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Primary Identification of *Microbacterium* spp. Encountered in Clinical Specimens as CDC Coryneform Group A-4 and A-5 Bacteria

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Over nearly two decades, 13 yellow- or orange-pigmented, fermentative gram-positive rods belonging to the genus *Microbacterium* were encountered in clinical specimens. All 13 strains, 10 of which came from blood cultures, were initially identified as CDC coryneform group A-4 and A-5 bacteria according to the scheme of Hollis and Weaver for the identification of gram-positive rods. The clinical isolates were compared with the type strains of the six species constituting the genus *Microbacterium* as well as with three *Microbacterium* strains isolated from hospital environments. By biochemical methods only 5 of 13 clinical isolates could be identified to species level. Peptidoglycan analysis proved to be a valuable tool for differentiation between *Microbacterium* spp. and related genera, whereas cellular fatty acid analysis did not allow species identification within the genus *Microbacterium*. The 22 *Microbacterium* strains studied were, in general, susceptible to antimicrobial agents used in the treatment of infections caused by gram-positive rods. This report is the first one concerning the isolation of *Microbacterium* strains from clinical specimens. The sources as well as the mode of transmission remain to be established.

In 1919 Orla-Jensen proposed the genus *Microbacterium*, but this genus underwent several changes within subsequent decades (3). These changes continued until 1983, when Collins et al. redefined the genus *Microbacterium* and reclassified *Brevibacterium imperiale* as *Microbacterium imperiale* and "*Corynebacterium laevaniformans*" as *M. laevaniformans* (4). In addition, *M. lacticum* was proposed as the type species of the redefined genus *Microbacterium* (4). The fourth species within the genus *Microbacterium*, *M. arborescens*, was reclassified from *Flavobacterium arborescens* by Imai et al. in 1984 (13). Most recently, Yokota et al. proposed *M. dextranolyticum* and *M. aurum* (24).

All *Microbacterium* strains described in the literature so far have been isolated from environmental sources. However, over nearly two decades the three laboratories involved in this study have isolated or received *Microbacterium* strains from clinical specimens. Initially, these yellow- or orange-pigmented, fermentative gram-positive rods (GPRs) were identified as CDC coryneform group A-4 and A-5 bacteria (12), but further investigations revealed that they belong to the genus *Microbacterium*. This report stresses again the value of chemotaxonomic methods for the identification of GPRs. To the best of our knowledge, this is the first report concerning the appearance of *Microbacterium* spp. in clinical specimens.

MATERIALS AND METHODS

Strains. The strains studied were isolated or received as reference strains between 1975 and 1993 by the three laboratories contributing to this study. Strain 15 was isolated from clinical material as described in detail earlier (8), and strains 16 to 18 were isolated from Septi-Chek blood culture bottles (Hoffmann-La Roche, Basel, Switzerland). For comparative biochemical and chemotaxonomic investigations we used the following type strains: *M. lacticum* CIP 69.3^T (ATCC 8180^T), *M. laevaniformans* CIP 100934^T (ATCC 15953^T), *M. dextranolyticum* IFO 14592^T (DSM 8607^T), *M. aurum* IFO 15204^T (DSM 8600^T), *M. imperiale* CIP 82.108^T (ATCC 8365^T), and *M. arborescens* CIP 55.81^T (ATCC 4358^T).

Biochemical profiles. Media (all from Becton Dickinson Microbiology Systems, Cockeysville, Md., unless stated otherwise) were prepared according to the methods of Nash and Krenz (16). All tests were performed at 37°C. The CAMP reaction was performed on Columbia agar with 5% sheep blood by using *Staphylococcus aureus* ATCC 25923. The commercial API 50CH and API ZYM systems (both from API bioMérieux, Marcy l'Etoile, France) were used according to the guidelines provided by the manufacturer.

Susceptibility studies. Eight antimicrobial agents (ciprofloxacin, clindamycin, erythromycin, gentamicin, penicillin G, rifampin, tetracycline, and vancomycin) used in the treatment of infections caused by GPRs were selected for determination of MICs by the agar dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (17, 18).

CFA profiles. Cells were grown for 48 h at 37°C with 5% CO_2 on Trypticase soy agar with 5% sheep blood, and cellular fatty acid (CFA) patterns were analyzed with the Microbial Identification System (Microbial ID, Inc., Newark, Del.) as described previously (23).

Peptidoglycan analysis. The methods used have already been outlined in detail by Barreau et al. (1) and Schleifer and Kandler (20). Briefly, cell wall hydrolysates as well as partial cell wall hydrolysates were separated by one- or two-dimensional thin-layer chromatography and then visualized with ninhydrin.

RESULTS

Of the 22 strains examined, 13 came from clinical materials and 10 of these were isolated from blood cultures (Table 1). All clinical strains except strain 6 grew in pure culture. Direct Gram stains of the clinical materials had not been performed. The clinical strains were compared with six reference strains and three strains isolated from hospital environments.

Table 2 lists the biochemical characteristics of all 22 strains tested. All strains were yellow or orange pigmented, the latter ones being identified as *M. imperiale* or *M. arborescens*. Only *M. imperiale* and *M. arborescens* strains were found to be motile. Nitrate reduction was positive in *M. lacticum* and in four of the clinical strains only. All strains were able to hydrolyze esculin (except *M. laevaniformans*), but this reaction was delayed in *M. lacticum* and *M. aurum*. Carbohydrate metabolism was fermentative in all strains. Fermentation reactions of five carbohydrates (glucose, maltose, sucrose, mannitol, and xylose)—applied in the routine testing of GPRs (12)—were positive for all *M. imperiale* and *M. arborescens* strains. The other *Mi-*

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TABLE 1. Origins of the strains studied

Strain no.	Reference no.[a]	Species	Source
1	CIP 69.3[T]	*M. lacticum*	NK (environmental)[b]
2	CIP 100934[T]	*M. laevaniformans*	Activated sludge
3	IFO 14592[T]	*M. dextranolyticum*	Soil
4	IFO 15204[T]	*M. aurum*	Corn steep liquor
5	CIP 82.108[T]	*M. imperiale*	*Eacles imperialis*
6	CIP 101583	*M. imperiale*	Vaginal swab
7	CIP 100644	*M. imperiale*	Blood culture
8	CIP 101188	*M. imperiale*	Blood culture
9	CCUG 31315	*M. imperiale*	Hospital humidifier
10	DMMZ 781	*M. imperiale*	Hospital air
11	CIP 55.81[T]	*M. arborescens*	NK (environmental)
12	CCUG 28797	*M. arborescens*	NK (clinical)
13	CIP 101307	*M. arborescens*	Blood culture
14	DMMZ 777	*M. arborescens*	Hospital air
15	DMMZ 102	*Microbacterium* sp.	Deep femoral wound
16	DMMZ 249	*Microbacterium* sp.	Blood culture
17	DMMZ 701	*Microbacterium* sp.	Blood culture
18	DMMZ 702	*Microbacterium* sp.	Blood culture
19	CIP 101901	*Microbacterium* sp.	Blood culture
20	CIP 100625	*Microbacterium* sp.	Blood culture
21	CIP 101305	*Microbacterium* sp.	Blood culture
22	CIP 101667	*Microbacterium* sp.	Blood culture

[a] CIP, Collection de l'Institut Pasteur; IFO, Institute of Fermentation Osaka; CCUG, Culture Collection of the University of Göteborg; DMMZ, Department of Medical Microbiology, University of Zürich.
[b] NK, not known.

TABLE 2. Biochemical characteristics of the strains studied

Organism (strain no.)[a]	Reaction or characteristic																		
						Fermentation of:										Enzymatic activity[b]			
	Pigment	Motility	Catalase	Nitrate reduction	Urea hydrolysis	Esculin hydrolysis	Glucose	Maltose	Sucrose	Mannitol	Xylose	L-Arabinose	Rhamnose	Melibiose	Melezitose	D-Raffinose	β-Galactosidase	β-Glucosidase	N-Acetyl-β-glucosaminidase	α-Mannosidase
M. lacticum (1)	Whitish-yellow	−	+	+	−	+[c]	+	+	−	+[c]	+	−	−	−	−	−	S	−	−	−
M. laevaniformans (2)	Yellow	−	+	−	−	−	+	+	+	+	−	−	−	−	−	+	−	−	M	−
M. dextranolyticum (3)	Yellow	−	+	−	−	+	+	+	+	−	−	−	−	−	−	−	−	W	S	−
M. aurum (4)	Yellow	−	+	−	−	+[c]	+	+	+	+[c]	−	−	−	−	−	−	S	−	−	W
M. imperiale (5–10)	Orange	+	+	−	−	+	+	+	+	+	+	+	+	+	−	+	S	M	M	M
M. arborescens (11–14)	Orange	+	+	−	−	+	+	+	+	+	+	+	+	+	+	−	S	M	M	M
Microbacterium sp. (15)	Yellow	−	+	+	−	+	+	+	+	+	−	+[c]	−	−	−	+	−	−	M	−
Microbacterium sp. (16)	Yellow	−	+	−	−	+	+	+[c]	+	+	+	−	−	−	−	−	−	−	−	−
Microbacterium sp. (17)	Yellow	−	+	+	−	+	+	+	+	+	+	−	−	−	−	−	S	W	M	−
Microbacterium sp. (18)	Yellow	−	+	+	−	+	+	+	+	+	+	−	−	−	−	−	S	W	M	−
Microbacterium sp. (19)	Yellow	−	+	−	−	+	+	+	+	+	−	−	−	−	−	−	−	W	M	−
Microbacterium sp. (20)	Yellow	−	+	−	−	+	+	+	+	+	−	−	−	−	−	−	−	W	M	−
Microbacterium sp. (21)	Yellow	−	+	−	−	+	+	+	+[c]	+	+	+	−	−	−	−	S	M	M	−
Microbacterium sp. (22)	Yellow	−	+	+	−	+	+	+	+	+	+	−	−	−	−	−	S	M	−	W

[a] Strain numbers are those of Table 1.
[b] W, approximately 5 nmol of substrate hydrolyzed; M, approximately 20 nmol of substrate hydrolyzed; S, 40 nmol of substrate hydrolyzed.
[c] Weak or delayed reaction.

crobacterium strains were able to ferment at least four of these five carbohydrates (Table 2). On the basis of these biochemical reactions (Table 2), all *Microbacterium* strains were initially assigned to CDC coryneform group A-4 (strains 6 to 10, 12 to 14, 16 to 18, 21, and 22) or A-5 (strains 15, 19, and 20). When the fermentation reactions of a further 44 carbohydrates were tested with the API 50CH system, the following reactions were positive for all strains examined: galactose, D-fructose, D-mannose, and cellobiose. None of the strains was able to ferment D-arabinose, D-fucose, L-fucose, L-arabitol, or 2-keto-gluconate. For the species diagnosis of *Microbacterium* strains fermentations of melezitose and D-raffinose were found to have the greatest differential potential (Table 2). The CAMP reaction was positive for 2 *M. arborescens* strains (11 and 13) and negative for the other 20 strains tested. Enzymatic reactions were relatively uniform for *Microbacterium* spp., with esterase (C_4), esterase lipase (C_8), and leucine arylamidase determined to be present in all strains. Strain 16 showed these few enzymatic reactions only, whereas all other strains were found to possess many of the enzymatic activities covered in the API ZYM system (Table 2).

All 22 strains examined were susceptible to rifampin, tetracycline, and vancomycin. Only strain 16 was resistant to ciprofloxacin. In contrast, only two strains (strains 9 and 15) were found to be susceptible to penicillin G when categories used for staphylococci were applied (18). Most *Microbacterium* strains were susceptible to the other antimicrobial agents tested (Table 3).

For all 22 strains tested, 12-methyltetradecanoic ($C_{a15:0}$) fatty acid methyl ester (FAME) and 14-methylhexadecanoic ($C_{a17:0}$) FAME were the predominant CFAs (Table 4). For *M. imperiale* and *M. arborescens* the amounts of $C_{a15:0}$ and $C_{a17:0}$ FAME taken together were greater than 75% of the total CFA content for all strains of these species tested. Otherwise, there were no species-specific CFA patterns within the genus *Microbacterium*.

TABLE 3. Antimicrobial susceptibility patterns of the strains studied

Antimicrobial agent	MIC(s) (μg/ml) for:													
							Microbacterium spp.[c]							
	M. lacticum	*M. laevaniformans*	*M. dextranolyticum*	*M. aurum*	*M. imperiale*[a]	*M. arborescens*[b]	Strain 15	Strain 16	Strain 17	Strain 18	Strain 19	Strain 20	Strain 21	Strain 22
Ciprofloxacin	0.5	0.5	1	0.5	0.5–1	0.5–1	0.5	>64	2	1	1	0.5	1	0.5
Clindamycin	0.5	<0.03	0.06	1	1–8	4–8	0.03	0.5	0.06	0.06	0.06	0.06	2	4
Erythromycin	0.06	0.06	0.06	0.06	0.06–16	0.06–8	0.06	1	0.06	0.13	0.06	<0.03	<0.03	0.06
Gentamicin	2	16	4	4	8–16	16–64	8	8	4	4	16	32	32	16
Penicillin G	2	2	0.25	0.25	0.06–1	0.25–2	0.06	0.25	1	1	0.5	4	0.25	1
Rifampin	<0.03	0.06	<0.03	1	<0.03–0.06	<0.03	<0.03	<0.03	0.5	0.5	0.25	0.25	<0.03	0.5
Tetracycline	0.13	0.5	1	0.5	0.5–1	1	1	2	1	1	1	1	1	1
Vancomycin	0.13	0.25	0.25	0.13	0.25	0.25	0.13	0.13	0.13	0.13	0.25	0.25	0.5	0.25

[a] Strains 5 through 10 in Table 1.
[b] Strains 11 through 14 in Table 1.
[c] Strain numbers are those of Table 1.

Peptidoglycan analysis revealed the presence of lysine as diamino acid and glycine$_2$-lysine or glycine-lysine as interpeptide bridges in all 22 strains included in this study.

DISCUSSION

Over nearly two decades *Microbacterium* strains were isolated and received at the three reference laboratories involved in this study. Strains able to ferment all five carbohydrates used in our routine panel (see above) were tentatively identified as CDC coryneform group A-4 bacteria, and those unable to ferment xylose were identified as CDC coryneform group A-5 bacteria according to the scheme of Hollis and Weaver (12). The presence of lysine as diamino acid of the cell wall peptidoglycan and glycine$_2$-lysine or glycine-lysine as interpeptide bridges clearly revealed that the strains were *Microbacterium* spp., as this combination of amino acids is not found in any other related genus (3, 20). By analysis of biochemical characteristics 5 of 13 clinical isolates could be assigned to the species *M. imperiale* (n = 3) or *M. arborescens* (n = 2). The other eight clinical isolates, however, could not be identified to the species level by the methods used. By using quantitative DNA-DNA hybridizations, the precise taxonomic positions of the remaining eight clinical *Microbacterium* strains, which represent a rather heterogenous group (Table 2), may be defined in the future.

All our phenotypical data indicate a very close relationship between *M. imperiale* and *M. arborescens*, as has already been reported (3, 13, 19). Distinguishing features include the presence of rhamnose (*M. imperiale*) or 6-deoxytalose (*M. arborescens*) as 6-deoxyhexoses within the cell wall (13). Surprisingly, we observed that two of four *M. arborescens* strains showed a positive CAMP reaction, whereas all *M. imperiale* strains had a negative reaction. Because the number of strains studied was much too limited, we cannot recommend the CAMP test for the differentiation of *Microbacterium* spp. at present. Fermentation of melezitose and fermentation of D-raffinose were, in fact, the only phenotypical reactions that discriminated between *M. imperiale* and *M. arborescens* (Table 2). In contrast to the results of Imai et al. (13), we found all *M. arborescens* strains (including the type strain) to be motile and we did not observe H_2S production (using Triple Sugar Iron agar) in *M. arborescens* strains.

For the routine work we recommend the following procedures in addition to use of the features outlined in Fig. 1. Yellow- or orange-pigmented GPRs should be suspected of being *Microbacterium* spp. Differential diagnosis includes *Aureobacterium* spp. (14), *Curtobacterium* spp. (15), and "*Coryne-*

TABLE 4. CFA patterns of the strains studied

Strain no.[a]	Organism	% of total fatty acid content[b]							
		$C_{i15:0}$	$C_{ai15:0}$	$C_{i16:0}$	$C_{16:0}$	$C_{i17:0}$	$C_{ai17:0}$	$C_{i18:0}$	$C_{18:0}$
1	*M. lacticum*	4	41	8	5	5	33		1
2	*M. laevaniformans*	7	37	10	11	4	28		1
3	*M. dextranolyticum*	1	44	15	6	1	31		1
4	*M. aurum*	1	28	10	4	1	52	1	2
5–10	*M. imperiale*[c]	1 ± 1	35 ± 4	13 ± 2	2 ± 1		48 ± 6		1 ± 1
11–14	*M. arborescens*[c]		33 ± 1	19 ± 4	2 ± 0		43 ± 3		
15	*Microbacterium* sp.	5	42	7	5	4	32		1
16	*Microbacterium* sp.	1	30	13	5	1	48		1
17	*Microbacterium* sp.	6	44	8	5	3	33		1
18	*Microbacterium* sp.	5	42	8	4	3	35		1
19	*Microbacterium* sp.	1	27	10	2	2	51		
20	*Microbacterium* sp.	2	32	10	3	2	47	1	1
21	*Microbacterium* sp.	1	26	13	1	2	46	2	1
22	*Microbacterium* sp.	1	27	11	5	1	46	2	2

[a] Strain numbers are those of Table 1.
[b] Amounts of less than 1% not reported.
[c] Data are means ± standard deviations.

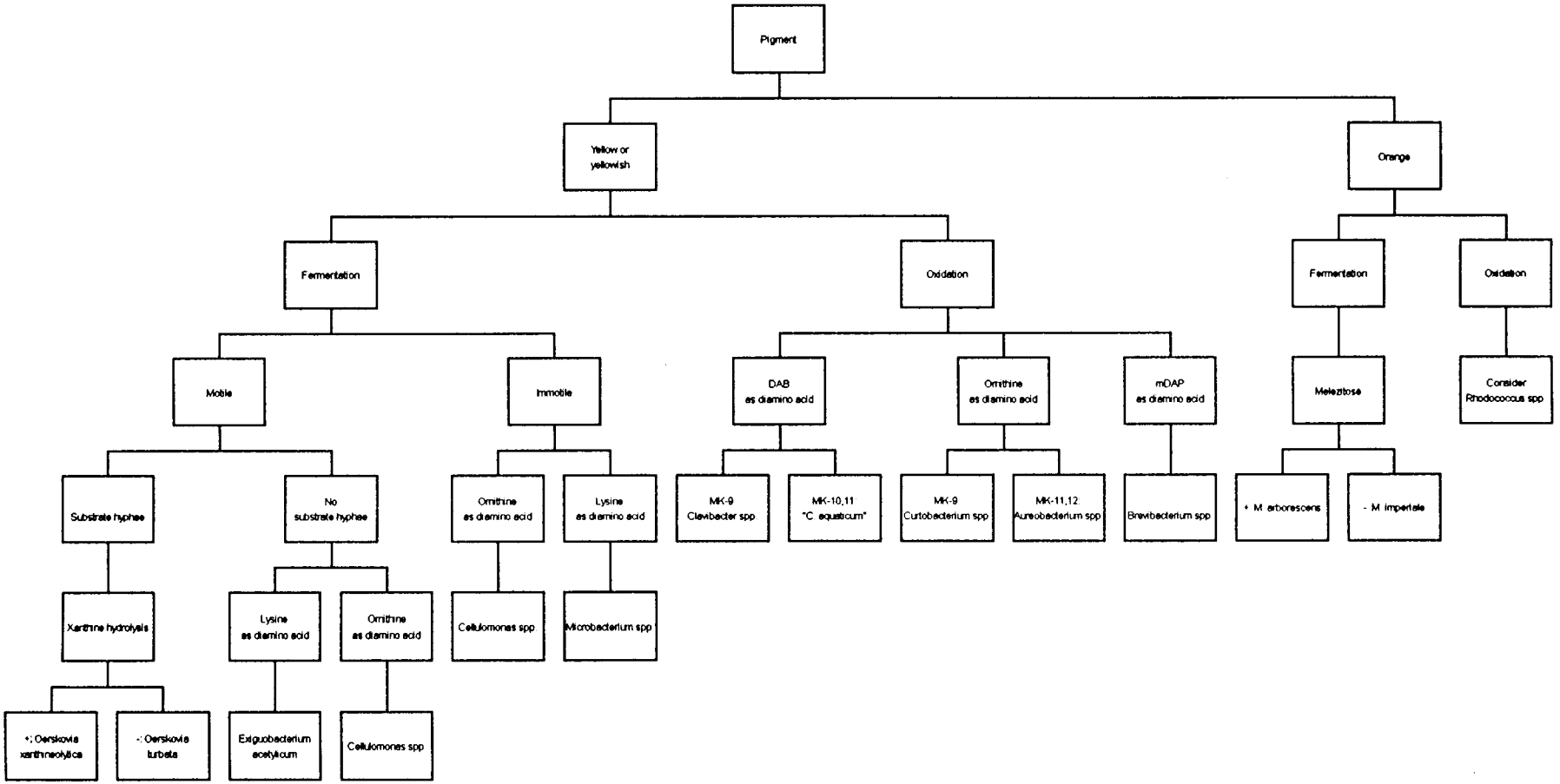

FIG. 1. Flow chart for differentiation of yellow- or orange-pigmented gram-positive rods encountered in clinical specimens. Data were compiled from references 3, 5–7, 9, 10, 12, and 22. DAB, diaminobutyric acid; mDAP, *meso*-diaminopimelic acid; MK, menaquinone; "*C. aquaticum*," "*Corynebacterium aquaticum*." *E. acetylicum* may also be orange pigmented.

bacterium aquaticum" (12), but these species do not grow under anaerobic conditions. The yellow-pigmented, fermentative species include *Oerskovia* spp. (21) and *Exiguobacterium acetylicum* (formerly *Brevibacterium acetylicum* [5]), but these are motile, whereas the yellow-pigmented *Microbacterium* spp. are all nonmotile. *Cellulomonas* spp. (22) can be differentiated from *Microbacterium* spp. by their cellulase activity. If CFA analysis is available, dominance of $C_{a15:0}$ and $C_{a17:0}$ FAME in *Microbacterium* spp. can be a further diagnostic aid in differentiation from *Oerskovia* spp., *E. acetylicum*, and *Cellulomonas* spp., which also have shorter CFAs (e.g., $C_{i14:0}$ or $C_{14:0}$) (2, 23).

It is most likely that CDC coryneform groups A-4 and A-5, as defined in the tables prepared by Hollis and Weaver (12), contain different genera. We, therefore, emphasize the value of chemotaxonomic methods (e.g., peptidoglycan analysis, analysis of menaquinone patterns, and polar lipid analysis) for the differentiation of GPRs in order to assign the isolates to the correct genera. However, we acknowledge that these techniques are reserved for the reference laboratory.

The mode of transmission of *Microbacterium* strains to humans remains to be elucidated. The roles of the three *Microbacterium* strains isolated from the hospital environment are unclear, and nosocomial transmission remains speculative.

Finally, after the demonstration that *Brevibacterium* spp. (7, 11) and *Aureobacterium* spp. (9) can be human pathogens, this report adds the genus *Microbacterium* to the list of already defined genera that the clinical microbiologist should be aware of, even though the number of strains isolated from clinical specimens is expected to be rather small.

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