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# *Gordona terrae* Central Nervous System Infection in an Immunocompetent Patient

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**The bacterial genus *Gordona* includes seven species of mycolic acid-containing actinomycetes which are cultured from environmental sources and rarely from clinical samples. They have been implicated in primary pulmonary diseases, nosocomial wound infections, and central nervous system infections in two immunocompromised patients. We isolated *Gordona terrae* from the cerebrospinal fluid sample of an immunocompetent patient with meningitis and multiple brain abscesses and detected *Gordona terrae* DNA in the abscesses. The outcome was good at the 4-year follow-up, after prolonged treatment with trimethoprim-sulfamethoxazole. Phenotypic identification of this isolate was confirmed by analysis of the 16S rRNA gene sequence, which shared 100% homology with that of *G. terrae* reference strains. Physicians and clinical microbiologists must be aware of the occurrence of *Gordona* species infection not only among immunocompromised patients but among all patients. Accurate identification of *Gordona* species may be accomplished by molecular techniques.**

The bacterial genus *Gordona* includes seven species of mycolic acid-containing actinomycetes (2) and belongs to a supragenic group including the genera *Mycobacterium*, *Corynebacterium*, *Nocardia*, *Rhodococcus*, *Tsukamurella* (21), and *Dietzia* (18). *Gordona* species were isolated from environmental sources and were sporadically associated with human infection. We herein report on an immunocompetent woman who developed central nervous infection due to *Gordona terrae.*

## MATERIALS AND METHODS

**Bacterial strain sources.** A clinical isolate previously identified as *G. terrae* on the basis of its phenotypic characteristics and ribotyping (6) was used as a reference strain (Collection de l'Institut Pasteur strain CIP104939). Isolate W5381 was cultured from a cerebrospinal fluid (CSF) sample of the patient described in this report.

**Isolation, culture, and phenotypic characterization of the isolate.** A CSF sample was obtained by lumbar puncture. Gram-stained of cytocentrifuged smears of CSF were microscopically observed at ×100 and ×1,000 powers. The sample was plated onto Trypticase soy agar (bioMérieux, Marcy-l'Etoile, France), chocolate agar (bioMérieux), and 5% sheep blood Columbia agar (bioMérieux), and the plates were incubated at 25, 35, and 45°C. The isolate was further characterized by plating onto Trypticase soy agar and 5% sheep blood agar at 35°C and was checked at 2 and 7 days for the presence of aerial hyphae. The morphology of the isolate was observed after Gram and Ziehl-Neelsen staining. Catalase production was demonstrated by emulsifying a colony in hydrogen peroxide and checking for the presence of microscopic bubbles within 1 min. The test for the presence of oxidase was done by using dimethyl-*para*-phenylenediamine oxalate disk (Diagnostic Pasteur, Marnes, France). Biochemical tests were performed by inoculation of API CORYNE, API ANA, and Biotype-99-carbon source strip (bioMérieux) with Biotype 2 assimilation medium (bioMérieux) according to the instructions of the manufacturer. Two readings were performed after 2 and 4 days of incubation at 35°C. Results of antibiotic susceptibility tests performed by the disk diffusion method on Mueller-Hinton medium were read after 24, 48, and 72 h of incubation at 35°C under aerobic conditions. The MICs of the following antibiotics were determined: penicillin G, amoxicillin, amoxicillin-clavulanic acid, cephalothin, ceftriaxone, imipenem, gentamicin, doxycycline, erythromycin, pefloxacin, ciprofloxacin, rifampin, trimethoprim-sulfamethoxazole, and vancomycin.

**Cellular fatty acids.** Colonies of the isolate grown on Trypticase soy agar (Becton Dickinson Microbiology, Meylan, France) at 28°C for 24 h were saponified, and cell wall fatty acids were extracted, methylated, and analyzed by capillary gas-liquid chromatography as reported previously (12). The profile was analyzed through MIDI software on the basis of the profiles determined for 139 *Rhodococcus* or *Gordona* species strains (12).

**16S rRNA gene sequencing and molecular detection.** The DNA was extracted from the isolate by using five cycles of freezing in liquid nitrogen and boiling as reported previously (19). The extracted DNA was amplified by using the PCR technology and universal primers fD1 and rp2 (Eurogentec, Seraing, Belgium) (28). PCR amplifications were performed in 50 ml by adding 100 mM (each) deoxynucleoside triphosphate, 0.2 mM (each) primer, and 0.4 U of *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, Conn.). Amplifications were carried out in a Perkin-Elmer 9600 thermal cycler by using 35 cycles consisting of denaturation at 90°C for 30 s, primer annealing at 55°C for 30 s, and extension at 72°C for 60 s. The quality of the amplification was checked by 1% agarose gel electrophoresis with ethidium bromide staining. Each experiment included sterile water (no DNA) as a negative control and *Escherichia coli* DNA as a positive control. The amplified products were sequenced by using 5′-fluorescein-labelled primers fD1, 800f (5′-ATT AGA TAC CCT GGT AG-3′), and 1050f (5′-TGT CGT CAG CTC GTG-3′) for direct sequencing and 5′-fluorescein-labelled primers rp2, GO1 (5′-GGG GCA TGA TGA CTT GAC GT -3′), GO2 (5′-CTG CTG GCA CGT AGT TGG C-3′), 1050r, and 800r for reverse sequencing. Primers 1050r and 800r are reverse complements of primers 1050f and 800f, respectively. *Tth* DNA polymerase and the AutoCycle sequencing kit (Pharmacia Biotech, Uppsala, Sweden) were used for electrophoresis, and the sequences were analyzed with an ALF DNA automatic sequencer (Pharmacia Biotech). The determined sequence was compared with those of *Gordona terrae* (GenBank accession number X79286), *Gordona bronchialis* (GenBank accession number X79287), *Gordona rubropertinctus* (GenBank accession number X80632), and *Rhodococcus equi* (GenBank accession number M29574) through Bisance (5) by using the Clustal package (7). Primers GO1 and GO2 were used to amplify DNA extracted from fixed brain tissue with Chelex (Bio-Rad, Richmond, Calif.) as reported previously (22). The amplicon was sequenced with the same primers, and the sequences were compared as reported above.

**Serology.** The agar-grown isolate, suspended in a 0.1% azide sodium solution, was used as an antigen for the microimmunofluorescence detection of immunoglobulin G (IgG)-, IgA-, and IgM-specific antibodies in the serum and CSF samples, following standard procedures (13).

## RESULTS

**Case report.** A previously healthy 40-year-old woman was hospitalized on 14 February 1992 with a 3-week history of progressive left hemiparesis. Her medical history was unremarkable. Physical examination at the time of admission revealed a florid, apyretic woman with left hemiparesis without any other clinical sign. Laboratory tests were unremarkable, with lymphocytes of $0.99 \times 10^9$/liter and a CD4/CD8 lymphocyte ratio of 1.96. Brain computerized tomography (CT) scan showed two right frontal lesions enhanced by contrast, and a nuclear magnetic resonance (NMR) scan disclosed multiple,

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TABLE 1. Microbiological characteristics of an isolate of *G. terrae* from CSF compared with those of a reference isolate

| Characteristic | Reference isolate[a] | W5381 |
|---|---|---|
| Morphology | | |
| Aerial hyphae | − | − |
| Colony color | Salmon pink | Red-brown |
| Growth | | |
| At 25°C | + | + |
| At 35°C | + | + |
| At 45°C | − | − |
| In lysozyme | − | − |
| Decomposition | | |
| Adenine | − | − |
| Casein | − | − |
| Acid production | | |
| Inositol | − | − |
| Maltose | − | + |
| Mannitol | + | − |
| Rhamnose | + | − |
| Sorbitol | + | − |
| Catalase activity | + | + |
| Oxidase activity | − | − |
| Percent 16S rRNA gene homology with *G. terrae* type strain | 100 | 100 |

[a] The reference isolate was described previously (6). Symbols: +, positive; −, negative.

bilateral white matter lesions which suggested abscesses on T2-weighted images. The CSF contained 75 mononuclear cells/ml, with a glucose concentration of 3.2 mmol/liter, a protein concentration of 340 mg/liter, and evidence of oligoclonal IgG intrathecal synthesis. Clinical and radiological searches for primary neoplasia remained negative, as did searches for the levels of various cancer-related serological markers. Direct and serological investigations for central nervous system pathogens, including mycobacteria, spirochetes, rickettsiae, and human immunodeficiency virus remained negative, but colonies of a gram-positive bacillus subsequently identified as *G. terrae* were isolated on the blood agar. Chloramphenicol was started at 3 g/day as a result of a provisional diagnosis of brain abscesses, and the neurological status of the patient improved. The patient was discharged 1 month later with a prescription for oral triamphenicol (3 g/day). Clinical follow-up examination on 8 April 1992 was normal, but the CT scan disclosed a few lesions. The CSF contained 18 mononuclear cells/ml, with a glucose concentration of 3.5 mmol/liter and a protein concentration of 280 mg/liter. Culture of the CSF was negative. The patient was hospitalized 1 week after she stopped the triamphenicol therapy for right spastic paraparesis, motor and sensory disability of the left hand, and a thoracic sensitivity level. The NMR scan revealed multiple new brain abscesses and two cervical medullary lesions. The CSF contained 20 mononuclear cells/ml, with a glucose concentration of 3.4 mmol/liter and a protein concentration of 340 mg/liter. A stereotaxic brain biopsy was performed. Histologic examination disclosed aspects compatible with suppurative encephalitis. Gram staining of the brain specimen revealed numerous gram-positive cocci and bacilli. A test for the presence of an antibiotic activity in the specimen (29) was positive, and culture of the specimen remained sterile. Treatment with trimethoprim-sulfamethoxazole at 3,200 and 640 mg/day, respectively, plus ofloxacin at 600 mg/day resulted in progressive improvement of the patient's neurological status, and the patient was discharged on 25 May 1992. Clinical and NMR scan follow-ups were performed every 6 months and were unremarkable up to June 1996.

**Isolation, culture, and phenotypic characterization.** A few red-brown colonies developed 5 days after the first CSF sample was plated onto 5% blood agar at 37°C in a $CO_2$ incubator. The isolate was a gram-positive, pleomorphic, weakly acid-fast, asporogenous coccobacillus which did not form aerial hyphae in 7 days. It grew on Trypticase soy agar and 5% sheep blood agar at 25 and 35°C, but not at 45°C. Growth was observed in the presence of 0.2% picric acid and 0.1% sodium salicylate, but not in presence of 5 mg of lysosyme per liter. Catalase activity was detected, but oxidase activity was not. The results of the biochemical tests presented in Table 1 were in agreement with the identification of the isolate as *G. terrae*. The MICs were >16 mg/liter for penicillin G, >64 mg/liter for amoxicillin, >64 mg/liter for amoxicillin-clavulanic acid, 128 mg/liter for cephalothin, 512 mg/liter for ceftriaxone, 2 mg/liter for imipenem, <0.25 mg/liter for gentamicin, 0.50 mg/liter for doxycycline, <1 mg/liter for erythromycin, <1 mg/liter for pefloxacin, <2 mg/liter for rifampin, 4 mg/liter for trimethoprim-sulfamethoxazole, and 2 mg/liter for vancomycin after 24, 48, and 72 h of incubation. The strain has been deposited in the Collection de l'Institut Pasteur, Paris, France, as strain CIP104940.

**Cellular fatty acids.** Gas chromatographic analysis of cell wall fatty acids yielded similar results for the patient's isolate and *Rhodococcus* species (Fig. 1). Percentages of fatty acids were 33% for $C_{16:0}$ acids, 22% for $C_{18:1\omega9c}$ acids, 19.7% for $C_{18:0}$ acids, and 16.5% for $C_{16:1}$ acids; in addition, to traces of other fatty acids were present.

**16S rRNA gene sequencing and molecular detection.** A total of 1,481 positions along the 16S rRNA gene of the patient's isolate were sequenced. This sequence was 100% similar to that of *G. terrae*, 98.5% similar to that of *G. rubropertinctus*, 98.3% similar to that of *G. bronchialis*, and 94.5% similar to that of *R. equi* (Fig. 2). DNA was amplified from fixed brain tissue by using the GO1 and GO2 primers, and the sequence of

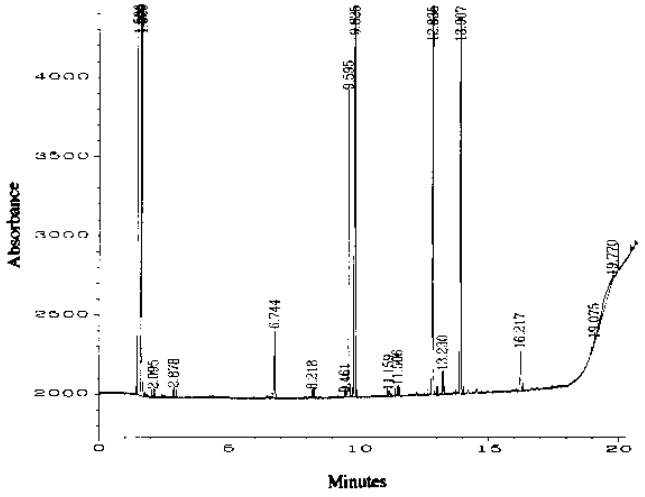


FIG. 1. Cell wall fatty acid chromatogram of strain W5381 by gas-liquid chromatography (12).

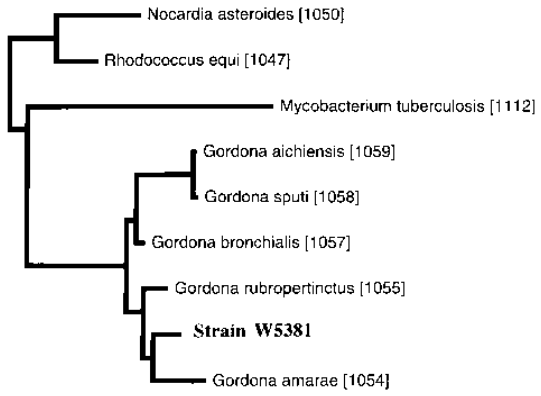


FIG. 2. A 16S rRNA similarity dendrogram indicating the position of isolate W5381. The isolate shared 100% sequence similarity with the sequence of *G. terrae*. Numbers in brackets indicate the reference number for sequences in the Ribosomal Database Project (11).

this amplicon shared 100% similarity with that of the isolate from the patient's CSF.

**Serology.** Specific antibodies were demonstrated in the serum samples, but not in the CSF, of the patient, by using the isolate as antigen and a microimmunofluorescence technique. The titers were 1:8 for IgG and 1:4 for IgM at the time of admission and 1:4 for IgG and 1:2 for IgM in two further samples drawn 2 weeks and 4 months, respectively, after admission.

## DISCUSSION

The pathogenic role of the strain described here was confirmed by its isolation in pure culture from a normally sterile fluid (CSF) which exhibited an inflammatory response, the observation of morphologically compatible bacteria in the brain biopsy specimen, the detection of a specific 16S rRNA sequence in that biopsy specimen, the presence of specific antibodies in the serum of the patient, the clinical course in response to the prescriptions of trimethoprim-sulfamethoxazole, and the absence of any other demonstrable pathogen or noninfectious etiology in the patient.

Coryneform bacteria may be regarded as contaminants without further identification, and conventional bench tests do not allow for the definite identification of coryneform bacteria except *R. equi*. Indeed, some recent reports mention the isolation of unidentified *Rhodococcus*-like bacteria (3, 4). Ribotyping was applied to the identification of *Rhodococcus* species (9), but failed to identify definitely our isolate. We therefore recommend 16S rRNA gene sequencing as the current technique for the accurate identification of non-*R. equi Rhodococcus* or *Gordona* species.

The genus *Gordona* now includes seven species (2), but the taxonomic history of this group is confusing because of renamings of original isolates and recent transfers of strains from the genus *Rhodococcus* or *Nocardia* to the genus *Gordona* (8). *G. terrae*, *G. rubropertinctus*, and *G. bronchialis* are derived from soil (23, 24), *G. amarae* has been isolated from foam formed on the surfaces of aeration tanks in industrial plants (10), and the latest described species, *G. hydrophobica*, has been isolated from the packaging material for biofilters (1, 2). As pathogens, *G. bronchialis*, *G. aichiensis*, *G. sputi*, and *G. rubropertinctus* have been isolated from the sputum of patients suffering from pulmonary lesions (24–26). A cluster of sternal wound infections due to *G. bronchialis* was reported in patients after coronary artery bypass surgery (20) and was traced to an operating room nurse. In addition to sternal wounds, *G. bronchialis* was isolated from the nurse's hands, scalp, and vagina, from her dogs, and from various places in and around the operating room where the nurse worked.

We previously reported on an immunocompromised child who developed a *G. terrae* brain abscess after he underwent neurosurgery for a malignant tumor (6). *R.* (*Gordona*) *aurantiacus*, identified on the grounds of morphological, cultural, and biochemical characteristics, was recovered from the CSF of a patient with hairy cell leukemia and a lymphocytic meningitis (17). The patient eventually died, and histological study of the brain revealed specific granulomatous tissue with giant cells of the Langhans type. No bacteria were demonstrable after Ziehl-Neelsen or Gram staining. *R. equi* was isolated in a few immunocompromised patients with brain abscesses (16, 27). At last, an unidentified, non-*R. equi Rhodococcus* species was isolated from the CSF of a previously healthy young woman with meningitis (4). *Gordona* species have been regarded primarily as opportunistic pathogens; a few cases of *Gordona* and unspecified *Rhodococcus* infections (4), however, have been reported in apparently immunocompetent patients.

In vitro (14) and experimental (15) data suggest that vancomycin should be included in the treatment of *R. equi*. Antibiotic susceptibility patterns have previously been determined for four *G. terrae* isolates (6), indicating common susceptibility to imipenem, aminoglycosides, and fluoroquinolones and variable susceptibility to other antibiotic families; one strain was resistant to vancomycin. The isolate described here exhibited an antibiotic susceptibility pattern identical to that previously reported for *G. terrae* strains, including susceptibility to vancomycin. However, this antibiotic was not used in our patient due to its poor intrathecal diffusion; instead, trimethoprim-sulfamethoxazole was apparently bacteriostatic, but it may not have achieved the eradication of *G. terrae* in our patient.

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