Susceptibilities of *Mycobacterium fortuitum* biovar. *fortuitum* and the Two Subgroups of *Mycobacterium chelonae* to Imipenem, Cefmetazole, Cefoxitin, and Amoxicillin-Clavulanic Acid

RICHARD J. WALLACE, JR.,* BARBARA A. BROWN, AND GRACE O. ONYI

Department of Microbiology, The University of Texas Health Center, Tyler, Texas 75710

Received 1 October 1990/Accepted 15 January 1991

MICs of imipenem, cefoxitin, cefmetazole, and amoxicillin-clavulanic acid were determined against 100 strains of *Mycobacterium fortuitum* and 200 strains of *Mycobacterium chelonae* subsp. *chelonae*. Imipenem and cefmetazole were more active against *M. fortuitum* than cefoxitin was, and imipenem (which inhibited 39% of strains at 8 μg/ml) was the only beta-lactam active against *M. chelonae* subsp. *chelonae*.

Rapidly growing mycobacteria cause a variety of infections, the majority of which involve skin and soft tissues (22). Antimicrobial therapy based on in vitro susceptibilities combined with surgical debridement are the indicated therapy for patients with serious cutaneous disease (9, 20). Long-term drug therapy of 3 to 6 months is usually needed (20). Amikacin is the most common drug used for serious disease, often in combination with cefoxitin (8, 9, 20). Because of toxicity (amikacin) and a relatively short half-life (cefoxitin), there has been continued interest in other potential drugs for therapy.

We chose to compare three beta-lactams (cefmetazole, imipenem, and amoxicillin-clavulanic acid) with cefoxitin for their in vitro activities against the common pathogenic species of rapidly growing mycobacteria, *Mycobacterium fortuitum* and *Mycobacterium chelonae*, after preliminary studies showed that the drugs have therapeutic potential (5, 17).

MICs were determined by using broth microdilutions with cation-supplemented Mueller-Hinton broth as described by Swenson et al. (16). Twofold dilutions of cefoxitin, cefmetazole, imipenem, and amoxicillin-clavulanic acid (2:1 ratio) were prepared and added to 96-well plates by using the Mini-Quick Spense II system (Dynatech Laboratories, Chantilly, Va.). Plates were inoculated with disposable inoculators with a dilution designed to obtain a final well concentration of 10^4 to 10^5 CFU/ml. The plates were covered, sealed in plastic bags, and incubated in room air at 30°C for 3 days. Quality control was performed by using *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218 (clavulanic acid), and *M. fortuitum* ATCC 6841. Moderately susceptible breakpoints, for imipenem (8 μg/ml) and amoxicillin-clavulanic acid (16/8 μg/ml) were the moderately susceptible breakpoints of the National Committee for Clinical Laboratory Standards (NCCLS) for aerobic bacteria (13). An MIC of 32 μg/ml rather than 16 μg/ml (the latter being the NCCLS breakpoint) has been used as the moderately susceptible breakpoint for cefoxitin against the rapidly growing mycobacteria (17, 20). Although not yet approved by NCCLS, the proposed moderately susceptible breakpoint for cefmetazole is also 32 μg/ml (10). Clinical isolates of *M. fortuitum* and *M. chelonae* submitted to the Mycobacterial/Nocardia Research Laboratory of the University of Texas between 1987 and 1990 were tested. Identification to the species level was performed by standard methods (14, 21), and identification to the subspecies level was by carbohydrate utilization tests and/or drug susceptibility patterns (14, 15).

One hundred isolates of *M. fortuitum* biovariant *fortuitum*, 141 isolates of *M. chelonae* subsp. *abscessus*, and 59 isolates of *M. chelonae* subsp. *chelonae* were tested. The results are given in Table 1. Imipenem was the most active beta-lactam, inhibiting 100% of *M. fortuitum* and approximately 50% of the two subspecies of *M. chelonae* at the susceptible breakpoint of 8 μg/ml. Amoxicillin-clavulanic acid was active only against *M. fortuitum*, with fewer than 5% of *M. chelonae* isolates being susceptible to 16/8 μg/ml. Cefmetazole inhibited 82% of *M. fortuitum* at 16 μg/ml compared with only 18% inhibition by cefoxitin. There was no difference between the two drugs in their activities against *M. chelonae*.

Previous studies have shown that cefoxitin has in vitro activity against *M. fortuitum* (1, 7, 9, 16, 17) and most isolates of *M. chelonae* subsp. *abscessus* (1, 16, 17), although the usual modal MIC for both organisms is 32 μg/ml, which is in the moderately susceptible category (as defined for these organisms) (10, 17). Results for cefoxitin from the current study were comparable.

Cefmetazole, like cefoxitin, is a 7-α-methoxycephalosporin or cephamycin, but the former has approximately 20% higher peak levels in serum and a longer half-life (90 versus 50 min) (11), which may allow for longer dosing intervals. The current study showed that cefmetazole has two- to fourfold greater activity than cefoxitin against *M. fortuitum* biovar. *fortuitum*, supporting similar findings in two previous studies by Cynamon and Palmer (5) (13 strains) and Casal et al. (3) (30 strains). (Isolates in the latter two studies were not identified to the biovariant level.) An MIC comparison of eight strains of the unnamed third biovariant complex of *M. fortuitum* also demonstrated two- to fourfold greater activity of cefmetazole compared with that of cefoxitin (19).

A previous study of cefmetazole against 20 strains of *M. chelonae* by Casal et al. (4) showed that it inhibits 40% of the strains at 16 μg/ml. The subspecies of the isolates was not determined. The current study showed that the activities of cefoxitin and cefmetazole against *M. chelonae* are comparable, but they have activities at clinically achievable levels only against *M. chelonae* subsp. *abscessus*. The isolates of *M. chelonae* subsp. *chelonae* were all highly resistant to...
both cephams (MICs, >128 μg/ml). Previous studies of cefotixin-resistant isolates of the unnamed third biovariant complex of \textit{M. fortuitum} showed that they are also cross-resistant to cefmetazole (19).

Imipenem was the most active beta-lactam studied, inhibiting 100% of \textit{M. fortuitum} isolates, 57% of \textit{M. chelonae} subsp. \textit{abscessus} isolates, and 39% of \textit{M. chelonae} subsp. \textit{chelonae} isolates at 8 μg/ml. The MICs for \textit{M. fortuitum} were comparable to those given in prior studies by Cynamon and Palmer (5) (12 strains) and Swenson et al. (17) (17 strains). The same MICs for 50% of isolated tested (2 μg/ml) and MICs for 90% of isolates tested (4 μg/ml) for imipenem observed here for the isolates of \textit{M. fortuitum} biovar. \textit{fortuitum} have been reported for 75 isolates of the unnamed third biovariant complex of \textit{M. fortuitum} (18). One-third of these latter isolates were resistant to cefotixin. Imipenem is the only known beta-lactam with activity against \textit{M. chelona} subsp. \textit{chelonae}.

Isolates of \textit{M. fortuitum} biovar. \textit{fortuitum} have a single chromosomal \beta-lactamase (19) which is partially susceptible in vitro to clavulanic acid (6, 12). Previous studies (2, 6, 12) showed that clavulanic acid produces a two- to fourfold decrease in MICs for \textit{M. fortuitum} when compared with that of amoxicillin alone. The current study showed an MIC for 90% of strains of 16/8 μg of the amoxicillin-clavulanic acid combination (2:1 ratio) per ml. By using the peak achievable level in serum of 2 μg of clavulanic acid per ml as a breakpoint, however, only 18% of strains were susceptible.

Imipenem was used successfully in the monotherapy of a case of \textit{M. chelonae} lung disease (MIC, 8 μg/ml) (23). No information is available on the clinical usefulness of amoxicillin-clavulanic acid and cefmetazole. However, both imipenem and cefmetazole have as good a potential for therapy as that of the only other proven beta-lactam, cefotixin, and all three drugs have sufficient in vitro activities to warrant clinical evaluation.

REFERENCES

15. Steele, L. C., and R. J. Wallace, Jr. 1987. Ability of ciprofloxacin but not pipemidic acid to differentiate all three biovariants

### TABLE 1. Antimicrobial susceptibilities of the three subgroups of rapidly growing mycobacteria to selected beta-lactams

<table>
<thead>
<tr>
<th>Drug</th>
<th>Subgroup (no. of isolates)</th>
<th>Cumulative % of strains susceptible at the following MICs (μg/ml):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotixin</td>
<td>\textit{M. fortuitum} biovar. \textit{fortuitum} (100)</td>
<td>2 18 77 100</td>
</tr>
<tr>
<td></td>
<td>\textit{M. chelonae} subsp. \textit{abscessus} (141)</td>
<td>1 29 87 99 100</td>
</tr>
<tr>
<td></td>
<td>\textit{M. chelonae} subsp. \textit{chelonae} (59)</td>
<td>2 100</td>
</tr>
<tr>
<td>Cefmetazole</td>
<td>\textit{M. fortuitum} biovar. \textit{fortuitum} (100)</td>
<td>5 55 83 100</td>
</tr>
<tr>
<td></td>
<td>\textit{M. chelonae} subsp. \textit{abscessus} (141)</td>
<td>3 27 76 96 100</td>
</tr>
<tr>
<td></td>
<td>\textit{M. chelonae} subsp. \textit{chelonae} (59)</td>
<td>5 100</td>
</tr>
<tr>
<td>Imipenem</td>
<td>\textit{M. fortuitum} biovar. \textit{fortuitum} (100)</td>
<td>16 37 55 93 100</td>
</tr>
<tr>
<td></td>
<td>\textit{M. chelonae} subsp. \textit{abscessus} (141)</td>
<td>1 4 27 57 89 96 100</td>
</tr>
<tr>
<td></td>
<td>\textit{M. chelonae} subsp. \textit{chelonae} (59)</td>
<td>3 14 39 73 92 100</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid (2:1)</td>
<td>\textit{M. fortuitum} biovar. \textit{fortuitum} (100)</td>
<td>1 18 62 90 96 100</td>
</tr>
<tr>
<td></td>
<td>\textit{M. chelonae} subsp. \textit{abscessus} (141)</td>
<td>1 2 3 4 100</td>
</tr>
<tr>
<td></td>
<td>\textit{M. chelonae} subsp. \textit{chelonae} (59)</td>
<td>2 2 100</td>
</tr>
</tbody>
</table>

* The concentration listed is that for amoxicillin.


