

## RESEARCH ARTICLE

# Antimicrobial Resistance of Enterococcus Species Isolated from Urine Cultures

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## Abstract

**Objective:** Enterococcus species are among the common causes of nosocomial urinary tract infections mainly, along with other infections and have been observed to have increasing rates of resistance against antimicrobial agents in recent years. The aim of this study is to determine the Enterococcus spp. isolated from urine cultures in our hospital and to determine antibiotic resistance rates.

**Methods:** Enterococcus isolates identification and antibiogram results obtained from urine samples sent to Ordu University Education and Research Hospital Microbiology laboratory from inpatients and outpatients were retrospectively evaluated. The identification and antimicrobial susceptibility tests of the isolates were completed on a VITEK 2 Compact (Biomérieux, Marcy l'Etoile, France) system. Resistance rates against ampicillin, ciprofloxacin, nitrofurantoin, tigecycline, linezolid, teicoplanin and vancomycin were analyzed.

**Results:** Our study identified 346 Enterococcus strains. These strains were defined as 195 *Enterococcus faecalis* (56%), 127 *Enterococcus faecium* (37%) and 24 other enterococci (7%). The antimicrobials with highest resistance were ciprofloxacin (51%), ampicillin (42%), nitrofurantoin (14%) and tigecycline (1%), in order, with no resistance encountered for linezolid, teicoplanin and vancomycin.

**Conclusion:** Glycopeptide resistance were not encountered among enterococci isolated from urine cultures in our hospital, with quinolone resistance at the fore. The results of antimicrobial susceptibility tests are important to select appropriate treatments.

**Key words:** antimicrobial resistance, Enterococcus species, urine culture

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## Introduction

With the reduction in the efficacy of antibiotics, hospitals around the world have seen increasing numbers of infections due to drug-resistant bacteria. Effective treatment of these infections is more difficult, which causes morbidity and mortality in the patient and increasing health care costs. The most common antimicrobial resistant hospital pathogens are *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacter species and are called ESKAPE (Rice, 2008). In humans the enterococcus species causing most infections are *Enterococcus faecalis* (80-90%) and one of the two gram positive

ESKAPE pathogens of *Enterococcus faecium* (5-10%). These bacteria are among the common causes of nosocomial urinary tract infections mainly, along with other infections (Aykut Arca et al., 2009). Enterococcus species carry a range of intrinsic and acquired resistance genes and may transfer these genes to other bacteria (van Harten et al., 2017). Enterococci may be resistant to commonly used antibiotics including ampicillin and vancomycin and currently resistance has begun to be determined against last-chance antibiotics like daptomycin and linezolid (Gonzales et al., 2001; Long et al., 2005; van Harten et al. 2017).

Due to increasing resistance against commonly used antibiotics, note should be taken of culture results for antimicrobial treatment of Enterococcus infections and the regional resistance phenotypes should be considered when deciding on empirical antibiotic treatment until culture results are obtained. This study aimed to identify the resistance status against a variety of antimicrobials of Enterococcus isolates from urine samples in Ordu University Education and Research Hospital microbiology laboratory.

### Methods

From January 2014 to June 2018, enterococci isolates identifications and antibiogram results obtained from urine samples sent to Ordu University Education and Research Hospital microbiology laboratory from inpatients and outpatients were retrospectively evaluated.

Midflow urine samples taken under appropriate conditions from patients with preliminary diagnosis of urinary tract infections were inoculated on the surface of 5% sheep's blood agar (Salubris, Istanbul, Turkey) using standard loops taking 1 µl urine. The media plates were incubated in an aerobic environment for 18-24 hours at 37 °C and cultures with single type proliferation and colony account 10<sup>5</sup> CFU/ml were taken for investigation. The identification and antimicrobial susceptibility

tests of the obtained isolates were completed in line with Clinical and Laboratory Standards Institute (CLSI) until January 2017 and then in line with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations on a VITEK 2 Compact (Biomérieux, Marcy l'Etoile, France) system. Resistance rates against ampicillin, ciprofloxacin, nitrofurantoin, tigecycline, linezolid, teicoplanin and vancomycin were analyzed. Strains with intermediate resistance were accepted as resistant. Only one strain from each patient was included in the study. For quality control, the *E. faecalis* ATCC 29212 standard strain was used.

### Results

Our study identified 346 enterococci strains. These strains were identified as 195 *E. faecalis* (56%), 127 *E. faecium* (37%) and 24 other enterococci (7%) (9 *E. gallinarum*, 7 *E. casseliflavus*, 3 *E. avium*, 3 *E. raffinosus*, 2 *E. durans*).

The *E. faecalis* strains were most common among outpatients, while *E. faecium* strains were isolated mainly from the intensive care units, with the clinical distribution of these strains given in Table 1.

**Table 1.** Distribution of *E. faecalis* and *E. faecium* strains according to clinics

Clinics	<i>E. faecalis</i>	<i>E. faecium</i>
Polyclinics	149	42
Intensive Care Units	20	51
Internal medicine Services	17	22
Surgical Services	6	7
Pediatric Services	3	5
Total	195	127

The antimicrobial resistance rates for the isolated *E. faecalis* and *E. faecium* strains are shown in Table 2.

## Resistance of Enterococcus Species

Table 2: Resistance rates of *E.faecalis* and *E.faecium* strains

Antibiotic	<i>E.faecalis</i>			<i>E.faecium</i>			Total*		
	Number of strains	Resistant n	Resistant %	Number of strains	Resistant n	Resistant %	Number of strains	Resistant n	Resistant %
Ampicillin	170	-	0	124	124	100	294	124	42
Ciprofloxacin	184	56	30	114	95	83	298	151	51
Tigecycline	182	-	0	103	3	3	285	3	1
Nitrofurantoin	36	3	8	15	4	27	51	7	14
Linezolid	192	-	0	122	-	0	314	-	0
Teicoplanin	184	-	0	124	-	0	308	-	0
Vancomycin	191	-	0	121	-	0	312	-	0

n :Number, %:Percent, \* Sum of *E.faecalis* and *E.faecium*

### Discussion

Enterococci are bacteria forming the normal flora in the gastrointestinal system, vagina and urethra of humans but may also cause a variety of infections. They may survive for long periods on inorganic material like stethoscopes, door handles, and beds in the hospital environment. As a result, enterococci may cause epidemics as a hospital infection vector carried on both inorganic materials and from patient to patient by health personnel (Butler, 2006). In recent years, the observation of an increase in vancomycin resistant strains in isolation and the variation in antibiotic susceptibility according to species have led to the requirement for species level identification. Studies about the topic have shown that in urine samples generally *E. faecalis* isolation rates are higher compared to *E. faecium* (Yuksel Ergin et al., 2013; Etiz et al., 2014; Yenisehirli et al., 2016). In our study the results were similar with 56% *E. faecalis* and 37% *E. faecium* identification rates.

Compared to other Enterococcus species, *E. faecalis* is found at higher rates in feces. Epidemiologic studies in recent years have shown that the presence of these bacteria in normal intestinal flora is a basic risk factor for the spread of enterococci from patient to patient and even between hospitals (Butler, 2006). Some studies have found higher rates of *E. faecium* strains among enterococci from hospital isolates compared to *E. faecalis* (Aykut Arca et al., 2009). In our study, *E. faecalis* was isolated more from outpatients, while *E. faecium* was isolated more from inpatients. This situation may be linked to the ability of *E. faecium*, found at high rates in intestinal microbiome, to spread between patients.

Treatment of Enterococcus infections has become complicated since the emergence of strains

with high levels of resistance to nearly all antibiotics used in clinical practice, especially aminoglycosides,  $\beta$ -lactams and glycopeptides. Enterococci are the most common vector for infections of the urinary tract. Enterococci are known to be more resistant to antimicrobials affecting inhibition of cell wall synthesis compared to other streptococci and the use of penicillin or ampicillin is recommended for susceptibility tests (Murray, 1997). Studies in Turkey have noted that enterococci are increasingly resistant to beta-lactam antibiotics and a variety of studies have reported ampicillin resistance from 16% to 84% (Baykan, 2001; Agus et al., 2006; Aktepe et al., 2011; Kalayci et al., 2011; Yuksel Ergin et al., 2013; Etiz et al., 2014). In our study, rates of 42% were between these two values.

Quinolones are found to have limited efficacy against enterococci. As a result, though they are effective in vitro, their use for treatment of infections caused by these bacteria is limited (Gordon et al., 1992). Ciprofloxacin is approved for use for both uncomplicated and complicated urinary tract infections, including cystitis, pyelonephritis, and chronic bacterial prostatitis (Andriole, 2005). Among enterococci infections, they are among alternative treatment choices only for urinary tract infections (Gordon et al., 1992). A variety of studies have identified ciprofloxacin resistance from 48% to 87% (Baykan, 2001; Yavuz et al., 2006; Aktepe et al., 2011; Kalayci et al., 2011; Yuksel Ergin et al., 2013; Etiz et al., 2014). In our study, ciprofloxacin resistance was close to the lower limit at 51%.

Tigecycline is an antibiotic derived from the first member of the glycyclines of minocycline and a promising new antibiotic of last resort, active against many bacteria including *Enterococcus* spp. (Tunger, 2012). Studies by Karaoglan et al. and

Aktepe et al. did not report resistance for enterococci against tigecycline, while Etiz et al. reported 0.3% resistance (Karaoglan et al., 2008; Aktepe et al., 2011; Etiz et al., 2014). In our study, *Enterococcus* spp. resistance to tigecycline was 1%, and all resistant isolates were observed to be *E. faecium*. Nitrofurantoin may be used for uncomplicated urinary tract infections (Tunger, 2012). The use of nitrofurantoin with appropriate indications will reduce the use of new antimicrobials and as a result their risk of resistance developing. In our study, 14% rates of resistance against nitrofurantoin were encountered and this resistance was observed more for the *E. faecium* isolates.

Glycopeptides are still known as the most effective antibiotics against enterococci, with increased rates of vancomycin and teicoplanin resistant strains reported (Agus et al., 2006; Yuksel Ergin et al., 2013; Etiz et al., 2014). In this study, vancomycin and teicoplanin resistance was not encountered among *E. faecalis* and *E. faecium* isolates.

Linezolid is an oxazolidinone group antibiotic effective against many gram positive bacteria including vancomycin-resistant enterococci (VRE) (Dilek et al., 2007). It is recommended for treatment of infections caused by VRE (Contreras et al., 2019). In our study, similar to many studies performed to date, linezolid resistance was not encountered among enterococci (Dilek et al., 2007; Aktepe et al., 2011; Yuksel Ergin et al., 2013). Contrary to this, Etiz et al. in a 2014 study found 5% resistance to linezolid and reported the necessity to use this agent with appropriate indications and sufficient doses by performing antibiotic susceptibility tests, as for other antimicrobials to prevent development of resistance (Etiz et al., 2014).

### Conclusion

Enterococci have become microorganisms threatening health and causing problems with treatment today. They are resistant to many drugs and the increase in this resistance will continue to increase problems like causing difficult clinical infections. As a result, as for all infection agents, rational antibiotic use is necessary for treatment of infections linked to *Enterococcus* species.

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