Brief Communication

Epsilometer test for determining in-vitro activity of tigecycline against rapidly growing mycobacteria from central Saudi Arabia

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Rapidly growing mycobacteria (RGM) are well-recognized causes of human infections, usually in association with defective protective mechanisms. The typical context for an RGM infection is primary or secondary immune deficiency, the presence of a foreign device, such as vascular catheter, disruption of epithelial barriers as a result of trauma or surgery, or structural abnormalities, such as those seen in patients with chronic airway diseases.[1] Although relatively uncommon, the clinical impact of RGM infections is considerable, given that they frequently affect individuals with significant co-morbidities. Furthermore, antimicrobial therapy options for RGM infections have for a long time been largely limited to macrolides and aminoglycosides.[2] Resistance to the former, de-novo, or emerging during treatment, and toxicity of the latter have often made clinical management of RGM infections very challenging.[2] Tigecycline, a glycylcycline derivative of minocycline, has a broad spectrum of anti-bacterial activity. It has been demonstrated to be clinically effective for the treatment of complicated skin and soft tissue infections, complicated intra-abdominal infections and community acquired pneumonia.[3] Using broth microdilution, investigators have shown that tigecycline has excellent in-vitro activity against many species of RGM.[4,5] This has encouraged some clinicians to use tigecycline, off-label for the treatment of refractory RGM infections, sometimes with good clinical results.[6] Antimicrobial susceptibility testing of RGM utilizing Epsilometer test (E-test [AB Biodisk, Solna, Sweden]) for agents such as amikacin, doxycycline, and macrolides is reasonably accurate and reproducible, although occasionally problematic.[7] More recently, this method was used successfully to determine RGM susceptibility to tigecycline.[8] Whereas access to susceptibility testing by broth microdilution is largely limited to reference laboratories and research facilities, E-test strips are available more widely and the methodology is relatively simple. The aim of this study is to examine the utility of E-test for susceptibility testing of RGM isolates from Saudi Arabia against tigecycline.

The study included 23 clinical non-tuberculous mycobacterial isolates collected prospectively from March 2011 to October 2012 at the TB Section, Division of Microbiology, Prince Sultan Military Medical City (PSMMC), Riyadh, Kingdom of Saudi Arabia. Species identification was performed by conventional bacteriological methods. The strains, which had been preserved in Trypticase Soy Broth with 15% glycerol (Oxoid, Basingstoke, England) and maintained at -70°C were sub-cultured onto Mycobacteria Growth Indicator Tube (Becton Dickinson, MD, USA), and then onto blood agar (Oxoid, Basingstoke, England). An additional blood agar plate (Oxoid, Basingstoke, England) was inoculated to confirm purity. American Type Culture Collection (ATCC) strains *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC29213 and *Escherichia coli* ATCC25922 were used as controls. Minimum inhibitory concentrations (MIC) of tigecycline against the study strains were determined using tigecycline E-test strips (BioMèrieux, Marcy L'Etoile, France) on Mueller-Hinton Agar (Becton Dickinson, NJ, USA) according to the manufacturer's instructions and the method previously described.[7]

The 23 test strains included 8 RMG strains, such as: *Mycobacterium chelonae* (3); *Mycobacterium abscessus* (3); and *Mycobacterium fortuitum* (2); and 15 slow growing mycobacteria (SGM) strains (*Mycobacterium avium complex* [10], and *Mycobacterium kansasii* [5]). The E-test MICs for tigecycline and organisms combinations are illustrated in **Table 1**.

Our results show that tigecycline was highly active against all strains of RGM included in the study with an MIC of 0.125 µg/mL or less. The opposite was noted for SGM, all of which had tigecycline MIC greater than 256 µg/mL. Both findings are consistent with those previously reported from other parts of the world.[4,5,8]

Our study is limited by the small number of isolates tested and the lack of MIC confirmation by broth microdilution. The results do, however, suggest that RGM strains from our region are likely to be highly susceptible to tigecycline. The use of E-test for MIC determination reflects actual practice in most diagnostic microbiology laboratories in the region, and is thereby, a closer reflection of its utility in clinical practice. Finally, although the tigecycline in-vitro activity against

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Table 1 - Tigecycline minimum inhibitory concentrations (MIC) for the study strains of non-tuberculous mycobacteria by Epsilometer test.

Species	No. of isolates tested	Number of isolates inhibited by MIC (µg/mL)								
		<0.016	0.032	0.047	0.125	1	8	64	256	>256
Mycobacterium avium complex	10	0	0	0	0	0	0	0	0	10
Mycobacterium kansasii	5	0	0	0	0	0	0	0	0	5
Mycobacterium abscessus	3	2	1	0	0	0	0	0	0	0
Mycobacterium chelonae	3	2	0	0	1	0	0	0	0	0
Mycobacterium fortuitum	2	2	0	0	0	0	0	0	0	0
Total	**23**	**6**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**15**

RGM is supported by some clinical reports of successful clinical therapy, this remains to be demonstrated in a controlled, prospective study.

In conclusion, tigecycline is highly active against RGM isolates from Saudi Arabia and is probably a useful therapeutic option to consider for patients with refractory RGM infections.

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