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Corynebacterium imitans sp. nov. Isolated from Patients with Suspected Diphtheria

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A 5-month-old boy of a Romanian family traveling via Ukraine to Poland developed a respiratory disease that resembled and that was initially diagnosed as pharyngeal diphtheria. The child recovered after treatment with antidiphtheria antitoxin. A coryneform bacterium had been isolated from a nasopharyngeal specimen from the child and was initially identified as an atypical *Corynebacterium diphtheriae* strain. Seven adults who had contact with either the child or an adult contact person also developed symptoms of pharyngeal diphtheria, were also treated with antitoxin, and recovered uneventfully. Coryneform bacteria similar to that originating from the index patient were also isolated from the throat swabs of three adults. Detailed biochemical and chemotaxonomic investigations revealed that the coryneform bacteria belonged to the genus *Corynebacterium* and could be differentiated from all other defined species of this genus. Ribotyping and pulsed-field gel electrophoresis demonstrated that all four patients' isolates were of clonal origin. The diphtheria toxin gene and its product were not detected either by PCR assays or by the Elek test, making a possible disease association of the *Corynebacterium* more unlikely. Comparative 16S rRNA gene sequence analysis revealed that the coryneform bacterium represented a new subline within the genus *Corynebacterium*, for which the name *Corynebacterium imitans* sp. nov. is proposed. The type strain is NCTC 13015 (DSM 44264; CCUG 36877).

During the 1990s there has been an ongoing diphtheria epidemic in the newly independent states of the former Soviet Union (6, 16). Accordingly, the situation has increased both clinical and microbiological awareness in the diagnosis of the disease. Despite increasing travel between the newly independent states and the rest of Europe, cases of diphtheria have only been sporadic within Western Europe, whereas more cases have been reported in the Eastern European countries. We report here on a coryneform bacterium that had been isolated from patients with suspected diphtheria in Poland. The index patient traveled from Romania to Poland via Ukraine. Applying both phenotypic and molecular genetic methods, we demonstrate that the coryneform bacterium isolated from the patients with suspected diphtheria represents a new *Corynebacterium* species for which the designation *Corynebacterium imitans* sp. nov. is proposed. Since *C. imitans* did not harbor the diphtheria toxin gene, its disease association remains uncertain.

CASE REPORT

In February 1996, a 5-month-old boy from Romania who had not been immunized against diphtheria became ill after a 7-day journey via Ukraine to Poland. He was diagnosed as having an illness resembling pharyngeal diphtheria, was admitted to the regional hospital of Przemysl, Poland, and received 20,000 U of antidiphtheria antitoxin. Bacteriological examination of a nasopharyngeal specimen yielded growth of a coryneform bacterium which was initially identified as an atypical *Corynebacterium diphtheriae* strain by the local laboratory. Cultures for virus were not performed. Eight days later, both the 48-year-old father of the boy and the 45-year-old-driver of the coach (bus) in which the Romanian family had traveled to Poland were admitted to the same hospital because they were also diagnosed as having pharyngeal diphtheria. Three days later, a 26-year-old female receptionist at the hotel where the family stayed was admitted to the hospital because of suspected diphtheria. A coryneform bacterium was isolated from the throat swabs of these three adult patients. Again, the bacterium was initially identified as an atypical *C. diphtheriae* strain. Another 3 days later, three other adults (an employee of the hotel bar, a hotel maid, and a second receptionist) were also admitted to the hospital for treatment of diphtheria. Finally, 2 days later, the mother of the first receptionist was also admitted to the hospital. No strain that was identified as *C. diphtheriae* or that resembled the atypical *C. diphtheriae* strain found in the other three adults was isolated from any of the last four patients. However, all adult patients received 30,000 to 40,000 U of antidiphtheria antitoxin and recovered uneventfully. Data on the patients' treatment with antimicrobial agents were not reported. Unfortunately, no photographs or any histopathological data regarding the patients' tonsillopharyngitis on initial examination were available.

MATERIALS AND METHODS

Strains. Four bacterial strains which had been isolated from four different patients with suspected diphtheria (see Case Report) and which were initially diagnosed as atypical *C. diphtheriae* had been submitted for further characterization to the Polish National Institute of Hygiene, Warsaw. Information regarding the primary procedures for isolation of the bacteria was not available. Strains NCTC 10648 (toxigenic *C. diphtheriae*), NCTC 3984 (toxigenic *C. diphtheriae*), NCTC 10356 (nontoxigenic *C. diphtheriae*), and NCTC 11397 (the nontoxigenic *C. diphtheriae* type strain) served as control strains in the PCRs, the Elek test, ribotyping, and pulsed-field gel electrophoresis (PFGE).

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Biochemical characteristics and stains. The strains were grown aerobically at 37°C in a 5% CO_2-enriched atmosphere on Columbia agar (Difco, Detroit, Mich.) supplemented with 5% sheep blood (SBA). The methods used for biochemical profiling of the bacteria have been outlined in detail before (8, 12). The commercially available API Coryne, API ZYM, and API 50CH (in conjunction with the 50CHE medium) systems (all from bioMérieux, Marcy l'Etoile, France) were used according to the manufacturer's instructions. Cells were incubated for 24 h in the API Coryne system, 4 h in the API ZYM system, and 48 h in the API 50CH system. For the detection of the end products of the glucose metabolism, cells were grown in brain heart infusion broth (Difco) supplemented with 1% glucose; the detection method by gas-liquid chromatography has been described previously (12).

Staining with Gram and Neisser stains was performed by published methods (18).

Antimicrobial susceptibility testing. The MICs of 40 antimicrobial agents were determined by a microdilution method. Ninety-six-well microtiter plates contained the antibiotics in lyophilized form with one starting concentration and 11 twofold dilutions (i.e., eight antimicrobial agents per plate) (Merlin Diagnostics, Bornheim-Hersel, Germany). A bacterial suspension (McFarland 0.5 standard) was prepared in 0.9% NaCl, 200 μl of the suspension was transferred into 10 ml of H medium (Merlin), and each well of the microtiter plates was filled with 100 μl. The plates were incubated at 35°C for 20 to 24 h. Bacterial growth was examined by reading the plates by eye. In a pragmatic approach, the breakpoints established by the National Committee for Clinical Laboratory Standards (NCCLS) for interpretation of susceptibility and resistance were applied, although it should be noted that NCCLS has not explicitly published particular breakpoints for coryneform bacteria (22). Susceptibility (i.e., the presence of any inhibition zone) to the vibriocidal compound O-129 (2,4-diamino-6,7-diisopropylpteridine) was tested on Mueller-Hinton agar (Becton Dickinson BBL, Cockeysville, Md.) plates supplemented with 5% sheep blood by placing a 150-μg O-129-containing disk (Oxoid, Basingstoke, United Kingdom) on the agar after the plates had been inoculated with cells grown to a density equivalent to that of a 0.5 McFarland standard in Trypticase soy broth (Becton Dickinson BBL). These plates were also incubated at 35°C for 20 to 24 h.

Chemotaxonomic investigations. Cellular fatty acid (CFA) patterns were generated by using the Sherlock Microbial Identification System (Microbial ID, Inc., Newark, Del.) (32). Thin-layer chromatography methods used for the determination of the cell wall diamino acid as well as for the detection of mycolic acids were performed as described previously (12). The technique used for determination of the DNA base composition has also been described before (13).

Ribotyping and PFGE. For ribotyping, chromosomal DNA preparations from the four patients' isolates and the four control strains were prepared as described by De Zoysa and colleagues (5). DNA was cleaved with *Bst*EII, and digests were electrophoresed in Tris-borate-EDTA (TBE) buffer at 27 V for 16 h. The gel was blotted onto a nylon membrane and hybridized with a biotin-labelled cDNA probe derived from the total rRNA of the type strain *C. diphtheriae* NCTC 11397 as described previously (5).

The preparation of bacterial DNA for PFGE and the separation of *Sfi*I restriction fragments with a CHEF-DRII system (Bio-Rad, Hertfordshire, United Kingdom) were also performed as described previously (5). Briefly, bacterial cells were incorporated into 0.8% (wt/vol) agarose plugs and incubated overnight at 37°C in lysis buffer. This was followed by deproteination in proteolysis buffer for 48 h. The DNA was cleaved with *Sfi*I, and PFGE was carried out in 0.5× TBE–1.5% agarose gels at 14°C.

Detection of diphtheria toxin. For PCR detection of the diphtheria toxin gene two independent methods were applied. The first was the method outlined by Pallen et al. (23), and the second was a modified protocol of the method outlined by Hauser et al. (17). In the second method, PCR was performed with 5 μl of the supernatant of a boiled bacterial cell suspension after removal of cell debris in a final volume of 100 μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (wt/vol) gelatin, 100 μM (each) the four deoxynucleoside triphosphates (Perkin-Elmer, Rotkreuz, Switzerland), 0.5 μM (each) primers DT1 and DT2, and 2.5 U of Ampli*Taq* Gold DNA polymerase (Perkin-Elmer). The vials were placed into a PCR GeneAmp 2400 apparatus (Perkin-Elmer), and the cycling program of Hauser et al. (17) was applied. Amplicons were detected by loading 10 μl of each reaction mixture on a 2% AmpliSize agarose gel (Bio-Rad, Hercules, Calif.) and electrophoresis. One microliter of a 100-bp ladder (Boehringer, Rotkreuz, Switzerland) was used as a molecular size marker.

The Elek test for the detection of the diphtheria toxin was performed by the method recently described by Engler et al. (9).

The in vivo subcutaneous test for virulence in guinea pigs was performed as described previously (8) by using the bacterial isolate from the index patient.

Determination of the 16S rRNA gene sequences and phylogenetic analyses. A large fragment of the 16S rRNA gene was amplified by PCR by using universal primers pA and pH* as described previously (19). The PCR products were purified by using a Prep-A-Gene kit (Bio-Rad) and were sequenced by using a *Taq* Dye Deoxy terminator cycle sequencing kit (Applied Biosystems, Inc., Foster City, Calif.) and a model 373A automatic sequencer (Applied Biosystems, Inc.). To establish the relatives of the clinical strains, preliminary searches in the EMBL/GeneBank Data Library were performed with the program FASTA. The sequences of the close relatives were retrieved and aligned with the newly determined sequences by using the program PILEUP (4). The rRNA alignment was corrected manually, and approximately 100 bases at the 5′ end of the molecule were omitted from further analyses because of alignment uncertainties due to hypervariable region V1. Percent sequence similarities were calculated and corrected for substitution rates by using Kimura's parameters. A phylogenetic tree was constructed by using the neighbor-joining method (27). The stability of relationships was assessed by bootstrap analysis by using the programs SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE of the PHYLIP package (10).

Nucleotide sequence accession number. The 16S rRNA gene sequence of strain NCTC 13015 (DSM 44264; CCUG 36877), i.e., the strain from the index patient, that was determined has been deposited in the EMBL Data Library under the accession number Y09044.

RESULTS

The four strains grew as white-grayish, glistening, creamy colonies of 1 to 2 mm in diameter with entire edges after 24 h of incubation on SBA. The isolates did not produce a brown halo on Tinsdale's medium but were tellurite reductase positive. Gram stains showed typical club-shaped diphtheroids that were arranged as single cells, in pairs, or that clustered with the appearance of Chinese letters. Staining with the Neisser stain was positive for polar bodies. The initial biochemical screening reactions for coryneform bacteria (31) were as follows: catalase positive; fermentative; nonmotile; nitrate reduction negative; urea hydrolysis negative; esculin hydrolysis negative; fermentation of glucose and maltose, but only very weak fermentation of sucrose and no fermentation of mannitol and xylose; nonlipophilic; and CAMP reaction positive (Table 1). The commercially available API Coryne system gave the numerical code 2100324 for all isolates (i.e., the isolates were positive for pyrazinamidase, alkaline phosphatase, and fermentation of glucose, ribose, and maltose; the isolates were negative for nitrate reduction, pyrrolidonyl arylamidase, β-glucuronidase, β-galactosidase, α-glucosidase, β-*N*-acetyl-glucosaminidase, esculin hydrolysis, gelatin hydrolysis, and fermentation of xylose, mannitol, lactose, sucrose, and glycogen), which, according to its present database, corresponded to the identification of the isolates as *C. minutissimum* (55.4% identification value; T value, 0.97), *C. jeikeium* (42.3% identification value; T value, 0.93), or "*Corynebacterium* group I" (1.5% identification value; T value, 0.67). Because the four strains were nonlipophilic, an assignment to the lipophilic species *C. jeikeium* could be ruled out. "*Corynebacterium* group I" bacteria have been shown to comprise *C. striatum* (former group I-1) and *C. amycolatum* (former group I-2) (15). Therefore, *C. minutissimum*, *C. striatum*, and *C. amycolatum* were phenotypically the closest relatives to the four clinical strains on the basis of biochemical reactions (Table 1). It should be emphasized that the pyrazinamidase reaction in the four strains was only weak, so that it is not unlikely that a false-negative result for the pyrazinamidase reaction might be obtained, leading to the assumption that the isolates belong to the pyrazinamidase-negative *C. diphtheriae* group of organisms (15). However, the four isolates were α-glucosidase negative, whereas strains of the *C. diphtheriae* group of organisms express this enzyme (1). The isolates could readily be distinguished from *C. striatum* (which does not ferment maltose) as well as from *C. minutissimum* and *C. amycolatum* (neither of which exhibits a positive CAMP reaction) (Table 1). In addition, both *C. striatum* and *C. minutissimum* hydrolyze tyrosine, but the four patients' isolates were negative for this reaction. Furthermore, the four isolates were resistant to O-129, whereas *C. striatum*, *C. minutissimum*, and *C. diphtheriae* are almost never resistant to this compound (11). Lactate and succinate were the main end products of the glucose metabolism (as is seen for *C. striatum* and *C. minutissimum* [11]), whereas in *C. amycolatum* and *C. diphtheriae*

TABLE 1. Characteristics that differentiate *C. imitans* from other fermenting, nonlipophilic *Corynebacterium* spp. encountered in clinical specimens[a]

Organism	Nitrate reduction	Urea hydrolysis	Esculin hydrolysis	Pyrazinamidase	Alkaline phosphatase	Acid produced from:			CAMP reaction	Other traits
						Glucose	Maltose	Sucrose		
C. imitans	−	−	−	W	+	+	+	W	+	Tyrosine negative
C. amycolatum	V	V	−	+	+	+	V	V	−	Mycolic acid negative
C. argentoratense	−	−	−	+	V	+	−	−	−	Chymotrypsin positive
C. coyleae	−	−	−	+	+	+	−	−	+	
C. diphtheriae	V	−	−	−	−	+	+	−	−	Cysteinase positive
C. glucuronolyticum	V	V	V	+	V	+	V	+	+	β-Glucuronidase positive
C. matruchotii	+	V	+	+	−	+	+	+	−	"Whip handle" (on Gram staining)
C. minutissimum	−	−	−	+	+	+	+	V	−	Tyrosine positive
C. pseudotuberculosis	−	+	−	−	V	+	+	V	REV	Glycogen negative
C. striatum	+	−	−	+	+	+	−	V	V	Tyrosine positive
C. ulcerans	−	+	−	−	+	+	+	−	REV	Glycogen positive
C. xerosis	V	−	−	+	+	+	+	+	−	Yellowish

[a] Data are from references 1, 13, and 15. Abbreviations: W, weakly positive; V, variable; REV, reverse CAMP reaction.

strains, propionate is the main end product (15). The four isolates were found to have *meso*-diaminopimelic acid as the diamino acid and to contain short-chain mycolic acids in their cell walls. The latter characteristic clearly separated the four isolates from mycolic acid-less *C. amycolatum* strains. On the basis of the phenotypic distinctiveness of the four strains, we considered them to represent a new *Corynebacterium* species.

The antimicrobial susceptibility patterns were identical for all four strains, with the following MICs: amikacin, 1 μg/ml; amoxicillin, 0.0625 μg/ml; azithromycin, 32 μg/ml; azlocillin, 0.5 μg/ml; aztreonam, >64 μg/ml; cefaclor, 0.125 μg/ml; cefazolin, ≤0.125 μg/ml; cefetamet, 32 μg/ml; cefotaxime, 0.25 μg/ml; cefoxitin, 1 μg/ml; ceftazidime, 4 μg/ml; ceftriaxone, 0.25 μg/ml; cefuroxime sodium, 0.125 μg/ml; chloramphenicol, 32 μg/ml; ciprofloxacin, 0.125 μg/ml; clarithromycin, 0.5 μg/ml; clindamycin, >32 μg/ml; doxycycline, 1 μg/ml; erythromycin, 4 μg/ml; fleroxacin, 1 μg/ml; fosfomycin, >256 μg/ml; fusidic acid, 0.0625 μg/ml; gentamicin, 0.25 μg/ml; imipenem, ≤0.03 μg/ml; kanamycin, >256 μg/ml; meropenem, ≤0.03 μg/ml; mezlocillin, 1 μg/ml; netilmicin, 0.25 μg/ml; ofloxacin, 0.25 μg/ml; oxacillin, 2 μg/ml; penicillin, 0.0625 μg/ml; piperacillin, 1 μg/ml; rifampin, 0.03125 μg/ml; sparfloxacin, 0.0625 μg/ml; streptomycin, 64 μg/ml; teicoplanin, 0.25 μg/ml; tetracycline, 2 μg/ml; ticarcillin, 0.5 μg/ml; tobramycin, 0.5 μg/ml; and vancomycin, 0.5 μg/ml.

CFA patterns were almost identical for all four strains, with $C_{14:0}$ representing 1% of total CFAs, $C_{16:1\omega9c}$ representing 2%, $C_{16:0}$ representing 50% (range, 50 to 51%), $C_{18:1\omega9c}$ representing 40% (range, 40 to 41%), and $C_{18:0}$ representing 5% (range, 5 to 6%) as the predominant CFAs. CFA $C_{16:1\omega7c}$ (/C_{15i2OH}), which is characteristic for strains belonging to the *C. diphtheriae* group (15) (when analyzed with the Sherlock system), was not detected. The G+C content was found to be 62 mol% for all four strains; this is substantially higher than the range of values reported for *C. diphtheriae* (52 to 55 mol%) (3).

Since all four strains came from closely related persons and since all strains exhibited identical phenotypic features they were considered to be of clonal origin. Ribotyping and PFGE (data not shown) demonstrated that the four isolates were indistinguishable (i.e., not a single fragment difference) (Fig. 1), and they were therefore considered to be identical (i.e., part of the outbreak) (30).

Using two independent PCR assays, we did not detect any portion of the diphtheria toxin gene in any of the four strains. These data were confirmed by negative Elek test results for all strains. In addition, at postmortem examination, the major organs, the spleen and adrenals, of the guinea pigs challenged with the isolate from the index patient did not show any pathological findings.

All biochemical and chemotaxonomical data were consistent with the assignment of the coryneform bacterium to the genus *Corynebacterium*. In order to establish the precise phylogenetic position and genealogical distinctiveness of the bacterium, the gene encoding the 16S rRNA was amplified by PCR and was subjected to sequence analysis. The almost complete 16S rRNA gene sequences (ca. 1,470 bp) of two isolates (the isolate from the index patient and the isolate from that patient's father) were determined. The two isolates shared 100% sequence similarity, thereby demonstrating their genealogical homogeneity. The unknown bacterium clustered within the genus *Corynebacterium* sensu stricto, where it formed a distinct subline loosely associated with, but distinct from, *C. afermentans*, *C. coyleae*, and *C. "genitalium"* (Fig. 2). The last three species showed approximately 96% 16S rRNA sequence relatedness with the unidentified clinical isolate, whereas *C. diph-*

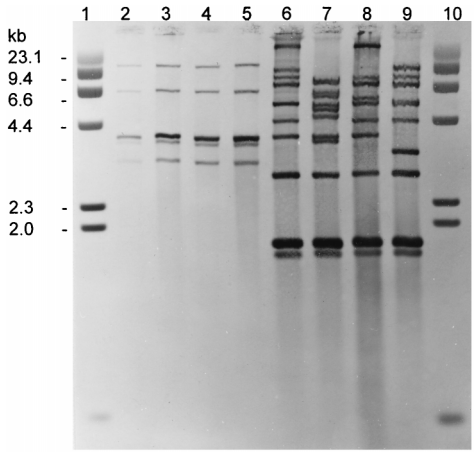

FIG. 1. *Bst*EII rRNA gene profiles of the atypical *C. diphtheriae* isolates from the four patients and from control strains. Lanes 1 and 10, bacteriophage lambda *Hin*dIII digests as a size marker (sizes are indicated on the left); lane 2, isolate from the index patient (see Case Report); lane 3, isolate from a hotel receptionist; lane 4, isolate from the coach (bus) driver; lane 5, isolate from the index patient's father; lanes 6 to 9, control strains NCTC 10648 (toxigenic *C. diphtheriae*), NCTC 10356 (nontoxigenic *C. diphtheriae*), NCTC 11397 (the nontoxigenic *C. diphtheriae* type strain), and NCTC 3984 (toxigenic *C. diphtheriae*), respectively.

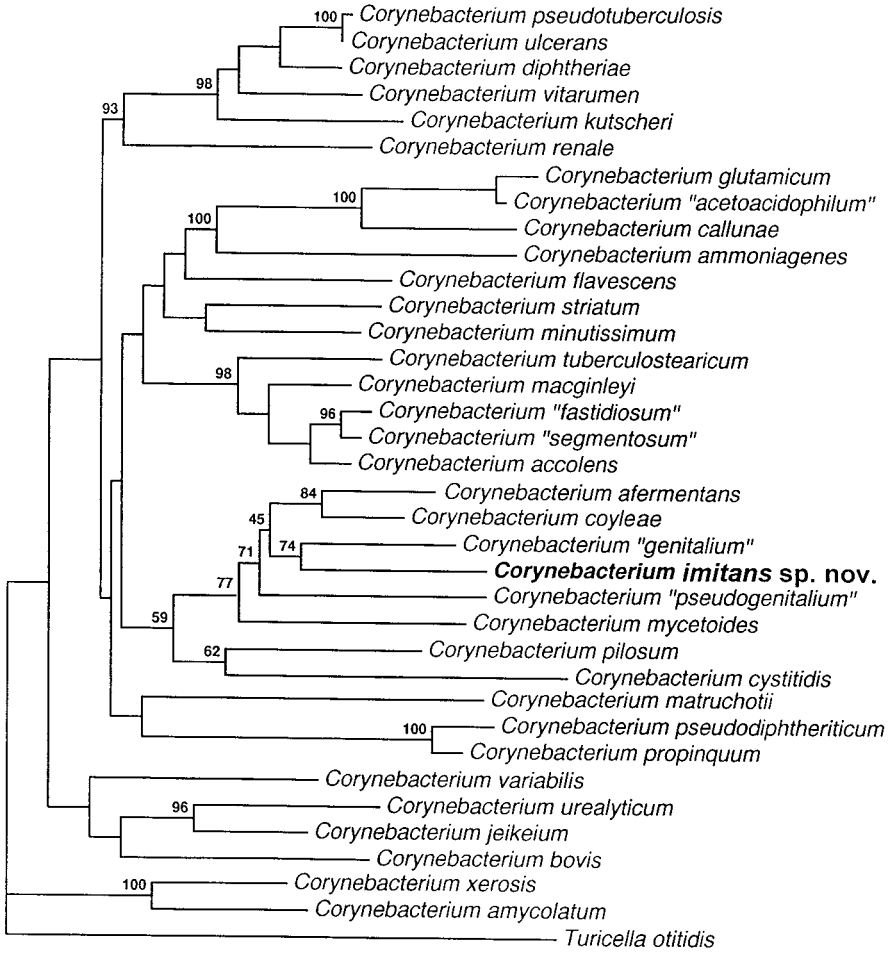

FIG. 2. Unrooted tree showing the phylogenetic position of *C. imitans* within the genus *Corynebacterium* sensu stricto. The tree was constructed by the neighbor-joining method and is based on a comparison of approximately 1,330 nucleotides. Bootstrap values, expressed as a percentage of 250 replications, are given at the branching points.

theriae and related species displayed significantly lower levels of relatedness (approximately 93%) (Table 2). On the basis of the phenotypic and molecular genetic data given here, we formally propose a new *Corynebacterium* species, *Corynebacterium imitans* sp. nov., for the isolate studied.

DISCUSSION

It is evident from the data presented here that the same bacterial strain was isolated from different patients and that this particular organism belongs to a new *Corynebacterium* species. All of the phenotypic and phylogenetic data are consistent with the assignment of the unknown bacterium to the genus *Corynebacterium* (3, 24). Since the unidentified bacterium shows >3% 16S rRNA sequence divergence from the 16S rRNA sequences of other members of this genus, it clearly represents a hitherto unknown species (29). Although it is in general not desirable to describe a new bacterial species on the basis of findings for a single strain, we believe that it is more likely that other clinical microbiologists will also identify this new species once its characteristics have been outlined. Moreover, it is not known whether other people or patients are carriers of *C. imitans*. This could be tested by using a selective medium for coryneform bacteria (e.g., one containing aztreonam and/or fosfomycin) and examining throat swabs for the presence of *C. imitans* or by performing PCR assays with these swabs by using a *C. imitans*-specific primer pair.

Since *C. imitans* does not possess the diphtheria toxin gene, its disease association is questionable. It is most likely that the patients had been treated as a result of clinical and microbiological misdiagnoses. Other nontoxigenic *Corynebacterium* species, namely, *C. argentoratense* and *C. pseudodiphtheriticum*, have also been isolated from patients with tonsillitis (26) and patients suspected of having diphtheria (28).

In addition, it is not unlikely that the positive test result obtained by staining with the Neisser stain and the weakly positive reactions for pyrazinamidase and sucrose fermentation (which might have been overseen) may have led to the initial diagnosis of atypical *C. diphtheriae* strains. However, it is important that almost all toxin-harboring *C. diphtheriae* strains reduce nitrate (15).

C. imitans showed an antimicrobial susceptibility pattern that is also observed in many other *Corynebacterium* species. In particular, resistance to macrolides (except clarithromycin) and clindamycin has been observed in *C. diphtheriae*, *C. striatum*, *C. auris*, *C. glucuronolyticum*, and CDC coryneform group G bacteria, with an rRNA methylase being a possible resistance mechanism (14). As has been the case for most other macrolide-resistant *Corynebacterium* species, the MIC of clar-

TABLE 2. Levels of 16S rRNA sequence similarity between *C. imitans* sp. nov. and other *Corynebacterium* species including *Turicella otitidis*

Species	% 16S rRNA sequence similarity with *C. imitans* sp. nov.	Species	% 16S rRNA sequence similarity with *C. imitans* sp. nov.
C. accolens	94.0	*C. minutissimum*	94.0
C. "acetoacidophilum"	92.4	*C. mycetoides*	95.5
C. afermentans	96.2	*C. pilosum*	94.2
C. ammoniagenes	93.3	*C. propinquum*	93.3
C. amycolatum	93.7	*C. pseudodiphtheriticum*	93.2
C. bovis	92.8	*C. "pseudogenitalium"*	95.1
C. callunae	91.9	*C. pseudotuberculosis*	93.9
C. coyleae	96.1	*C. renale*	93.9
C. cystitidis	93.8	*C. "segmentosum"*	93.5
C. diphtheriae	93.1	*C. striatum*	93.5
C. "fastidiosum"	93.6	*C. "tuberculostearicum"*	93.7
C. flavescens	93.5	*C. ulcerans*	94.1
C. "genitalium"	96.2	*C. urealyticum*	92.9
C. glutamicum	92.6	*C. variabilis*	93.1
C. jeikeium	93.2	*C. vitarumen*	93.5
C. kutscheri	94.0	*C. xerosis*	92.7
C. macginleyi	93.8	*T. otitidis*	90.6
C. matruchotii	92.4		

ithromycin was significantly lower than those of the other macrolides (11).

It is interesting that person-to-person transmission of *C. imitans* must have occurred between the four patients. Community-acquired transmission of *Corynebacterium* species other than *C. diphtheriae* has only very rarely been described. In contrast, nosocomial person-to-person transmission of *Corynebacterium* species, namely, *C. jeikeium* (20, 25) and *C. striatum* (2, 21), has been well documented.

Our present study indicates that (i) clinical microbiologists must be cautious not to misdiagnose other *Corynebacterium* species as probable *C. diphtheriae*, a problem that has recently been addressed by others (7, 34), and that (ii) it is worthwhile to identify *Corynebacterium* species to the species level since clinical microbiologists may encounter new *Corynebacterium* species, a point that has also been addressed before (33).

***Corynebacterium imitans* sp. nov.** *Corynebacterium imitans* (i′mi.tans. L. part. adj. *imitans*, imitating, copying, indicating that this bacterium was isolated from patients with an illness that was imitating the clinical picture of pharyngeal diphtheria and also indicating that the biochemical profile of the newly described bacterium imitates the biochemical profiles of other *Corynebacterium* species). Cells are gram-positive, nonmotile, asporogenous diphtheroids. Colonies are white-grayish, glistening, circular, convex, creamy, and 1 to 2 mm in diameter with entire edges after 24 h of incubation on sheep blood agar at 37°C. The organism is catalase positive. Acid is produced from D-arabinose, ribose, D-glucose, D-fructose, D-mannose, maltose, lactose, and L-fucose and weakly from sucrose but not from glycerol, erythritol, L-arabinose, xylose, adonitol, β-methylxyloside, galactose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, α-methylmannoside, α-methylglucoside, *N*-acetylglucosamine, amygdaline, arbutine, salicin, cellobiose, melibiose, trehalose, inulin, melezitose, D-raffinose, glycogen, xylitol, β-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, gluconate, and 2-ketogluconate. Nitrate is not reduced. Urea, esculin, and tyrosine are not hydrolyzed. The CAMP reaction is positive. Pyrazinamidase (weakly), alkaline phosphatase, esterase (C_4), esterase-lipase (C_8), acid phosphatase, and phosphoamidase activities are detected, but lipase, pyrrolidonylarylamidase, leucine arylamidase, valine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are not detected.

The cell wall contains *meso*-diaminopimelic acid. Mycolic acids are present. The main straight-chain saturated fatty acids are palmitic and stearic acids; oleic acid is the predominant unsaturated fatty acid. The DNA base composition is 62 mol% G+C. The strain is isolated from humans. The type strain has been deposited in the National Collection of Type Cultures, London, United Kingdom, as NCTC 13015, in the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, as DSM 44264, and in the Culture Collection of the University of Göteborg, Göteborg, Sweden, as CCUG 36877.

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