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# Antimicrobial Resistance of the Genus Enterococcus in Cantonal Hospital of Zenica, Bosnia and Herzegovina

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**Abstract**

Among *Enterococcus spp*, only the virulence gene harboring strains of *Enterococcus faecalis* and *E. faecium* are associated with human infections, including urinary tract infections (UTI), pelvic, blood, intraabdominal, and skin and soft tissue infections (SSTI). Over the past decades, enterococcal antimicrobial resistance has escalated in many regions of the world, leading to ominous outcomes. The rising incidence of Healthcare-Associated Infections (HCAIs) secondary to Vancomycin-resistant strain (VRE) resulted in high morbidity and mortality, as well as substantial challenges in control, prevention, and management). The aim of this study is to examine the antimicrobial resistance *of E. faecalis* and *E. faecium* species in different human samples. The study included 184 clinical samples over a period of 6 months. *E. faecalis* was identified in 95.65% and *E. faecium* in 4.35% of cases. *E. faecalis* isolates showed resistance to gentamicin in 40.9% of cases and to ampicillin in 1.7% of cases. Resistance to nitrofurantoin and ciprofloxacin was observed in 6.1% and 35.7% of *E. faecalis* isolates. VRE was isolated in 1.1% of *E. faecalis* isolates tested for this antibiotic. Resistance of *E. faecium* isolates to ampicillin and gentamicin was observed in 87.5% of cases in both antibiotics. All urinary isolates of *E. faecium* were resistant to ciprofloxacin. All *E. faecium* isolates were sensitive to vancomycin. Based on the results of our study, the growing importance of *Enterococcus spp*. as a causative agent of hospital infections and infections in the general population, and its antimicrobial resistance to various drugs were observed.

**Keywords**: antimicrobial resistance; *Enterococcus; E. faecalis*; *E. faecium*; antibiogram

**Резюме**

Сред *Enterococcus* spp. само щамовете на *Enterococcus faecalis* и *E. faecium*, притежаващи ген за вирулентност, се свързват с инфекции при човека, включително инфекции на пикочните пътища (ИПП), тазови инфекции, инфекции на кръвта, интраабдоминални инфекции и инфекции на кожата и меките тъкани (ИКМТ). През последните десетилетия антимикробната резистентност на ентерококите ескалира в много региони на света, което води до зловещи последици. Нарастващата честота на инфекциите, свързани с медицинското обслужване (ИСМО), вторично причинени от щам, резистентен към ванкомицин (VRE), доведе до висока заболеваемост и смъртност, както и до значителни предизвикателства в контрола, превенцията и управлението). Целта на това проучване е да се изследва антимикробната резистентност на видовете *E. faecalis* и *E. faecium* в различни човешки проби. Проучването включва 184 клинични проби за период от 6 месеца. *E. faecalis* е идентифициран в 95.65%, а *E. faecium* - в 4.35% от случаите. Изолатите на *E. faecalis* показват резистентност към гентамицин в 40.9% от случаите и към ампицилин в 1.7% от случаите. Резистентност към нитрофурантоин и ципрофлоксацин се наблюдава при 6.1% и 35.7% от изолатите *E. faecalis*. VRE е изолиран при 1.1% от изолатите *E. faecalis*, изследвани за този антибиотик. Резистентност на изолатите *E. faecium* към ампицилин и гентамицин се наблюдава в 87.5% от случаите и при двата антибиотика. Всички уринарни изолати на *E. faecium* са резистентни към ципрофлоксацин и чувствителни към

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ванкомицин. Въз основа на резултатите от нашето проучване се наблюдава нарастващото значение на *Enterococcus* spp. като причинител на болнични инфекции и инфекции в общата популация, както и неговата антимикробна резистентност към различни лекарства.

## Introduction

Bacteria of the *Enterococcus* genus are gram-positive and facultative anaerobic organisms. Enterococci are usually characterized by individual, paired, or short-chain, catalase-negative cocci. To date, more than 40 *Enterococcus* species have been described and constitute a widespread group of bacteria (Yin *et al*., 2018). These bacteria are normal inhabitants of the gastrointestinal tract, oral cavity, and female genital tracts in both humans and animals. Human enterococcus species are gastrointestinal commensals with low pathogenicity but capable of causing invasive diseases with significant morbidity and mortality, particularly in hospitalized patients with premorbid conditions, invasive procedures, and the immunocompromised (Ali *et al*., 2022).

Several virulence and pathogenicity factors were described from the genus *Enterococcus* such as those that enhance their ability to colonize human tissues, increase resistance to antibiotics, and aggravate infection outcomes (Toru *et al*., 2018). Recently, *Enterococcus* species emerged as important pathogens causing hospital-acquired and community-acquired infections (García-Solache and Rice, 2019). Moreover, the relative importance of the bacteria increased with the occurrence of high-level resistance to multiple antimicrobials, which is a serious problem for clinical anti-infective therapy. The emergence of vancomycin-resistant *Enterococci* species (VRE) is alarming for the global community because the options for treatment are sparse (Ahmed and Baptiste, 2018).

The two main pathogenic species, *Enterococcus faecalis* and *E. faecium* account for most of the clinical pathology with the former being widely associated with the community while the latter with Healthcare-Associated Infections (HCAIs). Among *Enterococcus* spp., only the virulence gene harboring strains of *E. faecalis* and *E. faecium* are associated with human infections, including urinary tract infections (UTI), pelvic, blood, intraabdominal, and skin and soft tissue infections (SSTI) (Tian *et al*., 2019).

The rising incidence of Healthcare-Associated Infections (HCAIs) secondary to Vancomycin-resistant strain (VRE) resulted in high morbidity and mortality, as well as substantial challenges in control, prevention, and management (Kirkizlar *et al*., 2020).

Due to the advanced and modern medical technology in more recent years, many patients with multiple comorbidities and immunosuppressive conditions have been subjected to a multitude of invasive interventions, which resulted in an increasing global prevalence of enterococcus bloodstream infections (BSIs) (Bhatt *et al.,* 2015).

The aim of this study is to examine the antimicrobial resistance of *E. faecalis* and *E. faecium* species in different human samples in inpatient and outpatient groups.

## Material and Methods

*Collection of specimens*

This study included a collection of 184 clinical samples from patients, processed and analyzed in the Microbiology laboratory of the Cantonal Hospital in Zenica, Bosnia and Herzegovina (BiH), over a period of 6 months (from September 2021 to February 2022). Clinical samples included inpatient and outpatient samples. Inpatient samples were taken from different departments of the Zenica Cantonal Hospital. Outpatient samples were taken from various Health Centers of the Zenica-Doboj Canton.

Clinical samples included urine culture, vaginal swab, wound swab, blood culture, cervical canal swab, vulva swab, sputum, sperm culture, urethral swab, axillary smear, groin smear, pleural and abdominal punctate. *E. faecalis* and *E. faecium* were isolated from these samples.

*Bacterial isolation and identification*

All samples were inoculated on MacConkey (bioMe´rieux; France), cysteine lactose electrolyte deficient agar (HIMEDIA, Mumbai, India), and sheep blood agar (SBA), incubated for 24 hrs at 37°C. Colony morphology and culture characteristics were observed macroscopically. Further identification was done with Gram stain, catalase production, esculin hydrolysis, salt tolerance (ability to grow in 6.5% NaCl broth), and L-pyrrolidonyl-b-naphthylamide (PYR) testing (England PH, 2014). *E. faecalis* ATCC 29212 was used as a positive control.

*Antimicrobial susceptibility testing*

The modified disc-diffusion method of Bauer and Kirby was used to determine sensitivity/resistance of *E. faecalis* and *E. faecium* isolates according to EUCAST (The European Committee on Antimicrobial Susceptibility Testing) standards to the following antibiotics: ampicillin (10 μg), ciprofloxacin (5 µg), vancomycin(30 µg), nitrofurantoin, and gentamycin 120 (120 µg).

Muller-Hinton agar plates were inoculated with 0.5 McFarland standard suspension of the strains, antimicrobial disks were placed into plates and then incubated at 37°C for 24 hours. Zone diameters were assessed according to the Clinical Laboratory Standard Institute guidelines (CLSI, 2019). All clinical samples were tested for ampicillin, vancomycin, and gentamicin, whereas only the clinical samples from patients with uncomplicated UTI were tested for ciprofloxacin and nitrofurantoin, as per the guidelines of EUCAST 2022. Nitrofurantoin is used in the treatment of UTIs caused by *E. fecalis*, and therefore only the urinary isolates of *E. fecalis* were tested for this antibiotic.

As stated in the EUCAST guidelines, if *E. fecalis* and *E. faecium* are resistant to ampicillin, the same can be applied to ureidopenicillins and imipenem. Although resistance to ampicillin predicts the result of the imipenem test, this is not the case when these bacteria are sensitive to ampicillin. Gentamicin 120 sensitivity test is a test for high-level resistance to aminoglycosides for the genus *Enterococcus*. A positive test means that the tested strain is highly resistant to gentamicin and other aminoglycosides, except for streptomycin, which requires special testing. A negative test means that the tested strain shows a low degree of intrinsic resistance to gentamicin, which does not necessarily apply to other aminoglycosides.

*Statistical analysis*

The collected data were processed in the software program Microsoft Excel 2010. To test hypotheses, the software program *MedCalc* Software was used, whereby the p-value was obtained using Chi2 - statistical test with 95% confidence interval - CI (Confidence interval).

**Results**

Out of a total of 184 samples, 45 (24.5%) were male and 139 (75.5%) were female. The largest number was isolated from patients over 50 years of age (94 isolates) and the lowest number of isolates was detected in patients aged 7-15 years. *E. faecalis* was isolated in 176 (95.65%) clinical samples, while *E. faecium* was isolated in 8 (4.35%) clinical samples.

We compared hospital samples (clinical samples taken from inpatients from different departments of the Zenica Cantonal Hospital) and outpatient samples (clinical samples that were taken from various Health Centers of the Zenica-Doboj Canton). Out of a total of 128 outpatient samples, *E. faecalis* was isolated from 126, and *E. faecium* from 2 outpatient samples. *E. faecalis* was isolated from 50, *E. faecium* from 6 hospital samples, out of a total of 56 hospital samples.

Table 1 shows the distribution of *Enterococcus spp*. isolates in hospitalized patients. Out of a total of 56 hospital isolates of *Enterococcus spp,* most were from the Department of Internal Medicine including hemodialysis patients (27 isolates or 48.2%).

The largest number of isolates were from urine culture (102), then from vaginal swabs (35), wound swabs (23), swabs of the cervical canal (6), vulval swabs (6), haemocultures (5) and others: sperm culture (1), sputum (1), urethral swab (1), pleural puncture (1), axillary swab (1), abdominal puncture (1) and swab groin (1).

Figure 1 shows a comparison of antibiotic resistance (%) of *E. faecalis* and *E. faecium* isolates. *E. faecalis* isolates showed a high-level gentamicin resistance, which was observed in 40.9% of cases, while 1.7% of cases were resistant to ampicillin, 6.1%, and 35.7% were resistant to nitrofurantoin and ciprofloxacin, respectively.

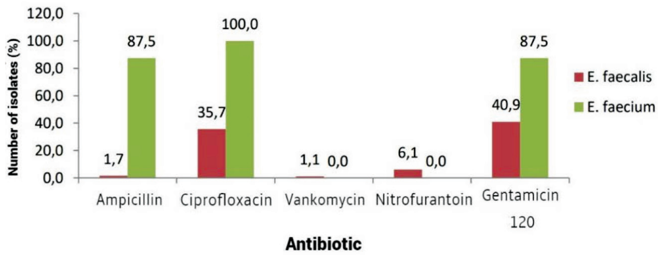


**Fig. 1.** Comparison of antibiotic resistance of *E. faecalis* and *E. faecium* isolates

A VRE strain was isolated in 1.1% of the isolates *E. faecalis*, while all *E. faecium* isolates were sensitive to vancomycin. Resistance of *E. faecium* isolates to ampicillin and gentamicin was observed in 87.5% of cases for both antibiotics. All *E. faecium* isolates were resistant to ciprofloxacin. It was observed that *E. faecium* shows significantly higher resistance to ampicillin, ciprofloxacin, and gentamicin compared *to E. faecalis* ($p<0.05$; 95% CI).

Based on a total number of resistant *E. faecalis* and *E. faecium* isolates, the *Enterococcus* genus showed a high level of resistance to gentamicin in 42.9% of cases, 38.2% of urinary isolates of *Enterococcus spp*. were resistant to ciprofloxacin, while resistance to ampicillin was observed in 5.4% of cases (Fig. 2).

Out of a total of 184 *Enterococcus spp*. isolates, 43 isolates were found to be resistant to two

**Table 1.** Distribution of *Enterococcus spp*. isolates in Zenica Cantonal Hospital departments

| Departments | Number | % |
|---|---|---|
| Department of internal medicine with haemodialysis* | | |
| Diabetology | 7 | 12.5 |
| Pulmonology | 6 | 10.7 |
| Haematology | 4 | 7.1 |
| Nephrology | 4 | 7.1 |
| Cardiology | 2 | 3.6 |
| Gastroenterology | 2 | 3.6 |
| Intensive care | 2 | 3.6 |
| Total | 27 | 48.2 |
| Department of infectious diseases | 7 | 12.5 |
| Department of anaesthesia, resuscitation and intensive care | 4 | 7.1 |
| Department of physical medicine and rehabilitation | 4 | 7.1 |
| Department for surgical diseases | 3 | 5.4 |
| Department of gynaecology and obstetrics | 3 | 5.4 |
| Department of orthopaedics and traumatology | 2 | 3.6 |
| Department of dermato-venerology | 2 | 3.6 |
| Department of plastic and reconstructive surgery | 2 | 3.6 |
| Department of psychiatry | 1 | 1.8 |
| Department of Otorhinolaryngology and Maxillofacial Surgery | 1 | 1.8 |
| Total | 56 | 100.0 |

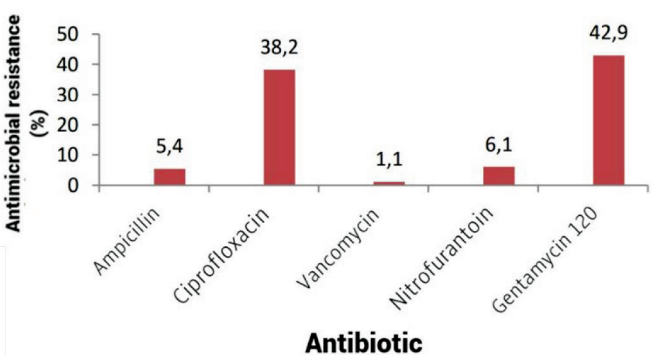


**Fig. 2.** Antibiotic resistance of *Enterococcus* genus.

or more antibiotics (Fig. 3). It was found that 35 isolates of *E. faecalis* were resistant to two antibiotics. Out of the above-mentioned isolates, 35 isolates were resistant to ciprofloxacin and gentamicin simultaneously (30), then to ciprofloxacin and nitrofurantoin (3), ampicillin and vancomycin (1) and vancomycin and gentamicin (1). Only 3 isolates of *E. faecium* were simultaneously resistant to ampicillin and gentamicin, and one *E. faecium* isolate was resistant to ampicillin, ciprofloxacin, and gentamicin. None of the E.fecalis isolates showed resistance to three antibiotics, and only one *E. faecalis* isolate and three *E. faecium* isolates showed resistance to four antibiotics: ampicillin, ciprofloxacin, nitrofurantoin, and gentamicin.

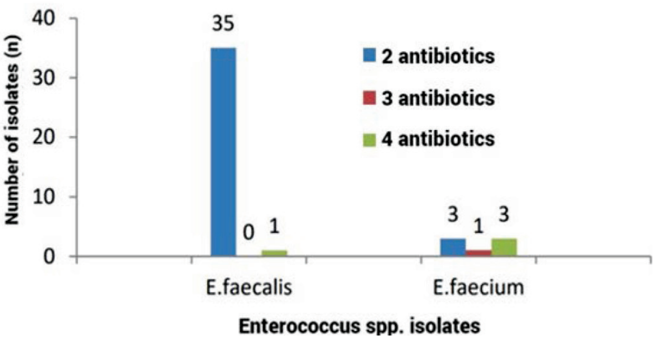


**Fig. 3.** Number of resistant *E. faecalis* and *E. faecium* isolates to two or more antibiotics

While comparing antibiotic resistance between inpatient and outpatient isolates, it was observed that inpatient isolates of *Enterococcus spp.* showed significantly higher resistance ($p<0.05$) to ampicillin, ciprofloxacin, and gentamicin compared to outpatient *Enterococcus spp*. isolates (Fig. 4). There were no inpatient vancomycin-resistant isolates of *Enterococcus spp*. There were 3.6% inpatient and 7.1% outpatient urinary isolates of *Enterococcus spp*. resistant to nitrofurantoin (only *E. faecalis* species).

## Discussion

This study examines the antibiotic resistance of *Enterococcus spp.* isolates (*E. faecalis* and *E. faecium*) found in inpatient and outpatient samples.

Bacteria of the genus *Enterococcus* are part

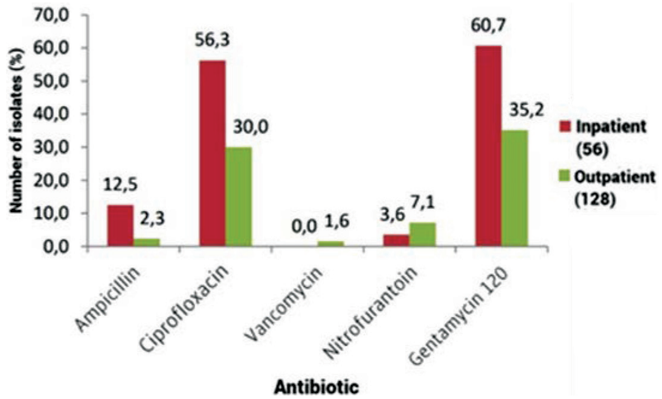


**Fig. 4.** Comparison of antibiotic resistance of inpatient and outpatient *Enterococcus spp*.

of the normal flora of the gastrointestinal tract, vagina, and urethra in humans, but can cause various infections (urinary tract infections, sepsis, bacteremia, endocarditis). Newborns and people with diabetes have an increased risk for infections caused by *Enterococcus* bacteria (Baghdayan *et al*., 2003; Butler, 2006; Fanaro *et al*., 2007; Kao and Kline, 2019; Krawczyk *et al*., 2020). *Enterococcus* bacteria represent a significant problem of the 21st century due to the easy spread of bacteria in hospital conditions, which is why this genus is in the main group of the causative agents of nosocomial infections (García-Solache and Rice, 2019; Rogers *et al*., 2021). In the last few years, an increase in enterococcal resistance to antimicrobial drugs has been observed (Miller *et al*., 2014; Zaheer *et al*., 2020). This was a result of the uncontrolled use of antibiotics in empiric treatment, but also their use for prophylactic purposes in hospitals.

In our study, *E. faecalis* species were isolated in 95.65% of clinical samples, while *E. faecium* species were isolated in 4.35% of clinical samples. Comparable results were observed in other studies from different countries (Udo *et al*., 2003; Emaneini *et al*., 2016). Data from a study done in Italy and other similar studies indicate that the *Enterococcus spp.* isolates are most often isolated from urine culture, vaginal swabs, and wound swabs (Salem-Bekhit *et al*., 2012; Boccella *et al*., 2021), which was also observed in our study.

The largest number of *E. faecalis* isolates were from clinical samples from the Department of Internal Medicine, followed by clinics, the infectious department, and the intensive care unit (Maleki *et al*., 2021). Our study showed similar results which can be associated with long-term use of antibiotics, critical conditions of patients, and patients with weakened immune systems (e.g., dialysis population).

In our study, *E. faecalis* showed resistance in 1.7% of cases, and *E. faecium* showed resistance in 87.5% of cases. This is in concordance with the results from various other studies. (Dan *et al*., 1999; Abamecha *et al*., 2015; Tambić Andrašević *et al*., 2021). These results can be explained by the fact that *E. faecium* is less sensitive to beta-lactam antibiotics compared to *E. faecalis* because penicillin-binding proteins of *E. faecium* species have a lower affinity for beta-lactam antibiotics, and some strains possess a plasmid-encoded beta-lactamase. Some similar studies showed that the frequency of *E. faecalis* resistance to ampicillin was much higher (Mathur *et al.,* 2003; Jabalameli *et al.,* 2009; Salem-Bekhit *et al*., 2012).

Almost all isolates in our study showed high sensitivity to vancomycin, in accordance with data from a similar study in India, Italy, and Turkey (Gupta *et al*., 2015; Çalgın and Çetinkol, 2019; Boccella *et al*., 2021). *E. faecium* showed variable resistance to vancomycin in different countries ranging from 15.8% in Brazil to 75% in Egypt (Al-Tonbary *et al*., 2010; Conceição *et al*., 2011; Kafil and Asgharzadeh, 2014; EFSA and ECDC, 2018; Andrašević *et al*., 2021).

Enterococci resistance to gentamicin is a good predictor of resistance to other aminoglycosides, except for streptomycin. In our study, the majority of *E. faecium* and *E. faecalis* isolates (87.5% and 40.9% respectively) showed resistance to gentamicin which is similar to the data from different countries (Abamecha *et al*., 2015; Labibzadeh *et al*., 2018). Aminoglycosides, such as gentamicin, have a synergistic effect in combination with penicillin or glycopeptides in the treatment of infections caused by enterococci. A synergistic effect is lost if the strain is highly resistant to aminoglycosides (Ascione *et al*., 2019; Aydemir *et al*., 2020). A high level of resistance to gentamicin is associated with higher mortality (Pinholt *et al*., 2014). Data from studies done in Italy and Croatia are different from the data in our study. These studies showed a significantly higher frequency of resistance to gentamicin in *E. faecalis* isolates compared to *E. faecium* isolates (Andrašević *et al*., 2021; Boccella *et al.,* 2021).

There are several reports of increased resistance of enterococci to ciprofloxacin (Kaçmaz and Aksoy, 2005). In our study, all *E. faecium* isolates were resistant to ciprofloxacin, while *E. faecalis* showed resistance to nitrofurantoin in 5.8% of cases and to ciprofloxacin in 27.7% of cases. Matching results were found in similar studies from Croatia and Turkey (Çalgın and Çetinkol, 2019; Andrašević *et al*., 2021). This may be due to the usage of these antibiotics as the first line of uncomplicated treatment of UTI. Quinolones are intended for the treatment of enterococcal infections only if they cause uncomplicated UTIs (Andrašević *et al.*, 2021).

Multi-resistant strains of enterococci show high resistance to penicillin, glycopeptides, fluoroquinolones, and aminoglycosides, which have been identified in most nosocomial infections (Asadi *et al*., 2021). According to a study in Ethiopia (Abamecha *et al*., 2015), the resistance of *E. faecium* inpatient isolates was higher compared to the resistance of inpatient isolates of *E. faecalis*, which corresponds with the data in this study. In our study, significantly higher resistance to ampicillin and ciprofloxacin was observed in *E. faecium* compared to *E. faecalis*, which coincides with data found in a study from China (Jia *et al*., 2014). *E. faecium* showed significantly higher resistance to ampicillin, ciprofloxacin, and gentamicin compared to *E. faecalis*, which is most likely a consequence of a greater ability to change genetic material-carrying genes responsible for antimicrobial resistance. Possible limitations of this study were short follow-up period and small geographical area.

## Conclusion

Based on the results of our study, the growing importance of *Enterococcus spp*. as a causative agent of hospital infections and infections in the general population, and its antimicrobial resistance to various drugs was observed. It can be concluded that *E. faecium* showed statistically significantly higher antimicrobial resistance compared to *E. faecalis*. Also, inpatient isolates showed statistically significantly higher antimicrobial resistance compared to outpatient isolates of the genus *Enterococcus*.

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