

# An evaluation of the burden of invasive Enterococcal infection in a large university hospital

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## ABSTRACT:

- **Objective:** Enterococci represent an important cause of health-care associated infections. The purpose of this paper was to investigate the burden of invasive vancomycin resistance in *Enterococcus* spp in a large University Hospital of the South Italy in order to assess the local situation and, eventually, to develop preventive action strategies.
- **Materials and Methods:** We performed a cross sectional study using the informative annual reports of the Microbiology Laboratory of the Messina University Hospital "G. Martino", focusing on the invasive enterococci isolated in positive blood cultures in the three-year period 2015-2017.
- **Results:** In the considered period, the number and percentages of blood cultures positive for enterococci were 28 (5.4%), 25 (3.6%) and 56 (7.9%) in the three years, respectively. Of these isolates, *Enterococcus faecalis* accounted for 76.8% while *Enterococcus faecium* for 23.1%. The majority of the invasive infections occurred in medical area (55.9%), followed by surgical (26.6%) and emergency ones (17.5%). The most interested wards were Nephrology, Metabolic Diseases and Internal Medicine for medical area and Vascular Surgery for surgical area. Only the 3.9% of invasive *E. faecalis* and the 3.7% of *E. faecium* were vancomycin resistant.
- **Conclusions:** In our context, enterococci represent a minor cause of invasive infections. *E. faecalis* are, far away, the most isolated enterococcal species in general and in positive blood cultures, followed by distance by *E. faecium*. Moreover, the vancomycin-resistance is poorly represented in our setting. The data show that the patients more interested by invasive enterococcal infections and towards which it is necessary focus the preventive actions, were those suffering from metabolic disorders and kidney diseases, for medical wards, and patients suffering from vascular pathologies, for surgical wards.
- **Keywords:** Enterococci, Epidemiology, Healthcare-associated infections, Blood infections, Vancomycin-resistance.

## INTRODUCTION

Enterococci are Gram-positive, catalase-negative, non-spore-forming, facultative anaerobic bacteria, normally colonizing human and animal bowel and the environment (soil, water, foods)<sup>1</sup>. These bacteria are resistant to several physicochemical conditions of the environmental settings and, because of this characteristic, they are very widespread in nature<sup>2,3</sup>. Usually, enterococci are slightly pathogenic for healthy people, causing occa-

sional urinary tract infections. However, these bacteria represent an important cause of health-care associated infections (HAIs)<sup>4</sup>. Clinically, the species of the highest interest are *Enterococcus faecalis* and *Enterococcus faecium*. Enterococci are common hospital-acquired pathogens, accounting for 7.4% of all healthcare-associated infections<sup>5</sup>. Their importance as nosocomial infectious agents is supported by their natural resistance to several antibiotics and an exceptional capacity to acquire and transfer genetic resistance determinants<sup>6</sup>. Particularly,

enterococci show an intrinsic resistance to some antibiotics such as clindamycin and trimethoprim/sulfamethoxazole and a low-level resistance to gentamicin<sup>7</sup>. In addition to intrinsic resistance and tolerance, enterococci are outstandingly capable to acquire resistance to virtually any antimicrobial agent used for clinical use. For instance, the introduction of chloramphenicol, erythromycin and tetracyclines was rapidly followed by the emergence of resistant strains, with a rapid preclusion of their empirical use. Moreover, all enterococci show a decreased susceptibility to penicillin in general, as well as high-level resistance to most cephalosporins as the result of expression of low-affinity penicillin-binding proteins (PBP). However, for many strains, their level of ampicillin resistance does not preclude the clinical use of this drug and ampicillin remains the treatment of choice for enterococcal infections<sup>8</sup>. In recent years, in the United States and Western Europe, the spread of vancomycin-resistant enterococci (VRE) occurred, representing a remarkable and still unsolved challenge for the therapeutic treatment and the infection control actions. Particularly, concerning the two most pathogenic enterococcal species, the rate of VRE is much higher among *E. faecium*<sup>9</sup>. Other species, such as *Enterococcus gallinarum* and *Enterococcus casseliflavus/flavescens* are intrinsically vancomycin-resistant but they account only for a slight portion of enterococcal infections<sup>10</sup>. Extensive use of antimicrobials in intensive breeding has played a remarkable role in the onset and spread of antimicrobial resistant bacteria in the environment, among which VRE<sup>11-16</sup>. Indeed, the use of antimicrobials in this activity leads to bacterial exposure to subtherapeutic concentrations of drugs and it can determine the expression of antibiotic resistance<sup>17</sup>. Asymptomatic gastrointestinal colonization by VRE is more common than clinical infection by a ratio of 10:1 and it can persist from months to years<sup>18</sup>. VREs are endemic in large hospitals and epidemics were also reported. In the United States, according to the National Healthcare Safety Network, from 2009 to 2010, 35.5% of enterococcal HAIs were resistant to vancomycin compared to 33% from 2006 to 2007. Moreover, VREs are the second most common causes of nosocomial infections in the United States, after *Staphylococcus* spp<sup>19,20</sup>. *E. faecalis* remains the most common strain, but the incidence of *E. faecium* has increased. Finally, hospitalization rates for VREs infection doubled from 2003 to 2006<sup>21</sup>. In Europe, according to the last antimicrobial report of the Europe-

an Center for Disease Control and Prevention (ECDC), Vancomycin resistance in *E. faecalis* remained low in most countries while the EU/EEA population-weighted mean percentage for vancomycin resistance in *E. faecium* was 14.9% in 2017, with a significant increase from 2014 when the percentage was 10.4%<sup>22</sup>. The reason why vancomycin-resistant *E. faecium* is increasing in Europe remains unclear. Some studies<sup>23-25</sup> carried out in individual EU/EEA countries showed highly regional spread with multiple hospital outbreaks. The purpose of this paper was to evaluate the rate of the invasive vancomycin resistance in *Enterococcus* spp in a large University Hospital of the South Italy in order to assess the local situation and, eventually, to develop preventive action to act.

## MATERIALS AND METHODS

We performed a cross sectional study using the informative annual reports of the Microbiology Laboratory of the Messina University Hospital "G. Martino". Particularly, we considered all the enterococci isolated in the three-year period 2015-2017 focusing on those invasive isolated in blood cultures. Then, we analyzed the antimicrobial reports focusing our attention on ampicillin and vancomycin resistance of the invasive isolates. The identification of the microbial isolates and relative anti-microbial resistance were obtained using the Vitek 2 automatic system (Biomerieux, Grassina, FI, Italy). The data were analyzed and the descriptive statistics (percentage and standard deviation) were obtained.

## RESULTS

In the considered period, *Enterococcus* spp represented 8.8%, 8.1% and 7.4% of the total microorganisms isolated in the whole hospital in the three years, respectively. The most isolated species were, by far, *E. faecalis* (mean in the three years 74.8% ± 1.85) and *E. faecium* (mean in the three years 22.9% ± 2.1). Other species (*E. casseliflavus*, *E. avium* and *E. durans*) were isolated very rarely with a mean in the three years equal to 2.2% ± 1.2.

In the considered period, the number and percentages of positive blood cultures were 522 (23.1%) in 2015, 691 (25.4%) in 2016 and 712 (26.7%) in 2017. The per-

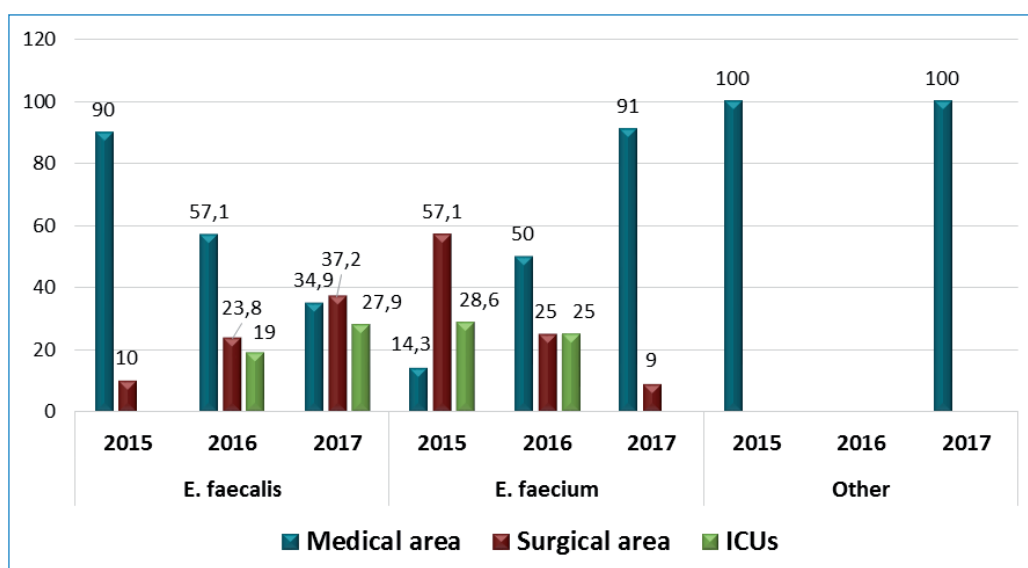
**Table 1.** Number and percentages (in brackets) of blood cultures positive for enterococci and relative species in the three-year period.

	Positive blood cultures	Blood cultures positive for <i>Enterococcus</i> spp*	Blood cultures positive for <i>E. faecalis</i> **	Blood cultures positive for <i>E. faecium</i> **	Blood cultures positive for other species**
<b>2015</b>	522 (23.1%)	28 (5.4%)	20 (71.4%)	7 (25%)	1 (3.6%)
<b>2016</b>	691 (25.4%)	25 (3.6%)	21 (84%)	4 (16%)	0 (0%)
<b>2017</b>	712 (26.7%)	56 (7.9%)	43 (76.8%)	11 (19.6%)	2 (3.6%)
<b>Mean</b>	1925 (25.1%)	109 (5.6%)	84 (77.4%)	22 (20.2%)	3 (2.4%)

\*Percentages calculated on total number of positive blood cultures;

\*\*Percentages calculated on total number of blood cultures positive for enterococci.

**Figure 1.** Percentages of invasive enterococci isolated from blood cultures in the three different areas.



percentages of blood cultures positive for enterococci and relative species are shown in Table 1.

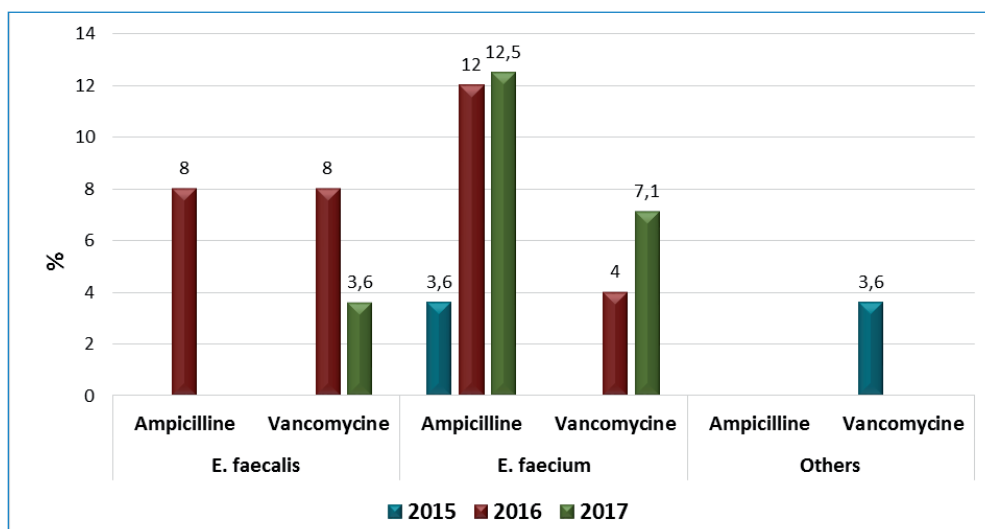
Grouping the different health activities in three areas (medical, surgical and emergency areas), the percentages of invasive enterococci isolated from blood cultures are shown in Figure 1 while in Table 2 are shown the percentages in the various wards.

Concerning the antimicrobial resistance of the isolates, the results are shown in Figure 2.

Concerning the wards in which the resistant invasive enterococci were isolated, in 2015 the interested wards were Oncology and Pediatric ICU, in 2016 Adult and Paediatric ICU, in 2017 Cardiology ICU, Vascular Surgery, Hepatology and Nephrology.

**Table 2.** Number and percentage (in brackets) of the total invasive enterococci isolated from blood cultures in the wards of the three areas during the considered period.

	2015			2016		2017				TOT
	<i>E. faecalis</i>	<i>E. faecium</i>	<i>E. casseliflavus</i>	<i>E. faecalis</i>	<i>E. faecium</i>	<i>E. faecalis</i>	<i>E. faecium</i>	<i>E. avium</i>	<i>E. durans</i>	
<b>MEDICAL AREA</b>										
Geriatry	0	0	0	1	0	0	1	0	0	2 (1.8)
Haematology	1	0	0	0	0	1	0	0	0	2 (1.8)
Hepatology	1	0	0	0	0	8	0	0	0	9 (8.3)
Infectious Diseases	3	0	0	0	0	0	0	0	0	3 (2.7)
Internal Medicine	4	0	0	4	1	1	1	0	0	11 (10.1)
Metabolic Diseases	6	0	0	0	0	3	4	0	0	13 (12)
Nephrology	2	1	0	2	1	2	4	1	1	14 (12.8)
Neurology	0	0	0	1	0	0	0	0	0	1 (0.9)
Oncology	1	0	1	3	0	0	0	0	0	5 (4.6)
Pulmonology	0	0	0	1	0	0	0	0	0	1 (0.9)
<b>Total</b>	18	1	1	12	2	15	10	1	1	61 (55.9)
<b>SURGICAL AREA</b>										
Geriatric Surgery	0	3	0	0	0	0	0	0	0	3 (2.7)
Neurosurgery	1	0	0	1	0	0	0	0	0	2 (1.8)
Oncologic Surgery	0	1	0	0	0	2	0	0	0	3 (2.7)
Urology	0	0	0	1	0	4	0	0	0	5 (4.6)
Vascular Surgery	1	0	0	3	1	10	1	0	0	16 (14.8)
<b>Total</b>	2	4	0	5	1	16	1	0	0	29 (26.6)
<b>EMERGENCY AREA</b>										
ICU	0	1	0	3	0	5	0	0	0	9 (8.3)
Paediatric ICU	0	1	0	1	1	2	0	0	0	5 (4.6)
Cardiology ICU	0	0	0	0	0	5	0	0	0	5 (4.6)
<b>Total</b>	0	2	0	4	1	12	0	0	0	19 (17.5)



**Figure 2.** Percentages of Ampicilline and Vancomycin-resistant Enterococci isolated in the considered period.

## DISCUSSION

The vancomycin resistance in enterococci is well known for a long time. This problem was first recognized in 1986, about thirty years after the introduction of this antibiotic<sup>26</sup>. Since then, the prevalence of VREs has been progressively increased, with the highest rates observed in the US. It is estimated that VREs are about one-third of *Enterococcus* isolates and they cause approximately 1,300 deaths each year<sup>27</sup>. Outbreaks with VREs have occurred in several settings, frequently associated with poor practises of infection prevention and control measures. Several previous studies focused the attention on the potential role of the contaminated surfaces and equipment in the transmission of these pathogens<sup>28-31</sup>. A recent VRE outbreak occurred in 2016 was likely caused by the lack of application of recommended control measures. Risk factors recognized for the transmission of VREs include hospitalization in the same unit in which a VRE carrier patient is present, age, presence of central venous catheter, surgery, hemodialysis and inappropriate antibiotic use<sup>32</sup>.

This research belongs to a line of research on the epidemiology and diffusion of the main AMR pathogenic microorganisms in our hospital settings<sup>33-35</sup>. The results highlight that, in our context, enterococci represent a minor cause of invasive infections representing a mean of  $5.6\% \pm 2.2$  of all bloodstream infections. However, the percentages slightly increased in the considered period with a percentage increase of 2.5%. *E. faecalis* is, far away, the most isolated enterococcal species in general and in positive blood cultures, followed by distance by *E. faecium*; other species were isolated very rarely. Concerning the areas, the medical one was the most interested in the isolation of these pathogens with a total percentage of isolation equal to  $55.9\% \pm 4.7$ , followed by the surgical one with a percentage of isolation of  $26,6\% \pm 5.4$ . The emergency area registered the lowest isolation rates ( $17.5\% \pm 2.1$ ). Moreover, in the medical area, we found an opposite situation between the two principal strains. Indeed, while *E. faecalis* remarkably decreased in medical area (percentage decrease 59.1%) increasing in surgical

one (percentage increase 27.2%), *E. faecium* showed an exactly opposed behavior, remarkably increasing in the medical area (percentage increase 76.7%) and decreasing in surgical one (percentage decrease 48.1%). The other strains (*E. casseliflavus*, *E. avium* and *E. durans*) were isolated only in medical areas. Concerning the wards, in the medical area we observed the highest isolation rate in the Nephrology Unit with a percentage of 12.8%, followed by the Metabolic Diseases and Internal Medicine Units with percentages equal to 12% and 10.1%, respectively. Concerning the surgical area, the Vascular Surgery Unit was, far away, the ward in which it was found the highest isolation rate of all healthcare structure (14.8%); the other wards registered much lower rates. In the emergency area, the highest percentage was found in the Pediatric ICU Unit, while the adult ICU wards registered approximately halved rates. These data show that the patients more interested by invasive enterococcal infections and towards which it is necessary focus the preventive actions, were those suffering from metabolic disorders and kidney diseases, for medical wards, and patients suffering from vascular pathologies, for surgical wards. Probably, in addition to the critical clinical conditions of these patients, to this epidemiology they also contribute the characteristics of these wards that often are overcrowded and understaffed.

Finally, concerning the antimicrobial susceptibility, the vancomycin resistance was found in rather low percentages both in *E. faecalis* and in *E. faecium* with an average percentage of  $5.8\% \pm 3.1$  for the first and  $5.5\% \pm 2.2$  for the second. In 2015, no vancomycin-resistant strains were isolated. However, the two species showed an opposite behavior because while for *E. faecalis* the resistance decreased of 4.4% from 2016 to 2017, for *E. faecium*, in the same years, there was an increase equal to 3.1%. In our setting, the percentage of *E. faecium* vancomycin-resistant is, then, much more lower than the European percentage (14.9%) but even in our territory we are assisting to a certain increase. Moreover, the ampicillin-resistance was much more represented in *E. faecium* with an important increase from 2015 to 2017 equal to 9.5%.

## CONCLUSIONS

Our data highlight that, although the enterococcal vancomycin resistance rate is still quite low, attention must be kept high and preventive measures to contain VREs invasive infections must be always implemented. During outbreaks, a bundle of prevention and control measures is able to contain the risk if used simultaneously. These measures comprise a cohort of patients, active surveillance cultures, environmental cultures, extensive environmental cleaning, education, and antimicrobial stewardship<sup>36</sup>. Concerning the latter, it has been demonstrated that a strict use of vancomycin is a part of a VRE outbreak control<sup>37</sup>.

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## AUTHOR CONTRIBUTIONS:

G.F.P., M.R.L.P.C and P.M. identified the endpoints analyzed and prepared the figures and tables; A.F., M.C. and E.V.R. designed the study, contributed to the acquisition and analyses of data; F.D.A., I.A.P., F.D.A. and D.M. recruited the patients and collected clinical data; A.F. wrote the paper. All authors have read and approved the final version of the manuscript.

## CONFLICT OF INTEREST:

The Authors declare that they have no conflict of interests.

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