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Distribution of antibiotic resistance among *Enterococcus* spp. isolated from 2017 to 2018 at the University Hospital "Luigi Vanvitelli" of Naples, Italy.

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ABSTRACT

In the last decade *Enterococcus* spp. has become one of the most important nosocomial pathogens. The prevalence of multi-resistant strains of *Enterococcus faecium* and *Enterococcus faecalis* responsible for hospital-acquired infections is associated with their ability to acquire and share antimicrobial resistance genes contained in Mobile Genetic Elements (MGE). This study investigated the distribution of antibiotic resistance in *Enterococcus* spp. isolated from clinical patients in the University Hospital "Luigi Vanvitelli" of Naples, Italy. The aim of the present study was to monitor the antimicrobial drug resistance and spread of nosocomial infection, to allow the optimal choice of antibiotic therapy. From January 2017 to December 2018, 351 *Enterococcus* spp. isolates were collected from different clinical samples, at the University Hospital "Luigi Vanvitelli". Bacteria identification was made using MALDI-TOF technology (Bruker Daltonics, Bremen, Germany). Susceptibility to 9 antibiotics was tested using BD Phoenix (Becton, Dickinson and Company). The results were compared with the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Data were analyzed using the statistical software SPSS v.22.0 (IBM SPSS Inc., New York, USA). Among the 351 collected samples, 88 (25.1%) were identified as *Enterococcus faecium* and 263 (74.9%) were *Enterococcus faecalis*. The *Enterococcus faecalis* showed the highest resistance rate to Tetracycline (73,5%) and Erythromycin (88,6%) than the *Enterococcus faecium*. The *Enterococcus faecium* has showed increase in resistance rates against Ciprofloxacin and Imipenem. This study showed the increased rate of resistant Enterococci in our University Hospital. Persistent surveillance of antimicrobial patterns was essential to adopt the empirical treatment guideline to treat infection caused by *Enterococcus* spp.

1. Introduction

Nowadays, antimicrobial resistance (AMR) is a severe threat to public health (Azimi et al., 2019; Pormohammad et al., 2019; Prestinaci et al., 2015; Zhang et al., 2019). The incorrect use of antibiotics, errors in prescribing practice and empirical treatment have caused the multidrug-resistant (MDR) bacteria advent, involved in 15.5% of Hospital Acquired Infections (HAIs) (Aga et al., 2015; Tangcharoensathien et al., 2018). Currently, AMR and HAIs represent the

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greatest global public health challenges. High morbidity and mortality are caused by nosocomial infections, impacting the health systems and increasing direct and indirect costs (Franci et al., 2018; MacDougall et al., 2019). European Centre for Disease Prevention and Control (ECDC) has estimated that 3.2 million patients acquire an HAI in Europe yearly and that 37,000 people die as a direct consequence of infection, due to a MDR bacteria (Caselli et al., 2018). In Italy, HAIs prevalence, caused by MDR bacteria, is 5 - 10%, with a mortality percentage of 20 - 30% (Kolpa et al., 2018). A significant proportion of this mortality is due to *Enterococcus* spp. infections. Enterococci are common residents of the gastrointestinal tract in different animal species (Kuang et al., 2016). Among the genus *Enterococcus* (Murray, 1990), *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*) are the species with more clinical relevance. *E. faecalis* causes 85 - 90% of enterococci infections, while, *E. faecium* only 5-10% (Jabbari Shiadeh et al., 2019). These bacteria act as opportunistic pathogens, causing different infections such as urinary, soft tissue, bloodstream infections, endocarditis and meningitis (Ferede et al., 2018; Fiore et al., 2019). Recent studies highlighted *Enterococcus* spp. as the third nosocomial pathogen (Bereket et al., 2012), after coagulase-negative Staphylococci and *Staphylococcus aureus*, involved in 9.6% of all nosocomial infections (Guzman Prieto et al., 2016). The high frequency of enterococcal infections is caused by the ability of these bacteria to resist the most common antibiotics used in clinical practice (Miller et al., 2014). Indeed, the indicated species are resistant to different antimicrobial agents due to intrinsic and acquired resistance mechanisms (Phukan et al., 2016). These bacteria have antibiotic intrinsic resistance to cephalosporins, aminoglycosides, lincosamides and streptogramins. In hospitalized patients with massive and long-term antibiotic treatments, enterococci accumulate together with other resistant bacteria in the gastrointestinal tract. This situation promotes the exchange of resistance genes, carried by plasmids or transposons (Sultan et al., 2018). Recently, enterococci resistant to numerous antimicrobial agents are isolated very frequently in the hospital setting. The emergence of vancomycin-resistant enterococci (VRE) has alerted the community health for the few treatment options available (Summers et al., 2019; Tendler et al., 1991). VRE spp. have been isolated in Europe (4%), Asia (11.9%), United States (35.5%) and South America (12.9%) (Alotaibi et al., 2017). This has led to an increase in the use of more recently developed antibiotics, such as daptomycin and linezolid (Narayanan et al., 2019). The difficulty in treating enterococcal infections is linked to antimicrobial resistance and the few treatment options available. The economic impact of VRE infection and colonization is considerable and can be reduced by infection control measures. Therefore, our study aimed to promote the surveillance of Enterococci infection and their resistance patterns, in the University Hospital "Luigi Vanvitelli" of Naples, to improve the management of this nosocomial concern. The resistance trends for common enterococci treatments observed in our region, highlights the urgent need of monitoring to understand the epidemiology and risk factor associated with infections.

2. Materials and Methods

351 clinical isolations of *Enterococcus* spp. were collected in the Complex Operative Unit (U.O.C.) of Virology and Microbiology of the University Hospital "Luigi Vanvitelli" of Naples from urine, wounds, catheters, bodily fluids, blood, and respiratory tract samples. Duplicate were excluded from the study.

2.1. Bacterial culture and identification:

The clinical samples were grown on BD Columbia Agar with 5% Sheep Blood (Becton Dickinson) and incubated at 37 °C for 24 hours. Pure colonies have been identified using MALDI-TOF technology (Bruker Daltonics, Bremen, Germany). Antibiotic susceptibility tests were performed with BD Phoenix (Becton, Dickinson and Company). The results were compared with EUCAST guidelines. The antimicrobial susceptibility for Gram-positive bacteria (GPB) was determined using the following antibiotics: Gentamicin (GM), Erythromycin (ERY), Vancomycin (VA), Teicoplanin (TEC), Ciprofloxacin (CIP), Ampicillin (AMP), Linezolid (LZD), Tetracycline (TET), and Imipenem (IPM). The results of the research were documented as susceptible (S) or resistant (R).

2.2. Statistical analysis

Descriptive statistical analysis was made for the patient's gender and type of specimens that as categorical data were expressed as numbers and percentages. Antibiotic susceptibility profiles were compared for *E. faecalis* and *E. faecium* and were expressed in percentages compared to continuous variables using the Student's t-test. P-value of 0.005 was considered significant. Data were analyzed using the statistical software SPSS v.22.0 (IBM SPSS Inc., New York, USA).

3. Results

In the present study, 351 clinical isolates from January 2017 to December 2018 (57.3% from male and 42.7% from female patients) were recovered by several sources. Pus, ulcers, wounds were the site of maximum percentage of isolation (28.5 %), followed by urine (28.2%), bile (25.9%), blood (16.0%), and others (1.4%) (Table 1). On the total *Enterococcus* spp. isolated, *E. faecalis* was the most representative (74.9%), while *E. faecium* represented only 25.1%. Enterococci had exhibited high resistance to Tetracycline (73.5%) and Erythromycin (88.6%). In contrast, they showed a reduced resistance to Vancomycin (3,1%) and Linezolid (2,6%) (Table 2). There was a significate difference in resistance to Tetracycline, Imipenem, Erythromycin, Ampicillin and Ciprofloxacin, between *E. faecium* and *E. faecalis* ($p < 0.005$). In contrast, no significant difference was shown to Gentamicin ($p = 0{,}269$), Vancomycin ($p = 0{,}032$), Teicoplanin ($p = 0{,}013$) and Linezolid ($p = 0{,}236$) (Table 3). The resistance rates analyzed in 2 years showed the increase in Vancomycin-resistant *E. faecium*, in detail, the percentages were 2.1% in 2017 and 12.2% in 2018. For *E. faecalis* all used antibiotics showed an overall reduction in resistance rates, except for Erythromycin which showed an increase in the resistance rate (Figure 1).

4. Discussion

This study examined the prevalence of antibiotic resistance among *Enterococcus* spp. isolated between 2017 and 2018 from clinical patients in the University Hospital "Luigi Vanvitelli" of Naples. *E. faecalis* was the more frequent isolates in 74,9% of the human clinical samples, followed by *E. faecium* found in 25,1% of the *Enterococcus* infections, like other reports (Beltrami et al., 2000; Karna et al., 2019; Shridhar et al., 2019). The present study highlighted a higher incidence in a group composed of over 65 years people (42,7%). This data is in accordance with Mathis et al., showing that the age could be a risk factor for VRE infection (Mathis et al., 2019).

However, the most common infection sites are the urinary tract, skin and soft tissues (Low et al., 2001). The global problem related to *Enterococcus* infections is due to i) the increase of infection rate and ii) the growing resistance to antimicrobial agents (Gholizadeh et al., 2000), as Beta-lactamases (Jacoby et al., 2005), aminoglycosides high-level (HLA) and more recently to glycopeptides, particularly in *E. faecium* (Mohanty et al., 2005). In the current study, *E. faecalis* had less resistance percentage in clinical samples to different tested antibiotics like Ampicillin, Imipenem, Ciprofloxacin, Erythromycin and Tetracycline than *E. faecium* ($p < 0.005$). Assessing the antibiotic resistance rates used in the treatment of *Enterococcus* spp. infections, they showed resistance to Erythromycin (88.6%), Ciprofloxacin (51.3%), Ampicillin (23.1%), Gentamycin (47.0%), Tetracycline (73.5%), Imipenem (24.9%), Linezolid (2.6%), Vancomycin (3.1%) Teicoplanin (3.4%). However, the isolates were highly sensitive to Teicoplanin (96.6%), Linezolid (97.4%) and Vancomycin (96.9%). The inverse results were reported in a study conducted in North West Ethiopia. Yilema et al. detected 41.7% of vancomycin resistant strains (Metz et al., 2019). Similar data were showed in a study conducted by Sader et al. They reported a susceptibility greater than 99.9% for Teicoplanin, Linezolid and Vancomycin (Sader et al., 2006). A study on the phenotypic profile of VRE strains showed that *E. faecalis* is related to genotype Van A for the high levels of resistance to all glycopeptides antibiotics (Pelosi et al., 1988). Instead, genotype Van B is linked to *E. faecium*, with sensibility to only Teicoplanin (Praharaj et al., 2013).

Table 1. Characteristics of the study population

Total Enterococci isolated	**n=351**
Sex	**n (%)**
M	201 (57.3)
F	150 (42.7)
Clinical isolated of Enterococci in different type of sample.	
Type of sample	
Urine	99 (28.2)
Blood	56 (16.0)
Bile	91 (25.9)
Wound, ulcer, pus	100 (28.5)
Other	5 (1.4)

Table 2. Resistance rates of the clinical isolates of *Enterococci* spp. to various antimicrobial agents.

Antibiotic	***E. faecalis* (n=263)**		***E. faecium* (n=88)**		**Total n (%) of isolates (n=351)**	
	R	**S**	**R**	**S**	**R**	**S**
Gentamicin-Sin	119	144	46	42	165 (47.0)	186 (53.0)
Ampicillin	10	253	71	17	81 (23.1)	270 (76.9)
Teicoplanin	5	258	7	81	12 (3.4)	339 (96.6)
Vancomycin	5	258	6	82	11 (3.1)	340 (96.9)
Erythromycin	263		48	40	331 (88.6)	40 (11.4)
Linezolid	5	258	4	84	9 (2.6)	342 (97.4)
Ciprofloxacin	102	161	78	10	180 (51.3)	171 (48.7)
Tetracycline	211	52	47	41	258 (73.5)	93 (26.5)
Imipenem	12	251	73	15	85 (24.9)	266 (75.8)

Table 4. Resistance percentage rates of tested antibiotic from clinical isolated collected at University Hospital of Naples “Luigi Vanvitelli”.

ANTIBIOTIC (%)	***E. faecium***	***E. faecalis***
AMPICILLIN	80,7	**3,8**
CIPROFLOXACIN	88,6	**38,3**
ERYTRIMICIN	54,5	**100**
GENTAMICIN-SIN	13,1	**33,9**
IMIPENEM	20,8	**3,4**
LINEZOLID	1,1	**1,4**
TEICOPLANIN	2,0	**1,4**
TETRACYCLINE	13,4	**60,1**
VANCOMICYN	1,7	**1,4**

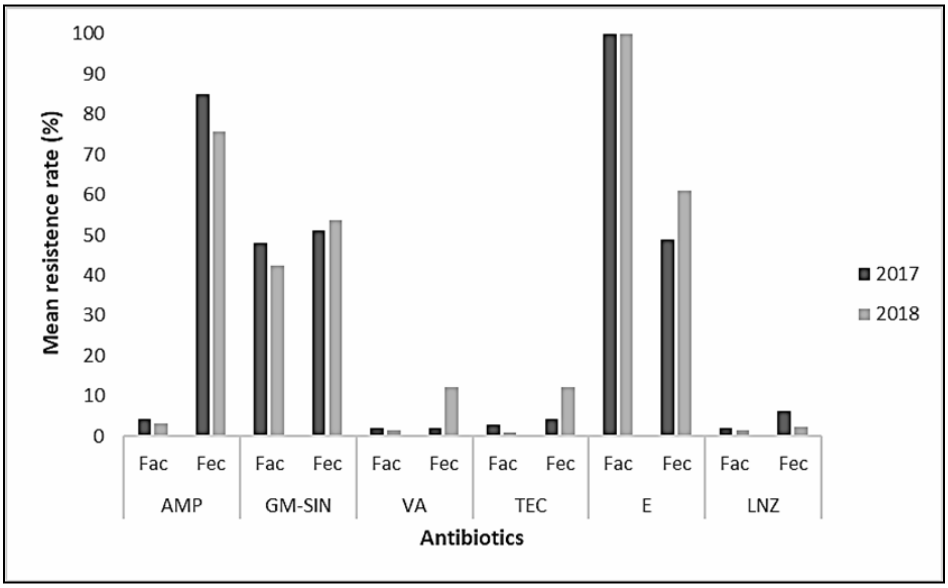

Figure 1: Comparison between mean resistance rates against most frequently used antibiotics in 2017 and 2018 among *Enterococcus faecalis* (Fac) and *Enterococcus faecium* (Fec).

Finally, the resistance to Vancomycin in *E. faecalis* decreases with a low percentage but without significative difference. In contrast, for *E. faecium* Vancomycin resistance rate in 2018 has increased, with a percentage of 12,2%. These data agree with the high average percentage reported for the EU/EEA population. To date, Vancomycin resistance in *E. faecium* was 17.3%, compared to 2015, when it was 10.5%. For several countries, the increase over the four years has been considerable. In an Iranian study by Talebi et al., the resistance pattern wasn't different from our results, where isolates were resistant to Teicoplanin (3%) and Vancomycin (9%), along with a few other antibiotics (Praharaj et al., 2013).

Enterococcus spp. represents a significant problem in nosocomial infections. This is caused by the massive use of antibiotics in hospitals that can produce changes in the intestinal microbiota of patients and subsequent systemic alterations. This study showed that *Enterococcus* spp. isolated from clinical samples in the U.O.C. of Virology and Microbiology of the University Hospital "Luigi Vanvitelli" of Naples had a high rate of resistance to different antimicrobial. These results are useful for monitoring the isolation and resistance rates of sentinel microorganisms, such as *Enterococcus* spp. Correct antibiotic usage and administration are essential for the treatment of bacterial infections (Aslam et al., 2018). Thus, inappropriate prescription and misuse of antibiotics could contribute to the emergence of AMR bacteria, restriction of therapeutic options, and increase of hospitalization time. A VRE specific control can reduce the treatment costs and saving cost (Li et al., 2018). AMR monitoring could improve different aspects: providing data on bacterial resistance rate, helping clinical to the selection of appropriate antibiotics and subsequently reduce the empirical therapy. Simple interventions to prevent the VRE infection, such as hand hygiene, environmental cleaning and antimicrobial stewardship program are fundamental to fight nosocomial infection (Mehta et al., 2014). Our study is restricted to a small number of patients, single-center and limited period. Its enrichment with data from other hospitals in our region obtained in an extended period could be our next goal. All these efforts could be necessary to monitor the Stewardship program progress and improve the empirical treatment guideline for *Enterococcus* spp. therapy.

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