INVITRO ACTIVITIES OF GLYCOPEPTIDES AGAINST ENTEROCOCCI COMPARING TWO DIFFERENT MEDIA

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Gazi Medical Journal 2000; 11: 73-76

SUMMARY

Purpose: *The incidence of nosocomial enterococcal infection has increased steadily over the past two decades. The treatment of patients with enterococcal infection has been complicated by the emergence of strains possessing high level resistance to aminoglycosides, penicillins, and most recently, glycopeptides. In this study in vitro activities of teicoplanin and vancomycin against 70 clinical isolates of enterococci were evaluated.* ***Methods:*** *The antimicrobial susceptibility of teicoplanin and vancomycin was tested with Kirby-Bauer Disc diffusion method and microbroth dilution methods; moreover, the effects of Mueller Hinton Broth (MHB) and Todd Hewitt Broth (THB) media on the microbroth dilution tests were compared.* ***Results:*** *By using MHB the susceptibility was found as 100 % and by using THB the susceptibility ratio was found as 95.7 % the intermediate susceptibility was 2.8 % and resistance was 1.5 % for teicoplanin. By using MHB, the susceptibility rate was found as 87.1 %, the intermediate susceptibility 10.1 % and resistance 2.8 %, and by using THB the susceptibility ratio was found as 78.6 %. The intermediate susceptibility rate was 11.4 % and resistance was 10 % for vancomycin.* ***Conclusion:*** *These results indicate that the utilisation of THB in the MIC determination studies of teicoplanin and vancomycin concerning enterococci is not recommended.*

Key Words: *Enterococci, Glycopeptide, Media.*

INTRODUCTION

Enterococci constitute a normal component of the human gut flora but may invade and provoke opportunistic infections in compromised patients. Serious enterococcal infections, including bacteremia or endocarditis, may be difficult to treat, the recommended regimen being a penicillin plus an aminoglycoside. However, enterococcal isolates are increasingly resistant to antibiotics and are also increasingly responsible for nosocomial infections (1). In patients in whom β-lactams can not be used, either because of infection with β-lactam resistant strains of enterococci or because of allergy to penicillins, glycopeptides often in combination with an aminoglycoside, may be the drug of choice (2,3). With the emergence of vancomycin resistance in 1986, enterococci can

now be resistant to all currently approved antimicrobial agents (4,5).

In this study, we evaluated the anti-enterococcal activity of glycopeptide antibiotics; vancomycin and teicoplanin and their in vitro efficacy comparing two different media.

MATERIALS AND METHODS

Teicoplanin (Hoechst, Paris, France) and vancomycin (Lilly, Indianapolis, USA) susceptibility of 70 clinical isolates of enterococci obtained from the Microbiology Laboratory of Gazi University Faculty of Medicine was determined by a broth microdilution method and Kirby-Bauer disc diffusion method as described by the National Committee for Clinical Laboratory Standards. For microbroth dilution tests, two different media, Mueller-Hinton Broth (MHB) (Difco, Detroit, USA) and Todd-Hewitt Broth (THB) (Difco, Detroit, USA) were used (6). The Enterococcus faecalis ATCC 29212 was used as a control strain. For MIC determinations, final inocula of $5x10^5$ CFU/ml were prepared from overnight cultures and the results were evaluated after incubation for 24h at 35°C.

RESULTS

MIC interpretive standards (µg/ml) for Enterococcus spp mentioned by NCCLS were as follows: ≤4 µg/ml indicates susceptibility for vancomycin and 8 µg/ml for teicoplanin; ≤8-16 mg/ml indicates intermediate susceptibility for vancomycin and 16 µg/ml for teicoplanin; ≥32 mg/ml indicates resistance to both vancomycin and teicoplanin.

The MIC values of teicoplanin and vancomycin comparing two different media are shown in Table 1 and 2 respectively.

The antimicrobial susceptibility results of teicoplanin and vancomycin comparing two different media are shown in Table 3.

For evaluating the susceptibility results of disc diffusion tests, the interpretive zone diameters mentioned by NCCLS were as follows: ≥17 mm indicates susceptibility to vancomycin and ≥14 mm to teicoplanin; 15-16 mm indicates intermediate resistance to vancomycin, 11-13 mm indicates intermediate resistance to vancomycin, ≤14 mm indicates resistance to vancomycin and ≤10 mm teicoplanin (7). The results obtained from the disc diffusion method using Mueller Hinton Agar (MHA) were the same as the results of microbroth dilution tests; the susceptibility rates for teicoplanin is 100% and for vancomycin 87.1%.

DISCUSSION

Because of their quasiconstant activity against enterococci, the glycopeptide antibiotics vancomycin and teicoplanin are useful as alternative drugs for the treatment of severe infections due to these organisms (8). However, glycopeptide-resistant enterococci have become a major threat especially to hospitalized patients (1). Like methicillin-resistant Staphylococcus aureus, vancomycin resistant enterococci can cause important nosocomial epidemics and increase morbidity, mortality, and costs related to admission to the hospital (9, 10). Little is known about the epidemiology of vancomycin-resistant enterococci (VRE) colonization outside the hospital environment. The investigations have suggested that VRE may be part of the intestinal microflora of patients inside and outside the hospital. However it is also shown that contamination of the environment from the hospital could not be excluded (11).

The development of vancomycin resistance was reported in clinical isolates in the late 1980s. By 1993, 7.9% of enterococcal infections reported to the Centers for Disease Control were due to VRE. Rates of faecal VRE carriage have ranged from 5 to 47% in tertiary care facilities (12). Many isolates are highly resistant to all standard anti-enterococcal drugs.

For infections caused by these strains, bactericidal therapy may not be possible, and even bacteriostatic therapy may be difficult to achieve. Analysis of the MICs of vancomycin and teicoplanin for the VRE reported to date indicates that resistance in these organisms falls into two categories. One category, which includes strains of Enterococcus faecalis, E. faecium, E. avium, is characterized by high level resistance to both vancomycin (MIC, 64 to > 2,000 mg/liter) and teicoplanin (MIC, ≥8 mg/liter) (2) while the other category is characterized by lower-level resistance to vancomycin (MIC, 32 to 64 mg/liter) and susceptibility to teicoplanin (MIC, ≤1 mg/liter). The accurate determination of

Table 1: The MIC values of enterococci for teicoplanin comparing two different media.

Medium	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	MIC_{100} (µg/ml)
MHB	1	8	8
THB	4	8	32

Table 2: The MIC values of enterococci for vancomycin comparing two different media.

Medium	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	MIC_{100} (µg/ml)
MHB	4	8	32
THB	4	16	32

Table 3: The antimicrobial susceptibility results of enterococci for teicoplanin and vancomycin comparing two different media (%).

Medium	Teicoplanin			Vancomycin		
	S	I	R	S	I	R
MHB	100	-	-	87.1	10.1	2.8
THB	95.7	2.8	1.5	78.6	11.4	10

glycopeptides is important for the clinical approach as the challenging difficulty is an important problem in the treatment of VRE.

In our country, there are similar results from different centers. In a study performed by Akdeniz University the resistance to both vancomycin and teicoplanin was found as 1.1% among 182 enterococcal clinical isolates (13). Another study from İstanbul University demonstrated 0.9% resistance to vancomycin among 111 clinical isolates of enterococci (14).

In our study, we obtained different results by using two different media in the determination of MICs by microbroth dilution method. Using MHB, the susceptibility was found as 100% and by using THB, the susceptibility ratio was found as 95.7%. The intermediate susceptibility was 2.8% and resistance was 1.5% for teicoplanin. Using MHB, the susceptibility was found as 87.1%, the intermediate susceptibility was 10.1% and resistance was 2.8% utilizing THB. The susceptibility ratio was found as 78.6%, the intermediate susceptibility was 11.4% and resistance was 10% for vancomycin. Taking these results into account, we recommend the using of MHB in the MIC determination studies.

Finally, because of the resistance that enterococci already exhibit to a variety of antimicrobial agents, the emergence of vancomycin resistance is troublesome. We believe that the use of antibiotics, in particular glycopeptides, should probably be dramatically restricted in order to avoid the selection of VRE, which are already part of the human microflora.

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