Activity of vancomycin combined with linezolid against clinical vancomycin-resistant *Enterococcus* strains

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Abstract

Introduction: Because multi-drug-resistant Gram-positive bacteria have been isolated frequently worldwide and are difficult to treat, alternative treatment choices are required. Combination antibiotherapies have a distinct advantage over monotherapies in terms of their broad spectrum and synergistic effect. In the present study, it was aimed to investigate the *in vitro* activity of vancomycin combined with linezolid against clinical vancomycin-resistant enterococci (VRE) strains with high-level aminoglycoside resistance.

Material and methods: A total of 30 randomly selected clinical VRE strains were studied. Susceptibility to agents tested was investigated using broth microdilution assay. The inoculum of strain was adjusted to approximately 5×10^5 CFU/ml in the wells. The results were interpreted in accordance with Clinical and Laboratory Standards Institute guidelines. *In vitro* activities of antibiotics in combination were assessed using the broth microcheckerboard technique. The fractional inhibitory concentration indexes (FICIs) were interpreted as follows: synergism, FICI ≤ 0.5; additive/indifference, FICI ≤ 0.5 – ≤ 4; antagonism, FICI > 4.

Results: All strains were resistant to vancomycin and susceptible to linezolid. The $MIC_{50,90}$ and MIC_{range} values of antimicrobials were 512, 512, and 512–1024 µg/ml for vancomycin; 2, 2, and 2–4 µg/ml for linezolid. The rate of synergy was found to be 46.6% (14/30) for linezolid combined with vancomycin. No antagonism was observed.

Conclusions: The results of the study suggest that this combination may contribute to the treatment of VRE infections for their synergistic effect and because no antagonism was observed.

Key words: vancomycin, linezolid, combination, vancomycin-resistant enterococci, high-level aminoglycoside resistance.

Introduction

Enterococcus faecalis and *E. faecium* can cause community-acquired and nosocomial infections. In recent decades, an increase in the occurrence of vancomycin-resistant enterococci (VRE) has been observed in Europe, with *E. faecium* being the most dominant species [1–3]. They have been isolated frequently worldwide and are difficult to treat [4–7].

Because enterococci have intrinsic resistance to some classes of commonly used antibiotics and the ability to acquire resistance to most of the current available antibiotics, either by mutation or by receipt of foreign genetic material, infections caused with multidrug-resistant enterococci are particularly difficult to treat [5, 8].

Although new antimicrobial agents designed to treat infections caused by multidrug-resistant pathogens have been introduced in the past few years, there has been a worldwide increase in the incidence of infections caused by VRE [4, 6, 7, 9]. Other choices for overcoming drug resistance include synergistic combinations of antimicrobials. Combination antibiotherapies have a distinct advantage over monotherapies in terms of their broad spectrum and synergistic effect at lower doses. They are sometimes used in an attempt to prevent or delay the *in vivo* emergence of drug-resistant subpopulations of pathogenic organisms [10, 11]. Linezolid is the first member of the structurally novel and totally synthetic antibiotic group named oxazolidinones, which acts by blocking protein synthesis at the ribosome. It was approved by the U.S. Food and Drug Administration in 2000. However, with the excessive use of linezolid during clinical trials and therapy, development of resistant isolates of *Enterococcus* spp. occurred [12–14].

Serious infections associated with enterococci are usually treated with a combination of penicillin/ampicillin with an aminoglycoside. The emergence of high-level resistance to aminoglycoside in enterococci, especially *E. faecium* and *E. faecalis*, seriously affected the therapeutic approach. Vancomycin is an agent acting on the cell wall. Because of the lack of reliable synergistic interaction between a cell wall active antibiotic and an aminoglycoside against high-level aminoglycoside-resistant (HLAR) *Enterococcus* strains, vancomycin became a first-line drug effective against these strains [15]. The options of therapy of infections caused by *Enterococcus* spp., which have resistance both to aminoglycosides and vancomycin, have been limited.

In the present study we aimed to investigate the *in vitro* activity of vancomycin combined with linezolid against VRE strains with high-level aminoglycoside resistance.

Material and methods

A total of 30 randomly selected clinical VRE strains were studied. Fourteen out of 30 strains were isolated from blood, and 16 from urine from different patients who were admitted to different clinics of the university's hospital.

Bacterial identifications of the strains were undertaken using conventional methods. They were identified as the genus *Enterococcus* if they had the following properties: Gram-positive; catalase negative; ability to grow in 6.5% sodium chloride and 40% bile; hydrolysed esculin; and positive results in pyrrolidonyl arylamidase tests (PYR; BD; USA). The *Enterococcus* species were identified using biochemical and physiological tests such as arginine dihydrolase, hippurate hydrolysis, growth in pyruvate, pigment production, motility, arabinose, and lactose utilisation, and other carbohydrate utilisation tests by using both a commercial identification system for enterococci (Microgen Strep ID, Microgen Bioproducts Ltd, UK) and inhouse products [15]. All strains were also tested for susceptibilities to ampicillin (10 µg: Oxoid, UK), imipenem (10 µg: BBL™, USA), and Quinupristin/Dalfopristin (Q/D) (15 µg: Oxoid, UK) using a disk diffusion test. *E. faecium* strains were resistant to ampicillin and imipenem and susceptible to Q/D, and *E. faecalis* strains had opposite results [4, 7, 16, 17]. Beta-lactamase enzyme production was also investigated by nitrocefin discs (BD BBL™, Cefinase, USA). The high-level resistance of aminoglycoside among VRE strains was investigated using 120 µg gentamicin and 300 µg streptomycin (BD BBLTM BENEX Ltd., Ireland) disks [18].

The antibiotics tested in the study were vancomycin (Multicell, USA) and Linezolid (Pfizer Inc., Groton, CT, USA). Teicoplanin (Glentham Life Sciences Ltd., UK) was also studied for phenotyping of the VRE strains. Susceptibility to agents against the strains tested was investigated using broth microdilution assay as described by the Clinical and Laboratory Standards Institute (CLSI) [18, 19]. They were prepared in accordance with the proposals of CLSI and the manufacturers. In all tests, cation-adjusted Mueller-Hinton II Broth (CAMHB) (BBL™, Becton, Dickinson and Company, France) were used for all experiments. The inoculum of each strain was adjusted to achieve a final inoculum of 10^5–10^6 CFU/ml in the wells of the plate. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic giving complete inhibition of visible growth, and was interpreted in accordance with the guidelines of the standards for antimicrobial susceptibility testing. Quality-control testing procedures were performed by also testing *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 as reference strains in each run [18–20].

In vitro activities of antibiotics in combination were assessed using a broth microcheckerboard [11]. The concentrations of antibiotics in combinations were based on two dilutions above and four dilutions below the MICs. The fractional inhibitory concentration (FIC) indexes (FICI) were calculated using the following formula: $FICI = FIC_A + FIC_B$. The FICI was interpreted as follows: synergism, $FICI \leq 0.5$; additive/indifference, $FICI \leq 0.5 - \leq 4$; antagonism, $FICI > 4$ [21].

Results

Twenty-eight of 30 VRE strains were identified as *E. faecium* and two as *E. faecalis*, depend-

Table I. The minimum inhibitory concentration (MIC) values of antimicrobial agents and susceptibility rates

Agent	MIC values (µg/ml)			Susceptibility, *n* (%)
	MIC_{50}	MIC_{90}	MIC_{range}	
LNZ	2	2	2–4	30 (100)
VAN	512	512	512–1024	0
TEC	64	128	16–128	0

LNZ – linezolid, VAN – vancomycin, TEC – teicoplanin. Susceptibility breakpoints: Lnz ≤ 4, Van ≤ 4, Teic ≤ 2 µg/ml [20].

Table II. The distribution of fractional inhibitory concentration indexes (FICI) values and interpreted FICI results of the combination against 30 VRE strains

Combination	Distribution of FICI values (*n* = 30)							Interpreted FICI results, *n* (%)		
	0.2	0.3	0.4	> 0.5	0.6	0.7	2	Syn	Add/Ind	Ant
VAN + LNZ	3	11	–	9	5	1	1	14 (46.6)	16 (53.4)	0

Syn – synergism, Add/Ind – additive/indifference, Ant – antagonism.

Table III. Distribution of aminoglycoside resistances and combination interactions by species in 30 VRE strains

VRE (*n* = 30)	HLAR	Non-HLAR	HLSR	Syn	Add/Int	Ant
Enterococcus faecium (*n* = 28)	24	0	4	14	14	0
Enterococcus faecalis (*n* = 2)	0	1	1	0	2	0

HLAR – high-level aminoglycoside resistant, HLSR – high-level streptomycin resistant, Syn – synergism, Add/Ind – additive/indifference, Ant – antagonism.

ing on conventional methods and antimicrobial results. All strains were found to be resistant to vancomycin and teicoplanin, and susceptible to linezolid by broth microdilution method. None of the strains detected beta-lactamase enzyme. The MIC values of antimicrobial agents and susceptibility rates are shown in Table I. The $MIC_{50,90}$ and MIC_{range} values were found as 2, 2, and 2–4 for linezolid, 512, 512, and 512–1024 for vancomycin, and 64, 128, and 16–128 µg/ml for teicoplanin. All strains had the VanA phenotype of glycopeptide resistance [15].

In this study, 24 (80%) of 30 VRE strains were identified as HLAR, five as high-level streptomycin resistant (HLSR), and one strain as non-HLAR. The rate of synergistic effect (FICI: ≤ 0.5) of vancomycin combined with linezolid against 30 VRE strains was found to be 46.6% (14/30) (Table II). One out of the 14 synergistic reactions belonged to the HLSR VRE strain, which was isolated from urine, and 13 to the HLAR VRE strains, which were isolated from both blood and urine samples. All synergistic reactions occurred against *E. faecium* strains. The rate of the additive/indifference effect (FICI: > 0.5–4) was found to be 53.4% (16/30). Two of them were *E. faecalis* that were isolated from urine samples. No antagonism was observed (Table III).

The MIC value distributions of each antimicrobial alone and in combination against 14 synergistic VRE strains are shown in Table IV. The MIC values of each antimicrobial alone against 14 strains given synergistic result were found as 512 µg/ml for vancomycin and 2, and 4 µg/ml for linezolid in microdilution method. However, in a combination of these antibiotics, the MIC concentration of each antibiotic was found as 32 µg/ml for vancomycin and 0.5 µg/ml for linezolid in 13 strains (32/0.5), and 32 µg/ml and 1 µg/ml in one strain (32/1) in the checkerboard method, respectively. The one strain had the HLAR.

Table IV. Comparative minimum inhibitory concentration (MIC) results of each antibiotic in both dilution and checkerboard tests against 14 synergistic VRE strains

VRE strains (*n* = 14)	MIC results (µg/ml)		
	Microdilution		Checkerboard
	MIC_{VAN}	MIC_{LNZ}	$MIC_{VAN/LNZ}$
13	512	2	32/0.5
1	512	4	32/1

Discussion

Linezolid is one of the last-resort antibiotics for the treatment of infections with VRE [17]. However, the increasing prevalence of linezolid resistance among clinical enterococ strains has been reported, especially during treatment of infections

[4, 22, 23]. Additionally, resistance to antibiotics that have been used to treat infections caused by VRE, such as tigecycline and daptomycin, has already been reported [4, 6].

Vancomycin is a bactericidal antimicrobial agent that is mainly active against Gram-positive cocci. Although vancomycin has been successfully used in therapy of Gram-positive bacterial infections for years, resistance has been increasing in recent years [4]. Because of the lack of reliable penicillin-aminoglycoside synergism among high-level aminoglycoside-resistant enterococci, vancomycin became a first-line drug effective against enterococci until the time when *Enterococcus* species–resistant to vancomycin were reported with increasing frequency [7, 15]. The synergistic effect of aminoglycosides and glycopeptide or beta-lactam antimicrobials is lost if there is high-level resistance to aminoglycosides [24]. Infections caused by *Enterococcus* spp. that have resistance both aminoglycosides and vancomycin have limited therapy options. Hence, it is important to introduce a new alternative method of treatment.

A high rate of resistance to antimicrobials in *Enterococcus* strains is obviously problematic, and a novel policy is needed to challenge the resistance in these microorganisms [25]. Additionally, the VRE strains with HLAR or HLSR that have aminoglycoside resistance have decreased the combination therapy alternatives to treat the infections caused.

In this study, 24 out of 30 VRE strains were found to have high-level aminoglycoside resistance (24/30). All these strains were *E. faecium*, which has high rate of resistance to antimicrobials [25]. The vancomycin concentrations alone in combination were found to be 32 mg/l in checkerboard test results (Table III). This concentration is reachable for vancomycin in human serum because it is inform that serum peak levels are reach to 30–40 mg/l in the administration of it at the treatment doses [13].

In conclusion, because of the synergistic results and lack of antagonism, the combination of vancomycin with linezolid can make an important contribution to the treatment of infections caused by VRE strains, especially VR–*E. faecium* with HLAR, which have limited numbers of alternative treatment choices if more *in vitro* experiments and *in vivo* applications on this combination are proven. Additionally, antibiotic combinations that have synergistic interaction have been used to treat infections in an attempt to prevent or delay resistant bacteria from arising.

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Conflict of interest

The author declares no conflict of interest.

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