ARTICLE ORIGINAL

Bacteriological and epidemiological profile of vancomycin-resistant *Enterococcus faecium* infections in Sahloul University Hospital.

Profil bactériologique et épidémiologique des infections à *Enterococcus faecium* résistant à la vancomycine au centre hospitalo-universitaire Sahloul.

Lamia Tilouche[1,2]
Nesrine Kalboussi[1,3]
Cherifa Chaouch[1,2]
Mohamed Ben Rejeb[4,5]
Balsam Kacem[1,3]
Soumaya Ketata[1,2]
Abdelhalim Trabelsi[1,2]
Noureddine Boujaafar[1,2]

[1] Faculty of Pharmacy of Monastir, University of Monastir, Tunisia

[2] Laboratory of Microbiology, CHU Sahloul de Sousse,Tunisia

[3] Pharmacy Service, Sahloul University Hospital of Sousse, Tunisia

[4] Prevention and care safety service, Sahloul University Hospital of Sousse, Tunisia

[5] Faculty of Medicine of Sousse, University of Sousse

Reçu le 19 octobre 2021 accepté le 7 juin 2022

Corresponding author :
Dr Tilouche Lamia

Address:
Laboratory of Microbiology, CHU Sahloul de Sousse,Tunisia

Email address:
lamia_312@hotmail.com

Abstract

Introduction: Antibiotics resistance is an emerging problem in isolates of *Enterococcus faecium* (*E. faecium)* which may develop resistance to vancomycin. The aims of this work were to establish the bacteriological profile of vancomycin resistant *E faecium* (VRE) infections, to describe the clinical characteristics of the corresponding patients and the evolution of the consumption of antibiotics that can select VRE.

Methods: It's a retrospective study conducted in the Microbiology laboratory of the Sahloul Hospital in Sousse, Tunisia. It concerned all non-redundant isolates of VRE isolated from positive cultures of all types of clinical samples taken from patients hospitalized from 2016 to 2018. The epidemiological and clinical characteristics of patients with at least one culture-positive VRE specimen were collected. Data regarding antibiotic consumption and the occupancy from 2015 to 2018 were collected from the Pharmacy department and statistics office of the hospital, respectively. A chi-squared test was used to compare the number of VRE cases during the years of study.

Results: There was a significant increase of VRE cases in 2017 (n=34) in comparison with 2016 (n=6) and 2018 (n=17). Most of VRE isolates had been isolated from urine samples and deep suppurations. They were multi-resistant to antibiotics. On the patient level, the main classes of antibiotics consumed during the same period were third generation cephalosporins and carbapenems in 20% of cases, teicoplanin and fluoroquinolones in 13% of cases. At the hospital level, excessive consumption of fluoroquinolones, of Amoxicillin–clavulanic acid and carbapenems was recorded during the study period.

Conclusion: VRE infections occur mainly in patients with underlying diseases, a history of antibiotic consumption and long term hospitalization. The abusive consumption of antibiotics seems to be incriminated in the selection and the increase of number of VRE.

Keywords: *Enterococcus faecium*, vancomycin, bacteriology, resistance, antibiotics

Résumé

Introduction: La résistance aux antibiotiques est un problème émergent chez les souches d'*Enterococcus faecium* (*E. faecium*) qui peuvent acquérir une résistance à la vancomycine. Les objectifs de ce travail étaient d'établir le profil bactériologique des infections à *E. faecium* résistant à la vancomycine (ERV), de décrire les caractéristiques cliniques des patients correspondants et l'évolution de la consommation d'antibiotiques pouvant sélectionner les ERV.

Méthodes: Il s'agit d'une étude rétrospective menée au laboratoire de microbiologie de l'hôpital Sahloul de Sousse en Tunisie. Elle a concerné toutes les souches non redondantes d'ERV isolées à partir de cultures positives de prélèvements à visée diagnostique réalisés chez des patients hospitalisés de 2016 à 2018. Les caractéristiques épidémiologiques et cliniques des patients ayant au moins un échantillon d'ERV positif en culture ont été recueillies. Les données relatives à la consommation d'antibiotiques et au nombre de journées d'hospitalisation de 2015 à 2018 ont été fournies respectivement par le service de Pharmacie et par la direction du système d'information et d'appui à la performance de l'hôpital. Un test du chi-deux a été utilisé pour comparer le nombre de cas d'ERV au cours des années d'étude.

Résultats: Il y avait une augmentation significative des cas d'ERV en 2017 (n=34) par rapport à 2016 (n=6) et 2018 (n=17). La majorité des isolats d'ERV avaient été isolés à partir d'échantillons d'urine et de suppurations profondes. Ils étaient multirésistants aux antibiotiques. A l'échelle des patients, les principales classes d'antibiotiques consommées au cours de la même période étaient les céphalosporines de troisième génération et les carbapénèmes dans 20% des cas, la teicoplanine et les fluoroquinolones dans 13% des cas. A l'échelle de l'hôpital, une consommation excessive de fluoroquinolones, d'amoxicilline-acide clavulanique et de carbapénèmes a été enregistrée pendant la période d'étude.

Conclusion: Les infections à ERV surviennent principalement chez les patients présentant des maladies sous-jacentes, des antécédents de consommation d'antibiotiques et une hospitalisation de longue durée. La consommation abusive d'antibiotiques semble être incriminée dans la sélection et l'augmentation du nombre de cas d'infections à ERV.

Mots-clés : *Enterococcus faecium*, vancomycine, résistance, antibiotiques, glycopeptides

INTRODUCTION

Antibiotic resistance is considered as an important emerging problem among clinical isolates of *E. faecium* which may develop resistance to vancomycin; a drug used to treat serious infections due to Gram positive bacteria (1).In the past two decades, *E. faecium* has rapidly evolved as a worldwide nosocomial pathogen. Its inherent tenacity to build resistance to antibiotics and environment stressors, allows the species to thrive in hospital environment (2). Furthermore, the selection pressure exerted by unreasonable prescriptions of broad-spectrum antibiotics is the main cause of the emergence and spread of VRE (2). Tunisian national studies on VRE infections remain extremely scarce: To date, few studies have reported the presence of VRE in hospital settings in our country (3–5). In this context, the aims of this work were to establish the bacteriological profile of VRE infections during the period of 2016-2018, to describe the demographic and clinical characteristics of the corresponding patients and to study the evolution of the consumption of antibiotics that can select VRE at Sahloul University Hospital for 4 years (2015-2018).

MATERIALS AND METHODS

Settings, study design and sample collection

This is a cross-sectional retrospective study carried out in the laboratory of Microbiology of Sahloul hospital, a 629-bed teaching hospital, in Sousse city. It concerned all non-redundant strains of VRE isolated from positive cultures of all types of clinical samples taken from patients hospitalized during the period of 2016 to 2018. VRE isolates detected in screening rectal swabs were excluded. An isolate was considered redundant if it was isolated from the same patient after a period of one month or less.

Microbiological methods

The VRE strains were isolated from positive cultures carried out on standard or enriched media according to the laboratory's current procedures. A biochemical identification using Vitek$^{\hat{a}}$2 identification cards (bioMerieux Inc., Marcy l'Etoile, Lyon, France) for Gram-positive cocci was conducted. The susceptibility testing was performed using the disc diffusion method and interpreted according to the standards issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for the year 2020 (6). All bacteria were tested against the following antimicrobial agents; Penicillin G, Ampicillin, Cefotaxim, Imipenem, Vancomycin, Teicoplanin, Tigecyclin, Gentamycin-hyper charged, Linezolid, Levofloxacin, Chloramphenicol, Erythromycin, Lincomycin and Pristinamycin. According to EUCAST recommendations, an *E. faecium* isolate is suspected of being resistant to vancomycin if the diameter of the vancomycin inhibition zone is less than 12 mm, or if the diameter was greater than 12 mm but the outline of the inhibition zone is blurred, or if colonies were present within the inhibition zone. In these cases, a determination of the minimum inhibitory concentration (MIC) of glycopeptides was carried out using the E-test® method.

Study population

The demographic and clinical characteristics of patients with at least one culture-positive VRE specimen were collected by consulting the request form for bacteriological analysis received by the laboratory of microbiology and properly completed by the clinicians, over the period lasting from 2016 to 2018. The studied variables were: age, hospitalization service (date, duration), date and nature of VRE positive sample, hospitalization history (institution, department, length of stay), personal history and underlying diseases, antibiotics used during hospitalization before isolation of a VRE strain (molecule and duration of use). Patients for whom demographic and clinical data were not available were excluded from the study**.**

Antibiotics consumption

Data regarding antibiotics consumption and the occupancy from 2015 to 2018 were collected from the Pharmacy department and statistics office of the hospital, respectively. We focused on antibiotics that could support the emergence and spread of VRE. The extracted data were converted to defined daily dose (DDD) *per* 1,000 in hospitalization days (HD) used according to the Anatomic Therapeutic Chemical classification system defined by WHO Collaborating Centre for Drug Statistics Methodology according to the ATC/DDD Index regularly revised (7).

Statistical Analysis

The statistical study was carried out using the IBM® SPSS® Statistics (version 21.0) software. Descriptive statistics was used for all the studied variables. A Chi-squared test was used to compare the number of VRE cases during the years of study. A P-value (P) below 0.05 was deemed statistically significant.

RESULTS

Bacteriological characteristics of Vancomycin Resistant *Enterococcus Faecium* isolates

In the microbiology laboratory, 57 non-redundant strains of VRE were isolated from clinical samples during the study period. The proportion of VRE isolates was calculated in relation to the total number of *E. faecium* isolated per year and were shown in Table 1.

Among all identified isolates of *E. faecium*, the proportion of VRE had increased in our university hospital; it

Table 1: Proportions of vancomycin-resistant isolates of *Entercoccus faecium*

Year	Number of *Enterococcus faecium* trains isolated	Number and percentage of vancomycin resistant Enterococcus *faecium* isolates		
		N	%	P-value
2016	43	6	13,95	
2017	79	34	43,04	P=0.001*
2018	88	17	19,32	P=0.0008**
Total	210	57	27,14	

*Value of p of chi2 between 2016 and 2017
**Value of p of chi2 between 2017 and 2018

was 13.95% in 2016 and it increased to 43.04% in 2017. It had relatively decreased in 2018 to 19.32%. There was therefore a significant increase of VRE cases in 2017 in comparison with 2016 (P=0.001) and 2018 (P=0.0008). The isolation of VRE strains was mainly obtained from urine samples (56%), deep suppurations (25%), blood cultures (14%), biomaterials (3%) and superficial suppurations (2%). VRE strains were isolated from monomicrobial cultures in 75% of cases. The antibiotics resistance profile of the isolated strains was illustrated in figure 1. We noted that the resistance rates to ampicillin, imipenem, erythromycin, levofloxacin and aminoglycosides were 100%. In contrast, all isolates were susceptible to chloramphenicol, linezolid and tigecycline. All *E. faecium* isolates had MICs to vancomycin and teicoplanin ≥ 256 mg/L.

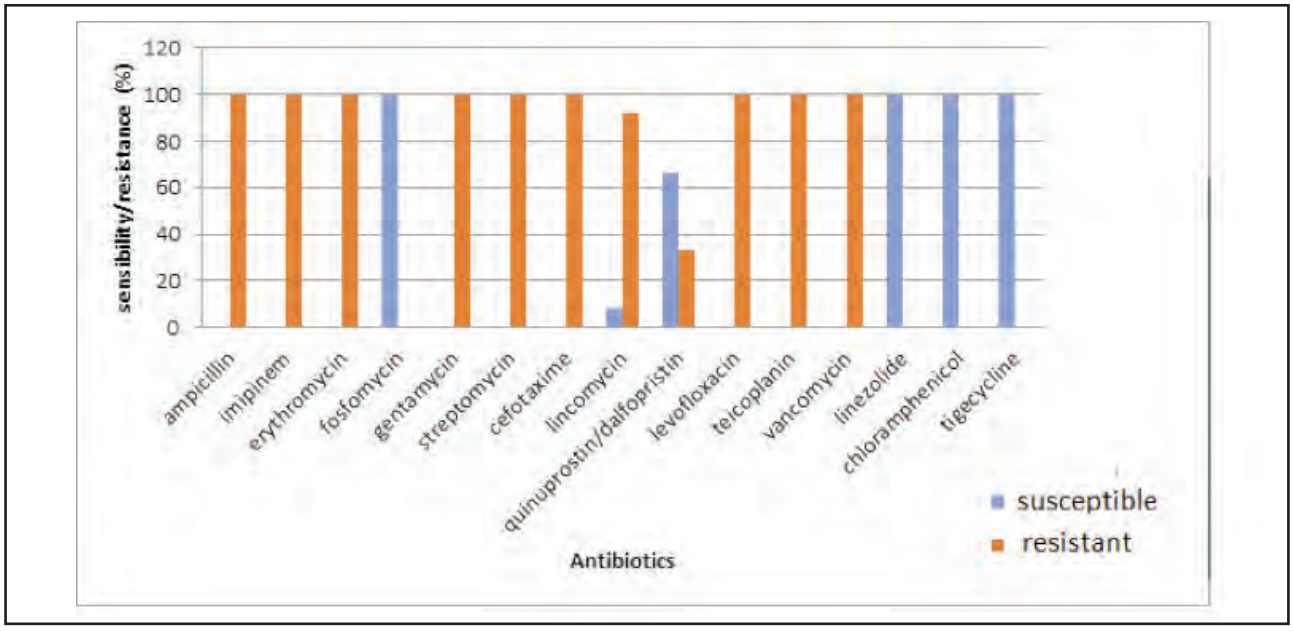

Figure 1: Antibiotics Susceptibility profile of vancomycin-Resistant isolates of Entercoccus faecium.

Demographic and clinical characteristics of patients with a clinical sample positive to Vancomycin Resistant *Enterococcus Faecium*

From 2016 to 2018, 48 patients with exploitable medical records were included in the study of the clinical and demographic characteristics. Among them, 44 patients had a single VRE infection episode whereas 4 had two separate clinical episodes of VRE. The distinction between VRE infection and colonization was made based on the clinical information provided. The number

of cases of VRE infections ranged from 6 to 34 cases per year with an average of 19. The demographic and clinical characteristics of patients are presented in Table 2. The average age of patients was 54 years with extremes ranging from 7 to 90 years. A history of hospitalization was noted in 73.3% (n=35) of the patients. The average

Table 2: Epidemiological and clinical characteristics of vancomycin resistant *Enterococcus faecium* positive patients

Epidemiological and clinical characteristics of patients (n=48)	**Percentage (%)**
Intensive Care Unit Transfer	33.3
Surgical Unit Transfer	**62.5**
Patient History :	
Endocrine Disorders	23
Diabetes	30.3
Cardiovascular Diseases	17
Renal Diseases	15
Cancers	15
Gastropathies	13
Hepatobiliary Diseases	4

length of hospitalization was 53.49 days with extremes ranging from 2 to 270 days. Most patients (n=40) presented an association of three to seven coexisting conditions. No cases without risk factors were noted. Patients with a history of surgery numbered 30 (62.5%). The nature of surgery performed in Sahloul University hospital (n=24) was as follows: abdominal and gastric surgery in 12 cases, orthopedic surgery in 4 cases, urological and renal surgery in 6 cases, cardiovascular and thoracic surgery in 1 case. During the hospitalization episode that led to the isolation of the VRE strain, 95.8% of the patients had consumed antibiotics. Antibiotics were prescribed in combination in 85% of cases and in monotherapy in only 15% of cases. These were third-generation cephalosporins, carbapenems in 20% of the cases, teicoplanin and fluoroquinolones in 13% of cases (Figure 2). The average duration of antibiotic therapy varied between 4.5 and 25.92 days. The longest duration of use was for vancomycin (25.92 days), followed by metronidazole (21.24 days) and fluoroquinolones (14.33 days).

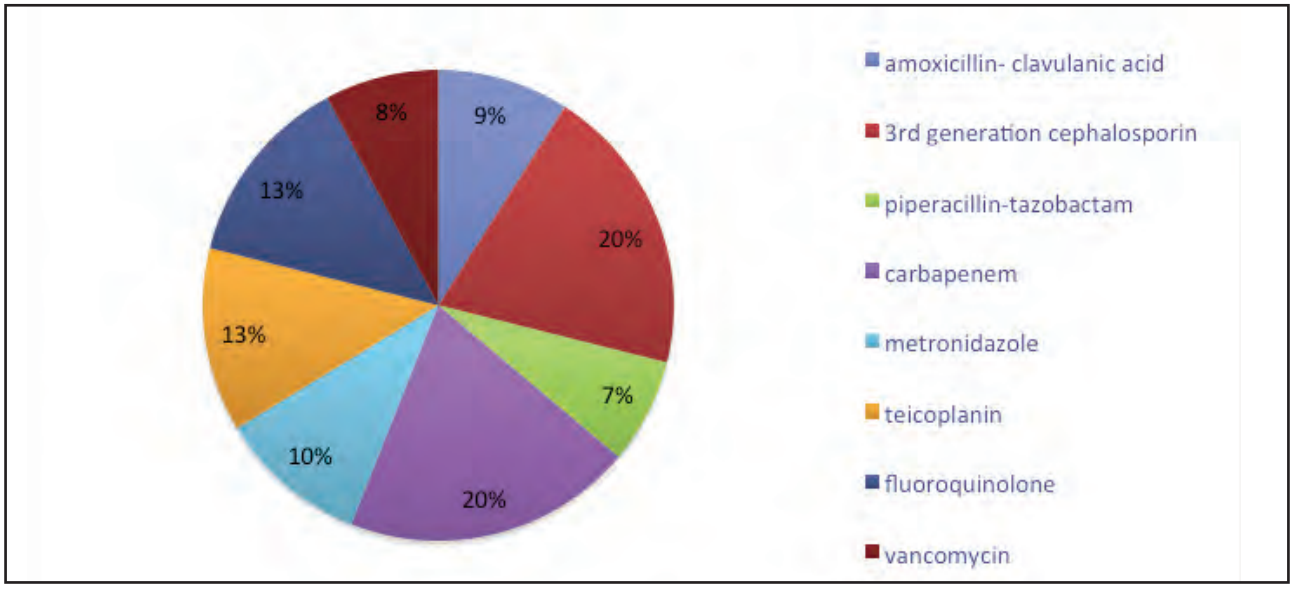

Figure 2: Patient antibiotic consumption prior isolation of the VRE strain

Antibiotics consumption in our hospital

The global average consumption of antibiotics over the whole study period (from 2015 to 2018) was 2165.8 DDD/1000 DH with a minimum consumption of 2060.89 DDD/1000 DH in 2016 and a maximum of 2206.05 DDD/1000 DH in 2017, with a slight decrease estimated at 1.07% from 2015 to 2018. Figure 3 showed excessive consumption of fluoroquinolones, specifically ciprofloxacin (with a maximum of 760DDD/1000 DH in 2015) and ofloxacin (with a maximum of 660DDD/1000 DH in 2018), as well as the combination amoxicillin-clavulanic acid (with a stable consumption rate around 300 DDD/1000 DH) and carbapenems (with a maximum of 180 DDD/1000 DH in 2017 for Imipenem).

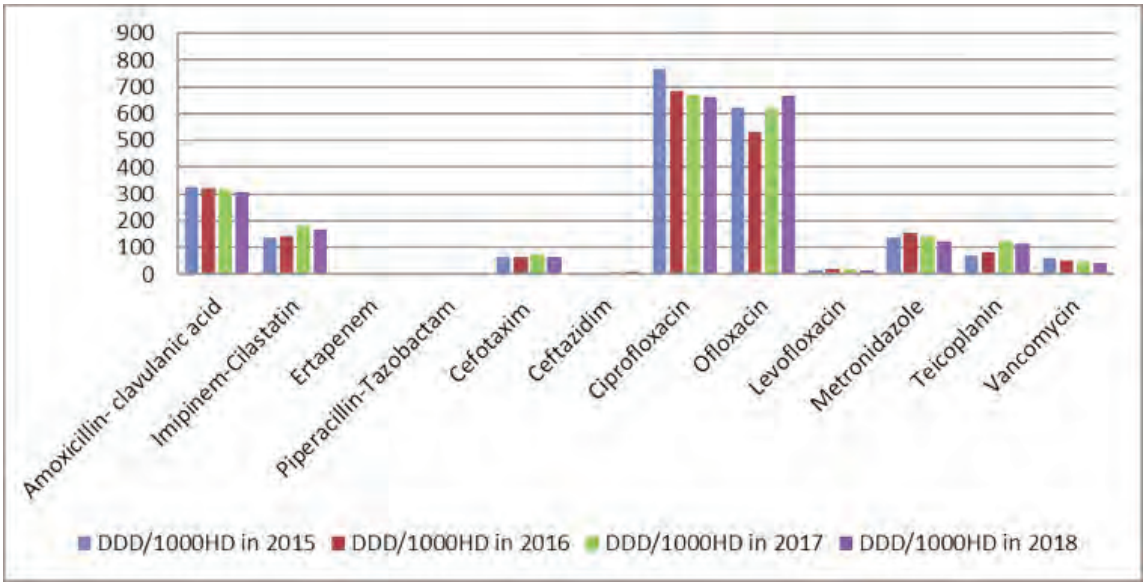

Figure 3: The antibiotics consumption at the Sahloul University Hospital during the study period.
DDD: defined daily dose; HD: hospitalization days

DISCUSSION

Resistance to vancomycin in *Enterococci* is still uncommon in Tunisia, and there are only a few previous reports of detection of VRE in hospitals in our country. Two clinical strains of VRE carrying the *vanA* gene were first isolated in Sfax (5). Thirteen isolates identified as VRE and carrying also the *vanA* gene were recovered from different patients in the Tunisian Military hospital of Tunis during 2012-2013. Blood cultures positive for VRE were obtained in all cases from immunocompromised or polytraumatic patients (4).

According to the result of the current study, the frequency of VRE had increased significantly in our university hospital center. At the national level, according to data from the Tunisian Antibiotic Bacterial Resistance Surveillance Network, vancomycin resistance in *E. faecium* was 26.2% in 2015, 37.5% in 2016 and 38.1% in 2017(8). These rates of vancomycin resistance among clinical isolates of *E faecium* were comparable to those recorded at the hospital centre El-Mansourah in Egypt, where the prevalence of VRE was 14.9% in early 2018, rising to 28.8% in a study published in 2020 (9). However, in other countries, registered rates are much lower; less than 1.1% in China (10), 1.7% in Ethiopia(11) and 8.7% in India (12). A study conducted at a medical center in Taiwan showed that 50 isolates of VRE were responsible for invasive infections over a 5-year period. The distribution of isolation sites was 54.2% from blood cultures, 37.5% from ascites punctures, 6.5% from pleural punctures and 2.1% from cerebrospinal fluid (13). The results of our study and those published in the literature showed that VRE were responsible for different types of healthcare associated infections ranging from superficial to invasive ones.

The presence of a subpopulation of *E. faecium* called hospital-associated (HA) *E. faecium* lineages responsible for nosocomial outbreaks and opportunistic infections in hospitalized patients had been recognized and confirmed during the two past decades (2). Genomic wide studies had showed that these HA isolates acquired a number of traits making them successful in the hospital environment such as an increase in antibiotics resistance genes and virulence genes enhancing biofilm formation and colonization (14). The study conducted in the Tunisian Military hospital of Tunis reported that five environmental strains hosted the same resistance genes and virulence as clinical isolates which can be explained by the transmission of a single strain VRE from one place to another by cross-contamination. Transmission of VRE can be achieved either by the patient to the envi-

ronment or of the environment to staff and visitors or from one patient to another (4). The role of the hospital environment as a reservoir of VRE in Tunisia has been investigated in a study conducted also in 2013. VRE were recovered from 14 samples (4.7%) among 300 environmental samples. Thirteen of the VRE strains were ascribed by PFGE and MLST to a novel clone (new ST910), and only one VRE strain was typed as ST80 included in CC17 (3).

The resistance profile of these bacteria sometimes leads to therapeutic deadlocks (9). Regarding the antibiotic resistance profile of the 57 VRE isolates in our study, we noted that almost all the antibiotics tested were ineffective against the VRE isolates with the exception of chloramphenicol, linezolid and tigecycline. Consistent with our findings, a prospective study conducted between 2016 and 2018 in Egypt showed that VRE isolates exhibited resistance to most antibiotics tested (9). VRE strains reported in previous studies conducted in Tunisia presented also a cross-resistance to all β-lactams tested. In addition, the other resistance markers were: high-level resistance to streptomycin, erythromycin (MLS_B-type A), and ciprofloxacin (4, 5). In February 2017, the World Health Organization (WHO) published a list of antibiotic-resistant pathogens for which the development of new antibiotics is a priority. Vancomycin resistance in *E. faecium* was included in the high priority category of this list (15). In addition, VRE are part of the ESKAPE group. These bacteria are characterized by potential antibiotics resistance mechanisms and are common causes of life-threatening hospital-acquired infections in severely ill and immunocompromised people (16). The notion of admission in an Intensive care unit (ICU) was reported in 33.3% of cases. Studies conducted in a medical center in Israel (17) and Taiwan (13) showed respectively that 28% and 50% of the patients stayed in an ICU, before isolation of the VRE isolate in a clinical sample. The study by Jackson *et al.*, outlines the different factors present in ICU that may favour the selection and emergence of VRE isolates (18). Surgery history was reported in 62.5% of patients in our study. Carmeli *et al*., showed that 29% of the patients included in their study had a history of surgery in the year prior to isolation of the VRE strain (17). In this work, patients were hospitalized for an average of 53.49 days prior to isolation of the VRE strain. Jiang *et al*., reported that the longer the length of hospitalization, the greater the risk of contracting VRE infection (13). Most patients in our study (n=40) had an association of three to seven underlying conditions. In a similar previous study, the most common underlying disease was a malignant tumor (22.6%), followed by diabetes and a viral or bacterial infection (21.5%) (19). The notion of contact with a person carrying an isolate of VRE and the presence of vascular access may increase the risk of contracting a VRE infection (16). According to Jiang *et al*., central venous catheterization and the use of a respirator appear to be risk factors for the development of VRE infections (13). It has been described that the overuse and intensive use of antibiotics has a very important role in VRE colonization because it causes changes in the intestinal microbiota. However, results regarding the effect of antibiotics on the nosocomial epidemiology of VRE vary from one study to another (20). Some studies showed that the more classes of antibiotics a patient consumes, the greater risk of developing an infection due to a VRE strain (13, 16). In our study, antibiotics were prescribed in combination in 85% of cases and in monotherapy in only 15% of cases. During their stay in hospital, the patients in our study had used antibiotics in 95.8% of the cases. The study by Carmeli *et al*., showed that only prior treatment with third-generation cephalosporins or metronidazole is a risk factor for isolation of a VRE strain from a clinical specimen, regardless of the duration of antibiotic therapy (17). The effect of third-generation cephalosporins is probably linked to their activity on the aerobic bacteria of the enteric flora with the exception of *enterococci* naturally resistant to third-generation cephalosporins (17). Indeed, studies have shown that the decrease in Gram-negative bacteria was linked to the increase in VRE colonization. The lipopolysaccharide of Gram-negative bacteria would stimulate the synthesis of REGIIIγ by Paneth cells. REGIIIγ being a lectin with anti-microbial effect against Gram-positive bacteria (21, 22).

The suppression of the gastrointestinal anaerobic flora is the mechanism presumed for the association between metronidazole consumption and the selection of a VRE isolate (17). Although the association between history of vancomycin treatment and VRE has been investigated in numerous studies, the true effect of oral or intravenous vancomycin exposure on the acquisition of VRE remains controversial (20). Fuller et al have suggested that exposure to vancomycin exerts selective pressure on the gut, increasing undetectable levels of pre-existing VRE to detectable levels (23). In our study, the duration of antibiotic therapy for the main classes of antibiotics consumed during hospitalization was also examined. A study in a Boston hospital showed that there was a linear relationship between the duration of exposure to fluoroquinolones and the selection of a VRE isolate in a clinical specimen ($p = 0.05$) (17). It also appears that the effect of fluoroquinolones on VRE acquisition was much greater in patients with an altered intestinal microbiota (20). Numerous studies had shown that at the patient level, the overconsumption of antibiotics plays a role in the selection of VRE (13, 16, 17).

In our work, the qualitative study of the global con-

sumption of antibiotics revealed an excessive consumption of broad-spectrum antibiotics, headed by fluoroquinolones, imipenem and the combination of amoxicillin and clavulanic acid during the whole study period. The impact of vancomycin resistance on clinical outcomes is controversial. Infections due to VRE have been found to be an independent risk factor for mortality resulting in approximately 1300 deaths in the United States each year (24). A retrospective study performed at the Phramongkutklao Hospital during the period from 2014 to 2018 confirmed the impact of VRE infection on mortality and hospitalization duration. The 30-day and 90-day mortality rates of patients infected with VRE *vs*. vancomycin susceptible *E. faecium* were 57.7% *vs*. 38.7% and 69.2% *vs*. 47.1%, respectively. The median length of hospitalization was significantly longer in patients with VRE infection (25). This underlines the urgent need to redouble efforts to find effective preventive strategies that could be useful in addressing this emerging problem. However, a study conducted at the University Hospital Heidelberg (Germany) showed that Vancomycin resistance did not influence outcome among patients with *E. faecium* bacteraemia. Only underlying severity of the disease predicted poor outcome among patients (26).

Our work had some limitations: On the one hand, case-control studies should be carried out to identify the risk factors for VRE infections in patients compared to a group of controls. On the other hand, for antibiotics consumption data, it is recommended to use extremely close iterative measurements such as monthly rather than annual data in order to study the correlation between antibiotic consumption and VRE emergence. Therefore, the use of sophisticated statistical tests would allow the analysis of monthly data and highlight this correlation. Despite these limitations, to the best of our knowledge, this is the first study conducted in Tunisia that provided valuable insights into the evolution of the consumption of antibiotics that can select VRE.

CONCLUSION

We conclude that in our institution, VRE isolates were multi-resistant to antibiotics and had been isolated from different clinical samples especially from urine and deep suppurations. VRE infections occur mainly in patients with underlying diseases, a history of antibiotics consumption that affects the enteric flora and who had been hospitalized for a long time. The excessive consumption of fluoroquinolones, amoxicillin-clavulanic acid and metronidazole seems to be incriminated in the appearance of VRE. Further studies will allow us to investigate the genetic determinism of vancomycin resistance in VRE isolates in our hospital, to study their virulence factors and to study the risk factors of VRE infections. VRE has the potential to become an emerging pathogen for nosocomial infections. Surveillance studies are essential for carefully monitoring trends. Active screening in the wards should be initiated in the future, to reduce the risk of invasive infection occurrence.

Competing interests

The authors declare no competing interest.

Authors' contributions

We certify that all authors have contributed to the work and write-up of the manuscript.

RÉFÉRENCES BIBLIOGRAPHIQUES

1. Patel S, Preuss CV, Bernice F. Vancomycin. StatPearls, Treasure Island (FL): Stat Pearls Publishing; 2021.

2. Zhou X, Willems RJL, Friedrich AW, Rossen JWA, Bathoorn E. *Enterococcus faecium*: from microbiological insights to practical recommendations for infection control and diagnostics. Antimicrobial Resistance & Infection Control 2020;9:130. https://doi.org/10.1186/s13756-020-00770-1.

3. Dziri R, Lozano C, Said LB, Bellaaj R, Boudabous A, Slama KB, *et al*. Multidrug-resistant enterococci in the hospital environment: detection of novel vancomycin-resistant E. faecium clone ST910. The Journal of Infection in Developing Countries 2016;10:799–806. https://doi.org/10.3855/jidc.8014.

4. Elhani D, Naouel K, Dziri R, Hassan M, Sélim A, Ben Said L, *et al*. VanA-containing *E. faecium* isolates of clonal complex CC17 in clinical and environmental samples in a Tunisian hospital. Diagnostic Microbiology and Infectious Disease 2014;79. https://doi.org/10.1016/j.diagmicrobio.2014.01.011.

5. Abbassi MS, Znazen A, Mahjoubi F, Hammami A, Ben Hassen A. Emergence of vancomycin-resistant *Enterococcus faecium* in Sfax: clinical features and molecular typing. Médecine et Maladies Infectieuses 2007;37:240–1. https://doi.org/10.1016/j.medmal.2006.09.011.

6. CASFM / EUCAST V1.2 Octobre 2020. Société Française de Microbiologie 2020. https://www.sfm-microbiologie.org/2020/10/02/casfm-eucast-v1-2-octobre-2020/ (accessed August 9, 2021).

7. WHOCC - Guidelines n.d. https://www.whocc.no/atc_ddd_index_
and_guidelines/guidelines/ (accessed August 9, 2021).
8. Résistance bactérienne n.d. https://infectiologie.org.tn/resistance.php (accessed August 9, 2021).
9. El-Kazzaz SS, Abou El-Khier NT. Effect of the lantibioticnisin on inhibitory and bactericidal activities of antibiotics used against vancomycin-resistant enterococci. J Glob Antimicrob Resist 2020;22:263–9. https://doi.org/10.1016/j.jgar.2020.02.031.
10. Jia W, Li G, Wang W. Prevalence and Antimicrobial Resistance of *Enterococcus* Species: A Hospital- Based Study in China. Int J Environ Res Public Health 2014;11:3424–42. https://doi.org/10.3390 /ijerph 110303424.
11. Abamecha A, Wondafrash B, Abdissa A. Antimicrobial resistance profile of *Enterococcus* species isolated from intestinal tracts of hospitalized patients in Jimma, Ethiopia. BMC Research Notes 2015;8:213. https://doi.org/10.1186/s13104-015-1200-2.
12. Praharaj I, Sujatha S, Parija SC. Phenotypic & genotypic characterization of vancomycin resistant *Enterococcus* isolates from clinical specimens. Indian J Med Res 2013;138:549–556.
13. Jiang H-L, Zhou Z, Wang L-S, Fang Y, Li Y-H, Chu C-I. The RiskFactors, Costs, and Survival Analysis of Invasive VRE Infections at a Medical Center in Eastern Taiwan. Int J Infect Dis 2017;54:18–24. https://doi.org/10.1016/j.ijid.2016.11.005.
14. Gao W, Howden BP, Stinear TP. Evolution of virulence in *Enterococcus faecium*, a hospital-adapted opportunistic pathogen. CurrOpin Microbiol 2018;41:76–82. https://doi.org/10.1016 /j.mib. 2017.11.030.
15. WHO publishes list of bacteria for which new antibiotics are urgently needed n.d. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed (accessed August 10, 2021).
16. Yeung C-S, Cheung C-Y, Chan Y-H, Chak W-L. RiskFactors and Outcomes of Vancomycin-Resistant *Enterococcus* Colonization in Patients on Peritoneal Dialysis: A Single-Center Study in Hong Kong. Perit Dial Int 2017; 37:556–561. https://doi.org/10.3747/pdi.2016.00278.
17. Carmeli Y, Eliopoulos GM, Samore MH. Antecedent treatment with different antibiotic agents as a risk factor for vancomycin-resistant *Enterococcus*. Emerg Infect Dis 2002;8:802–807. https://doi.org/10.3201/eid0808.010418.
18. Jackson SS, Harris AD, Magder LS, Stafford KA, Johnson JK, Miller LG, *et al*. Bacterial burden is associated with increased transmission to health care workers from patients colonized with vancomycin-resistant *Enterococcus*. Am J Infect Control 2019;47:13–7. https://doi.org/10.1016/j.ajic.2018.07.011.
19. Franyó D, Kocsi B, Bukta EE, Szabó J, Dombrádi Z. Assessing the intestinal carriage rates of vancomycin-resistant enterococci (VRE) at a tertiary care hospital in Hungary. FoliaMicrobiol (Praha) 2020;65:483–90. https://doi.org/10.1007/s12223-019-00751-x.
20. Harbarth S, Cosgrove S, Carmeli Y. Effects of Antibiotics on Nosocomial Epidemiology of Vancomycin-Resistant Enterococci. Antimicrob Agents Chemother 2002;46:1619–1628. https://doi.org/10.1128/AAC.46.6.1619-1628.2002.
21. Brandl K, Plitas G, Mihu CN, Ubeda C, Jia T, Fleisher M, *et al*. Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. Nature 2008;455:804–7.
https://doi.org/10.1038/nature07250.
22. Kinnebrew MA, Ubeda C, Zenewicz LA, Smith N, Flavell RA, Pamer EG. Bacterial flagellin stimulates Toll-like receptor 5-dependent defense against vancomycin-resistant Enterococcus infection. J Infect Dis 2010;201:534–543. https://doi.org/10.1086/650203.
23. Fuller RE, Harrell LJ, Meredith FT, Sexton DJ, Colvin LG. Vancomycin-resistant enterococci: risk related to the use of intravenous vancomycin in a university hospital. Infect Control Hosp Epidemiol 1998;19:821–823. https://doi.org/10.1086/647736.
24. CDC. The biggest antibiotic-resistant threats in the U.S. Centers for Disease Control and Prevention 2022. https://www.cdc.gov/drugresistance/biggest-threats.html (accessed May 10, 2022).
25. Hemapanpairoa J, Changpradub D, Thunyaharn S, Santimaleeworagun W. Does Vancomycin Resistance Increase Mortality? Clinical Outcomes and Predictive Factors for Mortality in Patients with *Enterococcus faecium* Infections. Antibiotics (Basel) 2021;10:105. https://doi.org/10.3390/antibiotics10020105.
26. Dubler S, Lenz M, Zimmermann S, Richter DC, Weiss KH, Mehrabi A, *et al*. Does vancomycin resistance increase mortality in *Enterococcus faecium* bacteraemia after orthotopic liver transplantation? A retrospective study. Antimicrobial Resistance& Infection Control 2020; 9:22. https://doi.org/10.1186/s13756-020-0683-3.