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Original Article

Microbiology Section

In-vitro Activity of Tigecycline versus Daptomycin against Clinical Isolates of *Enterococcus* Species

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ABSTRACT

Introduction: Tigecycline is a potential therapeutic agent for multidrug resistant gram positive and gram negative organisms. The clinical efficacy of synergic combination of daptomycin with betalactam antibiotics have been described against various species of *Enterococcus* isolates.

Aim: To detect the in-vitro activity of tigecycline and daptomycin against *Enterococcus* species and to compare their antimicrobial activity by Vitek 2 automated system.

Materials and Methods: This was a cross-sectional study conducted at Department of Microbiology, Vinayaka Mission's Kirupananda Variyar (VMKV) Medical College and Hospitals, Salem, Tamil Nadu, India. Isolates of *Enterococcus* obtained over a period of two years from March 2016 - March 2018 from various clinical samples were identified by standard biochemical method and their antimicrobial susceptibility pattern was determined. Minimum Inhibitory Concentration (MIC) of tigecycline and daptomycin was determined by Vitek 2 automated system. Analysis of data was done by using frequency distribution and percentage.

Results: Out of 211 *Enterococcus* isolates studied, 23 (10.90%) isolates showed decreased susceptibility to daptomycin with MIC of ≥8 µg/mL. All isolates showed 100% susceptibility to tigecycline.

Conclusion: The present study showed potent antimicrobial activity of tigecycline against various species of *Enterococcus*. Decreased susceptibility to daptomycin has been observed among the study isolates. Further clinical investigations are required to know the potential benefits of these agents in therapy.

Keywords: Minimum inhibitory concentration, Multidrug resistance, Vancomycin resistant *Enterococcus*

INTRODUCTION

Antibiotic resistance among *Enterococcus* represents a clinical challenge due to availability of limited sources of drugs for therapy. The standard treatment regime for Enterococcal infection depends on optimised dosage and combination of antibiotics. Tigecycline has demonstrated potent in-vitro activity against a wide range of clinically important gram positive cocci, gram negative organisms, anaerobes and atypical microorganism [1]. Tigecycline remains effective against *Enterococcus* expressing one or more vancomycin resistant determinants. Tigecycline acts on Vancomycin Resistant *Enterococcus* (VRE) by inhibiting 30S ribosomal subunit, thereby blocking protein synthesis. Monotherapy with tigecycline has been proven to be non inferior to other standard treatments for complicated skin and intra-abdominal infections [2,3]. The high volume distribution of tigecycline in serum makes its concentration inadequate for treating blood stream infection. Tigecycline does not require dose adjustments in patients with impaired renal function. Synergic combination of tigecycline with other antimicrobial agents such as vancomycin, gentamicin, rifampin and daptomycin have been recommended for the treatment of complex deep seated infections caused by *Enterococcus* [4].

Daptomycin is a novel cyclic lipopeptide antimicrobial agent that exhibits a rapid bactericidal concentration dependent action on cells through membrane lysis. Daptomycin has been shown to be a potential antimicrobial agent against *Enterococcus*. The major drawback for its use in VRE infection is the development of resistance during therapy. Daptomycin is not indicated for the treatment of pneumonia because of its inhibition by pulmonary surfactants [5]. As per Clinical Laboratory Standards Institute (CLSI) guidelines, in-vitro susceptibility to daptomycin is defined by MIC of ≤4 µg/mL, whereas isolates with MIC of >4 µg/mL is considered as daptomycin non susceptible [6]. Synergic combination of daptomycin with betalactam antibiotics have been shown to be effective against VRE species in-vitro. Beta lactams causes a reduction in cell wall positive charge by releasing lipoteichoic acid, results in destabilisation. This allows the cationic daptomycin complex to bind more effectively to cell wall [7].

There are various study reports on the efficacy of daptomycin and tigecycline against VRE [5,7-9]. However, treatment failure with daptomycin is described even in isolates with susceptible MIC for daptomycin [10]. There have been only a few study data from India on the antimicrobial susceptibility of *Enterococcus* towards daptomycin and tigecycline [4,11,12]. Hence, the present study was undertaken to determine the in-vitro activity of tigecycline and daptomycin against various species of *Enterococcus*.

MATERIALS AND METHODS

This cross-sectional study was done on clinical samples received at Department of Microbiology, VMKV Medical College and Hospitals, Salem, Tamil Nadu, India. A total of 15,504 clinical samples over a period of two years from March 2016-March 2018 were screened for *Enterococcus*. Out of which 211 samples yielded *Enterococcus*. This consists of 205 urine samples, three pus samples, two blood samples and one Ear swab. This study was approved by Institutional Ethical Committee (IEC) (IEC/VMMC/Microbiology/01/2015 Dated 25.03.2015).

Inclusion criteria: Clinical samples received at laboratory for routine culture and sensitivity were included. A colony count of >100,000 Colony Forming Unit (CFU)/mL from urine samples were included in the study.

Exclusion criteria: Gram positive cocci, morphologically similar to *Enterococcus*, but belong to other genus were excluded based on biochemical parameters. Bacterial growth from sites which were not clinically relevant due to its commensal nature was excluded.

Sample Processing

Samples were inoculated onto Blood agar and MacConkey agar and incubated overnight at 37°C. Isolates were further identified by gram stain morphology, catalase test and growth in bile esculin agar.

Detection of MIC by Vitek 2 Automated Method

Turbidometrically, controlled bacterial pure growth suspended in sterile physiological saline was used for Vitek identification and antimicrobial susceptibility testing cards. For biochemical identification of *Enterococcus* by Vitek 2 automated system, the following parameters were used: Growth in 6.5% Sodium chloride (NaCl), β-glucuronidase, trehalose, arginine dihydrolase, D-sorbitol, urease, raffinose, D-galactose, D-mannitol, sucrose, β-galactosidase, salicin, L-pyrrolidonylarylamidase, D-xylose, D-maltose, methyl- β-D-glycopyranoside, D-ribose, α-glucosidase, α-mannosidase, lactose, phosphatase etc. MIC break point of tigecycline and daptomycin was determined by Vitek 2 automated method.

STATISTICAL ANALYSIS

Data obtained was analysed by using frequency distribution and percentage.

RESULTS

Out of 211 *Enterococcus* isolates studied, 188/211 (89.09%) isolates showed decreased susceptibility to daptomycin whereas all isolates tested were susceptible to tigecycline. Majority of the isolates were obtained from patients in the age group of 41-60 years [Table/Fig-1]. Of the total isolates studied from urine, pus, blood and ear swab, 197 (93.37%) were *E. faecalis* followed by 10 isolates of *E. faecium* (4.74%), 3 isolates of *E. avium* (1.42%) and 1 isolate of *E. durans* (0.47%). The most predominant isolate obtained from urine sample was *E. faecalis*, 192/205 (93.66%) followed by *E. faecium*, 9/205, *E. avium*, 3/205 and *E. durans*, 1/205. Three out of 197 isolates of *E. faecalis* were from pus sample. Nine out of 10 *E. faecium* isolates were from urine sample and one (10%) isolate of *E. faecium* was from blood sample [Table/Fig-2].

Age group (years)	Gender n (%)	
	Male (92)	Female (119)
0-20	0	14 (11.77)
21-40	14 (15.21)	40 (33.61)
41-60	60 (65.22)	25 (21.01)
61-80	18 (19.57)	31 (26.05)
81-100	0	9 (7.56)

[Table/Fig-1]: Age and gender distribution.

Samples	*Enterococcus* species isolated			
	E. faecalis	*E. faecium*	*E. avium*	*E. durans*
Urine (205)	192 (93.66)	9 (4.39)	3 (1.46)	1 (0.49)
Pus (3)	3 (100)	0	0	0
Blood (2)	1 (50.00)	1 (50.00)	0	0
Ear swab (1)	1 (100)	0	0	0
Total (211)	197 (93.37)	10 (4.24)	3 (1.42)	1 (0.47)

[Table/Fig-2]: Distribution of *Enterococcus* from clinical samples.

All isolates (100%) were susceptible to tigecycline. A total of 183 (86.73%) out of 211 isolates showed susceptibility to tigecycline with MIC of ≤0.12μg/mL in all the *Enterococcus* species. The tigecycline MIC for 28 (13.27%) out of 211 isolates were found to be 0.25 μg/mL. 169 (85.79%) *Enterococcus faecalis* out of 197 isolates and 10 isolates (100%) of *Enterococcus faecium* showed susceptibility to tigecycline with MIC of ≤0.12 μg/mL [Table/Fig-3]. A single isolate each from urine 1/205 (0.49%) and pus sample 1/3 (33.33%) was found to be resistant to penicillin, aminoglycoside, fluoroquinolone, linezolid, daptomycin and glycopeptides but showed susceptibility to tigecycline. Out of 211 isolates studied, 8 (3.79%) showed susceptibility to penicillin, 58 (27.49%) were susceptible to fluoroquinolones and 70 (33.18%) to aminoglycosides. A total of 198 (93.84%) were susceptible to teicoplanin, 207 (98.10%) to vancomycin and 206 (97.63%) showed susceptibility to linezolid.

Twenty three out of 211 isolates (10.90%) isolates showed decreased susceptibility to daptomycin with an MIC of ≥8 μg/mL and 89.10% isolates showed susceptibility to daptomycin (MIC 0.25-4 μg/mL) [Table/Fig-4].

Among the *E. faecalis* isolates studied from clinical samples, 174 isolates (88.32%) out of 197 showed daptomycin MIC in the susceptible range of 0.25-4 μg/mL. Twenty two *E. faecalis* isolates from urine and one isolate from pus sample showed decreased susceptibility to daptomycin (MIC ≥8 μg/mL) [Table/Fig-4].

Out of 23 daptomycin resistant isolates in this study, three isolates showed intermediate resistance to vancomycin with MIC of 8-16 μg/mL. Nine isolates (39.13%) showed MIC of 1-4 μg/mL to vancomycin. The vancomycin MIC for 11 daptomycin resistant isolates were ≤0.5 μg/mL. A total of 23 daptomycin resistant isolates showed MIC of teicoplanin in the range of 1-4 μg/mL. Four out of 23 isolates were resistant to linezolid with MIC of ≥8 μg/mL and 15 isolates showed linezolid MIC in the range of 1-28 μg/mL.

DISCUSSION

Tigecycline is a tetracycline-class antibacterial agent developed for the treatment of polymicrobial infections caused by multidrug resistant organisms. Tigecycline effectively penetrates body fluids and tissues and achieves therapeutic concentration [4]. Isolates in current study showed 100% susceptibility to tigecycline. The tigecycline MIC break points for these isolates were in the range of 0.12 μg/mL-0.25 μg/mL.

Two (0.95%) out of 211 isolates in this study, were resistant to multiple antibiotics tested, penicillins, aminoglycosides, fluoroquinolones, linezolid, glycopeptides and daptomycin, but showed good in-vitro activity against tigecycline. Out of two isolates, one each was obtained from pus and urine sample. These isolates showed tigecycline MIC of ≤0.12 μg/mL and 0.25 μg/mL, respectively. Santimaleeworagun W et al., have reported the therapeutic potential of tigecycline (MIC susceptible breakpoint, ≤2 μg/mL) against VRE isolates from intra abdominal, skin and soft tissue infection [8].

Yemisen M et al., have reported the in-vitro activity of tigecycline against *E. faecalis* and *E. faecium* isolates with MIC of 0.12 μg/mL from clinical samples [9]. In the present study, tigecycline exhibited good in-vitro activity (100% susceptibility) against *E. faecalis*, *E. faecium*, *E. avium* and *E. durans* isolated from various clinical samples.

The high in-vitro activity of tigecycline against *Enterococcus* have been reported by Manoharan A et al., (100%) and Veeraraghavan B et al., (60%) [4,11]. In the present study three isolates with intermediate resistance to Vancomycin with MIC of 8-16 μg/mL were

Samples	Minimum Inhibitory Concentration (MIC) of Tigecycline							
	E. faecalis (197)		*E. faecium* (10)		*E. avium* (3)		*E. durans* (1)	
	≤0.12 μg/mL	0.25 μg/mL	≤0.12 μg/mL	0.25 μg/mL	≤0.12 μg/mL	0.25 μg/mL	≤0.12 μg/mL	0.25 μg/mL
Urine (205)	164 (85.42%)	28 (14.59%)	9 (100%)	0	3 (100%)	0	1 (100%)	0
Pus (3)	3 (100%)	0	0	0	0	0	0	0
Blood (2)	1 (100%)	0	1 (100%)	0	0	0	0	0
Ear swab (1)	1 (100%)	0	0	0	0	0	0	0
Total (211)	169 (85.79%)	28 (14.21%)	10 (100%)	0	3 (100%)	0	1 (100%)	0

[Table/Fig-3]: Minimum Inhibitory Concentration (MIC) of Tigecycline against various species of *Enterococcus*.

Samples	Minimum Inhibitory Concentration (MIC) of Daptomycin											
	E. faecalis (197)			*E. faecium* (10)			*E. avium* (3)			*E. durans* (1)		
	<0.25 µg/mL	0.25-4 µg/mL	8 µg/mL	<0.25 µg/mL	0.25-4 µg/mL	8 µg/mL	<0.25 µg/mL	0.25-4 µg/mL	8 µg/mL	<0.25 µg/mL	0.25-4 µg/mL	8 µg/mL
Urine (205)	0	170 (88.54%)	22 (11.46%)	0	9 (100%)	0	0	3 (100%)	0	0	1 (100%)	0
Pus (3)	0	2 (66.67%)	1 (33.33%)	0	0	0	0	0	0	0	0	0
Blood (2)	0	1 (100)	0	0	1 (100)	0	0	0	0	0	0	0
Ear swab (1)	0	1 (100%)	0	0	0	0	0	0	0	0	0	0
Total (211)	0	174 (88.32%)	23 (11.68%)	0	10 (100.%)	0	0	3 (100.00%)	0	0	1 (100.00%)	0

[Table/Fig-4]: Minimum Inhibitory Concentration (MIC) of Daptomycin against *Enterococcus* spp isolates.

also resistant to daptomycin (≥8 µg). A study conducted by Chitnis S et al., have shown the in-vitro activity of daptomycin against VRE isolates with MIC in susceptibility range of 0.19- 3 µg/mL [12].

Reduced daptomycin susceptibility and tolerance is associated with mutation in the LiaFSR three-component system which regulates cell membrane stress response in *Enterococcus* and for isolates with mutations to the LiaFSR system, daptomycin binding was found to be enhanced by the addition of ampicillin, ceftaroline, and beta-lactams [13-16]. In present study, 89.10% isolates showed susceptibility to daptomycin (MIC 0.25-4 µg/mL). However, Campeau SA et al., have reported treatment failures in patients infected with daptomycin-susceptible isolates with MIC in the susceptible range 2-4 µg/mL [17].

In the present study, daptomycin resistance was observed among glycopeptide susceptible as well as glycopeptide resistant strains of *Enterococcus*. Tigecycline was found to be effective against isolates of *Enterococcus* when compared with in-vitro activity of daptomycin.

Limitation(s)

During the study period, majority of the isolates obtained were from urine samples, as the isolation rate of *Enterococcus* from other samples were significantly less in the study. Despite this study detects the efficacy of these drugs against various species of *Enterococcus*, 93.36% isolates in present study were *Enterococcus faecalis*. Even though the efficacy of tigecycline and daptomycin against multidrug resistant *Enterococcus* was proved by in-vitro method, clinical efficacy of this drugs need to be assessed by monitoring patient therapy.

CONCLUSION(S)

Tigecycline showed good in-vitro activity against multidrug resistant Enterococci. Daptomycin was shown to be active against isolates of Enterococci resistant to glycopeptides and Linezolid. Isolates of *Enterococcus* with susceptible MIC for glycopeptides, but decreased susceptibility to daptomycin were also observed in the present study. Further studies are required to investigate the clinical efficacy of these drugs against *Enterococcus*.

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