TURKISH JOURNAL OF VETERINARY RESEARCH
TJVR 2018;2(1):14-18
Published online at www.dergipark.gov.tr/tjvr
Research Article

The Presence and Prevalence of *Enterococcus faecalis* and *Enterococcus faecium* Strains in Urine and Stool Samples

Ismail Hakki EKİN[1] Sevil ATES[1] Gülşah TOLLU[2] Ozgul GULAYDIN[1]

Kemal GURTURK[1] Ziya ILHAN[3] Cihat OZTURK[1]

[1]Van Yuzuncu Yıl University, Faculty of Veterinary Medicine, Department of Microbiology, Van, Turkey

[2] Mersin University,Technical Sciences Vocational School, Mersin, Turkey

[3] Balıkesir University, Faculty of Veterinary Medicine, Department of Microbiology, Balikesir, Turkey

Received: 18.02.2018 Accepted: 24.03.2018

ABSTRACT

Objective: In this study, it was aimed to investigate the presence and the prevalence of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from the urine and stool samples.

Materials and Methods: A total of 500 routine urine and feces samples were used for testing as the study materials, and a total of 349 *Enterococcus* spp. were collected for investigation. For the isolation, blood agar and bile esculin agar were used. DNA isolations of the 24-hour growth cultures of possible enterococci were carried out using a DNA isolation kit.

Results: Out of 350 routine urine and 150 stool samples taken with the approval of the patients, 235 (67.1%) and 114 (76%) *Enterococcus* spp. were isolated respectively. Using the multiplex PCR method with species specific primers, 136 (57.8%) of urine and 22 (19.2%) of stool originated enterococcal strains were identified as *Enterococcus faecalis*; on the other hand, 17 (7.2%) of urine and 61 (53.5%) of stool originated enterococci were identified as *Enterococcus faecium*.

Conclusion: As a result of the study in Van, Turkey, the isolation rate of *Enterococcus faecalis* and *Enterococcus faecium* strains were found to be lower than other regions.

Keywords: Stool, Urine, *Enterococcus faecium*, *Enterococcus faecalis*, Prevalence

INTRODUCTION

Enterococci that are at high rates in the gastrointestinal tract in humans, survive as normal flora and fewer rate on the genitourinary system and oral cavity (Huycke et al., 1998). Enterococci frequently lead to intra-abdominal infection, endocarditis and commonly less meningitis, skin and soft tissue infections. Bacteria can survive in varying temperature, pH and even in the presence of some bactericidal detergents outside the host. Enterococci are increasingly prevalent, especially in nosocomial infections and even in some cases which held responsible for nosocomial bacteremia (Lautenbach et al., 1999; Patterson, 2000; Shepard and Gilmore, 2002).

Although enterococci are low virulent microorganisms, they are important in community-

Corresponding author: *Ozgul GULAYDIN* ***E-mail***: *ozgul.1988@hotmail.com*

based and especially hospital-acquired infections. Cytolysin produced by some strains of *E. faecalis* and *E. faecium*, shows hemolysin activity for human and animal erythrocytes (Moellering, 2005; Yıldırım, 2007).

There are many reasons for enterococci to become important pathogens (Butler, 2006). Increased rate of sensitive population (immunodeficiency and critical diseases), increased intravascular catheter use, prolonged hospitalization and intensive use of antibiotics (especially cephalosporins and anti-staphylococcal penicillins) have made enterococci naturally resistant to antimicrobial agents. Enterococcal infections were previously thought to be endogenous infections originating from the patient's own flora. However, studies have shown that enterococci may also spread by exogenous pathways. Resistant enterococcal species that can be transmitted to the patient from hospitalized patient or by the colonized hospital staff, can easily spread within the hospital or between hospitals (Moellering, 1992; Herwaldt and Wenzel, 1995).

Among the Enterococcus species, *E. faecalis* and *E. faecium* were reported to be the most isolated and *E. durans, E. gallinarum, E. avium, E. casseliflavus, E. raffinosus, E. solitarius* and *E. hirae* as less isolated species (Schouten et al., 1999; Rodrigues et al., 2002).

E. faecalis and *E. faecium* which have also been isolated from the mouth, jugular vein system, vagina, food, vegetables, feed, urinary system infections, wounds, peritoneal fluid, deep pelvic abscess, endocarditis and blood cultures are members of the gastrointestinal flora in humans and cattle (Barrie et al., 1990; Chenoweth and Schaberg, 1990; Eliopoulos and Eliopoulos, 1990; Koneman et al., 1997). They are also found in the gastrointestinal tract of warm-blooded animals and humans, in insects, plants as well as in soil, water and food contaminated with feces. Therefore, they are used as indicator microorganisms in fecal contamination in drinking and using waters. In human feces *E. faecalis* (10^5-10^7 cfu/gr) is more common than *E. faecium* (10^4-10^5 cfu/gr), while *E. faecium* is more resistant to antibiotics, especially in the hospital setting (Fisher and Phillips, 2009; Hijazi et al., 2009).

Enterococci have been reported to be the second most common causative agents of nosocomial infections in various countries. *E. faecalis*, as the most common cause of infectious diseases, constitutes 85-95% of enterococci isolated from clinical specimens (Zouain and Araj, 2001).

Some studies have reported the isolation rate of *E. faecalis* between 44-74% and the *E. faecium* between 19-49% (Kaçmaz et al., 2003; Yazgı et al., 2003; Baylan et al., 2011; Özseven et al., 2011; Ergin et al., 2013; Vural et al., 2014).

This study aims to investigate the presence and prevalence of *Enterococcus faecalis* and *Enterococcus faecium* strains in routine urine and stool specimens brought to the microbiology laboratory of Van Region Training and Research Hospital and Erciş State Hospital using multiplex PCR technique.

MATERIALS AND METHODS

Urine and feces samples: In the study, a total of 500 routine urine and feces samples submitted to the microbiology laboratory of the Van Region Training & Research Hospital and Erciş State Hospital, Van, Turkey for testing on suspicion of urinary tract and gastrointestinal tract infection were used as the study material.

Bacteria isolates and reference strains: In the study, a total of 349 *Enterococcus* spp. were collected for investigation. *E. faecalis* ATCC® 29212, *E. faecalis* ATCC® 51299, and *E. faecium* ATCC® 19434 reference strains (MicroBiologics®, MediMark Europe/France) were used as quality control and standard strains for the identification.

Bacterial isolation: For the isolation of *E. faecalis* and *E. faecium* strains, urine and feces samples were inoculated with sterile swabs on blood agar and bile esculin agar. Cultures were incubated 24-48 hours at 37°C under aerobic conditions. S-type, esculin-positive and black-colored colonies were assessed (Koneman et al. 2005).

Preliminary identification: Isolates selected for assessment were examined using the Gram staining method. Gram-positive and catalase-negative chain shaped cocci were pre-identified as possible *Enterococcus* spp. (Koneman et al., 2005).

DNA isolation: E. faecalis ATCC® 29212, *E. faecalis* ATCC® 51299, and *E. faecium* ATCC® 19434 reference strains were used as positive control in the test. DNA isolations of the 24-hour growth cultures of possible enterococci were carried out using a DNA isolation kit (Thermo GeneJET Genomic DNA Purification Kit – K0722 Lithuania) and in accordance with the protocol proposed by the manufacturer for Gram-positive bacteria.

Primer design: Using the GenBank database, primers were verified and designed based on 16S and/or 23S rRNA gene-coding specific DNA sequences (Table 1) (Jackson et al., 2004). Multiplex PCR processes were carried out following the method proposed by Kariyama et al. (2000) and Jackson et al. (2004).

Species	Oligo	Amplicon lenght (bp)
E. faecalis	**F:** 5'ACT TAT GTG ACT AAC TTA ACC 3' **R:** 5' TAA TGG TGA ATC TTG GTT TGG 3'	360
E. faecium	**F:** 5' GAA AAA ACA ATA GAA GAA TTA T 3' **R:** 5' TGC TTT TTT GAA TTC TTC TTT A 3'	215

Table 1 Species-specific primers used in the study

Amplification: For the analyses of *E. faecalis* and *E. faecium* isolates, the multiplex PCR method was employed using species-specific primers. Mastermixes (Thermo PCR Mastermix 2x – K0171 Lithuania) were used for the amplification step. Accordingly, 2 µl of bacterial DNA and 1-µl forward and 1-µl reverse primers were added into the mastermix of 25 µl and then, the volume of the mixture was brought to 50 µl by adding PCR grade water. The PCR process comprised a pre-denaturation at 96°C for 10 min, followed by 35 cycles of a protocol involving 45-s denaturation at 95°C, 45-s annealing at 49 °C, 60-s extension at 72 °C and a final extension of 10 s at 72°C.

Agarose gel electrophoresis: The products obtained after the amplification step were run on a 1% agarose gel with the positive controls and examined using an imaging system (Genesis®). When evaluating the bands produced after electrophoresis, whether the *E. faecalis* positive isolates produced a band of 360 bp and whether *E. faecium* positive isolates produced a band of 215 bp were investigated (Jackson et al., 2004).

RESULTS

Isolation and Preliminary Identification

The Gram-positive, catalase-negative and esculin-positive cocci isolated from the urine and feces samples were regarded as possible *Enterococcus* spp. A total of 349 *Enterococcus* spp. were isolated and identified, including 235 (67.1%) from 350 urine and 114 (76%) from 150 stool specimens investigated in the study (Table 2).

Samples		***Enterococcus* spp. isolation**	
Type	n	n	%
Urine	350	235	67.1
Feces	150	114	76
Total	500	349	69.8

Table 2 Distribution of Enterococcus spp. cultures isolated from the urine and stool samples

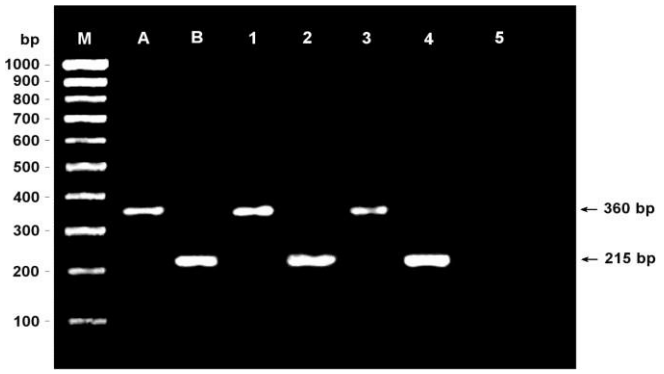

Figure 1: Agarose gel image of the isaletes with the multiplex PCR method (M: PCR ranger 100 bp DNA marker; A: E. faecalis-positive control; B: E.faecium-positive control; 1: E.faecalis-positive feces sample; 2: E.faecium-positive feces sample; 3: E

Identification of the *E. faecalis* and *E. faecium* with multiplex PCR

According to the identifications carried out using the PCR method with species-specific primers (Figure 1), a total 158 *E. faecalis* strains, 136 (57.8%) from urine and 22 (19.2%) from stool samples and a total 78 *E. faecium* species, 17 (7.2%) from urine and 61 (53.5%) from stool samples, were identified (Table 3, Figure 2).

Enterococcus spp		***Enterococcus faecalis***		***Enterococcus faecium***	
Source	**n**	**n**	**%**	**n**	**%**
Urine	235	136	57.8	17	7.2
Feces	114	22	19.2	61	53.5
Total	349	158	45.2	78	22.3

Table 3 Distribution of E. faecalis and E. faecium cultures according to Enterococcus spp isolates

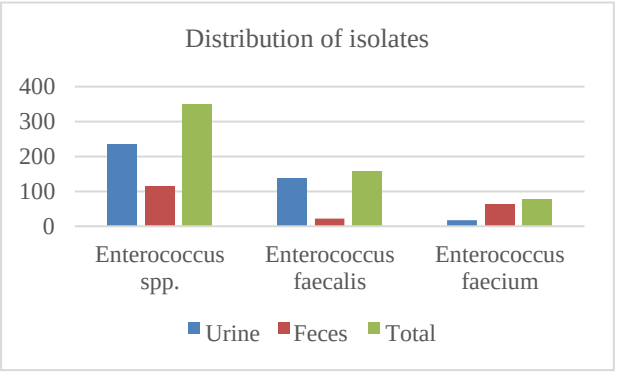

Figure 2 Distribution of E. faecalis and E. faecium cultures according to Enterococcus spp. isolates

DISCUSSION

In addition to surviving as normal flora in the gastrointestinal tract of humans and animals, enterococci also inhabit the skin, oral cavity, lower respiratory tract, and urogenital system (Huycke et al., 1998). Despite their low virulence, after a disturbance in the immunity of the host, opportunistic characteristics of enterococci emerge as primary or secondary agents in infections such as urinary tract infections, meningitis, endocarditis, intraabdominal, skin, and soft tissue infections (Schouten et al., 1999; Devriese et al., 2006).

Studies have shown that enterococci are the second major cause of nosocomial infections in different countries. Of the enterococci isolated from samples, 85 - 95% are E. faecalis, one of the most frequently encountered infectious agents, while 5-10% are E. faecium (Teixeria and Facklam, 2003).

Different isolation ratios were obtained in clinical samples used in different studies from Turkey. Özseven et al. (2011) reported in their study of 124 enterococci cultures isolated from urine samples, 48% were E. faecalis and 49% were E. faecium; Baylan et al. (2011) reported in their study of 91 enterococci strains isolated from the urine samples collected, 64.8% were identified as E. faecalis and 34.1% as E. faecium; Yazgı et al. (2003) reported in their study of 116 enterococci strains isolated from the rectal swab samples from 163 patients, 57.7% were identified as E. faecalis, while 38.8% were identified as E. faecium; Kaçmaz et al. (2003) reported in their study of 62 enterococci isolates from various clinical samples, 74% were E. faecalis and 19% were E. faecium; Ergin et al. (2013) reported in their study of enterococci strains isolated from urine samples, 44.7% were identified as E. faecalis and 38.3% as E. faecium.

In this study, in the isolations carried out with the multiplex PCR method using species-specific primer, E. faecalis was identified in 38.8% of 350 urine samples and 14.6% of 150 feces samples, while E. faecium was identified in 4.8% of 350 urine samples and 40.6% of 150 feces samples. In the study, E. faecium isolation ratio in the urine samples and E. faecalis isolation ratio in the feces samples were considerably low, which attributes to the differences in isolation methods, infection status, and hygiene habits of the individuals.

In conclusion, the present study conducted in Van, Turkey, demonstrates lower isolation rate of E. faecalis and E. faecium strains compared to the other regions in Turkey.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

Barrie PS, Christou NV, Patchen DE (1990). Patologenicity of the enterococcus in surgical infections. *Annals of Surgery,* 212, 155-159.

Baylan O, Nazik H, Bektöre B, Çitil BE, Turan D, Öngen B, Özyurt M, Açikel CH, Haznedaroğlu T (2011). Üriner enterokok izolatlarının antibiyotik direnci ile virulens faktörleri arasındaki ilişki. *Mikrobiyol Bul*, 45, 3, 430-445.

Chenoweth C, Schaberg D (1990). The epidemiology of enterococcus. *Eur J Clin Microbiol Infect Dis,* 9, 80-89.

Devriese L, Baele M, Butaye P (2006). The Genus Enterococcus: Taxonomy. *Prokaryotes.*4, 163-174.

Eliopoulos GM, Eliopoulos CT (1990). Therapy of Enteococcal Infections. *Eur J Clin Microbiol Infect Dis,* 9,118-126.

Ergin ÖY, Bayram ED, Uzun B, Güngör S, Demirdal T (2013). İdrar kültürlerinden izole edilen *Enterococcus* türleri ve antibiyotik dirençleri. *ANKEM Derg,* 27, 4, 173-178.

Fisher K, Phillips C (2009). The ecology, epidemiology and virulence of *Enterococcus.* Microbiol, 155, 1749-1757.

Herwaldt LA, Wenzel RP (1995). Dynamics of hospital-acquired infection. Manual of Clinical Microbiology. 6th edition, Washington D.C.: American Society for Microbiology, pp: 169-181.

Hijazi N, Elmanama AA, Al-Hindi A (2009). Vancomycin-resistant enterococci in fecal samples from hospitalized patients and non-hospitalized individuals in Gaza City. *J Public Health*, 17, 243-249.

Huycke MM, Sahm DF, Gilmore MS (1998). Multiple-drug resistant enterococci: the nature of the

problem and an agenda for the future. *Emerg Infect Dis,* 4, 2,239-249.

Jackson CR, Fedorka-Cray PJ, Barrett JB (2004). Use of a genus- and species-specific multiplex PCR for identification of *Enterococci*. *J Clin Microbiol*, 42, 8, 3558-3565.

Kaçmaz B, Akça G, Çağlar K, Sultan N (2003). Enterokoklarda antimikrobiyel duyarlılık. *ANKEM Derg,* 17, 28-32.

Kariyama R, Mitsuhata R, Chow JW, Clewell DB, Kumon H (2000). Simple and reliable multiplex PCR assay for surveillance isolates of vancomycin-resistant enterococci. *J Clin Microbiol*, 38, 3092-3095.

Koneman EW, Allen SD, Janda WM (1997). The Gram Positive Cocci Part II Streptococci, Enterococci and The Streptococci Like Bacteria. In Color Atlas and Textbook of Diagnostic Microbiology, 5th Ed. Philadelphia: Lippincott, pp: 577-629.

Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Procop G, Woods G (2005). Color Atlas and Textbook of Diagnostic Microbiology. Sixth edition. Philadelphia: JB Lippincott Co 700-711 pp.

Lautenbach E, Bilker W, Brennan P (1999). Enterococcal bacteremia: Risk factors for vancomycin resistance and predictors of mortality. *Infect Control Hosp Epidemiol* 20, 318-323.

Moellering RC (1992). Emergence of *Enterococcus* as a significant pathogen. *Clin Infect Dis,* 14, 1173-1178.

Moellering RC (2005) *Enterococcus* species, *Streptococcus bovis* and *Leuconostoc* species. In: Mandell GL, Bennett JE, Dolin R, Ed. Principles and Practice of Infectious Diseases. Fifth edition. New York. Churchill Livingstone, pp: 2411-2421.

Özseven AG, Sesli Çetin E, Cicioğlu Arıdoğan B, Çiftçi E, Özseven L (2011). Çeşitli klinik örneklerden izole edilen enterokok suşlarının antibiyotik duyarlılıkları. *Ankem Derg,* 25,4,256-262.

Patterson JE (2000). New Gram positive agents in nosocomial infection. *Curr Opin Infect Dis*, 13, 6, 593-598.

Rodrigues J, Poeta P, Martins A, Costa D (2002). The importance of pets as reservoirs of resistant *Enterococcus* strains, with special reference tovancomycin. *JVet MedB*, 49, 6, 278-280.

Schouten MA, Vose A, Hoogkamp-Karstanje JAA (1999). Antimicrobial susceptibility patterns of enteroccocci causing infections in Europe. *Antimicrob Agents Ch,* 43, 10, 2542-2546.

Shepard BD, Gilmore MS (2002). Antibiotic resistant enterococci: the mechanisms and dynamics of drug introduction and resistance. *Microbes Infect,* 4, 215-224.

Teixeira LA, Facklam RR (2003). Enterococcus. In: Murray PR, Baron EJ. Pfaller MA, Tenover FC, Yolken RH (eds). Manual of Clinical Microbiology. Eighth edition, Washington. ASM Pres. pp: 422-433.

Vural DG, Temiz H, Aktar GS, Onur A, Ayaydın Z, Turhanoğlu M, Vural H (2014). Çeşitli klinik örneklerden izole edilen enterokok suşlarının antibiyotik direnç oranları, 29.Ankem Antibiyotik ve Kemoterapi Kongresi, Bodrum, 28-31 Mayıs 2014, *ANKEM Derg,* 28, Ek 1, 26.

Yazgı H, Ertek M, Uslu H, Kadanalı A, (2003). Enterokoklarda yüksek düzey aminoglikozid direnci ile beta laktamaz üretimi ilişkisi. *Türk Mikrobiyol Cem Derg,* 33, 4, 333-336.

Yıldırım M (2007). Enterokoklar ve enterokoklarla gelişen infeksiyonlar. *Düzce Üniv Tıp Fak Derg*, 2, 46-52.

Zouain MG, Araj GF (2001). Antimicrobial resistance of enterococci in Lebanon. *Int J Antimicrob Agents,* 17, 3, 209-213.