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Investigation of Linezolid, Daptomycin, Quinupristin-Dalfopristin and Tigecycline Susceptibilities against Vancomycin Resistant Enterococcus Isolates

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The incidence of nosocomial infections due to vancomycin-resistant enterococci(VRE) has been increasing dramatically and the treatment of these infections has been challenging. The aim of this study was to evaluated the activity of linezolid, daptomycin, quinupristin-dalfopristin and tigecycline against VRE isolates. One hundred VRE isolates collected from surveillance and clinical specimens of patients in the intensive care units(ICUs) and pediatric clinics of our hospital between April 2007 and February 2010, and as a control group, 30vancomycin-sensitive enterococci(VSE) isolates from blood or urine cultures were included in this study. All of the VRE isolates and 16(53.3%) of 30VSE isolates were identified as *E.faecium,* while the remaining 14(46.7%) isolates were identified as *E.faecalis.* The susceptibility patterns of the isolates to linezolid, daptomycin, quinupristin-dalfopristin and tigecycline were determined by the E-test method, and the MIC values were interpreted according to CLSI and for tigecycline EUCAST recommendations. The rates of susceptible isolates were 100%for daptomycin, 81%for linezolid, 75%for tigecycline and 20%for quinupristin-dalfopristin. All of the VSE isolates were susceptible to linezolid and daptomycin; 53%of them were susceptible to tigecycline; 47%were susceptible to quinupristin-dalfopristin. The most active agent against VRE isolates was daptomycin, followed by tigecycline and linezolid in our unit.

Key words: Vancomycin resistant enterococci, Linezolid, Daptomycin, Quinupristin-dalfopristin, Tigecycline.

Although enterococci have low virulence, they have become one of today's important nosocomial pathogens because of its frequent isolation as infectious etiology, increase of multi-antibiotic resistant strains in recent years, and the limited treatment options. In the treatment of nosocomial infections caused by vancomycin-resistant enterococci (VRE), multi-antibiotic resistance is an important issue. The main antibiotics used to treat VRE infections are quinupristin dalfopristin, linezolid, tigecycline, daptomycin, mupirocin, ramoplanin, LY3328 (a new glycopeptide derivative) and currently ongoing clinical studies of drugs, such as dalbavancin[1-3]. Daptomycin and linezolid are known to be the most effective drugs in VRE infections[4-5]. In our study, we aimed to investigate the susceptibilities of linezolid, quinupristin-dalfopristin, tigecycline, and daptomycin against VRE isolates from surveillance and clinical samples.

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MATERIALS AND METHODS

In April 2007, isolation of vancomycin-resistant *Enterococcus faecium* in the blood culture of a newborn in neonatal care unit of our hospital lead to initiation of active VRE surveillance for in patients in neonatal care units and intensive care units (ICUs). Rectal swab samples were taken in the first 72 hours of hospitalization of all patients

in to these units and once a week during hospitalization and sent to our hospital's clinical microbiology laboratory. In the period of April 2007 - February 2010, a total of 550 VRE isolates were obtained from rectal swab samples. Among these samples, clinically randomized 100 VRE isolates selected evenly over years and for comparison 30 vancomycin-susceptible enterococci (VSE) isolates from different clinical samples in the same time period were included in the study. Repeated isolates from the same patient were excluded. Of the VRE isolates from surveillance cultures, 91 were from patients of neonatal and adult ICUs and 9 from patients of pediatric clinics. Of the VSE isolates, 24 were from urine, 5 from blood, and one from wound samples sent to our hospital's laboratory by different clinics.

Rectal samples were taken by sterile swab and inoculated on the Enterococcosel agar plate with vancomycin 8 µg/mL(Salubris, Turkey). Clinical samples were inoculated on 5% sheep blood agar plate, chocolate agar plate, and Eosin Methylene Blue agar plate. Plates were incubated at 37°C for 24-48 hours. Colonies growth on enterococcosel agar plate and suspicious enterococcus colonies isolated from clinical samples were identified as *Enterococcus spp.* by testing for production of catalase, growth in the presence of 6.5% NaCl, ability to hydrolyze esculin in the presence of 40% bile, and production of pyrrolidonyl arylamidase (PYR) and species identification was performed by API 20 Strep System (bioMerieux, France).

Minimum inhibitory concentrations (MICs) of linezolid, quinupristin-dalfopristin, daptomycin, and tigecycline against VRE and VSE isolates from all samples were determined by the E-test (bioMerieux, France) method and MIC values were evaluated with respect to CLSI and EUCAST–for tigecycline recommendations. [6],[7] As a control strain, *Enterococcus faecalis* ATCC 29212 was used.

RESULTS

All VRE isolates included in the study were *E.faecium,* whereas 16 (53.3%) out of 30 VSE isolates were *E.faecium* and 14 (%46.7) were *E.faecalis.*

The $MIC_{50}$and MIC_{90}values and the susceptibility rates of antibiotics tested against our VRE isolates are displayed in Table 1. Daptomycin was the most *in vitro* active (100%) agent followed by linezolid (81%) and tigecycline (75%). The susceptibility rate of quinupristin-dalfopristin was only 20%.

The MIC_{50} and MIC_{90} values and the susceptibility rates of antibiotics tested against our VSE control isolates are displayed in Table 2. Daptomycin and linezolid were the most *in vitro* active (100%) agent followed by tigecycline (53%) and quinupristin-dalfopristin (47%). Of VS*E.faecium* isolates, 87.5% were susceptible to tigecycline and 75% were susceptible to quinupristin-dalfopristin. Of VS *E.faecalis* isolates, 14.3% were susceptible to tigecycline and 14.2% were susceptible to quinupristin-dalfopristin (Table 2).

In our study the order of most effective *in vitro* antibiotics against VRE isolates were: daptomycin ,linezolid and tigecycline; where quinupristin-dalfopristin was the least effective antibiotic against VREs. In all of VSE isolates, daptomycin and linezolid were the most effective antibiotics; quinupristin-dalfopristin and tigecycline had higher efficacy on *E.faecium* but very low efficacy on *E.faecalis.*

Table 1. The MIC_{50} and MIC_{90} values and the rates of antibiotic susceptibilities of VRE isolates

	Linezolid	Daptomycin	Quinupristin/ dalfopristin	Tigecycline
Susceptible (%)	81	100	20	75
Intermediate (%)	18	-	75	18
Resistant (%)	1	-	5	7
MIC_{50} (µg/ml)	1.5	1.5	2	0.19
MIC_{90} (µg/ml)	4	3	3	0.5

Table 2. The MIC_{50} and MIC_{90} values and the rates of antibiotic susceptibilities of VSE isolates

	Linezolid	Daptomycin	Quinupristin/ dalfopristin	Tigecycline
VSE (n=30)				
Susceptible (n,%)	30(100)	30(100)	14(46.7)	16 (53.3)
Intermediate (n,%)	-	-	4(13.3)	9(30)
Resistant (n,%)	-	-	12(40)	5 (16.7)
MIC_{50} (µg/ml)	2	1	2	0.25
MIC_{90} (µg/ml)	3	2	3	0.75
VS *E.faecium*				
Susceptible (n,%)	16(100)	16(100)	12(75)	14(87.5)
Intermediate (n,%)	-	-	4(25)	2(12.5)
Resistant (n,%)	-	-	-	-
MIC_{50} (µg/ml)	2	2	0.75	0.19
MIC_{90} (µg/ml)	3	3	3	0.25
VS *E.faecalis*				
Susceptible (n,%)	14(100)	14(100)	2(14.2)	2(14.3)
Intermediate (n,%)	-	-	-	7(50)
Resistant (n,%)	-	-	12(85.7)	5(35.7)
MIC_{50} (µg/ml)	2	0.38	6	0.5
MIC_{90} (µg/ml)	3	1.5	12	0.75

DISCUSSION

Enterococci, despite having low virulence, are among important pathogens of today due to frequent isolation as infectious agent; intrinsically resistant feature to clindamycin, fluoroquinolone, trimethoprim/sulfamethoxazole, low level penicillin and low level aminoglycoside; and acquired resistant feature to tetracycline, erythromycin, rifampin, chloramphenicol, high level beta-lactam, high level aminoglycoside and vancomycin. Following the first report of VRE isolate in our country in 1998 VRE colonization and infection rates increased gradually. [8]

*E.faecalis*is the most commonly isolated infectious agent among enterococci; but *E.faecium* is more resistant to antibiotics than *E.faecalis.* In our study, all of 100 VRE isolates were identified as *E.faecium.*

The effect of linezolid against vancomycin-resistant *E.faecalis*and *E.faecium* is bacteriostatic. In a study conducted in 28 centers of the USA in 2002, 616 (88.4%) of 697 VRE strains isolated from urinary tract infections were *E.faecium* and 81(%11,6) were *E.faecalis.* In vancomycin-resistant *E.faecium* strains resistance to linezolid was 0.3%, chloramphenicol was 0.3%, and nitrofurantoin was 0.5%[9]. In a study conducted in 2007 with 5591 Gram positive clinical isolates from 23 countries, MIC_{50}=1 µg/mland MIC_{90}=2 µg/ mlfor 906 enterococcus isolates; and an intermetiate-resistance to linezolid was reported at the rate of 0.1-0.6%[10].

In another study, *in vitro* linezolid susceptibility in clinical MRSA and VRE isolates were investigated by the E test method, and all of 101 (100%) VRE isolates were found to be susceptible to linezolid. [11] In our study, linezolid susceptibility in rectal colonized VRE isolates were found as 81% and in VSE isolates as 100%.

Daptomycin is an effective antibiotic that can be used especially in the infections caused by methicillin-resistant *S.aureus* (MRSA) and VREs. Jevitt *et al* investigated *in vitro* activities of daptomycin, linezolid, quinupristin/dalfopristin against 90 enterococci isolates and daptomycin was determined as the most effective agent against *E.faecalis* with 92% susceptible (MIC_{90}=4 µg/ml)[12]. In a multi-center study all of 754 (100%) enterococci isolates were found as susceptible to daptomycin and 99.9% to linezolid. In another multi-center study in Europe, daptomycin and linezolid were found as the most effective antibiotics against VRE isolates from skin and soft-tissue infected

patients[13-14]. In our study all of VRE (MIC_{90} = 3 µg/ml) and VSE (MIC_{90} = 2 µg/ml) isolates were detected as daptomycin- susceptible.

In the treatment of infections caused by VRE, quinupristin/dalfopristin is an antimicrobial agent approved by FDA (Food and Drug Administration). This drug consists of two synergic parts called quinupristin (streptogramin B) and dalfopristin (streptogramin A). Its effecton *E.faecalis* is very low since *E.faecalis* strains are intrinsically resistant to streptogramin A[15]. In a study conducted in Turkey in 2004 including 38 MRSA and 55 VRE strains, no linezolid resistance was observed, four of VRE strains manifested enhanced quinupristin/dalfopristin resistance[16]

In a study of 23 centers from different countries, varying ratios of vancomycin resistance was found in *E.faecium* isolates. The highest MIC value for *E.faecalis* was 2µg/ml; where the highest MIC value for *E.faecalis* was 4 µg/ml. In the study, daptomycin (MIC_{90}= 0.5 µg/ml), ampicillin (MIC_{90}= 2µg/ml) and linezolid (MIC_{90} = 2µg/ml) were found as the most effective *in vitro* agents for *E.faecalis* and vancomycin resistance was determined as 0.9%. Vancomycin resistance of *E.faecium* was found as 17.9%. In the results of the study, 72.7% of vancomycin-resistant *E.faecium* isolates and 70.2% of *E.faecium* isolates were susceptible to quinupristin-dalfopristine[17]. In our study, 20 (20%) of rectal colonized VR *E.faecium* isolates, 2(14.2%) of VS *E.faecalis* isolates, and 12(75%) of VS *E.faecium* isolates were found susceptible.

Tigecycline is a novel antibiotic used in the treatment of multi-drug resistant, mild to moderate infections. In a study conducted in Argentina between 2005-2006, tigecycline susceptibilities of VRE and VSE isolates were tested by disk diffusion method and showed equal efficacy[18].

In another study by Rathe *et al*, *in vitro* activity of vancomycin, linezolid, daptomycin, and tigecycline were investigated by microdilution method in VRE and VSE isolates and linezolid, daptomycin, and tigecycline were found susceptible in all of them[19].

In Korea between years 1998-2005, mupirosin, daptomycin, linezolid, tigecycline, and quinupristin-dalfopristin susceptibilities against vancomycin-resistant *E.faecium* and *E.faecalis* were investigated. Although these antibiotics are rarely used in Korea, VREs isolated in 2005 were highly resistant to quinupristin-dalfopristin; resistant to linezolid and daptomycin; and almost all of them found to be resistant to mupirosin; tigecycline was determined as the most effective agent among other antibiotics[20].

In our study, daptomycin was found as the most susceptible antibiotic in both VRE and VSE isolates. Linezolid was the second with 100% suspectibility on VSE isolates and 81.2% in VRE isolates. Tigecycline was the third with 75% in VRE isolates, 87.5% in VS *E.faecium* isolates, and 14.3% susceptibility in *E.faecalis* isolates.

Quinupristin-dalfopristin was the most resistant antibiotic with 20% in rectal colonized VRE isolates, 75% in VS *E.faecium* isolates, and 14.2% in VS *E.faecalis* isolates. However, most of our isolates belonging to rectal colonization, despite the fact that we excluded recurrent isolates from the same patients in the same period, did not exclude the possibility of belonging to the same clone. For this reason, in order to make a better evaluation it is considered that making genetic typing and assessing the susceptibilities of isolates shown not to belong to the same clone will put forth more realistic resistance patterns.

As a result, today as the studies continue to research new and effective antibiotics it should not be forgotten that resistance may develop to this new antibiotics over time. One of the most important risk factors for VRE development is in proper use of glycopeptide antibiotics. To avoid this, enterococci have to be nomenclatured at species level, as in all important nosocomial pathogens; susceptibility pattern should be determined, and must be treated first-line with the antibiotic for which they are susceptible.

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