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In Vitro Comparison of Ertapenem, Meropenem, and Imipenem against Isolates of Rapidly Growing Mycobacteria and *Nocardia* by Use of Broth Microdilution and Etest

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We compared the activities of the carbapenems ertapenem, meropenem, and imipenem against 180 isolates of rapidly growing mycobacteria (RGM) and 170 isolates of *Nocardia* using the Clinical and Laboratory Standards Institute (CLSI) guidelines. A subset of isolates was tested using the Etest. The rate of susceptibility to ertapenem and meropenem was limited and less than that to imipenem for the RGM. Analysis of major and minor discrepancies revealed that >90% of the isolates of *Nocardia* had higher MICs by the broth microdilution method than by Etest, in contrast to the lower broth microdilution MICs seen for >80% of the RGM. Imipenem remains the most active carbapenem against RGM, including *Mycobacterium abscessus* subsp. *abscessus*. For *Nocardia*, imipenem was significantly more active only against *Nocardia farcinica*. Although there may be utility in testing the activities of the newer carbapenems against *Nocardia*, their activities against the RGM should not be routinely tested. Testing by Etest is not recommended by the CLSI.

Treatment of infections due to rapidly growing mycobacteria (RGM) and *Nocardia* remains difficult in part because of resistance to first-line antituberculous agents (for RGM) and other antimicrobial agents (for both RGM and *Nocardia*) (1, 2). Previous studies with the carbapenems have shown that these agents have limited activity against most pathogenic RGM, but few data on their activity against *Nocardia* exist (3, 4). Although ertapenem and meropenem have been in use against clinically significant bacteria for several years, there has been a paucity of data on the activities of these agents against RGM and *Nocardia*. Thus, we undertook a comparative study of the *in vitro* susceptibilities to imipenem, meropenem, and ertapenem of the most commonly encountered species of RGM and *Nocardia*, including the *Mycobacterium fortuitum* group (*M. fortuitum*, *M. senegalense*, *M. porcinum*); *M. chelonae*; the *M. abscessus* complex, including *M. abscessus* subsp. *abscessus* (formerly *M. abscessus* and here referred to as *M. abscessus*), *M. abscessus* subsp. *bolletii* (here referred to as *M. bolletii*), and *M. abscessus* subsp. *massiliense* (here referred to as *M. massiliense*); the *M. mucogenicum/M. phocaicum* group; *M. neoaurum*; *M. goodii*; *M. immunogenum*; *Nocardia cyriacigeorgica*; members of the *N. nova* complex; *N. farcinica*; *N. brasiliensis*; *N. abscessus*; *N. otitidiscaviarum*; members of the *N. transvalensis* complex; and *N. pseudobrasiliensis*. The taxonomy of the *M. abscessus* complex is currently controversial (5). However, for clarity, we have chosen to use the taxonomy proposal made prior to 2011 to combine the species *M. massiliense* and *M. bolletii* into one subspecies (i.e., *M. abscessus* subsp. *bolletii*). We also compared MICs for selected isolates from several of the clinically significant species using the Clinical Laboratory and Standards Institute (CLSI)-recommended broth microdilution method and the Etest.

MATERIALS AND METHODS

Organisms. Clinical isolates of RGM and *Nocardia* submitted to the University of Texas Health Science Center at Tyler, TX (UTHSCT), for susceptibility testing from 2006 to 2008 were selected for testing. This set included 180 isolates of RGM (67 *M. abscessus* isolates, 11 *M. massiliense* isolates, 3 *M. bolletii* isolates, 38 *M. fortuitum* isolates, 10 *M. porcinum* isolates, 7 *M. senegalense* isolates, 21 *M. chelonae* isolates, 16 *M. mucogenicum/M. phocaicum* group isolates, and 7 other isolates of RGM, including 2 isolates of the *M. neoaurum-M. lacticola* group, 3 *M. goodii* isolates, and 2 *M. immunogenum* isolates). The 170 isolates of *Nocardia* tested included 26 *N. cyriacigeorgica* isolates, 57 *N. nova* complex isolates, 13 *N. abscessus* isolates, 23 *N. brasiliensis* isolates, 19 *N. farcinica* isolates, 18 *N. transvalensis* complex isolates, 8 *N. otitidiscaviarum* isolates, 1 *N. pseudobrasiliensis* isolate, and 5 *Nocardia* sp. isolates.

Isolates of RGM and *Nocardia* were identified to the species level by molecular methods, including PCR restriction enzyme analysis (PRA) of a 441-bp sequence of the 65-kDa *hsp* gene (6, 7), and their antimicrobial susceptibility patterns (2, 8–15). Isolates that were not identifiable by PRA were subjected to 16S rRNA (16) and/or multigene target (*hsp65*, *secA1*, *rpoB*, etc.) sequence analysis (3, 17–20).

Susceptibility testing. The MICs of imipenem, meropenem, and ertapenem were determined by broth microdilution using the CLSI-recommended procedure and interpretive criteria for RGM and *Nocardia* (21) with imipenem. MIC panels were custom manufactured to include meropenem and ertapenem by Thermo Fisher (formerly Trek Diagnostics, Inc.). Since no interpretive criteria for mycobacteria and *Nocardia* with ertapenem are currently available, the CLSI-recommended intermediate (I) breakpoint (4 μg/ml) for testing of bacteria was employed (8). These bacterial breakpoints were also applied to testing of the *Nocardia* with meropenem and ertapenem, which has not been addressed by the CLSI. Additional testing by Etest (AB Biodisk, Uppsala, Sweden) was performed as previously described (22, 23), and the Etest results were compared to the broth microdilution results for 102 selected isolates of RGM and 87 isolates of *Nocardia* spp. For Etest MICs that fell between doubling dilutions, the results were rounded up

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TABLE 1 Acceptable MIC ranges[a] and numbers of quality control tests performed within those ranges

Quality control strain	Imipenem		Meropenem		Ertapenem	
	Acceptable MIC range (μg/ml)	No. of tests	Acceptable MIC range (μg/ml)	No. of tests	Acceptable MIC range (μg/ml)	No. of tests
Enterococcus faecalis ATCC 29212	0.5–2	225/225	2–8	225/225	4–16	225/225
Mycobacterium peregrinum ATCC 700686	2–16	250/250	2–16	250/250	NA[b]	NA[b]
Staphylococcus aureus ATCC 29213	0.015–0.06	34/34	0.03–0.12	34/34	0.06–0.25	31/31
Pseudomonas aeruginosa ATCC 27853	1–4	84/84	0.25–1	32/32	2–8	84/84

[a] Acceptable MIC ranges have been described previously (8, 21).
[b] NA, not available.

to the next 2-fold value, as recommended by the manufacturer. For both methods, results were read at 100% inhibition.

Quality control. Quality control for the carbapenems was performed by using *M. peregrinum* ATCC 700686, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853. CLSI-recommended quality control ranges were those shown in Table 1. Quality control of the broth microdilution assays and Etests was performed at the time of performance of each test.

RESULTS

Quality control. All quality control values for all three carbapenems were within acceptable limits with all reference isolates tested (Table 1).

Broth microdilution MICs. The susceptibilities of the most commonly encountered pathogenic species of RGM and *Nocardia* to the three carbapenems are shown in Tables 2 and 3, respectively. For the most commonly encountered RGM, the best activity of the carbapenems was noted against isolates of the *M. fortuitum* group and the *M. mucogenicum/M. phocaicum* group. Imipenem, meropenem, and ertapenem were active against 100% of 16 isolates of the *M. mucogenicum/M. phocaicum* group. The MICs of imipenem (MIC_{50} = 4 μg/ml) and meropenem (MIC_{50} = 8 to 16 μg/ml) were ≤16 μg/ml for 100% of the isolates in the *M. fortuitum* group (including *M. senegalense* and *M. porcinum*) (Table 2). Only 11% (1/9) of the isolates of *M. porcinum*

TABLE 2 Comparison of MIC ranges, MIC_{50}s, MIC_{90}s, and percentage of isolates susceptible/intermediate to imipenem, meropenem, and ertapenem for RGM isolates by broth microdilution

Complex or species and drug	Intermediate breakpoint (μg/ml)	No. of isolates tested	MIC (μg/ml)			% susceptible/ intermediate
			Range	50%	90%	
M. fortuitum						
Imipenem	8–16	38	2–8	4	8	100
Meropenem	8–16	38	4–16	8	16	100
Ertapenem	4[a]	38	8–32	16	>32	0
M. porcinum						
Imipenem	8–16	10	2–16	4	8	100
Meropenem	8–16	9	2–16	16	16	100
Ertapenem	4[a]	9	1–16	8	16	11
M. abscessus subsp. *abscessus*						
Imipenem	8–16	67	4–>16	>16	>16	66
Meropenem	8–16	67	8–>16	>16	>16	12
Ertapenem	4[a]	67	8–>32	>32	>32	0
M. massiliense						
Imipenem	8–16	11	8–>16	>16	>16	73
Meropenem	8–16	11	≥16	>16	>16	9
Ertapenem	4[a]	11	≥32	>32	>32	0
M. chelonae						
Imipenem	8–16	21	8–16	16	≥16	52
Meropenem	8–16	21	>16–>32	>16	>16	0
Ertapenem	4[a]	21	>16–>32	>32	>32	0
M. mucogenicum/M. phocaicum group						
Imipenem	8–16	16	≤0.5–4	2	4	100
Meropenem	8–16	16	≤0.5–8	4	8	100
Ertapenem	4[a]	16	2–4	2	4	100

[a] Based on the CLSI breakpoint for bacteria.

TABLE 3 Comparison of ranges, MIC_{50}s, MIC_{90}s, and percentage of isolates susceptible/intermediate to imipenem, meropenem, and ertapenem for *Nocardia* isolates by broth microdilution

Complex or species and drug	Intermediate breakpoint (μg/ml)	No. of isolates tested	MIC (μg/ml)			% susceptible/ intermediate
			Range	50%	90%	
N. cyriacigeorgica						
Imipenem	8	25	≤1–32	8	>16	60
Meropenem	8	25	4–>16	8	>16	68
Ertapenem	4[a]	26	2–>16	8	>16	15
N. nova complex						
Imipenem	8	57	≤0.5–8	≤1	2	100
Meropenem	8	54	≤0.5–16	≤1	4	94
Ertapenem	4[a]	57	0.5–16	2	4	96
N. abscessus						
Imipenem	8	13	2–32	>16	32	23
Meropenem	8	11	1–8	2	4	100
Ertapenem	4[a]	13	0.5–4	2	4	100
N. brasiliensis						
Imipenem	8	23	16–>32	>16	>32	0
Meropenem	8	23	4–>16	>16	>16	48
Ertapenem	4[a]	23	4–>16	>16	>16	26
N. farcinica						
Imipenem	8	19	≤1–>16	8	>16	63
Meropenem	8	18	4–>16	8	>16	33
Ertapenem	4[a]	19	4–>16	8	16	21
N. transvalensis complex						
Imipenem	8	18	4–>32	16	>32	22
Meropenem	8	18	2–16	8	16	83
Ertapenem	4[a]	18	2–>16	>16	>16	22

[a] Based on the CLSI breakpoint for bacteria (8).

had MICs indicating that they were susceptible (S)/intermediate (I) to ertapenem, similar to the results for both *M. fortuitum* and *M. senegalense* (0% of which were S/I) (data not shown).

Other clinically significant but less commonly encountered species included two isolates of the *M. neoaurum-M. lacticola* group and three isolates of *M. goodii*, which had 100% susceptibility to all three carbapenems (data not shown). In contrast, the ertapenem MICs for two isolates of *M. immunogenum* were >32 μg/ml (data not shown). The activity of meropenem was tested against only one isolate of *M. immunogenum* (MIC > 16 μg/ml); one isolate of *M. immunogenum* was resistant (R) (MIC > 32 μg/ml) and one was I (MIC = 16 μg/ml) to imipenem.

Sixty-six percent of 67 isolates of *M. abscessus* and 73% of 11 isolates of *M. massiliense* had imipenem MICs indicating that they were S/I, whereas meropenem had activity against ≤12% and ertapenem had activity against 0% of isolates in both groups. Meropenem and ertapenem showed no activity against three isolates of *M. bolletii*, in contrast to the I imipenem MICs (data not shown). Among 21 isolates of *M. chelonae*, 52% (11/21) had S/I imipenem MICs, and all isolates were R to meropenem and ertapenem, with MIC_{50}s of >16 μg/ml (Table 2).

For the *Nocardia*, only the members of the *N. nova* complex had MIC_{90}s in the S range for all three carbapenems (Table 3). One hundred percent of the isolates of the *N. abscessus* complex exhibited S/I meropenem MICs (11/11) and S/I ertapenem MICs (13/13), but only 23% were S/I to imipenem. The only other taxon against which any carbapenem had >80% activity was the *N. transvalensis* complex, with 83% (15/18) having S/I meropenem MICs (MIC_{50} = 8 μg/ml); in contrast, only 22% (4/18) had S/I imipenem and ertapenem MICs. The MIC_{90}s of all three carbapenems for all other species of *Nocardia* were ≥16 μg/ml. Only 48% (11/23) and 26% (6/23) of the isolates of *N. brasiliensis* were S/I to meropenem and ertapenem, respectively. One isolate of *N. pseudobrasiliensis*, eight isolates of the *N. otitidiscaviarum* complex, and three isolates unable to be identified to the species level (*Nocardia* spp.) had MIC_{90}s in the R interpretive category for all three carbapenems (data not shown).

Comparison of broth microdilution and Etest MICs. The MICs for a total of 197 isolates of RGM (n = 102) and *Nocardia* (n = 95) obtained by the broth microdilution and Etest methods were compared. Table 4 provides a comparison of the very major, major, and minor errors in each taxon studied with isolate numbers of ≥10. The CLSI defines very major errors (VME) to be an interpretive category change from R by the reference method (i.e., in this case, broth microdilution) to S by the method being evaluated (i.e., Etest). A major error is defined as an interpretive category change from S by broth microdilution to R by Etest. Minor errors are those in which one result is I and the other is S or R. In general, most discrepancies were considered minor (interpretive category change from S to I or vice versa or R to I or vice versa).

TABLE 4 Comparison of Etest MICs and broth microdilution MICs of imipenem, meropenem, and ertapenem for isolates of RGM and *Nocardia*

Species or complex and drug	No. of isolates tested	% error		
		Very major	Major	Minor
M. fortuitum				
Ertapenem	22	5	0	0
Meropenem	22	0	0	86
Imipenem	22	0	5	36
M. abscessus subsp. *abscessus*				
Ertapenem	44	0	0	0
Meropenem	44	0	0	14
Imipenem	44	0	7	48
M. chelonae				
Ertapenem	13	0	0	0
Meropenem	12	0	0	0
Imipenem	12	0	0	75
N. cyriacigeorgica				
Ertapenem	10	0	0	10
Meropenem	10	0	0	20
Imipenem	11	0	36	18
N. nova complex				
Ertapenem	32	0	3	43
Meropenem	31	0	0	9
Imipenem	32	0	0	0
N. brasiliensis				
Ertapenem	13	0	0	31
Meropenem	13	0	8	31
Imipenem	13	0	0	8
N. transvalensis complex				
Ertapenem	12	33	8	17
Meropenem	12	8	8	42
Imipenem	12	8	0	25

The discrepancies between the broth microdilution and Etest susceptibilities for the RGM showed rare (≤7%) major errors in the three major groups (*M. fortuitum*, *M. abscessus*, *M. chelonae*) (Table 4), except for *M. chelonae*, 75% of 12 isolates of which tested showed minor errors. Only one of five (20%) isolates of *M. porcinum* showed major discrepancies with ertapenem (data not shown). In all but one case (*M. fortuitum*), the broth microdilution MICs indicated resistance whereas the Etest reads indicated higher susceptibility than the broth MICs.

Only 1 of 22 isolates of *M. fortuitum* had a very major error with ertapenem, with the broth microdilution MIC indicating resistance at 8 μg/ml but the Etest indicating susceptibility at 1 μg/ml. Among six isolates of the *M. mucogenicum/M. phocaicum* group, one isolate had a major error with meropenem (data not shown).

Minor errors were most commonly seen in *M. fortuitum* with meropenem and imipenem (86% and 36%, respectively). For 15 of 16 isolates (94%), meropenem broth microdilution MICs were 8 μg/ml (intermediate), but Etest MIC reads were ≥16 μg/ml (resistant). Similarly, 3 of 44 isolates of *M. abscessus* had major errors with imipenem, in which the broth microdilution MICs were ≤4 μg/ml (S) but the Etest MICs were read to be >32 μg/ml (R). Forty-eight percent and 14% of the *M. abscessus* isolates had minor errors with imipenem and meropenem, respectively. One hundred percent of four isolates of the related group, the *M. massiliense* group, had major errors with imipenem, and 25% of the same group had minor errors with meropenem. One of four *M. senegalense* isolates had a broth microdilution imipenem MIC of 8 μg/ml but a susceptible Etest MIC read of 0.5 μg/ml (data not shown). All of the 13 isolates of *M. chelonae* were resistant by broth microdilution and Etest, and none of the isolates exhibited any minor or major errors with meropenem and ertapenem, but 75% had minor errors with imipenem. In general, ≥80% of the errors (both major and minor) were due to lower broth microdilution MICs rather than lower Etest MICs.

Of the 95 isolates of *Nocardia* compared by Etests, the majority of very major and major errors with ertapenem were seen with the *N. transvalensis* complex (Table 4). Of 12 isolates of the *N. transvalensis* complex tested, 4 isolates (33%) exhibited very major errors with ertapenem and 8% of the isolates showed very major errors with each of meropenem and imipenem. Eight percent of the 12 isolates showed major errors with each of ertapenem and meropenem, while 42% and 25% had minor errors with meropenem and imipenem, respectively. There were also major errors for 4 of 11 (36%) isolates of *N. cyriacigeorgica* with imipenem. In all four isolates, the broth microdilution MIC indicated resistance (>16 μg/ml), whereas the Etest MICs were ≤4 μg/ml.

For the *N. nova* complex, there were only rare major errors (1/32, or 3%) for ertapenem. Again, the broth microdilution MICs were higher than the Etest MIC reads.

For the one major error noted with 13 isolates of *N. brasiliensis* with meropenem, the broth microdilution MIC was susceptible (4 μg/ml), whereas the Etest MIC reading was resistant (16 μg/ml).

Analysis of both the major and minor errors for the nocardiae revealed that >90% of the isolates of *Nocardia* had higher MICs by broth microdilution than by the Etest. This finding was in contrast to the lower broth microdilution MICs seen for >80% of the RGM.

A comparison of the discrepant results obtained with taxa with >10 isolates tested by broth microdilution and Etest is seen in Table 4. There were significant discrepant results with several species of RGM and *Nocardia*, but in all cases, the numbers of tests performed by Etest were less than those performed by broth microdilution. Strikingly, different results by both methods were primarily seen with imipenem and were less commonly seen with meropenem. By broth microdilution, 100% of the isolates of *M. fortuitum* were S/I to all three carbapenems. However, by Etest only, 77%, 27%, and 5% of the isolates were S/I to imipenem, meropenem, and ertapenem, respectively. Another obvious discrepancy was noted with susceptibility to meropenem among isolates of *M. senegalense*, 100% of which were S/I by broth microdilution but only 25% were S/I by Etest, although the number of isolates compared was less than 10 (data not shown). Similarly, 100% of the nine isolates of *M. porcinum* tested were S/I to meropenem by broth microdilution, whereas only 33% were S/I to meropenem by Etest. Only 11% of the isolates of this species were S/I to ertapenem by broth microdilution, whereas 50% were S/I to ertapenem by Etest (data not shown). Of the isolates of *M. abscessus* and *M. massiliense* tested, 66% and 73%, respectively, were susceptible to imipenem by broth microdilution, whereas 0%

were susceptible by Etests (data not shown for *M. massiliense*). Additionally, 100% of the isolates of the *M. mucogenicum/M. phocaicum* group showed susceptibility to meropenem by broth microdilution, whereas only 67% showed susceptibility by the Etest method.

Among the *Nocardia*, the most striking discrepancies were again mostly with meropenem and imipenem. Fifty-four, 68, and 15% of the *N. cyriacigeorgica* isolates were S/I to imipenem, meropenem, and ertapenem, respectively, by broth microdilution, whereas 0, 36, and 55% were susceptible to the same agents, respectively, by Etest. Another difference was noted with imipenem and ertapenem and isolates of the *N. transvalensis* complex. By broth microdilution, 22% of the isolates were S/I to both carbapenems; in contrast, 42% were S/I to both carbapenems by Etests. Although only eight isolates were tested, the MICs of meropenem for isolates of the *N. otitidiscaviarum* complex showed a wide discrepancy, with 43% of isolates being S/I by Etest but only 25% being S/I when broth microdilution was performed (data not shown). Likewise, 48% of the isolates of *N. brasiliensis* were S/I to meropenem by microdilution, whereas only 23% were S/I to meropenem by Etest. Isolates of *N. farcinica* were more susceptible to meropenem and ertapenem (33% and 21%, respectively) when they were tested by the broth microdilution method than when they were tested by Etest (both only 13%), although the MICs of imipenem were equivalent (63% of isolates were S/I to imipenem) by both methods.

DISCUSSION

Treatment of infections due to RGM and *Nocardia* is often difficult because of the lack of antimicrobials with activity against these species. Additional complications arise due to the need for injectable antibiotics for most serious infections. Imipenem has been useful for the treatment of infections caused by most common pathogenic species of RGM and *Nocardia*, although some species of *Nocardia*, including *N. abscessus* and *N. brasiliensis*, and some isolates of *M. chelonae* and the *M. abscessus* complex are resistant. The necessity for the administration of imipenem two to three times daily creates problems for long-term therapy, which is required for the treatment of infections with RGM and *Nocardia*.

The results of this study indicate that neither the broth microdilution nor Etest MICs of imipenem are able to consistently predict susceptibility or resistance to meropenem and ertapenem. This fact was illustrated in this study with isolates of *M. abscessus*, in which imipenem had activity against 66% (44 of 67) of the isolates but neither meropenem nor ertapenem had significant activity by both the broth microdilution and Etest methods. Furthermore, although imipenem and meropenem showed activity against all isolates of the *M. fortuitum* group by broth microdilution, ertapenem was not active against any of these isolates.

For the *Nocardia* spp., only the isolates of the *N. nova* complex were uniformly susceptible or intermediate to all three carbapenems. Although isolates of the *N. abscessus* group were typically resistant to imipenem (10/13, or 77%), interestingly, 100% were S/I to meropenem and ertapenem. Among the isolates of the *N. transvalensis* complex, meropenem was the most active carbapenem by broth microdilution (22% [4/18] were S/I to ertapenem and imipenem, whereas 83% [15/18] were susceptible to meropenem).

Previous studies have demonstrated the instability of imipenem and meropenem related to the prolonged incubation (greater than 3 to 4 days) sometimes required by isolates of mycobacteria and *Nocardia* (24). Meropenem was also previously noted to be more stable, with an approximately 50% reduction in activity of the agent at 24 h, in comparison to an 85% loss of activity of imipenem at 24 h (24). This instability likely contributes to the high *in vitro* MICs seen in susceptibility testing of the carbapenems with these organisms. However, a practical solution for the testing of these agents in the laboratory has not been developed. No similar studies have been performed to test the stability of ertapenem.

Also intriguing was the fact that 48% (11/23) of the isolates of *N. brasiliensis* were S/I to meropenem, whereas they were completely resistant to imipenem and only marginally susceptible to ertapenem (6/23, or 26%) (Table 2). These results suggest the possibility of some therapeutic potential for meropenem and/or ertapenem against infections involving some groups of *Nocardia* (*N. cyriacigeorgica*, *N. nova* complex, *N. abscessus*, *N. transvalensis* complex, and *N. brasiliensis*). Although less therapeutic potential for these newer carbapenems against isolates of *N. brasiliensis* and *N. cyriacigeorgica* exists, the percentage of isolates S/I to meropenem (11/23 [48%] and 17/25 [68%], respectively) may indicate a possible alternative treatment, especially in serious infections with these groups. For isolates of *N. farcinica* and the *N. otitidiscaviarum* complex, the most active carbapenem was imipenem. Less than 6/18, or 35%, of the isolates of *N. farcinica* were S/I to meropenem and ertapenem and only 2/8 (25%) of the isolates of *N. otitidiscaviarum* were S/I to meropenem and ertapenem. The results of the current study are in concordance with those of a previous Japanese study of the MICs of imipenem and meropenem for these species (25). One possible explanation, according to Sato et al., for this difference in activity between imipenem and meropenem is the presence of a β-lactamase which inactivates imipenem in both *N. brasiliensis* and *N. otitidiscaviarum* (26).

Previous large-scale studies (3) focused on susceptibility testing results obtained by the broth microdilution method with imipenem, meropenem, and ertapenem with RGM but did not analyze the MICs of these agents against the *Nocardia* or differentiate the newly described species or subspecies of RGM (i.e., *M. massiliense* or *M. bolletii*, *M. porcinum*, and *M. senegalense*). Importantly, imipenem remains the carbapenem of choice for the treatment of infections due to *M. abscessus*, *M. massiliense*, and *M. chelonae*.

Testing of both the RGM and *Nocardia* by Etest was problematic, with hazy partial zones of inhibition (heavier marginal growth with lighter growth of inside colonies) that were difficult to interpret being detected. A similar observation has recently been reported by Chihara and colleagues when testing isolates of *M. abscessus* by Etest (27). In the current study, partial zones of inhibition were most often seen with imipenem with more susceptible isolates, such as the *M. mucogenicum/M. phocaicum* group, *M. fortuitum* group, *N. nova* complex, and *N. cyriacigeorgica*, although some resistant isolates, such as *N. abscessus* isolates, also posed difficult interpretations when Etest MICs were compared to broth microdilution MICs. Further investigation of the susceptibility to the carbapenems using Etests appears to be warranted before specific recommendations can be made.

Although imipenem has been the carbapenem most commonly used for the treatment of both mycobacterial and nocardial infections, the option of once daily administration of ertapenem makes this newer carbapenem an attractive alternative (28). How-

ever, this study indicates that appropriate MIC testing is necessary to ascertain specific susceptibility before meropenem or ertapenem is administered to ensure that the treatment regimen is effective. These studies also suggest that the usage of meropenem should be limited to the treatment of infections due to the *M. fortuitum* group and the *M. mucogenicum/M. phocaicum* group. Previous studies have indicated that meropenem has good penetration in lung, bronchial mucosa, and pleural tissues and may thus be useful in serious infections involving these species (29).

There is a paucity of laboratory and clinical data from studies with ertapenem and meropenem. However, a recent case of *M. fortuitum* infection in a surgical wound of a patient undergoing tendon repair surgery was successfully treated with a combination regimen of clarithromycin, trimethoprim-sulfamethoxazole, and ertapenem for 6 months. Unfortunately, no details, including the laboratory identification method or susceptibility to the newer carbapenems, were published (30).

Among the most commonly encountered *Nocardia* spp., meropenem showed greater *in vitro* activity against the *N. nova* complex, *N. abscessus*, and the *N. transvalensis* complex than other groups of *Nocardia*. Except for infections involving the *N. nova* complex and *N. abscessus*, this study suggests that patients should be treated with meropenem only if *in vitro* testing shows susceptibility to meropenem. Moreover, on the basis of the findings of this study, the use of ertapenem should be considered only with isolates of the *M. mucogenicum/M. phocaicum* group, the *N. nova* complex, and the *N. abscessus* group unless susceptibility testing shows that ertapenem has *in vitro* activity against these organisms. Thus, larger studies and comparative clinical data from studies with meropenem and ertapenem are needed to make further treatment recommendations for infections caused by RGM and *Nocardia*.

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REFERENCES

1. **Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ, Jr, Winthrop K.** 2007. An official ATS/IDSA statement: diagnosis, treatment and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med **175:** 367–416. http://dx.doi.org/10.1164/rccm.200604-571ST.
2. **Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ, Jr.** 2006. Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. Clin Microbiol Rev **19:**259–282. http://dx.doi.org/10.1128/CMR.19.2.259-282.2006.
3. **Brown-Elliott BA, Crist CJ, Spencer BG, Wallace RJ, Jr.** 2005. Comparison of *in vitro* activity of imipenem (IPM), meropenem (MER), and ertapenem (ERT) against isolates of rapidly growing mycobacteria (RGM), p 569. Abstr 105th Gen Meet Am Soc Microbiol, Atlanta, GA. American Society for Microbiology, Washington, DC.
4. **Crist CJ, Brown-Elliott BA, Mann LB, Wallace RJ, Jr.** 2003. Susceptibility of newer agents including gatifloxacin, moxifloxacin, meropenem, and tigecycline against the genus Nocardia, p 648. Abstr 103rd Gen Meet Am Soc Microbiol, Washington, DC. American Society for Microbiology, Washington, DC.
5. **Leao SC, Tortoli E, Euzeby JP, Garcia MJ.** 2011. Proposal that *Mycobacterium massiliense* and *Mycobacterium bolletii* be united and reclassified as *Mycobacterium abscessus* subsp. *bolletii* comb. nov., designation of *Mycobacterium abscessus* subsp. *abscessus* subsp. nov. and emended description of *Mycobacterium abscessus*. Int J Syst Evol Microbiol **61**(Pt 9):2311–2313. http://dx.doi.org/10.1099/ijs.0.023770-0.
6. **Telenti A, Marchesi F, Balz M, Bally F, Böttger EC, Bodmer T.** 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J Clin Microbiol **31:** 175–178.
7. **Steingrube VA, Gibson JL, Brown BA, Zhang Y, Wilson RW, Rajagopalan M, Wallace RJ, Jr.** 1995. PCR amplification and restriction endonuclease analysis of a 65-kilodalton heat shock protein gene sequence for taxonomic separation of rapidly growing mycobacteria. J Clin Microbiol **33:**149–153. (Erratum, **33:**1686.)
8. **Clinical and Laboratory Standards Institute.** 2011. Performance standards for antimicrobial susceptibility testing, 21st informational supplement. CLSI document M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.
9. **Brown-Elliott BA, Wallace RJ, Jr.** 2002. Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. Clin Microbiol Rev **15:**716–746. http://dx.doi.org/10.1128/CMR.15.4.716-746.2002.
10. **Springer B, Stockman L, Teschner K, Roberts GD, Böttger EC.** 1996. Two-laboratory collaborative study on identification of mycobacteria: molecular versus phenotypic methods. J Clin Microbiol **34:**296–303.
11. **Swenson JM, Thornsberry C, Silcox VA.** 1982. Rapidly growing mycobacteria: testing of susceptibility to 34 antimicrobial agents by broth microdilution. Antimicrob Agents Chemother **22:**186–192. http://dx.doi.org/10.1128/AAC.22.2.186.
12. **Wallace RJ, Jr, Tsukamura M, Brown BA, Brown J, Steingrube VA, Zhang Y, Nash DR.** 1990. Cefotaxime-resistant *Nocardia asteroides* strains are isolates of the controversial species *Nocardia farcinica*. J Clin Microbiol **28:**2726–2732.
13. **Wallace RJ, Jr, Brown BA, Onyi GO.** 1991. Susceptibilities of *Mycobacterium fortuitum* biovar. *fortuitum* and the two subgroups of *Mycobacterium chelonae* to imipenem, cefmetazole, cefoxitin, and amoxicillin-clavulanic acid. Antimicrob Agents Chemother **35:**773–775. http://dx.doi.org/10.1128/AAC.35.4.773.
14. **Wallace RJ, Jr, Brown BA, Silcox VA, Tsukamura M, Nash DR, Steele LC, Steingrube VA, Smith J, Sumter G, Zhang Y, Blacklock Z.** 1991. Clinical disease, drug susceptibility, and biochemical patterns of the unnamed third biovariant complex of *Mycobacterium fortuitum*. J Infect Dis **163:**598–603. http://dx.doi.org/10.1093/infdis/163.3.598.
15. **Wallace RJ, Jr, Brown BA, Tsukamura M, Brown JM, Onyi G.** 1991. Clinical and laboratory features of *Nocardia nova*. J Clin Microbiol **29:** 2407–2411.
16. **Patel JB, Leonard DGB, Pan X, Musser JM, Berman RE, Nachamkin I.** 2000. Sequence-based identification of *Mycobacterium* species using the MicroSeq 500 16S rDNA bacterial identification system. J Clin Microbiol **38:**246–251.
17. **Conville PS, Brown JM, Steigerwalt AG, Lee JW, Anderson VL, Fishbain JT, Holland SM, Witebsky FG.** 2004. *Nocardia kruczakiae* sp. nov., a pathogen in immunocompromised patients and a member of the "*N. nova* complex." J Clin Microbiol **42:**5139–5145.
18. **Adékambi T, Colson P, Drancourt M.** 2003. *rpoB*-based identification of nonpigmented and late pigmented rapidly growing mycobacteria. J Clin Microbiol **41:**5699–5708. http://dx.doi.org/10.1128/JCM.41.12.5699-5708.2003.
19. **Tortoli E.** 2003. Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. Clin Microbiol Rev **16:**319–354. http://dx.doi.org/10.1128/CMR.16.2.319-354.2003.
20. **Adékambi T, Drancourt M.** 2004. Dissection of phylogenetic relationships among nineteen rapidly growing mycobacterium species by 16S rRNA, *hsp65*, *sodA*, *recA*, and *rpoB* gene sequencing. Int J Syst Evol Microbiol **54:**2095–2105. http://dx.doi.org/10.1099/ijs.0.63094-0.
21. **Clinical and Laboratory Standards Institute.** 2011. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard, 2nd ed. CLSI document M24-A2. Clinical and Laboratory Standards Institute, Wayne, PA.

22. **Biehle JR, Cavalieri SJ, Saubolle MA, Getsinger LJ.** 1994. Comparative evaluation of the E test for susceptibility testing of *Nocardia* species. Diagn Microbiol Infect Dis **19:**101–110. http://dx.doi.org/10.1016/0732-8893(94)90120-1.
23. **Woods GL, Bergmann JS, Witebsky FG, Fahle GA, Boulet B, Plaunt M, Brown BA, Wallace RJ, Jr, Wanger A.** 2000. Multisite reproducibility of Etest for susceptibility testing of *Mycobacterium abscessus*, *Mycobacterium chelonae*, and *Mycobacterium fortuitum*. J Clin Microbiol **38:**656–661.
24. **Watt B, Edwards JR, Rayner A, Grindey AJ, Harris G.** 1992. *In vitro* activity of meropenem and imipenem against mycobacteria: development of a daily antibiotic dosing schedule. Tuber Lung Dis **73:**134–136. http://dx.doi.org/10.1016/0962-8479(92)90145-A.
25. **Yazawa K, Mikami Y, Obashi S, Miyaji M, Ichihara Y, Nishimura C.** 1992. In-vitro activity of new carbapenem antibiotics: comparative studies with meropenem, L-627 and imipenem against pathogenic Nocardia spp. J Antimicrob Chemother **29:**169–172. http://dx.doi.org/10.1093/jac/29.2.169.
26. **Sato K, Fujii T, Okamoto R, Inoue M, Mitsuhashi S.** 1985. Biochemical properties of β-lactamase produced by *Flavobacterium odoratum*. Antimicrob Agents Chemother **27:**612–614. http://dx.doi.org/10.1128/AAC.27.4.612.
27. **Chihara S, Smith G, Petti CA.** 2010. Carbapenem susceptibility patterns for clinical isolates of *Mycobacterium abscessus* determined by the Etest method. J Clin Microbiol **48:**579–580. http://dx.doi.org/10.1128/JCM.01930-09.
28. **Anonymous.** 2002. Ertapenem (Invanz)—a new parenteral carbapenem. Med Lett Drugs Ther **44:**25–26.
29. **Byl B, Jacobs F, Roucloux I, de Franquen P, Cappello M, Thys J-P.** 1999. Penetration of meropenem in lung, bronchial mucosa, and pleural tissues. Antimicrob Agents Chemother **43:**681–682.
30. **Hetsroni I, Rosenberg H, Grimm P, Marx RG.** 2010. *Mycobacterium fortuitum* infection following patellar tendon repair: a case report. J Bone Joint Surg Am **92:**1254–1256. http://dx.doi.org/10.2106/JBJS.I.01083.