Correspondence

Comparative in vitro Antimicrobial Activity of Tigecycline Against Clinical Isolates of Vancomycin-Resistant Enterococcus

Dear Editor,

In last four decades, tetracyclines have been widely used due to their broad-spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria. However, indications for the use of tetracyclines have been limited due to the emergence of resistant strains. Tigecycline,[1] former GAR-936, is the first representative of a new class of antimicrobial agents known as glycylcyclines. Tigecycline may be an alternative for the treatment of Vancomycin-resistant Enterococcus (VRE), one of the most important and emerging causes of human nosocomial infections. In this study, we evaluate the In vitro activity of tigecycline in comparison to other antimicrobial agents against VRE isolates obtained from the patients followed in our university hospital.

About 22 *Enterococcus faecalis* and six *Enterococcus faecium* isolates were obtained from clinical materials (19 urine, nine blood) of hospitalised patients. Patients with VRE blood stream infection were followed in the intensive care unit, had central venous catheter and severe underlying diseases. Isolates were identified to genus level by Gram staining, L-pyrrolindonyl-b-naphthylamide reaction, catalase reaction, bile esculin test, salt tolerance and streptococcal grouping. Identification to species level was performed using the API Rapid ID 32 Strep system (BioMe´rieux, Marcy l'Etoile, France). Antibiotic susceptibility testing was performed by disk diffusion, as recommended by the Clinical Laboratory Standard Institute[2] (CLSI, formerly NCCLS). For isolates not fully susceptible to vancomycin, the MIC of vancomycin was determined by Etest (AB Biodisk, Solna, Sweden), using the high-inoculum method on Mueller-Hinton agar. All 28 isolates, obtained from cultures were stored in brain-heart infusion broth at -70ºC.

In this study, antimicrobial susceptibility testing was performed against eight antimicrobial agents by using broth microdilution method, as described by the CLSI. Minimal inhibitory concentration (MIC) was defined as the lowest concentration of antimicrobial agent producing no visible growth of microorganism at 37ºC after overnight incubation. MIC 50 and MIC 90 was determined by the concentrations of antimicrobial agent at which 50% and 90% of microorganism are inhibited, respectively. We decided to determine both MIC 50 and MIC 90 values to show the potency of anibiotics. The following quality control strains were concurrently tested by using *E. faecalis* ATCC 29212 and *E. faecium* ATCC 6057.

Results of the In vitro susceptibility testing are presented in Table. The antimicrobial activity of tigecycline was compared to those selected antimicrobial agents. Tigecycline was highly active against VRE. MIC 50 and MIC 90 values were 0.06 and 0.12 µg/mL, respectively and same for E. faecalis and E. faecium isolates. Only one islolate showed a MIC value of 0.5 µg/mL over 0.25 µg/mL and the tigecycline MIC range of the isolates was between 0.03 and 0.5 µg/mL. The vast majority of strains showed high MIC values against other antibiotics. Linezolid was another antibiotic with low MIC 50 and MIC 90 values, which was 4 µg/mL for both.

In conclusion, VRE is an emerging problem and antibiotic sources are limited. Our study agrees with previous In vitro studies[3,4] and shows that tigecycline has high In vitro activity against VRE. These results suggest that further prospective clinical studies must be performed and

Table: The distribution of antibiotic minimal inhibitory concentrations

Agents	Number of vancomycin-resistant enterococcus isolates inhibited at MIC (µg/mL)									
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
Amoxycillin/clavulanate	-	-	-	-	1	1	-	1	-	25
Ampicillin	-	-	-	-	-	1	1	1	-	25
Imipenem	-	-	-	-	-	1	2	-	-	25
Levofloxacin	-	-	-	-	-	1	1	2	1	23
Linezolid	-	-	-	-	-	-	8	20	-	-
Penicillin	-	-	-	-	-	-	-	1	-	27
Piperacillin/tazobactam	-	-	-	-	-	-	-	-	1	27
Tigecycline	5	13	9	-	1	-	-	-	-	-

MIC: Minimum inhibitory concentration

tigecycline may have an important role in the treatment of severely ill patients infected with VRE.

Acknowledgments

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Pilot Evaluation of Commercial Liquid Culture Method for Isolation of Mycobacteria in Resource-Poor Settings

Dear Editor,

High infrastructural costs and lack of trained manpower make setting up of automated mycobacterial culture facilities difficult. Amongst those offering culture, great disparity exists in techniques used. Though a few reference laboratories and hospitals offer automated rapid culture technology, the high cost prevents their widespread use. A vast majority of mycobacteriology laboratories continue to use the conventional Lowenstein-Jensen (LJ) media. Though the LJ media is highly specific for mycobacteria, the long time taken for growth delays diagnosis and hampers initiation of early treatment.[1,2] Evaluating cheaper and rapid culture methods is, thus, vital in tuberculosis control and management.[3] We undertook a pilot study to compare mycobacterial recovery rates and detection time of the Bio FM culture medium (M/s Bio-Rad Ltd), the mycobacteria growth indicator tube (MGIT) 960 system (M/s BD Ltd.) and LJ medium(M/s EOS labs, Mumbai, India). The Bio FM medium, a Middlebrook 7H9 medium with OADC (Oleic Acid - Albumin Fraction V, Bovine -Dextrose - Catalase (beef) - Sodium Chloride) and VCA (Vancomycin - Colistin - Amphotericin B) supplements, contains a chromogenic indicator that changes to dark blue/violet in response to mycobacterial growth. A total of 20 sputum specimens with known smear findings were included, four specimens from each category (negative, scanty, 1+, 2+ and 3+) of smear grading (Revised National Tuberculosis Control Programme). Specimen digestion and decontamination was done by the NaOH (Sodium Hydroxide) -NALC (N-acetyl-L-cysteine) method, followed by centrifugation for 20 minutes at 3000 revolutions/minute. 0.5 ml of each concentrate was added into the 3 media. MGIT vials were monitored by the MGIT 960 system while the Bio FM and LJ media were examined upto 42 days and 56 days respectively for mycobacterial growth. Isolates were identified by P-nitro-a-acetylaminob-hydroxypropiophenone (NAP) testing. 17 of the 20 specimens turned culture positive while three smear negative specimens remained culture negative. All isolates were identified as *M. tuberculosis* complex. Surprisingly, no discrepancy was seen in the recovery rates of the 3 media. The time to mycobacterial detection was shortest with MGIT and longest with LJ media [Table 1]. The detection time of BioFM was comparable with MGIT and outperformed the LJ media, similar to other international study findings.[4,5]

Our pilot study, thus, indicates that BioFM media

Table 1: Detection time of Mycobacteria by MGIT, BioFM and LJ

	Mean time for detection (in days)	
	Mean	Range
MGIT	10.23	4 - 20
BioFM	10.47	6 - 19
LJ	21.82	14 - 35