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Comparative Drug Resistance of *Mycobacterium abscessus* and *M. chelonae* Isolates from Patients with and without Cystic Fibrosis in the United Kingdom

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The isolation of rapidly growing mycobacteria (RGM), particularly *Mycobacterium abscessus*, from individuals with cystic fibrosis (CF) is associated with poor clinical outcome due to broad drug resistance and the difficulty of eradicating the organisms. Susceptibility testing is recommended to guide therapy. A disc diffusion method is used in the United Kingdom, whereas in the United States, the CLSI (Clinical and Laboratory Standards Institute) recommends the broth dilution method. The purpose of this study was to investigate whether the two methods produced comparable drug resistance profiles and to test the hypotheses that the disc diffusion method overscores resistance and that isolates of *M. abscessus*/*M. chelonae* from CF patients are more likely than those from non-CF patients to show drug resistance, as a result of CF patients' greater exposure to antibiotic therapy. A total of 82 isolates (58 *M. abscessus* and 24 *M. chelonae* isolates) were tested blindly against 15 antimicrobials by broth dilution and the disc diffusion method. Isolates tested by the broth microdilution showed high levels of resistance; susceptibility to amikacin, clarithromycin, tobramycin (only in *M. chelonae*), and cefoxitin (only in *M. abscessus*) was shown. Tigecycline results varied widely depending on which breakpoint was used. Agreement between methods for a few drugs (e.g., cefoxitin and amikacin) was poor. Although there were drug resistance differences between CF and non-CF isolates, these did not reach statistical significance. The CLSI method provided more robust breakpoints, standardization, and reproducibility. An analysis of the implementation of the CLSI method demonstrated ease of use and similar drug resistance findings for the two species.

Nontuberculosis mycobacteria (NTM) have taken on great clinical and public health importance due to their increasing association with patients with cystic fibrosis (CF) and AIDS. The NTM include rapidly growing mycobacteria (RGM), of which the most clinically significant species are the *Mycobacterium fortuitum* group, *M. chelonae*, and the *M. abscessus* group (1, 2); *M. abscessus* has the greatest capacity to colonize the respiratory tract and cause disease in patients with CF and is often associated with a poor clinical outcome (3). Treatment of NTM infections is very difficult and in patients with CF is arguably more resistant to antimicrobial therapy due to their constant exposure to antibiotics. The American Thoracic Society currently recommends at least annual screening of CF patients for NTM (4).

Historically, *M. chelonae* and *M. abscessus* were classed under the same name: *M. chelonae/abscessus* complex. However, it became apparent that they differ biologically and that differentiation to a species level was necessary because treating *M. chelonae* infections is potentially easier than treating those caused by *M. abscessus* (4). However, both species are resistant to multiple antimicrobial agents. Recently it has been argued that *M. abscessus* should be divided into two or three clinically significant subgroups (*M. abscessus*, *M. bolletii*, and *M. massiliense*) (5–7).

At the UK Health Protection Agency, National Mycobacterium Reference Laboratory (NMRL), which analyzes approximately two-thirds of mycobacterial cultures in the United Kingdom, drug susceptibility tests (DSTs) for NTM were conducted using the disc diffusion method following guidelines of the British Society for Antimicrobial Chemotherapy (8). This method, however, may have problems with standardization across laboratories (e.g., variation in inoculum size, media, and incubation conditions).

The aims of this study were to test the hypotheses that the disc method might overscore resistance and that drug resistance in isolates from CF patients may be more extensive due to multiple previous exposures to antibiotics. We compared the accuracy and reliability of the broth microdilution method for RGM, as described by the CLSI (9), with the established disc diffusion method of BSAC (8) for drug susceptibility testing of *M. abscessus* and *M. chelonae* isolates as a prelude to a replacement of the disc method at the United Kingdom national center.

Additionally, we investigated the extent to which *M. abscessus* isolates might be inducibly resistant to clarithromycin (10, 11).

MATERIALS AND METHODS

Isolates. For the evaluation of the broth microdilution assay in CF patients, 82 RGM isolates from United Kingdom hospital patients with and without underlying CF disease (58 *M. abscessus* and 24 *M. chelonae* isolates), over a 3-year period from January 2007 to December 2009, were analyzed blindly. These included all the CF-associated NTM received during this period. Drug susceptibility testing by the disc diffusion and microdilution methods was performed by different scientists, and each was blind to results obtained using the other method. All were blind to patient data.

Antimicrobial agents. Isolates were tested against 15 antimicrobial agents: amikacin, amoxicillin-clavulanic acid, cefepime, cefoxitin, ceftriaxone, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid,

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TABLE 1 Breakpoints used for RGM drug susceptibility testing by the broth dilution with antimicrobial concentration range for broth dilution and disc diffusion method[a]

Antimicrobial	MIC (μg/ml) for broth dilution			Broth dilution range (μg/ml)	Disc diffusion content (μg)
	Susceptible	Intermediate	Resistant		
AMI[b]	≤16	32	≥64	1–64	30
AUG2[c]	≤8/4	16/8	≥32/16	2/1–64/32	NA
FEP[c]	≤8	16	≥32	1–32	NA
FOX[b]	≤16	32–64	≥128	4–128	30
AXO[c]	≤8	16–32	≥64	4–64	NA
CIP[b]	≤1	2	≥4	0.12–4	5
CLA[b]	≤2	4	≥8	0.06–16	15
DOX[b]	≤1	2–8	≥16	0.12–16	30
IMI[b]	≤4	8	≥16	2–64	10
LZD[b]	≤8	16	≥32	1–32	10
MIN[c]	≤1	2–4	≥8	1–8	30
MXF[e]	≤1	2	≥4	0.25–8	1
TGC[d]	NA	NA	NA	0.015–4	15
TOB[b]	≤4	8	≥16	1–4	10
SXT[c]	≤2/38	NA	≥4/76	0.25/4.75–8/152	NA

[a] AMI, amikacin; AUG2, amoxicillin-clavulanic acid; FEP, cefepime; FOX, cefoxitin; AXO, ceftriaxone; CIP, ciprofloxacin; CLA, clarithromycin; DOX, doxycycline; IMI, imipenem; LZD, linezolid; MIN, minocycline; MXF, moxifloxacin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole; NA, not tested by the disc diffusion method.
[b] These breakpoints are recommended by CLSI for rapidly growing mycobacteria (12).
[c] These breakpoints have not yet been established by CLSI for RGM. The values are recommended by CLSI for *Nocardia* and other *Actinomycetes* (12).
[d] For breakpoints for TGC, see Tables 4 and 5.
[e] These breakpoints are recommended by CLSI for Gram-positive bacteria (9).

minocycline, moxifloxacin, trimethoprim-sulfamethoxazole, tigecycline, and tobramycin. Table 1 shows the antimicrobial concentration range for the disc diffusion and the broth dilution methods with breakpoints for 15 antimicrobials used in this study.

Susceptibility test method. The standard broth dilution method with cation-supplemented Mueller-Hinton broth recommended by the CLSI was used. Microtiter plates with antibiotics were purchased from Trek Diagnostics UK (Trek Diagnostic Systems Limited, East Grinstead, United Kingdom) and were provided together with demineralized water and Mueller-Hinton broth. Susceptibility testing using the broth microdilution method was performed according to the CLSI guidelines described in document M24-A (12).

Preparation of inoculum. Colonies were swept off a Lowenstein-Jensen slope with a sterile loop, transferred to Sensititre demineralized water (Trek Diagnostic Systems Limited), and adjusted to a 0.5 McFarland standard using a nephelometer. The McFarland 0.5 is equal to a bacterial load of 1.5×10^8 CFU/ml. A 50-μl aliquot of the suspension was transferred into a tube of the cation-adjusted Mueller-Hinton broth with TES (Tris, EDTA, and NaCl) buffer to reach an inoculum density of 5×10^5 CFU/ml (range, 1×10^5 to 1×10^6 CFU/ml). The broth was poured into a sterile seed trough, and the susceptibility plate was inoculated using an electric multichannel pipette, with 100 μl added to each well of the microtiter plate, according to the manufacturer's guidelines. Plates were covered with a sterile adhesive seal and incubated at 37°C ± 2°C in a non-CO_2 incubator for 72 to 120 h.

Reading. MIC breakpoints indicating susceptible or resistant isolates were interpreted according to the guidelines established by CLSI. The MIC was the lowest concentration of drug that inhibited visible growth. The exception was trimethoprim-sulfamethoxazole, for which the endpoint was the well with 80% inhibition of growth compared to the growth in the control well with no drug.

Breakpoints for eight of 15 antimicrobials were established by CLSI specifically for RGM in document M24-A in 2003 (12). The breakpoints for remaining antimicrobials are those recommended by CLSI for *Nocardia* and other *Actinomycetes* with the exception of moxifloxacin and tigecycline. Breakpoints for moxifloxacin were those recommended by CLSI for Gram-positive bacteria. Breakpoints for tigecycline have not been determined, and various studies have used different resistance cutoff values. We presented our results for tigecycline with resistance breakpoints at 0.5, 1, 2, and 4 μg/ml. The 0.5-μg/ml breakpoint is recommended by the EUCAST (European Committee on Antimicrobial Susceptibility Testing), which proposed non-species-related breakpoints for tigecycline (TGC) with susceptibility (S) at a concentration of ≤0.25 μg/ml and resistance (R) at >0.5 μg/ml (13). However, RGMs tend to have much higher breakpoints than usual for most antimicrobials; for example, Wallace and colleagues (14) used the resistance breakpoint tentatively recommended by the manufacture of ≥8 μg/ml.

Disc diffusion method. The results obtained from the broth microdilution method were compared with results obtained from the disc diffusion method performed on the same isolates on blood agar plates according to the BSAC method.

Quality control. All plates included positive-control wells without antibiotics. Tests were invalid when no growth in a positive-control well appeared. Additionally, *S. aureus* ATCC 29213 was used as a quality control. The plate was inoculated with *S. aureus*, and MICs were read according to the CLSI guidance for quality control breakpoints. The test was valid if the breakpoints were within an indicated range.

Implementation. Following analysis of the data, we implemented the new method for RGM into the United Kingdom national center from 1 January 2012. A postimplementation analysis of 145 clinical isolates comprising *M. abscessus* ($n = 119$) and *M. chelonae* ($n = 25$), from January to June 2012, was conducted.

We made one change to the method, which was to include prolonged incubation for 10 days for strains which were identified as *M. abscessus* and which were clarithromycin susceptible, in order to detect inducible clarithromycin resistance, which has been reported for some strains and subspecies of *M. abscessus* (10, 11).

RESULTS

MIC susceptibility using broth microdilution. In total, 82 isolates for the CF study of *M. abscessus* ($n = 58$) and *M. chelonae* ($n = 24$) were tested for antimicrobial susceptibility using the broth microdilution method described in CLSI document M-24A (12).

Based on the MIC results, isolates were classified into three

TABLE 2 Susceptibility results for *M. abscessus* and *M. chelonae* isolates from CF study samples by broth dilution method against 14 antimicrobial agents

	M. abscessus isolates (58)						*M. chelonae* isolates (24)					
	Susceptible		Intermediate		Resistant		Susceptible		Intermediate		Resistant	
Drug	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
AMI	30	52	18	31	10	17	5	21	8	33	11	46
AUG2	0	0	0	0	58	100	1	4	0	0	23	96
FEP	0	0	0	0	58	100	0	0	0	0	24	100
FOX	5	9	37	64	16	28	0	0	1	4	23	96
AXO	0	0	0	0	58	100	0	0	0	0	24	100
CIP	2	3	1	2	55	95	0	0	1	4	23	96
CLA	33	57	3	5	22	38	20	83	0	0	4	17
DOX	1	2	0	0	57	98	1	4	0	0	23	96
IMI	0	0	2	3	56	97	0	0	0	0	24	100
LZD	17	29	11	19	30	52	5	21	6	25	13	54
MIN	0	0	0	0	58	100	0	0	0	0	24	100
MXF	2	3	1	2	55	95	0	0	1	4	23	96
TOB	0	0	5	9	53	91	13	54	4	17	7	29
SXT	5	9	0	0	53	91	0	0	0	0	24	100

[a] AMI, amikacin; AUG2, amoxicillin-clavulanic acid; FEP, cefepime; FOX, cefoxitin; AXO, ceftriaxone; CIP, ciprofloxacin; CLA, clarithromycin; DOX, doxycycline; IMI, imipenem; LZD, linezolid; MIN, minocycline; MXF, moxifloxacin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole.

categories for each drug: susceptible, intermediate, and resistant. In general, isolates were highly resistant to most antimicrobials. Tables 2 and 3 summarize MIC results against 14 antimicrobial agents (TGC is presented later with various MICs). All plates were read after 3 to 5 days of incubation. Previous studies (15) and our experience have indicated 72 to 120 h as an optimum time to examine the growth of RGM.

Tables 3 and 4 show DST results of clinical laboratory samples after the implementation of the CLSI method between January and June 2012.

The present study, using CF and non-CF patient samples (2007-2009), showed that the most active antimicrobials among the aminoglycosides were amikacin and tobramycin, according to the CLSI method (Table 2): amikacin was moderately active against *M. abscessus* and *M. chelonae*, with 83% and 54% of isolates being susceptible and moderately susceptible (intermediate), respectively. Tobramycin had poor activity against *M. abscessus* (only 9% isolates were susceptible) but had better activity than amikacin against *M. chelonae*, with 71% of isolates being susceptible or moderately susceptible.

Macrolides like clarithromycin proved to be more active against *M. chelonae* than *M. abscessus*, with 83% and 62% of isolates being susceptible, respectively.

Linezolid had moderate activity against both species, with nearly 50% of isolates being susceptible or moderately susceptible.

Cefoxitin was also active against *M. abscessus*, with 73% of isolates being susceptible (although the majority of these isolates [64%] were considered to be of moderate sensitivity, or border-

TABLE 3 Susceptibility results for *M. abscessus* and *M. chelonae* isolates by the broth dilution method against 14 antimicrobial agents in clinical laboratory evaluations (January to June 2012)

	M. abscessus isolates (119)						*M. chelonae* isolates (25)					
	Susceptible		Intermediate		Resistant		Susceptible		Intermediate		Resistant	
Drug	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
AMI	67	56	25	21	27	23	11	46	10	42	3	12
AUG2	0	0	1	1	118	99	0	0	0	0	24	100
FEP	0	0	0	0	119	100	0	0	0	0	24	100
FOX	2	2	93	78	24	20	0	0	0	0	24	100
AXO	1	1	1	1	117	98	0	0	0	0	24	100
CIP	0	0	4	3	115	97	0	0	1	4	23	96
CLA	54	45	14	12	51	43	22	92	1	4	1	4
DOX	0	0	2	2	117	98	0	0	0	0	24	100
IMI	1	1	1	1	117	98	0	0	0	0	24	100
LZD	26	22	18	15	75	63	13	54	7	29	4	17
MIN	0	0	2	2	116	98	0	0	0	0	24	100
MXF	2	2	9	8	108	90	0	0	1	4	23	96
TOB	4	3	12	10	103	87	21	88	1	4	2	8
SXT	2	2	3	2	114	96	0	0	1	4	23	96

[a] AMI, amikacin; AUG2, amoxicillin-clavulanic acid; FEP, cefepime; FOX, cefoxitin; AXO, ceftriaxone; CIP, ciprofloxacin; CLA, clarithromycin; DOX, doxycycline; IMI, imipenem; LZD, linezolid; MIN, minocycline; MXF, moxifloxacin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole.

TABLE 4 Rate of susceptibility to tigecycline at different MICs among strains in the clinical laboratory implementation

Species (no. of isolates)	% of isolates (no.) with MIC of: ≤0.5 μg/ml	≤1 μg/ml	≤2 μg/ml	≤4 μg/ml
M. abscessus (119)	28 (35)	41 (51)	56 (69)	68 (85)
M. chelonae (25)	12 (3)	64 (16)	84 (21)	96 (24)

line). *M. chelonae* strains were highly resistant to this agent. Cefepime and ceftriaxone were not active against either *M. abscessus* or *M. chelonae*.

Quinolones used to be popular in the treatment of RGM. In this study, we tested ciprofloxacin and moxifloxacin, and both showed no activity in more than 95% of isolates. Doxycycline and minocycline also showed poor or no activity.

In this study, amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole showed almost no activity against the RGM isolates tested.

Among tetracyclines, only tigecycline showed activity which profoundly depended on resistance breakpoint values; Tables 4 and 5 give the susceptibility results for tigecycline with different published breakpoints. In the CF study, *M. abscessus* and *M. chelonae* isolates showed high resistance to tigecycline at a MIC of ≤0.5 μg/ml, with 19% and 12% susceptible strains, respectively. At the highest MIC, ≤4 μg/ml, based on the work of Wallace et al. (14), the majority of isolates (96% of both species) were susceptible to tigecycline. These data demonstrate the difficulty of testing and interpreting DST results for TGC and likely clinical efficacy.

Comparative DST results between CF and non-CF patients. Table 6 shows susceptibility results among patients with *M. abscessus* or *M. chelonae* infection with and without underlying CF. For the purpose of the comparisons below, the "intermediate" results were classified in the "resistant" group. Although there was some variation in drug susceptibility, none reached statistical significance (two-tailed Fisher exact test). Linezolid had better activity against isolates from CF patients than against those from non-CF patients. Table 7 shows the tigecycline susceptibility pattern among the same groups of patients, with various published resistance breakpoints.

Comparison of DST by broth microdilution and disc diffusion. We compared results from the broth microdilution method with those obtained by the disc diffusion method. The disc diffusion method was conducted on 71 of the 82 isolates tested by the broth method, comprising 51 *M. abscessus* and 20 *M. chelonae* isolated tested against the same 11 antimicrobial agents. Eleven isolates failed to generate results with the disc method due to contamination. Table 8 shows the agreement between those methods

TABLE 5 Rate of susceptibility to tigecycline at different MICs among strains in the CF study

Species (no. of isolates)	% of isolates (no.) with MIC of: ≤0.5 μg/ml	≤1 μg/ml	≤2 μg/ml	≤4 μg/ml
M. abscessus (58)	19 (11)	48 (28)	71 (41)	96 (56)
M. chelonae (24)	12 (3)	37 (9)	79 (19)	96 (23)

TABLE 6 Susceptibility pattern of RGM in CF patients[a]

Drug	*M. abscessus* (*n* = 58) CF (*n* = 38) No.	%	Non-CF (*n* = 20) No.	%	*P*[b]	*M. chelonae* (*n* = 24) CF (*n* = 10) No.	%	Non-CF (*n* = 14) No.	%	*P*[b]
AMI	31	82	17	85	1	6	60	7	50	1
AUG2	0	0	0	0	1	1	10	0	0	0.41
FEP	0	0	0	0	1	0	0	0	0	1
FOX	27	71	15	75	1	1	10	0	0	0.41
AXO	0	0	0	0	1	0	0	0	0	1
CIP	3	8	0	0	0.54	0	0	1	7	1
CLA	23	61	13	65	0.78	8	80	12	86	1
DOX	1	3	0	0	1	0	0	1	7	1
IMI	2	5	0	0	0.54	0	0	0	0	0.41
LZD	22	58	6	31	0.06	7	70	4	29	0.09
MIN	0	0	0	0	1	0	0	0	0	1
MXF	3	8	0	10	0.54	1	10	0	0	0.41
TOB	4	11	1	50	0.65	5	50	12	86	0.08
SXT	4	11	1	0	0.65	0	0	0	0	1

[a] Data are numbers and percentages of susceptible isolates. AMI, amikacin; AUG2, amoxicillin-clavulanic acid; FEP, cefepime; FOX, cefoxitin; AXO, ceftriaxone; CIP, ciprofloxacin; CLA, clarithromycin; DOX, doxycycline; IMI, imipenem; LZD, linezolid; MIN, minocycline; MXF, moxifloxacin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole.
[b] Determined by a two-tailed Fisher's exact test.

for 10 antimicrobials. Table 9 demonstrates the agreement between both methods for TGC with various MICs.

Agreement varied considerably for some antimicrobials (Table 8). Overall, agreement for *M. abscessus* isolates was best for doxycycline, linezolid, minocycline, moxifloxacin, and tobramycin, with more than 94% agreement. Concordance was more than 95% for *M. chelonae* isolates with amikacin, clarithromycin, doxycycline, cefoxitin, minocycline, and moxifloxacin.

For *M. abscessus*, correlation between these two methods was poor for amikacin and cefoxitin (61% and 57% agreement, respectively). Overall there was greater susceptibility to these agents when the broth-based method was used. However, the opposite was true with ciprofloxacin and imipenem, for which susceptibility by the broth method was much lower than that by the disc method and agreement was 71% and 55%, respectively.

For *M. chelonae*, major discrepancies between methods were observed with ciprofloxacin and tobramycin. The 45% agreement with ciprofloxacin was caused by a much lower number of susceptible isolates with the broth method. The 70% agreement with tobramycin was caused by much higher number of susceptible

TABLE 7 Tigecycline susceptibility pattern of RGM in patients with CF and without CF for different resistance breakpoints

TGC MIC (μg/ml)	*M. abscessus* (*n* = 58) CF (*n* = 38) No.	%	Non-CF (*n* = 20) No.	%	*P*[a]	*M. chelonae* (*n* = 24) CF (*n* = 10) No.	%	Non-CF (*n* = 14) No.	%	*P*[a]
0.5	10	26	1	5	0.08	2	20	1	7	0.55
1	21	55	7	35	0.17	4	40	5	38	1
2	29	76	12	60	0.23	8	80	11	79	1
4	36	94	20	100	0.54	9	90	14	100	0.4

[a] Determined by a two-tailed Fisher's exact test.

TABLE 8 Comparison of susceptible and resistant strains of *M. chelonae* and *M. abscessus* between broth dilution and disc diffusion by the standard proportion method for 10 antimicrobials[a]

Organism (no. of isolates)	Drug	No. of isolates				Agreement (%)
		S by both methods	R by both methods	S in broth, R in disc	R in broth, S in disc	
M. chelonae (20)	AMI	10	10	0	0	100
	CIP[c]	1	8	0	11	45
	CLA	18	2	0	0	100
	DOX	1	19	0	0	100
	FOX	0	20	0	0	100
	IMI	0	18	0	2	90
	LZD[b]	4	12	4	0	80
	MIN	0	19	0	1	95
	MXF	1	19	0	0	100
	TOB[b]	8	6	6	0	70
M. abscessus (51)	AMI[b]	22	9	20	0	61
	CIP[c]	3	33	0	15	71
	CLA[c]	35	10	0	6	88
	DOX	1	48	0	2	96
	FOX[b]	13	16	22	0	57
	IMI[c]	2	26	0	23	55
	LZD	0	48	3	0	94
	MIN	0	49	0	2	96
	MXF	0	49	2	0	96
	TOB	1	48	2	0	96

[a] AMI, amikacin; AUG2, amoxicillin-clavulanic acid; FEP, cefepime; FOX, cefoxitin; AXO, ceftriaxone; CIP, ciprofloxacin; CLA, clarithromycin; DOX, doxycycline; IMI, imipenem; LZD, linezolid; MIN, minocycline; MXF, moxifloxacin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole; S, susceptible; R, resistant.
[b] Isolates are more likely to be sensitive in the broth than in the disc diffusion method.
[c] Isolates are more likely to be sensitive in the disc than the broth method.

TABLE 9 Comparison of tigecycline susceptibility of *M. chelonae* and *M. abscessus* between broth dilution and disc diffusion for different resistance breakpoints

Organism	MIC (μg/ml)	No. of isolates				Agreement (%)
		S by both methods	R by both methods	S in broth, R in disc	R in broth, S in disc	
M. chelonae (20)	0.5	3	0	0	17	15
	1	7	0	0	13	35
	2	16	0	0	4	80
	4	19	0	0	1	95
M. abscessus (49)[a]	0.5	11	0	0	38	22
	1	25	0	0	24	51
	2	37	0	0	12	76
	4	49	0	0	0	100

[a] Only 49 of 51 isolates were tested with TGC.

isolates by the broth method. Good correlation was observed with minocycline and imipenem.

The greatest variation in susceptibility testing occurred for tigecycline (Table 9). Agreement for *M. abscessus* was 22% and 100% for MICs at 0.5 and 4 μg/ml, respectively. Agreement for *M. chelonae* varied from 15% to 95% for MICs at 0.5 and 4 μg/ml, respectively.

Finally, to confirm the reproducibility of the analysis using the microdilution method, we blindly tested 20 isolates of *M. abscessus* twice. The MICs for all isolates in the first test were in concordance with those in the second test except for trimethoprim-sulfamethoxazole: for 6 out of 20 isolates, we observed lower MICs, but these were still in the "resistant" reporting range.

Implementation. Following on from the initial analysis we introduced the CLSI method (Tables 3 and 4). Implementation of the method in the United Kingdom national center was in a completely unselected population, but results were broadly in concordance with the pilot CF study with the exception of those for amikacin, linezolid, and tobramycin. Resistance was less likely to be seen in the unselected population study.

Thirty clinical isolates of *M. abscessus* were included from the completely unselected population, which were identified as clarithromycin susceptible; inducible resistance to clarithromycin was found in 15 of 30 isolates previously classified as susceptible.

DISCUSSION

Rapidly growing mycobacteria are increasingly being recognized as important human pathogens. Susceptibility testing of RGM is likely to be of increasing importance in selecting an optimal and effective drug therapeutic regimen, as the resistance pattern varies with different species. The most clinically significant ones are *M. chelonae* and *M. abscessus* (and its subspecies), for which drug susceptibility is likely to influence outcome. The latter species is considered more drug resistant (16) and is extremely difficult to treat and eradicate. However, there is a lack of a clear correlation between DST results and clinical outcome.

In 2003, the CLSI recommended the MIC broth microdilution method as a standard DST for RGM. This technique seemed to be easy to adopt for routine testing because it is standardized, reproducible, and accurate, mostly due to the availability of commercial media. Results are expected to be more reliable if variables such as incubation conditions and time, inoculum size, and media are consistent across different laboratories.

The NMRL has conducted DSTs for rapidly and slowly growing mycobacteria using the disc diffusion method following the BSAC recommendation for many years. Results over time demonstrated that a large proportion of the isolates tested were resistant to a number of drugs. This study showed that overall this remained true regardless of the method employed, although results did vary for some key drugs.

Results confirmed high levels of resistance in both *M. abscessus* and *M. chelonae*. In patients with *M. abscessus* (regardless of CF status), the majority of isolates were susceptible to amikacin (AMI), cefoxitin (FOX), clarithromycin (CLA), and linezolid (LZD), with a proportion being susceptible to tigecycline. These isolates would also in practice be susceptible to imipenem (as imipenem instability in broth makes these results difficult to interpret). For *M. chelonae*, the majority of strains were susceptible to amikacin, clarithromycin, and linezolid, with a proportion being susceptible to tigecycline.

Among the most active drugs were the aminoglycosides, which were previously used successfully in the treatment of *M. abscessus* and *M. chelonae* infections. This was in agreement with previous studies (15) that showed that amikacin had one of highest rates of activity among all tested drugs. Tobramycin was active against *M. chelonae* but not against *M. abscessus*. It was observed by previous investigators (17) that results obtained with tobramycin were not reproducible with *M. abscessus* (16). The CLSI in document M24-A did not recommend testing this species with tobramycin (12).

Newer macrolides are important antimicrobial agents used for treatment. Brown et al. (1) reported clarithromycin as the most powerful among them, and later, Park et al. (18) reported 91% of *M. abscessus* isolates were susceptible to this drug. Our susceptibility results showed that clarithromycin was effective, with 83% of *M. chelonae* and 57% of *M. abscessus* (all subspecies) isolates being susceptible. However, in the national center implementa-

tion phase, inducible clarithromycin resistance was seen in 50% of isolates. As the *M. massiliense* subspecies/subgroup of *M. abscessus* does not have a functioning *erm* gene responsible for inducible clarithromycin resistance (10), the identification of *M. massiliense* (and *M. bolletii*) subgroups has been advocated. However, their taxonomic status is not entirely clear, and although all strict *M. abscessus* strains have an *erm* gene, it does not appear to be functional in all strains.

Cefoxitin is another drug considered to have good activity among RGM. This study confirmed cefoxitin activity against *M. abscessus* (73% of isolates susceptible) but not *M. chelonae.* Other cephalosporins tested by the broth method (cefepime and ceftriaxone) were not active against tested isolates.

Our strains were highly resistant to sulfamethoxazole which was with agreement with previous data. Swenson and colleagues (15, 19) reported that almost all *M. chelonae* subspecies were resistant to this drug. Additionally, we found a reading of the endpoints at 80% of inhibition of growth very subjective, which could generate "false" resistance.

Tetracyclines used to be very popular therapeutic drugs, but their value has declined over the last 20 years due to the buildup of drug resistance among other species of rapid growers (14). Our data have shown that tigecycline susceptibility results were the most varied from previous studies. Tigecycline was reported to have very good activity in the study by Wallace et al. (14). The growth of 90% of *M. abscessus* and *M. chelonae* isolates was inhibited at MICs of ≤0.25 μg/ml and <0.12 μg/ml, respectively. Because resistance breakpoints have not been established for RGM by the CLSI, we presented our susceptibility results for different MICs. At a MIC of ≤0.5 μg/ml, 19% of *M. abscessus* and 12% of *M. chelonae* isolates were susceptible to tigecycline. However, at a MIC of ≤4 μg/ml, tigecycline activity was very high, 96%, for both species.

The other tetracyclines, minocycline and doxycycline, had even poorer activity against both strains, with MICs of ≥8 μg/ml and ≥16 μg/ml, respectively. Swenson et al. (15) and Wallace et al. (14) also reported mycobacteria as resistant to those drugs, with MICs of >8 μg/ml.

Linezolid was moderately active, with 48% and 46% of *M. abscessus* and *M. chelonae* isolates being inhibited by MICs of ≤16 μg/ml, respectively. The drug was reported to be active against *M. abscessus* and *M. chelonae*, with 48% and 94% of isolates being inhibited at drug concentrations of 16 μg/ml or less (sensitive and moderate sensitive) (20).

All strains were very resistant to imipenem, amoxicillin-clavulanic acid, and the quinolones ciprofloxacin and moxifloxacin. However, imipenem cannot be reliably tested by the broth method due to stability problems, and reporting of results was not recommended by CLSI (12). Therefore, imipenem should be a part of the empirical treatment regiment for *M. abscessus* regardless of the broth assay results.

We hypothesized that strains from patients with CF might exhibit a greater degree of antimicrobial resistance than those from non-CF patients due to the multiple antibiotic exposures experienced by these patients; CF patients are exposed to a great number of drugs for long periods, so it was possible that isolates from these patients might become more resistant. However, this study did not identify statistically significant variations in susceptibility between CF and non-CF patients.

In general, the greatest susceptibility of *M. abscessus* isolates from CF patients was shown for amikacin (82%), cefoxitin (71%), and clarithromycin (61%). Our results are comparable to those reported from a Virginia hospital in patients with CF infected with *M. abscessus*, where 90% of isolates were susceptible to amikacin and clarithromycin, with lower rates of susceptibility shown for cefoxitin (30%) (21).

We did not encounter any major problems with the broth method. The only minor problem was with some interpretation of results, which was also highlighted by Wood et al. (16). The growth of RGM does not appear as a button in the bottom of the well but as a hazy suspension, and therefore, training might be required for the reader to acquire sufficient experience to make an accurate determination. However, our recommendation is that plates be read by two persons to avoid misinterpretation until sufficient experience has been obtained. We also realized that visual adjustment of the inoculum could be quite problematic. Bacterial microparticles form clumps rather than uniform dispersions, making visual interpretation difficult. This variable could potentially influence the reading, very often by creating a higher bacterial load. Therefore, all isolates were adjusted to 0.5 McFarland with a nephelometer only.

Our study was not designed to determine which of the two methods was more "accurate" or predictive of clinical outcome. In CF, patients have frequent episodes of pulmonary infection, and it is not always clear what was the etiological agent of any exacerbation; even when organisms are isolated, it is not always clear if they are colonizing or pathogenic. Similarly, treatment protocols often involve multiple therapeutic options, and so it is not always clear what agent led to recovery or improvement. Nevertheless this study is likely to lead to changes in prescribing practice in the United Kingdom; e.g., cefoxitin is rarely used, and arguably this may have been reinforced by the higher rates of "resistance" seen for this drug by the BSAC method than by the broth method.

The implementation of the broth microdilution method will certainly enable drug resistance and patient outcome data from cohorts of CF patients in the United Kingdom and the United States to be combined retrospectively and/or prospectively, facilitating the development of more effective therapeutic drug regimens. This will be of particular value for CF patients, as ongoing *M. abscessus* infection is a barrier to successful lung transplantation, the final life-preserving strategy for these patients.

Implementation. In summary, the broth microdilution method is a reliable DST for RGMs and can be performed with confidence by individuals with only modest training to obtain reliable and reproducible results across different laboratories. It is standardized, with all components being commercially available, and easy to perform in the routine testing environment. For the future, because six of 15 antimicrobial agents recommended for testing do not have clearly established breakpoints for RGM, clinical and outcome data should be gathered to demonstrate correlation between *in vitro* susceptibility results and clinical efficacy.

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