a secure database.

2. Results:

One hundred thirty five strains of *Enterococcus* were isolated during the study period. The two main clinical wards from where most enterococcal strains are isolated were the Internal medicine (47.4%) followed by the intensive care unit ICU(31.9%) from which 107 out of the 135 studied strains were collected. Of all enterococcal isolates, *Enterococcus faecalis* were the most predominant isolates representing 60% (n=81/135), followed by *E. faecium* (n=54/135).The bacterial isolates were obtained from five different specimens with the following proportion representations: urine (45.9%),blood (35.6%), pus (8.1%), and respiratory secretions (5.2%). The predominant species detected in female sex was *E. faecalis* (n = 61; 75. 3%) with the average age of patients 50 years (IQR 51-89years), while the majority of patients whose *E. faecium* (n = 39) was isolated were male with the average age of patients 53.1 years (IQR 51-89years).

We isolated VRE from thirty seven Patients during the hospital stay. Demographic and clinical data of patients are shown in Table 1. The main age of patients harbouring VRE was 57years (IQR 51-89years). The rate of nosocomial infections due to VRE is much higher in intensive care units (p-value=0.044538 <0.05 / Odds ratio of 17.45) with a significant mortality compared to other strains .Pus specimens were the most common among the 37 single specimens that yielded this organisms (45.9%), followed by blood specimens (40.5%), and urine specimens

(13.5%). There was a statistically significant difference between these groups (Enterococci susceptible to vancomycin and VRE) related to co-morbidities such as cancer, diabetes, Chronic renal disease, respiratory disease (Covid 19) .About 24.3% of VRE-infected patients died of which odds ratio of mortality was OR, 2.071 [95% CI, 1.022-2.225].

All the 37 vancomycin-resistant Enterococcal strains showed high level of resistance to vancomycin (MICs 32-256 µg/ml) and teicoplanin (MICs 16- 256µg/ml). in addition to glycopeptides, all these strains were Multi-Drug-Resistant (resistant to more than three classes of antimicrobial agents) and presented levels of resistance rates higher than 85% to erythromycin, quinupristin-dalfopristin, tetracycline, levofloxacin, high-level gentamicin and high-level streptomycin, Cotimoxazol ,Nitrofurantoin . However, tigecycline, Linezolide showed good activity against VRE strains with MICs ≤ 3 µg/ml and ≤ 8 µg/ml respectively.

3. Discussion

This study focuses on the epidemiology and the resistance profile of isolated Enterococci in Algeria, via different samples from five departments of the Biskra Hospital, Algeria. Two predominant Enterococci species have been found:

E. faecalis (60%), followed by*E. faecium* with 40%. This species distribution was comparable to a study realized by Rahmoun in Tlemcen University Hospital Centre where *E. faecalis* was predominant over *E. faecium*isolates in hospitalised patients[11] . Enterococci are important hospital-acquired pathogens,inwhich the isolates of *E faecalis* and *Efaecium* are the third- to fourth-most prevalent nosocomial pathogen worldwide[12],[13].

Our enterococci strains are found mainly in urine samples (45.9%)and blood (35.6%), followed by purulent samples with a rate of less than 9%. This distribution is higher than that observed in a recent study carried out in hospitals in south-eastern Romania by Iancu et al. by isolating 38.4% and 32.6% of bacterial strains in urine and blood cultures respectively [14]. Our results were comparable to those found by El Ghazawy et *al* who reported rates of 64.6% of isolates came from urine samples [15]. While Yadav and Agarwal found a percentage highest (86.2%) of samples of urinary origin [16]. This is consistent with enterococci being one of the main causes of urinary tract infections due to the increased and prolonged use of urinary catheters in hospitals. On the other hand, enterococci are capable of adapting to a wide range of environmental conditions, including those present in the urinary tract. Their ability to survive in a relatively hostile environment may contribute to their predominance in the urine when an infection develops and in this case, this resistance may limit the effectiveness of traditional antibiotic treatments [17]. While the thirty-seven strains resulting from infection with Enterococci are mainly obtained during a COVID-19 pandemic, where seriously affected patients may have a weakened immune system, making them more vulnerable to bacterial infections leading to sepsis (bacteremia or serious infections) and in this case the infection can

spread quickly through the blood system, allowing different strains of bacteria to colonize the blood. Additionally, patients with severe COVID-19 may have a weakened skin barrier due to medical interventions such as intubations, catheters, etc. This may facilitate the entry of pathogenic bacteria, including VRE, into the bloodstream, which could be detected by an elevated level in blood cultures [18]. The results confirm the multidrug resistance state of these strains of Enterococci, The development of resistance to vancomycin is a significant concern because vancomycin is one of the last-resort antibiotics for treating serious infections caused by Gram-positive bacteria, including methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococci (VRE).The correlation between vancomycin resistance and multidrug resistance can be explained by several factors:genetic elements like plasmids or transposons, these elements can also carry resistance genes to other classes of antibiotics, contributing to multidrug resistance [19].In other hand ,selective pressure can lead to the co-selection of resistance to other antibiotics, especially if the genes for different resistances are clustered together[20].In addition to Cross-resistance mechanisms in which some of the mechanisms that confer resistance to vancomycin may also provide resistance or tolerance to other antibiotics[21].

Table 1.Demographic and clinical data of patients.

Charasteristics	Enterococcus (%)	VRE (%)	P-value
Total of patients	98	37	
demographic data			
Male/Female, sex ratio	46/52	19/18	0,824
Age (y), mean	50 (51-89)	57(51-89)	0,99
Clinical outcome			
Mortality rate	4(1,6)	9(24,3)	<0,001
Isolation site			
Blood	27(35.6)	13(40.5)	0,025
pus	19(8.1)	17(45.9)	0,492
urinary tract	38(45.9)	6(13.5)	0,037

respiratory secretions	14(5.2)	1(0,7)	0,049
Clinical wards			
Internal medicine	25(26,1)	5(1,2)	0,009
Intensive care unit	20(16,3)	26(6,4)	0,044
Pediatric	20(16,3)	2(0,5)	0,69
Cardiology	17(12,6)	2(0,5)	0,045
Surgery	16(12,3)	1(0,2)	0,66
Comorbidities			
Cancer	16(16,03)	6(1,62)	0,025
Covid19	20(20)	20(54)	<0,001
Diabetes mellitus	48(48,64)	20(54)	<0,001
renal failure	18(18,75	13(35,1)	<0,001
Heart disease	31(32)	11(29,7)	0,039

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