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Identification of *Turicella otitidis* Isolated from a Patient with Otorrhea Associated with Surgery: Differentiation from *Corynebacterium afermentans* and *Corynebacterium auris*

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Turicella otitidis **is a newly described coryneform bacterium isolated from middle ear fluids. We report here on the diagnosis of a strain isolated from otorrhea. The API Coryne system (bioMérieux, Marcy l'Etoile, France) used alone failed to differentiate *T. otitidis*, *Corynebacterium afermentans*, and *Corynebacterium auris* (ANF group). Biochemical tests such as DNase, enzymatic reactions (API ZYM; bioMérieux), and carbon substrate assimilation tests (Biotype 100; bioMérieux) allow presumptive identification. However, only chemotaxonomy and molecular biology can achieve unequivocal differentiation among these three species.**

A number of authors have emphasized the importance of nonfermenting coryneform bacteria in ear infections. Funke et al. (5) isolated eight samples of a nonfermenting coryneform bacterium (two acute otitis media, one chronic otitis media, one otitis media, and four otitis media perforata). In five of the cases, the isolates were monomicrobial. In the other three cases, other bacilli were observed in addition to the coryneform bacterium. The gram-positive bacteria were identified as *Corynebacterium afermentans* (group ANF-1 [absolute nonfermenter 1; Centers for Disease Control and Prevention classification]) by their biochemical characteristics, but as they were mycolic-acid-free, they could not be classified in the genus *Corynebacterium*. Moreover, the presence of the bacterium in the polymicrobial compounds raised doubt as to its pathogenicity. Simonet et al. (12) isolated in pure culture from middle ear fluids collected by tympanocentesis 16 strains devoid of mycolic acid which were very similar to *C. afermentans*. Notable differences in colony morphology and the presence of DNase and trypsin activities led the authors to tentatively name the strain ANF-1. On the other hand, the strains isolated in pure culture by tympanocentesis from patients with acute otitis media have been considered responsible for pathogenesis. In 1994, Funke et al. (6) clearly delineated *C. afermentans* and the ANF-1-like corynebacteria by comparing the partial 16S rRNA gene sequences and proposed a new genus for the ANF-1-like bacteria, *Turicella*, that contained only the *Turicella otitidis* species. In a more recent article, Funke et al. (4) described a coryneform bacterium responsible for otitis media, close to *C. afermentans* and *T. otitidis*, which differed only in its assimilation pattern and in the presence of mycolic acid. The phylogeny of the bacterium based on the comparison of the 16S rRNA gene sequence assigned it to the genus *Corynebacterium*, and a new species, *C. auris*, was proposed. The ANF-3 group is now known as *C. propinquum* (10), but it is easily differentiated from the other nonfermenting coryneform bacteria because of the presence of nitrate reductase.

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In our clinical case, a 6-month-old girl was seen at regular intervals by an otorhinolaryngologist. Three months earlier she had undergone three surgical repairs for a bilateral maxillolabiopalatine cleft with mucopurulent discharge. Four months after the final intervention, she returned to the hospital with a serous otitis of the right ear and spontaneous otorrhea of the left ear. She greatly improved after administration of an amoxicillin-clavulanic acid therapy.

We studied only one pus sample from serous otitis. Microscopic examination of the pus revealed the presence of numerous polynuclear cells, some gram-negative bacilli that were later identified as *Escherichia coli* (because of the small number of colonies obtained on culture media, this bacterium was not considered responsible for the infection), and numerous gram-positive coryneform bacilli, which were sometimes coccobacillary, sometimes longer in size and club shaped, and often showed a V-like arrangement. The bacilli were sometimes discolored. Gram stain did not evoke *T. otitidis* because bacilli were not branched (2). The colonies were nonhemolytic on blood agar. After a 48-h CO_2 incubation at 37°C, they measured approximately 2 mm. They were smooth, round, raised, and pale yellow (the colonies are usually described as creamy) (2). The strain did not require addition of Tween 80 to the medium and was therefore nonlipophilic. It was oxidase negative, catalase positive, nitrate negative, urease negative, and esculin negative. It failed to acidify glucose, ribose, maltose, sucrose, and glycogen, attributes placing it in the ANF-1 group (2). It did not produce gelatinase, allowing differentiation from a nitrate-negative *Brevibacterium* sp. (2). Pyrazinamidase and alkaline phosphate were the only positive reactions in the API Coryne system (3), allowing a 79.8% identification of *Corynebacterium* group ANF. ANF-1 corynebacteria do not produce acid from any sugars and do not possess urease activity. It seemed interesting to us to further discriminate between *C. afermentans* (group ANF-1), *T. otitidis* (group ANF-1-like), and *C. auris*.

Besides the biochemical characteristics revealed by the API Coryne system, the following complementary tests were carried out. DNase, examined on a DNA medium (bioMérieux), was positive. Enzyme activity was determined with an API ZYM system (bioMérieux) according to the manufacturer's instructions, i.e., after a 4-h incubation. These tests were per-

TABLE 1. Enzymatic reactions of *T. otitidis*, *C. afermentans*, and *C. auris*

Reaction	*T. otitidis*			*C. afermentans*[a]	*C. auris*[b]
	Isolate	Type strain	Ref. 4[c]		
Alkaline phosphatase	+	+	+	+	+
Esterase C4	+	+	+	+	+
Leucine arylamidase	+	+	+	−	+
Acid phosphatase	+	+	+	+	+
Phosphoamidase	+	+	V	−	+
Esterase lipase C8	+	+	+	−	+
Lipase C14	−	−	−	+	+
Cystine arylamidase	−	−	V	−	−

[a] The data are from reference 11.
[b] The data are from reference 4.
[c] Ref., reference; V, variable.

TABLE 2. Results of carbon substrate assimilation tests

Substrate	*C. afermentans* (3 strains)	*C. auris* (type strain)	*T. otitidis* (2 strains)
L-(−)-Malate	V[a]	+	+
Citrate	−	−	V
2-Keto-D-gluconate	−	−	+
Phenylacetate	−	−	V
Caprylate	−	+	−
Succinate	V	−	+
Fumarate	V	+	+
3-Hydroxybutyrate	−	+	−
L-Glutamate	−	+	+
L-Proline	−	−	+
D-Alanine	−	−	+
L-Serine	−	+	+
Propionate	−	−	V
α-Ketoglutarate	−	−	+

[a] V, variable.

formed on the clinical isolate discussed here and on the type strain of *T. otitidis* (CIP 104075; Collection de l'Institut Pasteur, Paris, France). The results were compared with those obtained by Funke et al. (4) using the same system for *C. auris* and Riegel et al. (11) using the same system for *C. afermentans*. The results in the first two columns of Table 1 pointed to *T. otitidis.*

To achieve definite identification of the isolate, we performed a chemotaxonomic study. Determination of cell wall constituents, sugar analysis of whole-cell extracts, mycolic acid detection, and polar lipid analysis were conducted by thin-layer chromatography as described by Barreau et al. (1). Total-protein was profiled (sodium dodecyl sulfate polyacrylamide gel electrophoresis) as previously described (1). Menaquinones were demonstrated by chemical reduction (8) followed by fluorimetric detection (7). The *N*-glycolyl groups were examined by the colorimetric method as described by Uchida and Aida (13). The strain contained *meso*-diaminopimelic acid but no mycolic acid. It also contained arabinogalactane, and the high-performance liquid chromatography peaks of the menaquinones (MK-10 and MK-11) migrated with similar retention times as those of the type strain of *T. otitidis*. It contained only a small amount of phosphatidylglycerol as polar liquids and was glycolipid free with the methods used in this study. The protein profile closely resembled (98% similarity) that of the type strain of *T. otitidis*. The peptidoglycan of the strain revealed *N*-glycolyl groups.

The results of the enzymatic study were in agreement with those of Funke et al. (6), as alkaline and acid phosphatase, esterase, leucine arylamidase, phosphoamidase, and lipase esterase were positive. On the other hand, and in agreement with the results of Funke et al., neither lipase nor valine arylamidase nor trypsin was positive under the normal incubation conditions of the system, although they had been found to be positive by Simonet et al. (12). This poses a problem, as trypsin is, with DNase, one of two characteristics that allow differentiation of ANF-1 (*C. afermentans*), trypsin-negative, DNase-negative bacteria from ANF-1-like (*T. otitidis*), trypsin-positive, DNase-positive bacteria (12). On the other hand, DNase was positive for both the strain isolated and the type strain of *T. otitidis*, thus confirming the importance of the discriminating characteristics. DNase was found to be negative for one type strain of *C. auris* (CIP 104632; Collection de l'Institut Pasteur de Paris, France). The data given in Table 1 suggest that leucine arylamidase, esterase lipase C8, and lipase may be additional major characteristics allowing differentiation between *T. otitidis* and the other two species.

The carbon substrate assimilation profiles were determined with the Biotype 100 system (bioMérieux), as described in the manufacturer's protocol. Interpretation of the results in Table 2 is difficult since we tested only three *C. afermentans* strains, one *C. auris* strain, and two *T. otitidis* strains (the type strain and the clinical strain). However, we noticed that *C. afermentans* strains utilized fewer carbon substrates than the strains from the other two species.

The data in Table 3 allowed presumptive identification of a small gram-positive bacillus belonging to group ANF according to the following characteristics: catalase positivity, nitrate negativity, urease negativity, esculin negativity, pyrazinamidase positivity, alkaline phosphate positivity, and failure to acidify glucose, ribose, maltose, sucrose, and glycogen.

TABLE 3. Differentiation between *C. afermentans*, *C. auris*, and *T. otitidis*

Characteristic or test	Result		
	C. afermentans	*C. auris*	*T. otitidis*
Colony	Flat, grayish-white, smooth	Circular, convex, dry, becoming slightly yellowish, slightly adherent to agar	Circular, convex, creamy (becoming pale yellow)
CAMP test	V[a]	+	+
DNase	−	−	+
Enzymes			
Leucine arylamidase	−	+	+
Esterase lipase C8	−	+	+
Lipase C14	+	+	−
Carbon substrate assimilation			
2-Keto-D-gluconate	−	−	+
Caprylate	−	+	−
3-Hydroxybutyrate	−	+	−
L-Glutamate	−	+	+
L-Proline	−	−	+
D-Alanine	−	−	+
L-Serine	−	+	+
α-Ketoglutarate	−	−	+

[a] V, variable.

Recent phylogenic studies tend to demonstrate that *T. otitidis* is very similar to the *Corynebacterium* species (4, 9). However, our chemotaxonomy results show that these species are nonetheless not as closely related as they seem. *T. otitidis* is the only coryneform bacterium that has a polar lipid profile with absence of glycolipids. Moreover, the presence of *N*-glycolyl groups is unknown in the genus *Corynebacterium*, but *N*-glycolyl is present in *Aureobacterium* and *Microbacterium* spp.

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REFERENCES

1. **Barreau, C., F. Bimet, M. Kiredjian, N. Rouillon, and C. Bizet.** 1993. Comparative chemotaxonomic studies of mycolic acid-free coryneform bacteria of human origin. J. Clin. Microbiol. **31:**2085–2090.
2. **Clarridge, J. E., and C. A. Spiegel.** 1995. *Corynebacterium* and miscellaneous irregular gram-positive rods, *Erysipelothrix*, and *Gardnerella*, p. 357–378. *In* P. R. Murray, E. J. Barron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
3. **Freney, J., M. T. Duperron, C. Courtier, W. Hansen, F. Allard, J. M. Boeufgras, D. Monget, and J. Fleurette.** 1991. Evaluation of API Coryne in comparison with conventional methods for identifying coryneform bacteria. J. Clin. Microbiol. **29:**38–41.
4. **Funke, G., P. A. Lawson, and M. D. Collins.** 1995. Heterogeneity within human-derived Centers for Disease Control and Prevention (CDC) coryneform group ANF-1-like bacteria and description of *Corynebacterium auris* sp. nov. Int. J. Syst. Bacteriol. **45:**735–739.
5. **Funke, G., G. E. Pfyffer, and A. von Graevenitz.** 1993. A hiherto undescribed coryneform bacterium isolated from patients with otitis media. Med. Microbiol. Lett. **2:**183–190.
6. **Funke, G., S. Stubbs, M. Altwegg, A. Carlotti, and M. D. Collins.** 1994. *Turicella otitidis* gen. nov., sp. nov., a coryneform bacterium isolated from patients with otitis media. Int. J. Syst. Bacteriol. **44:**270–273.
7. **Haroon, Y., D. S. Bacon, and J. A. Sadowski.** 1986. Liquid-chromatographic determination of vitamin K1 in plasma, with fluorometric detection. Clin. Chem. **32:**1925–1929.
8. **Haroon, Y., D. S. Bacon, and J. A. Sadowski.** 1987. Reduction of quinones with zinc metal in the presence of zinc ions: application of post-column reactor for fluorometric detection of vitamin K compounds. Biomed. Chromatogr. **2:**4–8.
9. **Pascual, C., P. A. Lawson, J. A. E. Farrow, M. N. Gimenez, and M. D. Collins.** 1995. Phylogenetic analysis of the genus *Corynebacterium* based on 16S rRNA gene sequences. Int. J. Syst. Bacteriol. **45:**724–728.
10. **Riegel, P., D. De Briel, G. Prévost, F. Jehl, and H. Monteil.** 1993. Proposal of *Corynebacterium propinquum* sp. nov. for *Corynebacterium* group ANF-3 strains. FEMS Microbiol. Lett. **113:**229–234.
11. **Riegel, P., D. De Briel, G. Prévost, F. Jehl, H. Monteil, and R. Minck.** 1993. Taxonomic study of *Corynebacterium* group ANF-1 strains: proposal of *Corynebacterium afermentans* sp. nov. containing the subspecies *C. afermentans* subsp. *afermentans* subsp. nov. and *C. afermentans* subsp. *lipophilum* subsp. nov. Int. J. Syst. Bacteriol. **43:**287–292.
12. **Simonet, M., D. De Briel, I. Boucot, R. Minck, and M. Veron.** 1993. Coryneform bacteria isolated form middle ear fluid. J. Clin. Microbiol. **31:**1667–1668.
13. **Uchida, K., and K. Aida.** 1977. Acyl type of bacterial cell wall: its simple identification by colorimetric method. J. Gen. Microbiol. **23:**249–260.