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Mycobacterium neoaurum and *Mycobacterium bacteremicum* sp. nov. as Causes of Mycobacteremia▽

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Reference isolates of *Mycobacterium neoaurum*, *Mycobacterium aurum*, and the nonvalidated species "*Mycobacterium lacticola*" were the focus of two recent molecular taxonomic studies. On the basis of this grouping, we identified 46 clinical pigmented, rapidly growing mycobacterial isolates. By 16S rRNA gene sequencing, only two major taxa were identified: *M. neoaurum* and a previously uncharacterized "*M. neoaurum*-like" group. The *M. neoaurum*-like group exhibited only 99.7% identity to *M. neoaurum* by 16S rRNA gene sequencing and 96.5% identity to *M. neoaurum* by *rpoB* sequencing and was named *M. bacteremicum*. No clinical isolates of *M. aurum* or *M. lacticola* were identified. Of isolates with known sources, 4/8 (50%) of *M. bacteremicum* isolates and 22/34 (65%) of *M. neoaurum* isolates were recovered from blood, and 35% of these were known to be from patients with catheter-related sepsis. MIC and clinical data on these 46 isolates of *M. neoaurum* and *M. bacteremicum* along with a review of 16 previously reported cases of infection with the *M. neoaurum-M. lacticola* group demonstrated that the isolates were highly susceptible to all drugs tested except clarithromycin, and most clinical cases were successfully treated. The clarithromycin resistance suggested the presence of an inducible *erm* gene reported in other species of rapidly growing mycobacteria. Sequencing studies are currently required to identify these two species. Strain ATCC 25791 (originally submitted as an example of *Mycobacterium aurum*) is proposed to be the type strain of *M. bacteremicum*.

Recently, we reported on the phylogenetic analysis of the *Mycobacterium neoaurum*-"*M. lacticola*" group and redescription of reference American Type Culture Collection (ATCC) isolates previously classified as *Mycobacterium aurum* (18). These pigmented, rapidly growing *Mycobacterium* spp. have been associated with clinical disease, especially catheter-related sepsis. In a previous study by Simmon and colleagues (18), complete 16S rRNA gene sequence analysis showed four reference strains of *M. aurum* to have 100% identity to the type strain of the nonvalidated species *M. lacticola*, which differed by 8 bp from the type strain of *M. neoaurum*. With the addition of *hsp65* and *rpoB* gene targets, these four isolates remained closely clustered as *M. lacticola*, with intraspecies variabilities being only 0.7% for *hsp65* and 1.5% for *rpoB*. One reference strain submitted as *M. aurum* (ATCC 25791) did not match either of these two taxa. All of these reference strains were from environmental sources (18).

Phylogenetic studies need to be performed with clinical isolates to determine their relatedness to these reference strains of *M. neoaurum*, *M. lacticola*, and ATCC 25791, which we tentatively named "*M. neoaurum*-like" (11, 18). We initiated a detailed study for 32 of the previously reported clinical isolates of *M. neoaurum-M. lacticola* group from the laboratory at the Associated Regional and University Pathologists (ARUP) Institute for Clinical and Experimental Pathology and the University of Texas Health Science Center at Tyler (UTHSCT), along with 14 additional isolates of the *M. neoaurum–M. neoaurum*-like group which were recovered following the submission of the first study.

As noted in the previous publication, there is a paucity of case reports of *M. neoaurum* and *M. lacticola* infections in the literature. The incidence of *M. neoaurum-M. lacticola* group infections likely has been underestimated, since most clinical laboratories have not been able to identify pigmented, rapidly growing mycobacteria of this species. Biochemical and cell wall analysis and high-performance liquid chromatography (HPLC) are not validated methods for such identification. Hence, we reviewed published cases of this pigmented group to determine how well they met current molecular taxonomic criteria and how they compared to the well-studied isolates in the present study.

MATERIALS AND METHODS

Organisms. Clinical isolates of pigmented, rapidly growing mycobacteria identified to be *M. neoaurum*, *M. neoaurum*-like, or *M. lacticola* by *hsp65* PCR restriction enzyme analysis (PRA) (at UTHSCT) or 16S partial gene sequencing (500 bp) (at ARUP) were the focus of this study (7, 11, 18, 19, 21). Isolates had been stored at −70°C after initial laboratory testing. They were later subcultured and underwent complete 16S rRNA gene sequencing as previously described (18). Isolates whose complete 16S rRNA gene sequence was a 100% match to the complete 16S rRNA gene sequence of the type strain of *M. neoaurum* (ATCC 25795) or *M. lacticola* (ATCC 9626) or that exhibited >99.5% identity (difference of 6 bp or less) to the complete 16S rRNA gene sequence of the *M.*

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TABLE 1. Demographic and phylogenetic information for isolates of *Mycobacterium neoaurum* and the *M. neoaurum*-like group from the current study[a]

Organism and laboratory strain no.	Age[b]	Sex	Source	Geog loc.	Underlying disease	Phylogenetic cluster[c]		
						16S rRNA	*hsp65*	*rpoB*
M. neoaurum-like group								
MO-2218	2	M	Central catheter	OH	Bone marrow transplant	C	H	S
MO-1171	31	F	Blood	TX	Unknown	C	E	N
MO-1875	54	M	Blood	IN	Unknown	C	G	P
MO-2886	36	M	Blood	LA	Retinitis/HIV infection	C	E	R
MO-2842	5	M	Toe wound	NY	Unknown	C	H	Q
AR-19	59	F	Sputum	TN	Unknown	C	G	P
AR-04	72	F	Finger	UT	Unknown	C	G	O
AR-08	38	M	Sputum	OH	Unknown	C	G	O
AR-01	39	M	Unknown	TX	Unknown	C	G	R
AR-25	38	M	Unknown	CA	Unknown	C	G	T
M. neoaurum								
MO-2355	5	M	Blood	PA	Unknown	B	A	H
MO-2441	4	4	Blood	CA	Myeloblastic leukemia	B	D	B
MO-2117	2	M	Central catheter	NC	Hemophilia	B	A	D
MO-2123	4	F	Blood	TX	Leukemia	B	A	A
MO-2432	61	M	Blood	MA	Unknown	B	A	D
MO-2442	61	F	Central catheter	MA	Pulmonary embolism	B	A	D
AS-544	67	F	Central catheter	OH	Unknown	B	A	H
MO-1110	67	F	Central catheter	ME	Unknown	B	D	B
MO-1302	32	F	Blood	FL	Systemic lupus erythematosus	B	A	C
MO-696	36	F	Central catheter	CA	Pulmonary hypertension septicemia	B	A	C
MO-2765	61	F	Blood	LA	Unknown	B	A	D
MO-2921	64	M	Blood (port also)	PA	End-stage renal disease on hemodialysis	B	A	H
MO-2933	33	F	Central catheter	KS	Unknown	B	A	L
AR-20	24	F	Central catheter	IL	Unknown	B	A	G
MO-2225	35	F	Sputum	IL	Unknown	B	A	H
MO-1351	86	F	Sputum	WI	Unknown	B	D	K
MO-1934	31	M	Sputum	NY	Unknown	B	A	H
MO-891	50	F	Sputum	TX	Unknown	B	C	J
MO-2573	52	M	Palm tissue	MA	Unknown	NA	NA	NA
MO-2496	NA	F	Sputum	MA	Unknown	NA	NA	NA
MO-1156	80	F	Urine	OH	Unknown	B	A	K
AR-21	84	M	Sputum	OR	Unknown	B	B	I
AR-22	81	F	Sputum	MA	Unknown	B	D	B
AR-18	30	M	Sputum	HI	Unknown	B	A	B
AR-11	22	M	Blood	CA	Unknown	B	A	C
AR-05	56	M	Blood	MN	Unknown	A	A	H
AR-14	11 mo	F	Blood	PA	Unknown	B	A	H
AR-09	4	F	Blood	CA	Unknown	B	B	H
AR-16	52	F	Blood	HI	Unknown	B	A	F
AR-15	10	M	Blood	PA	Unknown	B	B	G
AR-12	10 mo	F	Blood	IL	Unknown	B	A	G
AR-10	61	F	Wound	MD	Unknown	B	D	B
AR-06	17	M	Wound	PA	Unknown	B	A	D
AR-02	55	M	Unknown	FL	Unknown	B	D	B
AR-03	81	F	Unknown	NY	Unknown	B	A	D
AR-24	1	F	Blood	WV	Unknown	B	D	E

[a] Geog loc., geographic location; NA, not available; M, male; F, female.
[b] Ages are in years unless indicated otherwise.
[c] A phylogenetic cluster is defined as a unique sequence for an individual gene target. Phylogenetic clusters correspond to the labels in Fig. 1 or 2.

neoaurum type strain (designated *M. neoaurum*-like) were included in the study.

Forty-six clinical isolates (21 from ARUP, 25 from UTHSCT) were studied. These included 32 isolates from the study of Simmon et al. (18) and 14 additional isolates recovered after the submission of the manuscript for the first study.

Patient demographics. Patient information, including geographical source, clinical site, age, sex, and associated or underlying disease or risk factor, were compiled for all isolates at submission (Table 1). Both of these studies were conducted under protocols approved by the institutional review boards (IRBs) at both UTHSCT and ARUP (18).

Sequencing. Forty-four of 46 (96%) of the clinical isolates were subjected to full sequencing of the 16S rRNA gene and partial sequencing of the *hsp65* and the *rpoB* genes as previously described (18, 23). Two isolates previously identified by *hsp65* PRA were not available for multigene sequencing.

Susceptibility testing. Susceptibilities to 14 antimicrobial agents were determined by broth microdilution using the recommended Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) guidelines for rapidly growing mycobacteria (26). The antimicrobials tested included those recommended by the CLSI: amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, sulfamethoxazole, tobramycin, and linezolid. Additional antimicrobials for which there are no current CLSI recommendations were tested and included

gatifloxacin, minocycline, moxifloxacin, tigecycline, and trimethoprim-sulfamethoxazole. The breakpoints for the last group of agents were those recommended by the CLSI in document M100-S18 for aerobic bacteria (4) for all agents except moxifloxacin and gatifloxacin, for which the breakpoints used were 2 and 4 μg/ml, respectively (1 and 2 dilutions higher, respectively, than the breakpoints recommended for bacterial isolates), and tigecycline, whose breakpoint has not been addressed by the CLSI. Because not all isolates were tested simultaneously, some isolates were not tested with all antimicrobials, and the concentrations of antimicrobials tested varied in the MIC panels.

Susceptibility to clarithromycin was read after incubation for 3 and 14 days in order to ascertain isolates that had inducible macrolide resistance (15, 26) (proposals for modification of the document cited in reference (26) have been submitted to the CLSI).

Sequence analysis. Sequence alignment and phylogenetic trees were constructed using the neighbor-joining method with Kimura's two-parameter distance correction model and 1,000 bootstrap replications in the MEGA (version 4) software package (20).

Nucleotide sequence accession numbers. The 16S rRNA, *hsp65*, and *rpoB* gene sequences of the clinical isolates were deposited in GenBank with accession numbers HM011124 to HM011258. The *M. lacticola hsp65* and *rpoB* gene sequences were deposited in GenBank with accession numbers HMO30495 and HMO30494, respectively.

RESULTS

Organisms. Forty-four isolates underwent DNA sequencing (18): 34 were identified as *M. neoaurum* and 10 were identified as *M. neoaurum*-like. Clinical information on all of the isolates was provided at the time of submission (Table 1). Of 42 isolates of the *M. neoaurum* and *M. neoaurum*-like groups from UTHSCT and ARUP with known sources, 26 (62%) were recovered from blood, 5 (12%) were recovered from tissue or wound specimens, 10 (24%) were recovered from sputum, and 1 (2%) was recovered from urine. Within the two groups, 4/8 (50%) of the *M. neoaurum*-like isolates and 22/34 (65%) of the *M. neoaurum* isolates with known sources were from blood (Table 1).

Patient demographics. There were 10 patients under age 6 years, and the remaining 36 patients were adolescents, adults, or of unknown ages. Patient ages and geographical locations along with their underlying diseases or conditions are presented in Table 1.

Underlying diseases or conditions were known for only nine (19%) of the cases (Table 1). Of the 26 isolates recovered from blood cultures, 35% were known to be associated with catheter-related sepsis.

The majority (60%) of patients were from California, Pennsylvania, Massachusetts, Texas, Ohio, New York, and Illinois; but overall, patients were also from 14 other states.

Sequencing. (i) 16S rRNA gene. By full 16S rRNA gene sequencing (1,413 bp), two major clusters with three unique sequences were identified among clinical isolates. The first cluster contained 34 clinical isolates whose sequences showed 99.9% to 100% identity to those of *M. neoaurum* type strain ATCC 25795 (10) and ATCC reference strains ATCC 27277, ATCC 25796, and ATCC 23072 (18) (Fig. 1A). A sole isolate in this cluster differed by a single nucleotide from the *M. neoaurum* type strain. A second cluster consisted of 10 clinical isolates, which shared 100% identity to ATCC 25791. This group does not match any recognized species. The sequence of these isolates differed by 4 bp from the sequence of the *M. neoaurum* (2 substitutions and 2 indels) and 4 bp from the sequence of *M. lacticola* (4 substitutions) type strains (99.7% identity). Compared to the sequence of *M. neoaurum*, *M. lacticola* had the same 4-bp substitutions as the ATCC 25791 group plus four additional unique substitutions.

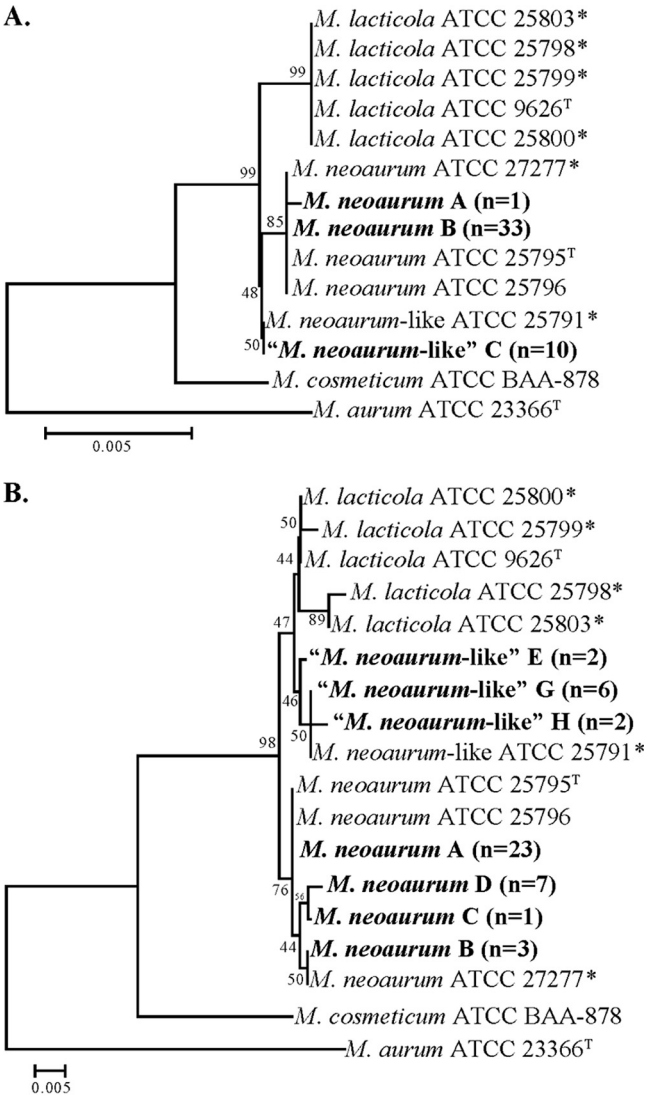

FIG. 1. Neighbor-joining trees of 16S rRNA (A) and *hsp65* (B) genes of *M. neoaurum*, *M. lacticola*, and *M. neoaurum*-like clinical isolates and culture collection strains. Branch support is recorded at the nodes as a percentage of 1,000 bootstrap iterations. *M. cosmeticum* is present as the most closely related species. *M. aurum* is included as an outgroup. *, annotated as a different species by the American Type Culture Collection (18).

Of interest, the full rRNA gene sequence of none of the clinical isolates matched the full 16S rRNA gene sequence of the nonvalidated species of *M. lacticola* (ATCC 9626) (11, 18).

(ii) *hsp65* gene. By *hsp65* partial gene sequencing (402 bp), the grouping of the clinical isolates was the same as that seen by 16S rRNA gene sequencing (Fig. 1B). Among the 34 clinical isolates whose 16S rRNA gene sequences had 100% identity to the 16S rRNA gene sequence of the *M. neoaurum* type strain, all formed a tight cluster of *hsp65* sequence variants (sequevars). The largest sequevar cluster consisted of 23 clinical strains whose *hsp65* sequences showed 100% identity to the *hsp65* sequences of *M. neoaurum* type strain ATCC 25795 and

reference strain ATCC 25796. The other three sequevars included 7 clinical strains, 3 clinical strains, and 1 clinical strain each, respectively. These four sequevars showed up to 0.5% intraspecies variability and differed by a G → T at position 112 and/or a C → T at position 211 compared to the sequence of the *M. neoaurum* type strain. Among the 10 clinical isolates whose 16S rRNA genes showed 100% sequence identity to the 16S rRNA gene of ATCC 25791, three sequevars of the *hsp65* gene were seen. The largest cluster consisted of six clinical isolates whose *hsp65* sequences had 99.2% identity (3 bp differences) and 99.8% identity (1 bp difference) to the *hsp65* sequences of the *M. neoaurum* and *M. lacticola* type strains, respectively. The other two sequevars included two clinical strains each. The sequences of these three sequevars showed up to 0.3% intraspecies variability and differed by 1 or 2 bp. All but one sequevar had the same position 88 C → T and position 115 G → C substitutions seen with *M. lacticola* compared to the sequence of *M. neoaurum*, and all sequevars had one additional unique position 367 C → G substitution. Thus, no *hsp65* sequences shared 100% identity to the *hsp65* sequence of the *M. lacticola* type strain. It should be noted the *hsp65* amino acid sequence was identical for all clinical strains.

(iii) *rpoB* gene. By *rpoB* partial gene sequencing (621 bp), the grouping of the clinical isolates was the same as that seen with the 16S rRNA gene, but with overall greater sequence heterogeneity. In total, for the two taxa, 19 sequence variants (sequevars) were seen among the clinical isolates by *rpoB* sequencing and 7 were seen for *hsp65*.

Among the 34 clinical isolates whose 16S rRNA gene showed 100% sequence identity to that of *M. neoaurum* type strain ATCC 25795, the same grouping uncovered with the *hsp65* gene was detected (Fig. 2). Eleven sequevars were identified among the 34 isolates that group with *M. neoaurum*, with no more than 8 isolates being in any one group (Fig. 2). The *rpoB* sequence of one sequevar of 6 clinical isolates matched that of ATCC 27277. Five sequevars contained only 1 strain, while the remaining six consisted of 8, 6, 6, 3, 3, and 2 clinical isolates each, respectively. Overall the 11 sequevars showed up to 1.1% intraspecies variability, with a total of 11 positions being variable.

Among the 10 clinical isolates that showed 100% 16S rRNA gene sequence identity to ATCC 25791, seven sequevars were identified which contained three groups of two isolates each and four isolates that each had a unique sequence. These seven sequevars showed up to 1.0% intraspecies variability in their *rpoB* sequences, which varied by 2 to 6 bp from each other. The closest identities observed for any of the 10 isolates to the *M. neoaurum* and *M. lacticola* type strains were 96.4 and 97.9%, respectively.

The 19 unique *rpoB* sequences seen among the clinical isolates resulted in only 3 unique amino acid sequences. Thirty-four clinical isolates shared 100% amino acid homology with the *M. neoaurum* type strain (Fig. 2). The 10 clinical isolates that showed 100% 16S rRNA gene sequence identity to ATCC 25791 shared 100% amino acid homology with *M. lacticola*.

The four reference strains that showed 100% identity to *M. lacticola* ATCC 9626 by 16S rRNA gene sequencing exhibited three *rpoB* sequevars that showed only 1.5% intraspecies variability and that exhibited a 3.9% difference from the *M. neoaurum* type strain and a 2.3% difference from *M. neoaurum*-like reference strain ATCC 25791.

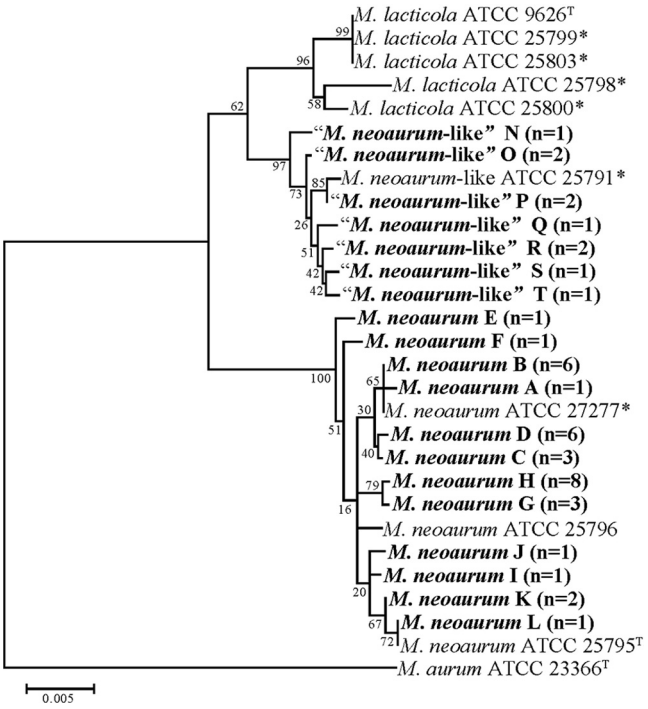

FIG. 2. Neighbor-joining trees of the *rpoB* gene of *M. neoaurum*, *M. lacticola*, and *M. neoaurum*-like clinical isolates and culture collection strains. Branch support is recorded at the nodes as a percentage of 1,000 bootstrap iterations. *M. aurum* is included as an outgroup. *, annotated as a different species by the American Type Culture Collection (18).

Susceptibility testing. Susceptibilities by broth microdilution were available for 46 isolates in this study. The majority of the 46 isolates tested in this study were pansusceptible, having MICs indicating susceptibility to amikacin (46/46), cefoxitin (46/46), tobramycin (46/46), ciprofloxacin (46/46), doxycycline (21/21), gatifloxacin (38/38), imipenem (46/46), linezolid (46/46), moxifloxacin (23/23), sulfamethoxazole (20/20), tigecycline (22/22), and trimethoprim-sulfamethoxazole (45/45).

The one exception was for clarithromycin. Clarithromycin susceptibility primarily exhibited a bimodal distribution with 3 days of incubation, with 12/46 (26%) isolates having highly resistant MICs of ≥16 μg/ml and 25/46 (54%) having susceptible MICs of ≤2 μg/ml. A total of 18/46 (39%) isolates had resistant clarithromycin MICs (≥8 μg/ml) at 3 days. With 14 days of incubation, most susceptible isolates remained susceptible or intermediate to clarithromycin, although 25/46 (54%) were resistant. Tables 2 and 3 show the antimicrobial susceptibility results by broth microdilution for the isolates in the current study. Of the 36 isolates of *M. neoaurum*, 12 (47%) were susceptible to clarithromycin at 3 days of incubation, whereas only 3 isolates (8%) were susceptible to clarithromycin at 14 days of incubation. Likewise, for the 10 isolates in the *M. neoaurum*-like group, 80% were clarithromycin susceptible at 3 days of incubation, whereas only 30% were susceptible after 14 days of incubation. There was no apparent difference

TABLE 2. Comparison of MIC ranges, MIC_{50}s, and MIC_{90}s and percent susceptible to antimicrobials for clinical and reference isolates of *M. neoaurum*-like group

Antimicrobial	No. of isolates tested	MIC (μg/ml)				% susceptible
		ATCC 25791	Range	50%	90%	
Amikacin	10	≤1	≤1–2	≤1	2	100
Cefoxitin	10	≤4	≤2–8	≤8	8	100
Ciprofloxacin	10	≤0.12	≤0.06–0.5	≤0.12	0.25	100
Clarithromycin, 3 days	10	0.5	≤0.06–>16	0.5	8	80
Clarithromycin, 14 days	10	4	1–>64	4	>64	30
Doxycycline	5	≤0.12	≤0.12–≤0.25	≤0.25	≤0.25	100
Gatifloxacin	10	ND[a]	≤0.03–0.12	≤0.06	0.12	100
Imipenem	10	≤2	≤0.5–1	≤0.5	≤1	100
Linezolid	10	≤1	≤1–≤2	≤1	1	100
Minocycline	10	≤1	≤0.06–≤0.5	≤0.5	≤0.5	100
Moxifloxacin	5	≤0.25	0.03–≤0.25	≤0.06	≤0.25	100
Sulfamethoxazole	5	ND	≤2–32	4	32	100
Trimethoprim-sulfamethoxazole	10	≤0.25/4.8	≤0.25/4.8–0.25/4.8	≤0.25/4.8	0.25/4.8	100
Tigecycline	4	0.03	0.06–0.12	≤0.03	0.12	—[b]
Tobramycin	10	2	≤2–4	2	4	100

[a] ND, not done.
[b] —, breakpoints for tigecycline have not been addressed by the CLSI.

in susceptibility to antimicrobials other than clarithromycin between the two groups of isolates.

DISCUSSION

The first reported human infection of *M. neoaurum* was in 1988 in Queensland, Australia, from a patient with a cystadenocarcinoma of the ovary with involvement of abdominal nodes and peritoneal metastases (22). Parenteral nutrition was provided via a Hickman catheter, from which the isolate was recovered. This report is of a study performed prior to the use of molecular identification techniques. The investigators identified this isolate by biochemical testing and thin-layer chromatography and compared the results for the isolate to those for the type strain of *M. neoaurum*. The results were identical to those for the *M. neoaurum* type strain, ATCC 27595 (5). Prior to that report, the species (including the type strain) had been isolated only from soil, dust, and water (22). Since the original report, 15 other human cases have been documented in Australia, the United States, Italy, Canada, and China (Table 4).

A review of the previously published cases of *M. neoaurum* and *M. lacticola* shows results similar to those obtained in the current study, in that 11/15 (73%) of the infections were associated with catheter- or line-related sepsis. Of these 11 cases, however, only three isolates were identified using molecular techniques (1, 11, 25). The first case of *M. neoaurum* infection identified using 16S rRNA sequencing was in 2000 in a 9-year-old girl with acute lymphoblastic leukemia in Hong Kong. Blood cultures performed with blood drawn through a Hickman catheter were positive for a pigmented, rapidly growing mycobacterium that was identified by complete 16S rRNA gene sequencing as *M. neoaurum* (25). In 2004, the first documented case of *M. lacticola*, a newly characterized but currently unvali-

TABLE 3. Comparison of MIC ranges, MIC_{50}s, and MIC_{90}s and percent susceptible to antimicrobials for clinical and reference isolates of *Mycobacterium neoaurum*

Antimicrobial	No. of isolates tested	MIC (μg/ml)				% susceptible
		ATCC 25795T	Range	50%	90%	
Amikacin	36	≤1	≤1–2	≤1	2	100
Cefoxitin	36	8	4–16	≤8	16	100
Ciprofloxacin	36	≤0.12	≤0.12–1	≤0.12	≤0.25	100
Clarithromycin, 3 days	36	4	0.5–>32	4	32	47
Clarithromycin, 14 days	36	8	2–>64	8	>64	8
Doxycycline	19	≤0.12	≤0.06–≤0.25	≤0.25	≤0.25	100
Gatifloxacin	29	ND[a]	≤0.06–0.25	≤0.06	≤0.12	100
Imipenem	36	≤2	≤0.5–≤2	≤0.5	1	100
Linezolid	36	≤1	≤0.5–2	≤1	2	100
Minocycline	35	≤1	≤0.25–≤1	≤0.5	0.5	100
Moxifloxacin	18	≤0.25	≤0.06–0.5	≤0.12	≤0.25	100
Sulfamethoxazole	15	ND	≤4–32	8	16	100
Trimethoprim-sulfamethoxazole	35	0.5/9.5	≤0.25/4.8–1/19	0.25/4.8	0.5/9.5	100
Tigecycline	18	0.06	≤0.03–0.25	0.06	0.12	—[b]
Tobramycin	36	2	≤1–4	2	4	100

[a] ND, not done.
[b] —, breakpoints for tigecycline have not been addressed by the CLSI.

TABLE 4. Published cases of *Mycobacterium neoaurum*, *M. neoaurum*-like, and *M. lacticola* infections[a]

Yr (reference)	Geographic location	Underlying disease	Age[b]	Sex	Catheter removed (type)	Antibiotics given	Length of antibiotic treatment	Resolution of infection	Isolate cultured	Method(s) of ID	Susceptibility pattern
1988 (5)	Australia	Ovarian adenocarcinoma	55	F	No (Hickman)	Gentamicin, cefoxitin	7 wk	Yes	Yes	TLC, Bios	Susceptible to amikacin, kanamycin, gentamicin, tetracycline, minocycline, doxycycline, SXT, cefoxitin, vancomycin, imipenem, streptomycin, capreomycin; resistant to ethambutol, isoniazid, cycloserine, cefotaxime, amoxicillin
1994 (10)	Australia	ALL (allogeneic bone marrow transplant)	17	M	Yes	Ticarcillin-clavulanate, amoxicillin-clavulanate, tobramycin	3 wk	Yes	Yes	Bios	Susceptible to ticarcillin clavulanate, amikacin, tetracycline, cefoxitin, imipenem, ciprofloxacin, erythromycin, clarithromycin, roxithromycin, azithromycin; resistant to TMP-SMX
1999 (6)	USA	i.v. drug abuse	NA	M	Yes	Piperacillin, gentamicin	6 wk	Yes	Yes[c]	HPLC, Bios	NA
1999 (6)	USA	Primary pulmonary hyper tension, recurring fevers	46	M	Yes (Hickman)	Vancomycin[d] Ceftriaxone[d] Amoxicillin-clavulanate[d]	3 days 3 days 10 days	Yes	Yes	HPLC, Bios	NA
2000 (25)	Hong Kong	ALL	9	F	Yes (Hickman)	Ceftazidime, amikacin	3 wk	Yes	Yes	16S rRNA sequencing	Susceptible to ampicillin, amoxicillin clavulanate, imipenem, meropenem, ofloxacin, ciprofloxacin, amikacin, tetracycline, doxycycline, co-trimoxazole
2000 (13)	USA	Diabetes mellitus hyper tension, renal failure	54	M	Yes (peritoneal)	Cefoxitin, ethambutol, rifampin, and clarithromycin; after ID changed to ciprofloxacin, amikacin, and rifampin	4 wk	Yes	Yes	TLC, HPLC	Susceptible to amikacin, ampicillin-sulbactam, ciprofloxacin, doxycycline, ofloxacin, rifampin; resistant to clarithromycin, ethambutol, ethionamide, isoniazid
2000 (27)	Italy	Recurrent UTI (progressive renal failure)	62	F	NA	NA	NA	Yes	Yes[e]	*hsp65* PRA, 16S rRNA, HPLC	Susceptible to streptomycin, vancomycin, imipenem, levofloxacin, ofloxacin; intermediate to amoxicillin; resistant to isoniazid, rifampin, ethambutol, ethionamide
2003 (1)	Canada	Diabetes mellitus, renal failure on hemo-dialysis	40	F	Removal not clear (graft/line infection)	Ciprofloxacin, doxycycline	~ 4 wk	Yes	Yes	16S rRNA sequencing	Susceptible to ciprofloxacin, doxycycline, rifampin, imipenem; resistant to ethambutol, isoniazid, clarithromycin
2004 (11)	USA	Autologous stem cell transplant	4	F	Yes (Hickman)	Azithromycin, rifampin	5 days	Yes	Yes	*hsp65* PCR, 16S rRNA sequencing	NA

2006 (14)	USA	Pulmonary gastro esophageal, reflux disease, corticosteroids	67	F	NA	Clarithromycin, amikacin	6 mo	Yes	Yes	16S rRNA sequencing	Susceptible to amikacin, kanamycin, tobramycin, cefoxitin, imipenem, doxycycline, ciprofloxacin, gatifloxacin, moxifloxacin, clarithromycin, SXT, linezolid, amoxicillin clavulanate
2007 (12)	Australia	Cutaneous infection, sarcoid	53	F	NA	Moxifloxacin, roxithromycin	4 mo	Yes	No	PCR of tissue	NA
2007 (24)											
Patient 1	USA	Neuroblastoma	32 mo	F	Yes (Hickman)	Meropenem[f] Amikacin[f] Clarithromycin[f] Ciprofloxacin Linezolid	7 days 7 days 7 days 3 wk 3 wk	Yes	Yes	HPLC, Bios	Resistant to clarithromycin
Patient 2	USA	Liver transplant	15 mo	F	NA	NA		Yes	Yes	HPLC, Bios	NA
Patient 3	USA	Rhabdomyosarcoma	3	M	Yes (Broviac)	Amikacin Clarithromycin Levofloxacin	12 days 6 wk 6 wk	Yes	Yes	HPLC, Bios	Resistant to clarithromycin
Patient 4	USA	Colon cancer, short gut syndrome	59	F	Yes (PICC)	Cefoxitin Levofloxacin Clarithromycin Ethambutol	2 wk 4 wk 4 wk 4 wk	Yes	Yes	HPLC, Bios	NA

[a] ID, identification; AST, antimicrobial susceptibility testing; TLC, thin-layer chromatography; i.v., intravenous; ALL, acute lymphocytic leukemia; M, male; F, female; NA, not available; Bios, biochemical testing, TMP, trimethoprim; SXT, trimethoprim-sulfamethoxazole; UTI, urinary tract infection; PICC, peripherally inserted central catheter.
[b] Ages are in years unless indicated otherwise.
[c] Polymicrobial infection consisting of two strains of *Pseudomonas aeruginosa* and *Comamonas acidovorans* with *M. neoaurum*.
[d] Prior to catheter removal.
[e] Two separate isolates were recovered (2 months apart).
[f] Initial treatment that was changed to ciprofloxacin and linezolid.

dated species was described in a patient with catheter-related sepsis following autologous stem cell transplantation (11). Identification was based on complete 16S rRNA gene sequencing. The third case was a patient in Canada with diabetic mellitus and renal failure on hemodialysis (1).

Four patients (27%) without catheters also developed infection with *M. neoaurum*, with three of these isolates being identified by molecular techniques. These cases included an Italian patient with recurrent urinary tract infections and renal failure (27), a patient from the United States with chronic pulmonary disease receiving long-term corticosteroids (14), a pediatric liver transplant patient from the United States (24), and an immunocompetent Australian patient with cutaneous infection of the scalp for whom cultures were negative but PCR of tissue detected *M. neoaurum* (12).

Recently, a case of meningoencephalitis followed by rapidly progressive dementia in which *M. neoaurum* was identified as the possible pathogen was reported (9). However, a subsequent investigation of the case suggested that the *M. neoaurum* DNA recovered in the previously reported case was likely a laboratory contaminant, and this case was excluded from the cases documented in Table 4 (8).

Susceptibility testing was recorded in 9 of 15 (60%) of the previously reported cases of infection due to *M. neoaurum.* The susceptibility testing results for the previously published cases, shown in Table 4, indicate susceptibility of most isolates to the antimicrobials recommended for treatment of infections caused by rapidly growing mycobacteria, including amikacin (5/5), doxycycline (5/5), cefoxitin (3/3), imipenem (6/6), ciprofloxacin (5/5), tobramycin (1/1), linezolid (1/1), and sulfonamides (3/4). Four of six (67%) of the isolates were resistant to clarithromycin. Isolates of the *M. neoaurum–M. neoaurum*-like group in the current study were also generally susceptible to the antimicrobials recommended by the CLSI for susceptibility testing of rapidly growing mycobacteria, including amikacin, cefoxitin, ciprofloxacin, doxycycline, imipenem, tobramycin, trimethoprim-sulfamethoxazole, and linezolid; but similar to the previously documented cases, almost half of the isolates showed resistance to clarithromycin (26).

The outcomes of the previous infections presumed to be caused by the *M. neoaurum-M. lacticola* group were good. In all 15 of the previously described cases, resolution of infection was documented. For the 14 patients who were treated with antimicrobials, various regimens were successful, although all of the regimens contained at least two antimicrobials. As with other serious nontuberculous mycobacterial infections, combination antimicrobial therapy is probably warranted. Since only one of nine patients (11%) maintained a catheter after discovery of the *M. neoaurum-M. lacticola* group infection, it seems expedient to recommend removal of the catheter when an *M. neoaurum-M. lacticola* group infection is diagnosed.

The resistance of *M. neoaurum* and *M. neoaurum*-like organisms to clarithromycin suggests the presence of an inducible *erm* gene. These genes have been shown to be the basis for intrinsic macrolide resistance in other mycobacterial species, including *M. tuberculosis* [*erm*(37)] (3), *M. smegmatis* [*erm*(38)] (15), *M. fortuitum* [*erm*(39)] (17), *M. mageritense* [*erm*(40)] (15), and most recently, *M. abscessus* [(*erm*(41)] (16). *M. smegmatis* [*erm*(38)], like *M. neoaurum* and the *M. neoaurum*-like group, is a pigmented, rapidly growing mycobacterial species.

As for identification of the majority of the other nontuberculous mycobacterial species, identification by molecular techniques is currently necessary for definitive identification of isolates of the *M. neoaurum–M. neoaurum*-like–*M. lacticola* group, as three closely related species or taxonomic groups (*M. neoaurum*, *M. neoaurum*-like, and *M. lacticola*) are present within the group and are associated with mycobacteremia. Moreover, in a previous report, Simmon et al. indicated that multigene target sequencing, including sequencing of *rpoB* and *hsp65* genes, in addition to DNA relatedness studies, may be necessary to differentiate the *M. neoaurum-M. lacticola* group from other closely related species (18). It is noteworthy that only five of the previously documented cases of infection caused by the *M. neoaurum-M. lacticola* group were confirmed by 16S rRNA gene sequencing (Table 4), and the percent similarities to other species in the previous studies was not indicated. Partial sequencing was performed in two of the five cases. Two of the cases used a >1,200-bp sequence, and in one case the number of base pairs sequenced was not specified.

Interestingly, the *M. neoaurum*, *M. neoaurum*-like group, and *M. lacticola* all appear to have pathogenicity similar to that of another rapidly growing mycobacterial species, *M. mucogenicum*, which is also primarily associated with bloodstream and central line-related infections (2, 22).

Thus, by using multilocus gene sequencing, this study confirms that two closely related taxa of pigmented, rapidly growing mycobacteria are associated with mycobacteremia and central catheter line-related sepsis. The study also demonstrates that these taxa are relatively susceptible to all drugs except the macrolides, and therapy is usually successful. No clinical cases of mycobacteremia due to *M. aurum* or the nonvalidated species *M. lacticola* were identified. Continued use of sequencing likely will identify other pathogenic pigmented, rapidly growing mycobacteria in the future.

Mycobacterium bacteremicum. *Mycobacterium bacteremicum* (pertaining to the organism's association with bloodstream infections) is an acid-fast bacillus that grows aerobically within 7 days on standard mycobacterial media. It is scotochromogenic (yellow). It is susceptible (100%) to amikacin, cefoxitin, imipenem, trimethoprim-sulfamethoxazole, doxycycline, minocycline, tigecycline, linezolid, ciprofloxacin, moxifloxacin, and gatifloxacin. It shows variable susceptibility to clarithromycin. It is an established cause of central venous catheter-related infections, and as its name suggests, it is most commonly recovered from blood. It has also been recovered from posttraumatic wound infections. The proposed type strain is ATCC 25791, which was originally submitted to the ATCC as an example of *M. aurum.* By complete 16S rRNA gene sequencing, ATCC 25791^T has a unique sequence that is the most closely related to the sequences of *M. neoaurum* (4 bp, or 99.7%) and *M. lacticola* (4 bp, or 99.7%) (GenBank accession number FJ172308) (21). By partial sequencing of the *hsp65* gene, three closely related sequence variants that showed 0.3% intraspecies variability were identified and were most closely related to *M. neoaurum* (99.2% identity) (GenBank accession number FJ172314). By *rpoB* partial gene sequencing, six closely related sequence variants that showed 0.8% intraspecies variability were identified. They were most closely related to *M. neoaurum* (3.6% difference) and *M. lacticola* (2.3%) (GenBank accession number FJ172329).

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