RESEARCH NOTE

THE EVALUATION OF ANTIMICROBIAL SUSCEPTIBILITY OF URINE ENTEROCOCCI WITH THE VITEK 2 AUTOMATED SYSTEM IN EASTERN TURKEY

Sibel AK[1], Köroğlu Mehmet[1] and Muharrem AK[2]

[1]Malatya State Hospital, Malatya; [2]Department of Family Medicine, Faculty of Medicine, Inonu University, Malatya, Turkey

Abstract. Antibiotic resistant enterococci are an emerging problem, especially in urinary tract infections. The aim of the present study was to evaluate the antimicrobial susceptibility of 118 enterococci isolates from urine samples of patients admitted to Malatya State Hospital, a secondary care hospital in eastern Turkey. The Vitek 2 automated sytem was used to identify the bacteria and detect antimicrobial susceptibility to ten antibiotics: ampicillin, imipenem, ciprofloxacin, moxifloxacin, quinupristin-dalfopristin, tetracycline, tigecyclin, linezolid, vancomycin, teicoplanin and high level aminoglycoside resistance (HLAR) against kanamycin, gentamicin and streptomycin. The predominant species was *Enterococci faecalis* (74.5%) followed by *Enterococcus faecium* (18.6%). The resistance rates for *Enterococcus faecalis* and *E. faecium*, were 54.5%/77.2% for ampicillin, 0/77.2% for imipenem, 18.1%/72.7% for both ciprofloxacin and moxifloxacin, 10.2%/9.1% for linezolid and 65.1%/5.2% for quinopristine-dalfopristin, respectively. Beta-lactamase production was detected in 54.5% of *E. faecalis* isolates. HLAR was also found in 54.5% of *E. faecalis* isolates and 36.3% of *E. faecium* isolates; kanamycin resistance comprised the highest proportions (39.7% and 9.1%) of these resistance rates. Five strains were resistant to and one had intermediate resistant to vancomycin. The highest resistance rates were against ampicillin, ciprofloxacin, moxifloxacin and tetracycline. Of the antimicrobial agents evaluated, vancomycin, teicoplanin and tigecycline had the lowest resistance rates.

Keywords: *Enterococcus* spp, urinary tract infection, antimicrobial susceptibility, HLAR, Vitek 2

INTRODUCTION

Enterococci are gram-positive, catalase negative, non-spore forming, facultative anaerobes that grow as diplococci in short chains. For many years these bacteria were believed to be harmless since they are normal residents of the gastrointestinal tract of humans (Franz *et al*, 1999). Recently, *Enterococcus* spp have become known as an important cause of both nosocomial and community acquired infections, owing to its increasing antibiotic resistance to different groups

Correspondence: Dr Sibel AK, Malatya State Hospital, Malatya, Turkey.
Tel: 090 422 326 1569; Fax: 090 422 325 34 38
E-mail: sibel_mr@hotmail.com

of antibiotics, including beta-lactams, aminoglygosides and glycopeptides (Patterson *et al*, 1995; Courvalin, 2006). Most enterococcal infections are associated with *Enterococcus faecalis (E. faecalis)* and *Enterococcus faecium (E. faecium)* at a ratio of 80-90% to 10-15%, respectively (Ruoff *et al*, 1990). Identification of the bacteria to the species level has become necessary due to the intrinsic antibiotic resistance that bacteria possess.

Although these bacteria can be isolated from polymicrobial wound infections, intraabdominal and pelvic abscesses; urinary tract infections (UTI) are the most common site of infection with enterococci in all age groups (Barros *et al*, 2009). Most UTI associated with enterococci are complicated and related to structural abnormalities or urinary tract instrumentation. Since untreated UTI can be a source for bloodstream infections, identification and appropriate antimicrobial are important for recovery (Winn *et al*, 2006).

We aimed to determine the antimicrobial susceptibility of *Enterococcus* spp isolated from urine samples.

MATERIALS AND METHODS

From June 2008 to September 2010, a retrospective study was carried out at Malatya State Hospital, a secondary care hospital in Malatya Province, Turkey. One hundred eighteen enterococci isolates were obtained from urine of 118 patients (inpatients and outpatients) and included in the study. Each isolate studied was from a different patient. Cultures which yielded $\geq 10^5$ CFU/ ml of urine were used for the evaluation.

Routine laboratory methods were used for urine analysis and cultures (Thomson, 2007). Identification of the bacteria was based on macroscopic and microscopic properties (Gram stain, colony morphology, type of hemolysis, catalase test and PYR test) and confirmed with the Vitek 2 automated system (bioMérieux, Marcy l'Etoile, France). *Enterococcus faecalis* reference strain, ATCC 29212 recommended by the Clinical Laboratory Standards Institute (CLSI), was used as a quality control for antimicrobial susceptibility tests. Susceptibility was tested for ten antibiotics: ampicillin, imipenem, ciprofloxacin, moxifloxacin, quinopristin-dalfopristin, tetracycline, tigecycline, linezolid, vancomycin and teicoplanin and high level aminoglycoside resistance (HLAR) was tested for kanamycin, gentamicin and streptomycin using Vitek 2 AST (Antibiotic Susceptibility Test) cards designed for gram-positive cocci. MIC (minimal inhibitory concentration) values for the antibiotics were evaluated according to CLSI recommendations for enterococci except for tigecycline, which was performed with the automated system (CLSI, 2008). MIC values for imipenem and moxifloxacin were determined using European Committee on Antimicrobial Susceptibility Testing (EUCAST). The Food and Drug Administration (FDA) set point was used for tigecycline (Brown and Traczewski, 2007).

RESULTS

One hundred eighteen enterococci isolates obtained from urine samples were included in the study. *E. faecalis* was the predominant enterococcus species (74.5%), followed by *E. faecium* (18.6%), *E. gallinarum* (4.2%) and other species (2.7%).

Beta-lactam resistance

Ampicillin resistant enterococci were identified in 71 isolates (60.1%). Acquired penicillinase was the leading cause (54.5%) of beta-lactam resistance

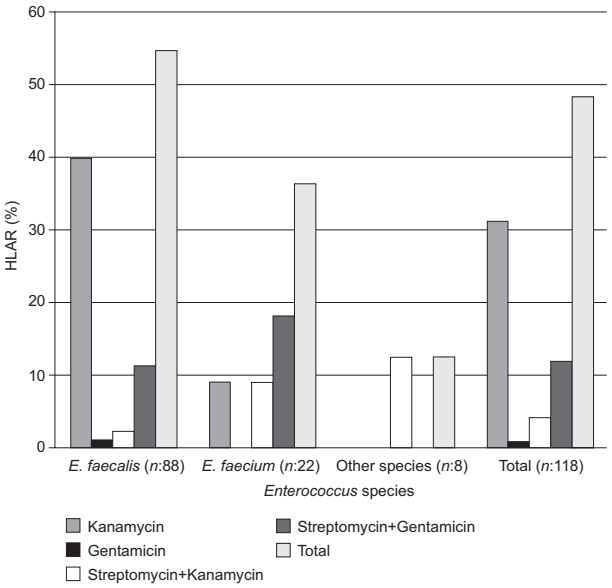

Fig 1–High level aminoglycoside resistance (HLAR) rates among isolates.

detected in *E. faecalis* isolates, followed by modified penicillin binding protein (mPBP), detected in 7 *E. faecium* isolates. All *E. faecalis* isolates were sensitive to imipenem, but 17 *E. faecium* isolates (77.2%) were resistant.

High level aminoglycoside resistance

Of the 118 isolates 57(48.3%) had HLAR. The most frequently resistance was against kanamycin, found in 35 of 48 *E. faecalis* isolates (72.9%) and 2 of 22 *E. faecium* isolates (9.1%). Ten of 88 *E. faecalis* isolates (11.3%) and 4 of 22 *E. faecium* isolates (18.1%) were resistant to both gentamicin and streptomycin. Of the isolates resistant to only gentamicin or streptomycin, there was only one isolate each (Fig 1).

Glycopeptide resistance

Of 88 *E. faecalis* isolates 4 were resistant to vancomycin (one with intermediate resitance; MIC:8 µg/ml). Of 22 *E. faecium* isolates, 1 was resistant to vancomycin and of 5 *E. gallinarum* isolates, 1 was resistant to vancomycin; 4 were VanA type also resistant to teicoplanin and 2 were VanB sensitive to teicoplanin.

The resistance rates against vancomycin, teicoplanin and tigecycline were 5.08, 3.3, and 0%, respectively (Table 1). While the resistance rates against ampicillin, ciprofloxacin, moxifloxacin and tetracycline were 60.1, 30.5, 28.8 and 66.9%, respectively.

DISCUSSION

Urinary tract infections (UTI) are encountered frequently in clinical practice (Kucheria *et al*, 2005). Although the most common agent responsible for this condition is *Escherichia coli*, enterococci are a leading gram-positive bacterium cause of UTI (Winn *et al*, 2006). The frequency of enterococcal UTI varies by study. Koeijer *et al* (2010) found enterococci were a cause of 9% of UTI among males. Daza *et al* (2001) found the frequency of enterococcal UTI was 6% for community acquired UTI. They (Daza *et al*, 2001) found *Enterococcus* spp were the most frequent gram-positive uropathogen. In our study, 6.8% of isolates were *Enterococcus* spp among 1,714 urine culture specimens. A possible reason for the low frequency may be patient characteristics. Enterococcal UTI are frequently associated with anatomical abnormalities or urinary catheterization (Bratcher, 2001). Most of our samples were collected from outpatients with uncomplicated UTI.

Although enterococci are susceptible on *in vitro* tests evaluating trimethoprim-sulfamethoxazole (SXT), intrinsic resistance can cause resistance to SXT since this antibiotic has been widely used as

Table 1
Antimicrobial susceptibility of isolates.

	Number of resistant or intermediate resistant isolates (%)				
	E. faecalis *n*=88	*E. faecium* *n*=22	*E. gallinarum* *n*=5	*E. durans*/*E. hirae* *n*=3	Total *n*=118
Ampicillin	48 (54.5)	17 (77.2)	5 (100)	1 (33.3)[a]	71 (60.1)
Imipenem	0	17 (77.2)	4 (80)	1 (33.3)[a]	22 (18.6)
Ciprofloxacin	16 (18.1)	16 (72.7)	3 (60)	1 (33.3)[a]	36 (30.5)
Moxifloxacin	16 (18.1)	16 (72.7)	1 (20)	1 (33.3)[a]	34 (28.8)
Tetracycline	74 (84.1)	2 (9.1)	2 (40)	1 (33.3)[a]	79 (66.9)
Vancomycin	4 (4.5)	1 (4.5)	1 (20)	0	6 (5.1)
Teicoplanin	3 (3.4)	0	1 (20)	0	4 (3.3)
Linezolid	9 (10.2)	2 (9.1)	1 (20)	0	12 (10.1)
Quinupristin/dalfopristin	56 (65.1)	1 (5.2)	0	0	57 (50.4)
Tigecycline	0	0	0	0	0

[a] The resistant isolate was *E. durans*

empirical treatment for UTI (Wisell *et al*, 2008). It should be remembered these bacteria are responsible for most UTI caused by gram-positive bacteria. Beta lactams used as second line treatment for community acquired UTI, are not effective against enterococci due to different resistance mechanisms. For *E. faecalis* isolates, resistance caused by beta-lactamases can be treated with beta-lactamase inhibitors, such as clavulanic acid or sulbactam. With *E. faecium*, beta-lactam resistance can give rise to resistance against imipenem. In our study 54.5% of *E. faecalis* isolates had ampicillin resistance probably due to acquired penicillinase. We did not find imipenem resistance in any of the 88 *E. faecalis* isolates. Of the 22 *E. faecium* isolates 17 (77.2%) were resistant to both ampicillin and imipenem. Fortunately these species are less commonly a cause of UTI.

Ampicillin resistance by *E. faecalis* in our study is different from the finding of other studies (Kaçmaz and Aksoy, 2005; Protonotariou *et al*, 2010). The reason for the higher ampicillin resistance by *E. faecalis* in our study is probably beta-lactamase activity detected by the Vitek 2 gram-positive susceptibility cards in all ampicillin resistant isolates.

Beta-lactams and aminoglycosides are usually combined to enhance penetration of the bacterial cell wall and to eliminate tolerance to the bactericidal effect of the beta-lactams (Çetinkaya *et al*, 2000; Adhikari, 2010). This synergystic effect is ineffective with acquired penicillinase or HLAR leading to treatment failure.

In the present study all isolates with acquired beta-lactamase had HLAR to at least one aminoglycoside. Rudy *et al* (2004) found HLAR rates of 17% and 29% among *E. faecalis* and *E. faecium* isolates, respectively. In contrast, we detected HLAR rates of 54.5% and 36.3% for *E. faecalis* and *E. faecium*, respectively. This difference may be due to the kanamycin resistance not evaluated in the study by Rudy *et al* (2004). In our study, 72.9% of *E. faecalis* and 25% of *E. faecium* isolates

had high level kanamycin resistance. HLAR rates were greater among *E. faecalis* than *E. faecium* isolates. All 48 beta-lactamase producing *E. faecalis* isolates also had HLAR concurrently. HLAR genes may be transferred with acquired penicillinase genes on plasmids.

Glycopeptide resistance among enterococci may cause 20% in clinical treatment failure and a 25% rise in mortality rates (Brown *et al*, 2006). Vancomycin resistance rates have been reported as 0.9-5% world-wide (Fluit *et al*, 2000; Turnidge *et al*, 2002; Deshpande *et al*, 2007). In our study six clinical isolates (5.1%) were resistant or intermediately resistant to vancomycin. The vancomycin resistance rates were 4.5% for both *E. faecalis* (4/88) and *E. faecium* (1/22). The remaining resistant isolate was *E. gallinarum*. Four of these isolates were encoded by *vanA* and two were encoded by *vanB*. The *vanA* isolates were resistant to teicoplanin and the *vanB* isolates were sensitive to teicoplanin. *VanA* and *vanB* vancomycin resistance genotypes are transmissible among bacteria, unlike chromosomally encoded *vanC* (Arthur and Courvalin, 1993). Three out of the 6 resistant isolates were susceptible to linezolid. The remaining three were susceptible to fluoroquinolones. No significant differences in susceptibility rates were found between ciprofloxacin and moxifloxacin. All six isolates were susceptible to tigecycline. Tigecycline is active against vancomycin susceptible *E. faecalis in vitro* and in clinical infections, but tigecycline is only active against *E. faecium in vitro*. The clinical effectiveness of tigecycline against *E. faecium* is still unclear.

In conclusion, of the 10 antibiotics studied, tigecycline was the only antimicrobial agent to which all the isolates were susceptible. Enterococci had the highest resistance rate (66.9%) against tetracycline. Antimicrobial susceptibility testing of enterococci having beta lactamase production and high levels of resistance should be conducted in order to prevent treatment failure.

REFERENCES

Adhikari L. High level aminoglycoside resistance and reduced susceptibility to vancomycin in nosocomial enterococci. *J Glob Infect Dis* 2010; 2: 231-5.

Arthur M, Courvalin P. Genetics and mechanisms of glycopeptide resistance in enterococci. *Antimicrob Agents Chemother* 1993; 37: 1563-71.

Barros M, Martinelli R, Rocha H. Enterococcal urinary tract infections in a university hospital: Clinical studies. *Braz J Infect Dis* 2009; 13: 294-6.

Bratcher DF. Vancomycin-resistant enterococci in the pediatric patient. *Pediatr Infect Dis J* 2001; 20: 621-2.

Brown DFJ, Brown NM, Cookson B, *et al*. National glycopeptide-resistant enterococcal bacteraemia surveillance Working Group report to the Department of Health – August 2004. *J Hosp Infect* 2006; 62 (suppl 1): 1-27.

Brown SD, Traczewski M, Cookson B, *et al*. Comparative *In vitro* antimicrobial activity of tigecycline, a new glycylcycline compound, in freshly prepared medium and quality control. *J Clin Microbiol* 2007; 45: 2173-9.

Çetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. *Clin Microbiol Rev* 2000; 13: 686-707.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; Eighteenth informational supplement. Document M100-S18. Wayne: CLSI, 2008.

Courvalin P. Vancomycin resistance in Gram-positive cocci. *Clin Infect Dis* 2006; 42 (suppl 1): S25-34.

Daza R, Gutiérrez J, Piédrola G. Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections. *Int J Antimicrob Agents* 2001; 18: 211-5.

Deshpande LM, Fritsche TR, Moet GJ, Biedenbach DJ, Jones RN. Antimicrobial resistance and molecular epidemiology of vancomycin-resistant enterococci from North America and Europe: a report from the SENTRY Antimicrobial Surveillance Program. *Diagn Microbiol Infect Dis* 2007; 58: 163-70.

Fluit AC, Jones ME, Schmitz FJ, Acar J, Gupta R, Verhoef J. Antimicrobial resistance among urinary tract infection (UTI) isolates in Europe: results from the SENTRY Antimicrobial Surveillance Program 1997. *Antonie Van Leeuwenhoek* 2000; 77: 147-52.

Franz CMAP, Holzapfel WH, Stiles ME. Enterococci at the crossroads of food safety. *Int J Food Microbiol* 1999; 47: 1-24.

Kaçmaz B, Aksoy A. Antimicrobial resistance of enterococci in Turkey. *Int J Antimicrob Agents* 2005; 25: 535-8.

Koeijers JJ, Verbon A, Kessels AGH, *et al.* Urinary tract infection in male general practice patients: Uropathogens and antibiotic susceptibility. *Urology* 2010; 76: 336-40.

Kucheria R, Dasgupta P, Sacks SH, *et al*. Urinary tract infections: new insights into a common problem. *Postgrad Med J* 2005; 81: 83-6.

Patterson JE, Sweeney AH, Simms M, *et al*. An analysis of 110 serious enterococcal infections. Epidemiology, antibiotic susceptibility, and outcome. *Medicine (Baltimore)* 1995; 74: 191-200.

Protonotariou E, Dimitroulia E, Pournaras S, Pitiriga V, Sofianou D, Tsakris A. Trends in antimicrobial resistance of clinical isolates of *Enterococcus faecalis* and *Enterococcus faecium* in Greece between 2002 and 2007. *J Hosp Infect* 2010; 75: 225-7.

Rudy M, Nowakowska M, WiechuBa B, Zientara M, Radosz-Komoniewska H. Antibiotic susceptibility analysis of *Enterococcus* spp. isolated from urine. *Przegl Lek* 2004; 61: 473-6.

Ruoff KL, de la Maza L, Murtagh MJ, Spargo JD, Ferraro MJ. Species identities of enterococci isolated from clinical specimens. *J Clin Microbiol* 1990; 28: 435-7.

Thomson Jr RB. Specimen collection, transport and processing: bacteriology. In: Murray P, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, eds. Manual of clinical microbiology. Vol 1. 9th ed. Washington, DC: American Society for Microbiology, 2007: 291-333.

Turnidge J, Bell J, Biedenbach DJ, Jones RN. Pathogen occurrence and antimicrobial resistance trends among urinary tract infection isolates in the Asia-Western Pacific Region: report from the SENTRY Antimicrobial Surveillance Program, 1998/1999. *Int J Antimicrob Agents* 2002; 20: 10-7.

Winn JW, Allen S, Janda W, *et al*. Koneman's color atlas and textbook of diagnostic microbiology. 6th ed. Chapter 13. Gram positive cocci. Philadelphia: Leppicott Williams & Wilkins, 2006: 672-764.

Wisell KT, Kahlmeter G, Giske CG. Trimethoprim and enterococci in urinary tract infections: new perspectives on an old issue. *J Antimicrob Chemother* 2008; 62: 35-40.