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THE INCREASE OF VANCOMYCIN-RESISTANT ENTEROCOCCUS FAECIUM IN URINE: A RETROSPECTIVE STUDY AT UNIVERSITY HOSPITAL OF BARI

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ABSTRACT

Recently, vancomycin-resistant strains have emerged as a significant threat to patients. This study describes the local epidemiological trend of vancomycin-resistant *E. faecium* (VR-*E. faecium*) strains isolated from urine, their antibiotic susceptibility to empirical treatment, patients and wards at increased risk. We retrospectively analyzed *E. faecium* isolates collected from January 2013 to December 2020 as part of standard patient care at the University Hospital of Bari, Italy. The antimicrobial susceptibility of *E. faecium* was assessed using an automated system. Out of 512 *E. faecium* isolates, 110 (21.48%, 95% CI: 18.06%-25.35%) were vancomycin-resistant. A relevant increase of resistance rates to vancomycin (1.45% Vs 21.48% Vs 38.78%, *p*-value <0.001, Cramer's V= 0.282) was observed during years 2013-2015, 2016-2017, and 2018-2020. A linearly increasing trend of vancomycin resistance rates was observed (*p*-value <0.001). The VR-*E. faecium* isolates were multidrug-resistant with resistance versus quinupristin/dalfopristin (9.18%) and linezolid (3.56%). A VanA phenotype was observed in 95.45% VR-*E.faecium* strains. The maximum prevalence of VR-*E. faecium* strains were found in the ICU. Vancomycin resistance was associated with the patient's age. This retrospective analysis of urinary E. faecium isolates revealed a progressive increase of vancomycin resistance that highlights the need for an active surveillance system and control strategies to avoid outbreaks inwards with critically ill patients.

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Introduction

Enterococci are part of the human gut microbiota, although they can become clinically relevant and cause principally urinary tract infections (UTIs), bloodstream infections, and endocarditis mainly in hospitalized patients [1].

In recent years, vancomycin-resistant *Enterococcus faecium* (VR-*E. faecium)* has emerged as a significant threat to patients [2]. The European Antimicrobial Resistance Network (EARS-Net) showed an increase in invasive VR-*E. faecium* strains from 10.5% in 2015 to 17.3% in 2018, with higher rates in several Eastern European countries and Ireland, followed by Italy, Czechia, Germany, and United Kingdom [3-5]. The Italian time series showed an increase in VR-*E. faecium* strains from 2013 (4.4%) and in 2018 the percentage (18.9%) was higher than the European average [6].

Several risk factors have been described for enterococcal infections, such as neutropenia, organ transplantation, hemodialysis, corticosteroid treatment, chemotherapy, long-term antimicrobial courses, parenteral nutrition, surgery, ICU stay, prolonged hospitalization, indwelling urinary catheters, and mucositis [7]. Even SARS-Cov-2 could be a risk factor for enterococcal infections as described by Kampmeier *et al*. [8]

Prolonged hospitalization and improper use of antibiotics have led to the emergence of VR-*E. faecium*, which currently belongs to the high priority group of pathogens because of its association with increased mortality and health-care costs due to the limited treatment options [9].

For Enterococci, the multidrug resistance arose from both intrinsic (beta-lactams and aminoglycosides) and acquired mechanisms (glycopeptides, quinolones, tetracyclines, macrolides, and streptogramin) through mobile elements like transposons and plasmids [10].

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For vancomycin, nine different genes (VanA, VanB, VanC, VanD, VanE, VanG, VanL, VanM, and VanN) have been associated with resistance, although VanA and VanB are the most clinically important [11]. Resistance mechanisms can be identified by both genotypical and phenotypical methods. In particular, the VanA phenotype provides resistance to both vancomycin (MICs >64 µg/mL) and teicoplanin (MICs >16 µg/mL), while the VanB phenotype presents modest-to-high levels of vancomycin resistance (range from 4 to >64 µg/mL), with retained susceptibility to teicoplanin (MICs <0.5 µg/mL) [12, 13].

Although discerning between colonization and infection can be difficult, Enterococci are one of the most common pathogens isolated from the urine of hospitalized patients.

VR- *E. faecium* is becoming a major cause of healthcare-associated UTIs and recognizing the local trend of multidrug-resistant pathogens is critical for optimizing outcomes and minimizing cost and inconvenience, given that nosocomial enterococcal bacteremia is commonly acquired from UTIs [14].

This study describes the local epidemiological trend of VR-*E. faecium* strains isolated from urine samples during 7 years, their antimicrobial susceptibility, patients and hospital wards at increased risk.

Materials and Methods

We conducted a retrospective study including 512 patients who had at least one positive urine culture for *E. faecium* during hospitalization at the University Hospital of Bari, Italy in the period from 2013 to 2020. Duplicated strains from the same patient were deleted by a 365-days filter. Five patients were removed from the analysis because of the absence of vancomycin data.

The strains were identified as *E. faecium* by MALDI TOF VITEK MS™ assay (BioMérieux, France) and antibiotic susceptibility was assessed using VITEK 2 System™ (BioMérieux, France) following the manufacturer's instructions. The MIC values were interpreted according to the European Committee on Antimicrobial Susceptibility Testing criteria. Intermediate strains have been classified as resistant [15].

This study follows the 305 ethical standards of the institutional and/or national research committee and the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Sample and patients' information (date of sampling, ward, type of specimen, testing results, sex) have been recorded in an anonymous database by transforming sensitive data into alphanumeric codes. No clinical data associated with these specimens were available.

Statistical Analysis

The independence of categorical variables was assessed by a two-tailed Fisher's exact test as appropriate. To evaluate the age differences, the two-tailed Kruskal-Wallis test was performed. Moreover, evaluation of the effect size was performed by Cramer's V and Odds Ratio. Cramer's V is a measure of association between two nominal random variables, which is also appropriate for tables larger than 2x2. The coefficient ranges between 0 (no relationship) and 1 (perfect relationship).

Cramer's V is computed by:

$$\sqrt{X^2/[nobs * (\min(ncols.nrows) - 1)]}$$
$$X^2 = derived\ from\ the\ Pearson's\ chi - square\ test \quad (1)$$
$$nobs = number\ of\ observations$$

ncols; nrows=number of columns and rows, respectively

Specifically, Cramer's V effect size was considered small (if Cramer's V was <0.2), medium (if Cramer's V was≥0.2 or <0.35), or large (if Cramer's V was ≥0.35).

To evaluate the resistance rates in years 2013-2015, 2016-2017, and 2018-2020 two-tailed Fisher's exact test was performed, and the *p-values* were subsequently corrected for multiple comparisons by Benjamini and Hochberg's procedure with false discovery rate (FDR) <5%.

The post-hoc analysis to evaluate the differences in the prevalence of *VR-E. faecium* strains among the hospital wards were performed by two-tailed Fisher's exact tests with the Benjamini and Hochberg's procedure.

Exploratory analysis of the monthly time trends of VR-*E. faecium* strains were performed by Lowess smoothing (Locally weighted scatterplot smoothing). The smoother span value was 0.6. Evaluation of a yearly increasing trend of VR-*E. faecium* strains were performed by Chi-squared trend test.

Finally, logistic regression analysis was performed to evaluate the presence of a yearly background trend of *VR-E. faecium* strains.

Calculations of all statistical tests were performed by the open-source environment R 3.5.2 (R Core Team) [16].

Results and Discussion

From January 2013 to December 2020, 512 *E. faecium* strains were isolated from urine samples of hospitalized patients. In particular, the number of isolates increased from 43 in 2013 to 104 in 2020. The monthly time series of *E. faecium* strains increased to a maximum value (12 strains) in August 2019, April 2020, and June 2020 **(Figure 1)**.

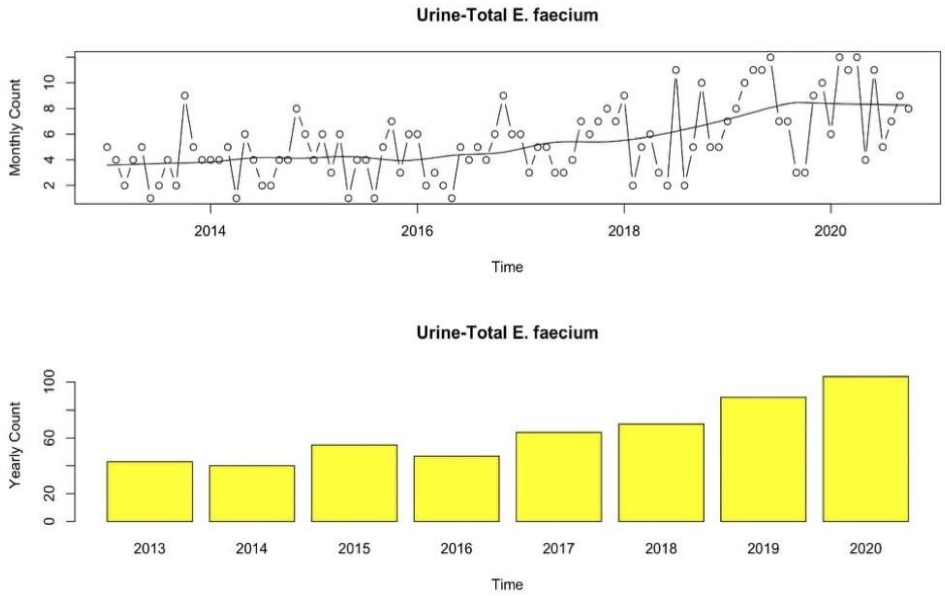
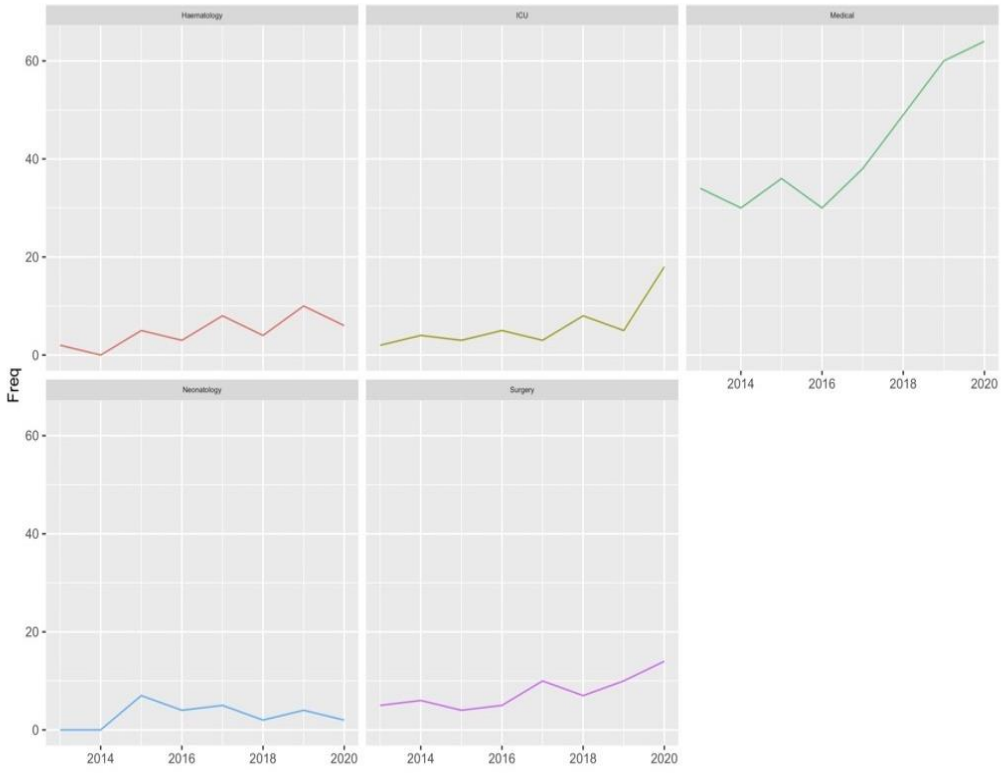
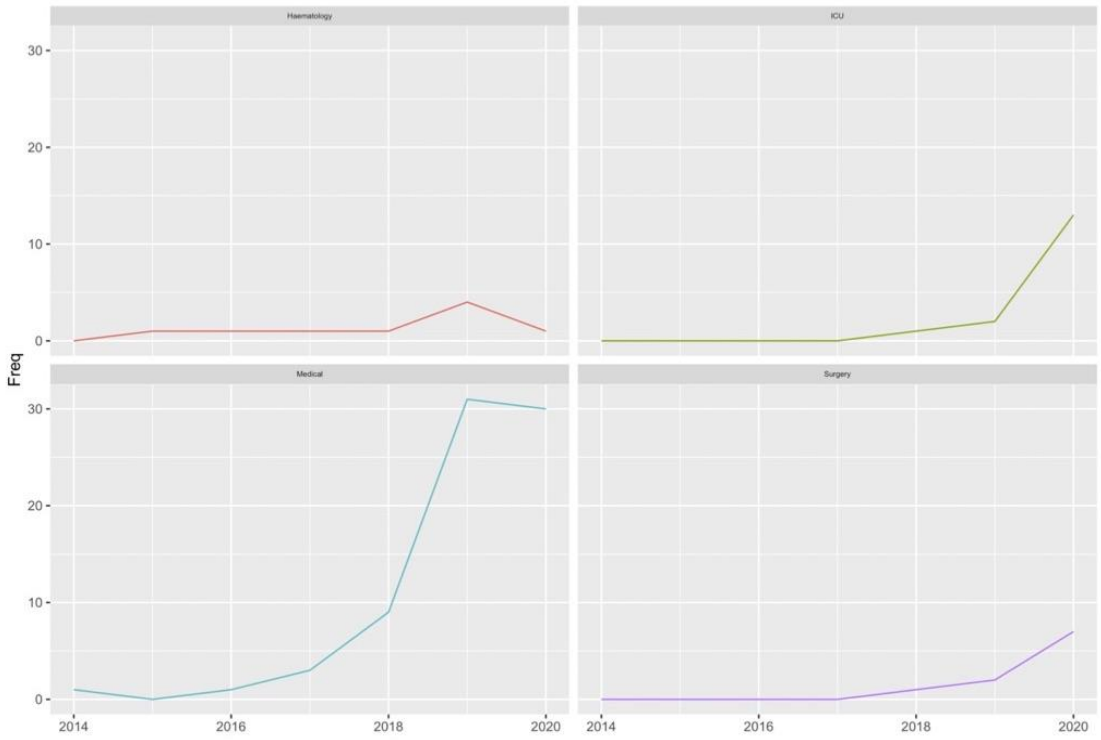


Figure 1. Monthly time series and yearly time series of the *E. faecium* strains isolated from urinary samples. The trend of the monthly time series was estimated by Lowess smoothing with smoothing span value 0.6 (black line).

The increase of the isolates mainly involved the medical wards starting from 2016 **(Figure 2a)**.



a)



b)

Figure 2. a) Yearly time series of the *E. faecium* strains isolated from urinary samples classified by typology of the ward. b) Yearly time series of the vancomycin-resistant *E. faecium* strains isolated from urinary samples classified by typology of the ward.

Regarding the vancomycin susceptibility, 110/512 (21.48%) strains (95% Confidence Interval: 18.06%-25.35%) were resistant. In particular, VR-*E. faecium* strains increased from 0 in 2013 to a maximum of 51 in 2020, and a statistically significant increasing trend was detected (Chi-squared for trend *p value*<0.001). A maximum of 8 VR-*E. faecium* strains was isolated in December 2020 **(Figure 3)**, and the medical wards were mainly involved also in this case **(Figure 2b)**. Globally, 105/110 (95.45%) vancomycin-resistant strains had a VanA phenotype.

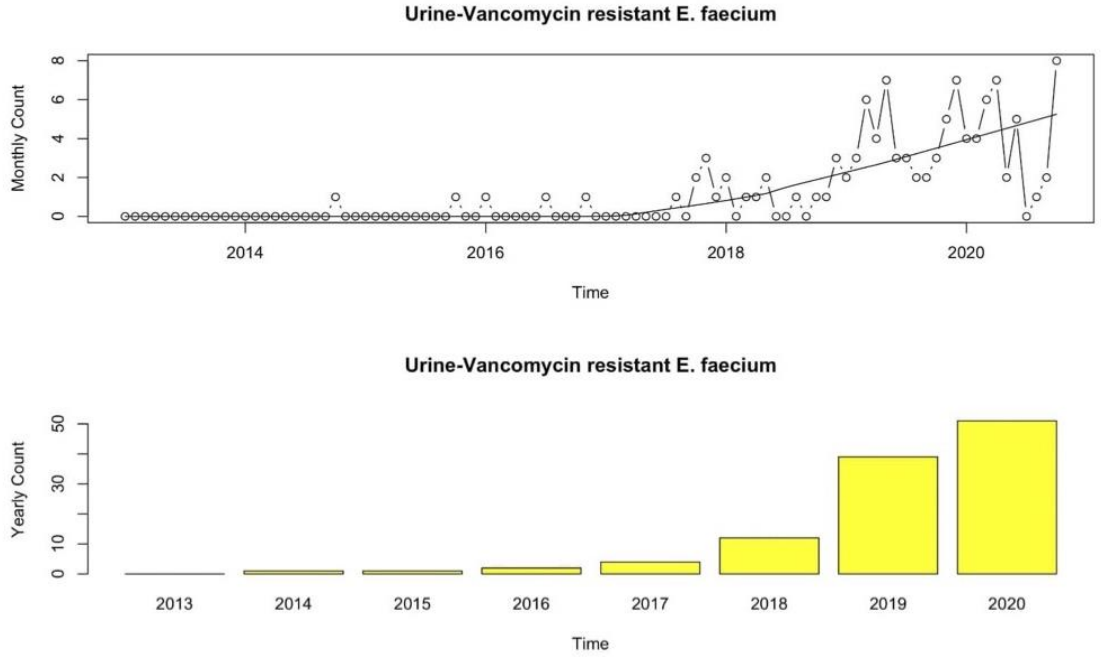
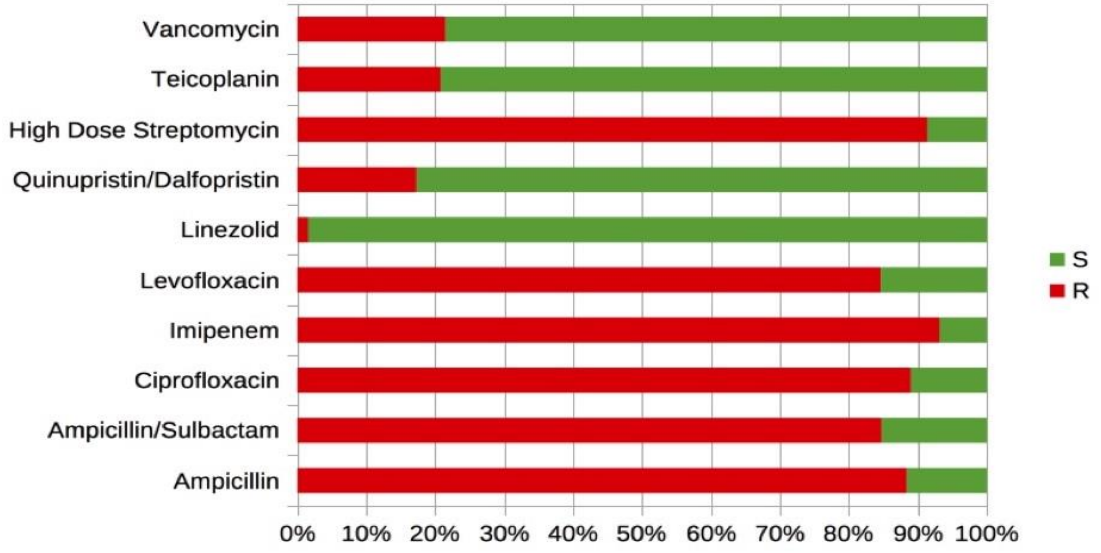
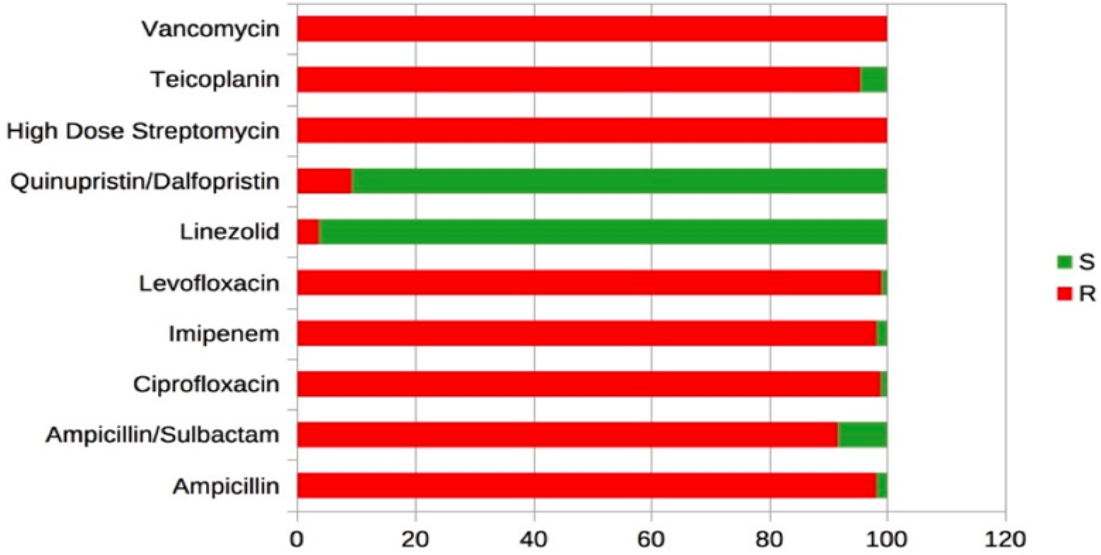


Figure 3. Monthly time series and yearly time series of the vancomycin-resistant *E. faecium* isolated from urinary samples. The trend of the monthly time series was estimated by Lowess smoothing with smoothing span value 0.6 (black line).

Regarding susceptibility results, *E. faecium* strains showed resistance to high-dose streptomycin (91.36%), levofloxacin (84.63%), imipenem (93.18%), ampicillin (88.41%), quinupristin/dalfopristin (17.26%), and linezolid (1.56%) **(Figure 4a)**. The cumulative antibiogram of the VR-*E. faecium* strains exhibited higher resistance levels than vancomycin-sensitive strains except for quinupristin/dalfopristin (9.18%) **(Figure 4b)**.



a)



b)

Figure 4. a) Cumulative antibiogram of the *E. faecium* strains isolated from urine. S: Sensitive; R: Resistant. b) Cumulative antibiogram of the Vancomycin-resistant E. faecium strains isolated from urine. S: Sensitive; R: Resistant.

The comparison of the resistance rates of *E. faecium* during 2013-2015, 2016-2017, and 2018-2020 showed statistically significant differences for ampicillin (81.75% Vs 88.41% Vs 93.18%, *p value*=0.003, Cramer's V= 0.115), imipenem (86.76% Vs 93.18% Vs 96.59%, *p value*=0.009, Cramer's V= 0.111), levofloxacin (78.99% Vs 84.63% Vs 90.53%, *p value*=0.005, Cramer's V= 0.107), teicoplanin (1.45% Vs 20.78% Vs 38.85%, *p value*<0.001, Cramer's V= 0.286), and vancomycin (1.45% Vs 21.48% Vs 38.78%, *p value*<0.001, Cramer's V= 0.282) **(Table 1)**.

Table 1. Evaluation of the resistance rates of *E. faecium* isolated from urines in the years 2013-2015, 2016-2017, and 2018-2020.

Antibiotic	2013-2015 (R%)	2016-2017 (R%)	2018-2020 (R%)	*P-value*	Corrected *P- value*	Cramer's V
Ampicillin	81.75	88.41	93.18	0.003	S	0.115
Ampicillin Sulbactam	80.88	84.77	90.00	0.283	NS	0.079

Ciprofloxacin	84.06	89.01	90.61	0.316	NS	0.064
Imipenem	86.76	93.18	96.59	0.009	S	0.111
Levofloxacin	78.99	84.63	90.53	0.005	S	0.107
Linezolid	0.00	1.56	2.65	0.131	NS	0.066
Quinupristin Dalfopristin	14.60	17.26	17.91	0.711	NS	0.030
Streptomycin High	89.71	91.36	92.80	0.622	NS	0.033
Teicoplanin	1.45	20.78	38.85	0.000	S	0.286
Vancomycin	1.45	21.48	38.78	0.000	S	0.282

Note. S: Statistically significant after Benjamini-Hochberg's correction; NS: Not statistically significant after Benjamini-Hochberg's correction; (R%): percentage of resistant strains.

VR-E. faecium strains were isolated from 56 females (19.51%) and 54 males (24.00%), with no statistically significant difference in the prevalence (*p value*=0.234). The median age of men and women was 70 years old (Interquartile range [IQR]: 54.00-81.00) and 72 years old (IQR: 57.00-80.00), respectively, with the age difference not statistically significant (Krsukal-Wallis *p value*=0.095). However, vancomycin resistance was associated with the patient's age in univariate analysis. In particular, among 2/41 (4.88%) *E. faecium* collected from patients younger than 20 years old were vancomycin-resistant, while among 107/470 (22.76%) strains collected from patients ≥ 20 years old were vancomycin-resistant (*p value*= 0.005, Odds ratio=0.174, 95% Confidence Interval: 0.020-0.693).

Considering the different hospital wards, it is worthwhile to consider that the maximum prevalence of VR-*E. faecium* strains was found in ICU (16/48, 33.33%), followed by hematology ward (9/38, 23.68%), medical wards (75/341, 22.00%), surgery wards (10/61, 16.39%), and neonatology (0/24, 0.00%) (p value=0.009).

The post hoc test by Fisher's exact test and Benjamini-Hochberg's correction revealed the statistically significant differences in the prevalence of neonatology compared to the other hospital wards) **(Table 2)**.

Table 2. Prevalence of the vancomycin-resistant *E. faecium* strains and comparison by Fisher's test. The p-values were corrected by Benjamini-Hochberg's correction for multiple *p-values*.

Ward	***E. faecium* Vancomycin-resistant N (%)**	*Hematology*	*Surgery*	*Medical*	*Neonatology*
Hematology	9 (23.68%)				
Surgery	10 (16.39%)	0.483^{NS}			
Medical	75 (22.00%)	0.837^{NS}	0.483^{NS}		
Neonatology	0 (0.00%)	0.032^{S}	0.111^{NS}	0.031^{S}	
ICU	16 (33.33%)	0.483^{NS}	0.111^{NS}	0.167^{NS}	0.007^{S}

Note. S: Statistically significant after Benjamini-Hochberg's correction; NS: Not statistically significant after Benjamini-Hochberg's correction; N: number; (%): percentage of resistant strains.

Finally, the logistic regression analysis confirmed the presence of a background trend (*p value*<0.001) for vancomycin resistance (OR: 2.223, 95%CI:1.858-2.725). On the contrary, age < 20 and neonatology ward were not confirmed as protective factors for vancomycin resistance **(Table 3)**.

Table 3. Evaluation of the association of the vancomycin resistance of the *E. faecium* strains with neonatology ward, sex, age of the patients, and year by logistic regression analysis.

Variable	**P-value**	**Odds Ratio**	**95% CI**
Intercept	<0.001	0.002	0.000-0.006
Ward: Neonatology	0.984	0.000	0.000-6157.528
Sex (M Vs F)	0.188	1.392	0.851-2.283
Age<20	0.150	0.303	0.043-1.323
Year	<0.001	2.223	1.858-2.725

Enterococci are considered one of the leading causes of hospital-acquired infections since they can be responsible for several clinical pathological conditions such as UTIs and bloodstream infections which have been associated with a significant burden of morbidity and mortality [17].

Because of the limited efficacy of monotherapy treatment, enterococcal infections are generally treated with antibiotic combinations. Ampicillin *plus* aminoglycoside is a common approach against susceptible strains. However, this treatment has become quite problematic for the increasing high-level resistance to the aminoglycosides [18]. *E. faecium* strains are

highly resistant to ampicillin and streptomycin (>80%) [19, 20]. To overcome this issue, other therapies have been actively investigated [21], such as vancomycin, but its inappropriate use has led to the emergence of vancomycin-resistant strains responsible for infections difficult to treat and consequently associated with high mortality [22]. For these reasons, *VR-E. faecium* has been cataloged as a high-priority pathogen in an attempt to incentive new active research to provide new antibiotic discoveries [9].

A prerequisite for the presence of *VR-E. faecium* infections are the intestinal colonization and the transmission patient-to-patient, highlighting, therefore, the need for quick surveillance. Xanthopoulou *et al.* reported that 263/16350 (1.6%) patients were colonized by *VR-E. faecium* between 2014 and 2018 and their prevalence steadily increased from 0.8% to 2.6% [23]. Bressan *et al.* in their study analyzed *VR-E. faecium* strains were collected from rectal swabs since May 2014 and reported a quick increase responsible for an endemic outbreak in the Trieste University Hospital. In particular, they detected a single major clone, belonging to the sequence type 17, with a likely low virulence, in fact only 5/104 *VR-E. faecium* patients got infected, but 3 of them died [24].

The retrospective analysis of *E. faecium* strains from urine samples revealed an alarming increase in vancomycin resistance in our hospital between 2013 and 2020. Increased resistance against vancomycin and teicoplanin (0.282, medium Cramer's V, and 0.286, medium Cramer's V, respectively) was found.

Although there was not an increase over time, the resistance against quinupristin/dalfopristin (17.26%) remained higher than the values reported by Deshpande *et al.* in their multi-center study (European and North American strains 10% and 0.6%, respectively) [25]. On the contrary, the rate of linezolid resistance remained low (1.56%) in agreement with other studies [26].

VR-E. *faecium* easily spreads in hospital settings through medical equipment and surfaces, as it survives and persists for a long time representing a continuous source of transmission if no regular preventive disinfection is performed. Another source of infection is colonized health workers and/or patients [27, 28]. For this reason, active screening of patients should be implemented for their isolation to limit the spread [29].

According to our data, vancomycin resistance does not appear to be related to gender but the patient's age (≥ 20 years) and specific hospital wards. The highest prevalence of VR-E. faecium strains were found in ICU, followed by the hematology department, medical departments, surgery departments, and neonatology. Clinicians should be aware of the local VR-*E. faecium* trend in vancomycin resistance to choose the best empirical antibiotic therapy for a better patients outcome, implement strategies to suppress emerging resistance and reduce all risk factors.

Although the increased incidence of *E. faecium* and VR-E. *Faecium* isolates in urine samples started in 2019, the peak was recorded at the time of the Sars-Cov-2 pandemic. Other previous pandemics of viral respiratory infections, such as Middle Eastern respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-1, have reported bacterial coinfection that complicates early viral respiratory disease [30, 31]. Regarding the Sars-Cov-2 pandemic, there is significant heterogeneity among studies evaluating the prevalence of bacterial infections in these patients [32]. The increasing trend could be explained by the additional difficulty for healthcare professionals in treating patients with COVID-19 wearing personal protective equipment. COVID-19 has also led to an increase in the use of biocides worldwide, which could contribute to the antibiotic pressure on microorganisms [33].

In this scenario, comparing the pre-pandemic levels of antibiotic resistance to today may be of particular interest to establish possible effects of treatment programs for bacterial infections. Despite the absence of clinical data and outcomes of the patients, the increasing trend of VR-*E. faecium* highlights the need for an active surveillance system and control strategies to avoid the uncontrolled outbreak.

Conclusion

The retrospective study of urinary *E. faecium* isolates revealed an increasing trend of vancomycin resistance in the University Hospital of Bari during the observed period from 2013 to 2020.

This highlights the need for an active surveillance system and control strategies to avoid outbreaks inwards and prevent infections in critically ill patients.

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Conflict of interest: None

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Ethics statement: This study follows the 305 ethical standards of the institutional and/or national research committee and the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Sample and patients' information (date of sampling, ward, type of specimen, testing results, sex) have been recorded in an anonymous database by transforming sensitive data into alphanumeric codes. No clinical data associated with these specimens were available.

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