Evaluation of antimicrobial susceptibilities of rapidly growing mycobacteria by Sensititre RAPMYCO panel

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Infections due to rapidly growing mycobacteria (RGM), involving the chronic lung infections, skin, soft tissues and skeletal infections, catheter infections and disseminated infections, have acquired great clinical importance in recent years as they may affect both immunocompromised and immunocompetent patients. Treatment of infections due to RGM remains difficult, because they are resistant to many of the first-line tuberculosis agents (Bicmen et al., 2010, Brown-Elliott et al., 2002a). In order to optimize susceptibility testing and facilitate the interpretation of susceptibility results, identification of isolates to the species level or, at a minimum, differentiation of the Mycobacterium fortuitum group from the Mycobacterium chelonae-abscessus group is recommended by the Clinical and Laboratory Standards Institute (CLSI). Susceptibility testing is indicated for any RGM considered clinically significant and made by standard broth microdilution method (CLSI, 2003).

Sensititre RAPMYCO panel (Trek Diagnostic Systems Limited, Imberhorne Lane, East Grindstead, West Sussex RH 19 IQX UK) is a standard-ordered broth microdilution panel which can be used for the testing of RGM against amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, sulfamethoxazole, tobramycin and tigecycline. This study used Sensititre RAPMYCO to test the activities of various antibiotics against 25 clinical isolates of rapidly growing mycobacteria (RGM), including the common disease producing species Mycobacterium abscessus, Mycobacterium chelonae, Mycobacterium fortuitum and Mycobacterium peregrinum. Analysis of the four different RGM species showed that isolates of M. fortuitum and M. peregrinum were more susceptible than M. abscessus and M. chelonae. Different antimicrobials showed a variable sensitivity in all strains. Therefore, each species and strain must be individually evaluated, and it is always advisable to perform in vitro sensitivity tests before the treatment of infections due to RGM.

KEY WORDS: Rapidly growing mycobacteria, Antimicrobial susceptibility, Broth microdilution, Sensititre RAPMYCO

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SUMMARY

This study used Sensititre RAPMYCO to test the activities of amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, sulfamethoxazole, tigecycline and tobramycin against 25 clinical isolates of rapidly growing mycobacteria (RGM), including the common disease producing species Mycobacterium abscessus, Mycobacterium chelonae, Mycobacterium fortuitum and Mycobacterium peregrinum. Analysis of the four different RGM species showed that isolates of M. fortuitum and M. peregrinum were more susceptible than M. abscessus and M. chelonae. Different antimicrobials showed a variable sensitivity in all strains. Therefore, each species and strain must be individually evaluated, and it is always advisable to perform in vitro sensitivity tests before the treatment of infections due to RGM.

Twenty-five clinical isolates of RGM obtained from 25 different patients were included in the study. Organisms had been previously identified to the species level by a PCR-reverse hybridization method GenoType Mycobacterium CM assay (Hain Lifescience GmbH, Nehren, Germany). The
test species used and the number of clinical test isolates (in parentheses) were as follows: *M. abscessus* (7); *M. chelonae* (7); *M. fortuitum* (6); *M. peregrinum* (5).

Sensititre RAPMYCO was used according to the instructions of the manufacturer. Inoculum suspensions were prepared in sterile water to a density of 0.5 MacFarland standard. Fifty microlitres of suspension were transferred to a tube of cation adjusted Mueller Hinton broth (CAMHBT) with TES buffer. One hundred microlitres of this suspension was transferred to each well of the sensititre CAMHBT plate containing antibiotics in appropriate dilutions. All the wells were covered with adhesive seal and incubated at 30°C in a non-CO₂ incubator for 72 hours. Growth appeared as turbidity or as a deposit of cells at the bottom of the well. If poor, plates were reincubated for up to a further 48 hours. The susceptible and resistant breakpoints used were those recommended by the CLSI guidelines (CLSI, 2003). Quality control assays were performed with *Staphylococcus aureus* ATCC 29213. The same inoculation method was used for *S. aureus* ATCC 29213 and incubated at 35°C for 18-24 hours.

Susceptibilities to amikacin, tobramycin, doxycycline, tigecycline, ciprofloxacin, clarithromycin, linezolid cefoxitin, imipenem and sulfamethoxazole were determined for 25 clinical isolates of RGM belonging to 4 species (*M. abscessus, M. chelonae, M. fortuitum* and *M. peregrinum*) by Sensititre RAPMYCO. The drug susceptibility patterns of the isolates are shown in Table 1.

It is well-known that the *M. fortuitum* group is much less drug resistant than *M. abscessus* and *M. chelonae* (Brown-Elliott et al., 2002a). In agreement with previous reports (Brown et al., 1992; Swenson et al., 1985; Wallace et al., 1990; Wallace et al., 1991; Wallace et al., 2001), analysis of the four RGM species showed that isolates of *M. fortuitum* and *M. peregrinum* were more susceptible than *M. abscessus* and *M. chelonae* isolates. This study found that the MICs of amikacin for *M. abscessus* and *M. chelonae* isolates were higher than *M. fortuitum* and *M. peregrinum* isolates. However, all isolates tested were susceptible or

### Table 1 - Broth microdilution interpretive criteria and antimicrobial susceptibility of rapidly growing mycobacteria.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>MIC breakpoints (µg/mL)</th>
<th>MIC (µg/mL) for isolates of RGM</th>
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<tr>
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<tr>
<td>Amikacin</td>
<td>≤16</td>
<td>32 ≥64</td>
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<tr>
<td>Tobramycin</td>
<td>≤4 8 ≥16</td>
<td>&gt;16, 16, &gt;16, 16, &gt;16, &gt;16</td>
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<tr>
<td>Doxycycline</td>
<td>≤1 2-8 ≥16</td>
<td>8, 0.12, 0.25, 0.12, 0.12, 0.25</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>- - -</td>
<td>0.5, 0.25, 0.25, 0.25, 0.5, 0.5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤1 2 ≥4</td>
<td>0.12, 0.12, 0.12, 0.12, 0.12</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>≤2 4 ≥8</td>
<td>0.06, 1, 1, 0.25, 1</td>
</tr>
<tr>
<td>Linezolid</td>
<td>≤8 16 ≥32</td>
<td>2, 2, 2, 32, 2, 2</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤4 8 ≥16</td>
<td>4, -2, 8, 4, -2, 8</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>≤16 32-64 ≥128</td>
<td>16, 8, 16, 8, 8, 16</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>≤32 ≥64</td>
<td>-4.75, -4.75, 9.5, 76, -4.75, 9.5</td>
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</table>
moderately susceptible to amikacin, and were inhibited by amikacin at concentrations of ≤32 µg/ml. Swenson (Swenson et al., 1985) reported amikacin susceptibilities 100% for M. fortuitum group, 98% and 97% for M. abscessus and M. chelonae isolates, respectively. The others also found that amikacin has good activity against RGM species (Fernández-Roblas et al., 2008; Gayathri et al., 2010; Shen et al., 2007).

Amikacin was more active against M. fortuitum, M. peregrinum and M. abscessus isolates than tobramycin. In agreement with the result of Swenson (Swenson et al., 1985) but not with those of Fernández-Roblas (Fernández-Roblas et al., 2008) and Shen (Shen et al., 2007), in the present study tobramycin was also more active against M. chelonae isolates than amikacin. For this reason, the CLSI has recommended that tobramycin is the aminoglycoside of choice for isolates of M. chelonae and amikacin results should be reported only if the isolates is resistant to tobramycin (CLSI, 2003).

Tigecycline was more active than doxycycline against all RGM isolates. In accordance with the results of previous studies (Fernández-Roblas et al., 2008; Wallace et al., 2002), in this study, tigecycline showed the good activity and all strains tested were inhibited by tigecycline at concentrations of ≤1 µg/ml. According to these findings tigecycline could be a potentially useful antibiotic for treating infections caused by these organisms. Wallace (Wallace et al., 2002) reported doxycycline susceptibility rates to be 5%, 15% and 56% for M. abscessus, M. chelonae and M. fortuitum isolates, respectively. Fernández-Roblas (Fernández-Roblas et al., 2008) found MIC\textsubscript{50} of doxycycline for isolates of M. abscessus, M. chelonae, M. peregrinum and M. fortuitum >64, 8, 8 and 4 µg/ml, respectively. Our findings were also the same as the previous studies (Swenson et al., 1985; Fernández-Roblas et al., 2008; Wallace et al., 2002) and this study found that among the isolates of RGM, M. fortuitum was the most susceptible species, and M. abscessus was the most resistant species to doxycycline.

Analysis of the four different RGM species showed that ciprofloxacin was most active against isolates of M. fortuitum and M. peregrinum while M. abscessus and M. chelonae isolates were the most resistant, these findings were also reported previously (Brown-Elliott et al., 2002b; Fernández-Roblas et al., 2000; Fernández-Roblas et al., 2008; Wallace et al., 1990). Previous studies reported ciprofloxacin susceptibility rates to be 6% and 0% for M. abscessus isolates and 19% and 8%, for M. chelonae isolates, respectively (Brown-Elliott et al., 2002b; Wallace et al., 1990). In the present study, all isolates of M. abscessus and 5 of 7 M. chelonae isolates were also resistant to ciprofloxacin.

In the present study, clarithromycin showed the best activity against isolates of M. chelonae and all strains tested were inhibited by clarithromycin at concentrations of ≤0.5 µg/ml. Clarithromycin also showed good activity against isolates of M. abscessus, and 5 out of 7 were inhibited by clarithromycin at concentrations of ≤2 µg/ml, and 100% were inhibited by clarithromycin at 4 µg/ml. However, 2 out of 5 M. peregrinum isolates were inhibited by clarithromycin at concentrations of 4 µg/ml or higher, and 5 out of 6 M. fortuitum isolates were inhibited by clarithromycin at 1 µg/ml, and one was inhibited by clarithromycin at 8 µg/ml. In the previous studies (Brown et al., 1992; Fernández-Roblas et al., 2000; Fernández-Roblas et al., 2008) MICs of clarithromycin among the RGM species were highly variable. While isolates of M. abscessus, M. chelonae and M. peregrinum were found susceptible to clarithromycin, isolates of M. fortuitum were the most resistant RGM species.

All isolates of M. chelonae and M. peregrinum and 5 out of 6 M. fortuitum isolates were susceptible to linezolid. In this study, the MICs of linezolid were higher for M. abscessus than for M. peregrinum, M. fortuitum and M. chelonae. In accordance with the previous report (Vera-Cabrera et al., 2006; Wallace et al., 2001) M. abscessus was the least susceptible to linezolid of the common species of RGM.

All isolates of M. chelonae were resistant to imipenem and cefoxitin, while M. fortuitum and M. peregrinum isolates susceptible or moderately susceptible to imipenem and cefoxitin. With regard to M. abscessus, all isolates of tested were resistant to imipenem and susceptible or moderately susceptible to cefoxitin. In disagreement with the result of Wallace (Wallace et al., 1991), who reported imipenem susceptibility rates to be 57% for M. abscessus isolates, and 39% for M. chelonae isolates, in the present study all isolates of M. abscessus and M. chelonae were found resistant to
imipenem. In agreement with previous reports (Fernández-Roblas et al., 2008; Swenson et al., 1985), in this study all isolates of M. chelonae and M. abscessus were resistant to sulfamethoxazole, and all isolates of M. fortuitum and M. peregrinum susceptible to sulfamethoxazole.

In conclusion, the treatment of serious infections with RGM is difficult and limited by the small number of available drugs. Different antimicrobials exists in all strains with variable sensitivity; therefore, each species and strain must be individually evaluated, and it is advisable always to perform in vitro sensitivity tests before using the drug for human therapy.

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REFERENCES


