JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1996, p. 2045–2047 Vol. 34, No. 8
0095-1137/96/$04.00+0

Bacteremia Due to *Gordona sputi* in an Immunocompromised Patient

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Received 29 January 1996/Returned for modification 23 April 1996/Accepted 17 May 1996

We report a case of bacteremia due to a strain identified as *Gordona sputi* in a patient with metastatic melanoma. The origin of infection remains unknown, but extensive cutaneous lesions due to interleukin-2 treatment may have been the portal of entry. The isolate was related to *G. sputi* on the basis of its biochemical and genomic properties but exhibited some differences from the type strain.

The genus *Gordona* was proposed by Tsukamura in 1971 for coryneform bacteria isolated either from the sputa of patients with pulmonary diseases or from soil (9). In recent years, *G. bronchialis*, *G. terrae*, and *G. rubropertincta* were recognized as the causes of catheter-related sepsis or abscesses which mainly occurred in immunocompetent hosts (5). We report the first case, to our knowledge, of a bacteremia due to a strain identified as *Gordona sputi*, in a patient with immune deficiency.

A 34-year-old man with a 6-year history of ocular melanoma was admitted in May 1993 to the hospital because of fever. In December 1992, hepatic metastases were detected by ultrasonographic examination and a treatment consisting of 70 mg of *cis*-platinum per day for three days in a month was started. In April 1993, in addition to this treatment the patient received a combination of intravenous interleukin-2 (IL-2), 36 M U per day on days 1, 2, 3, and 4 on a 2-week cycle, and subcutaneous alpha interferon, 9 M U per day on days 1, 3, and 5 each week. After 1 month of this treatment, the temperature of the patient rose to 40°C and one blood aerobic culture incubated in Bactec NR860 (Becton Dickinson, Cockeysville, Md.) became positive after 5 days of culture for a gram-positive coryneform bacterium. Antimicrobial susceptibility tested by using a 48-h disk diffusion method on Mueller-Hinton plates incubated in an ambient-air incubator revealed that this isolate was susceptible to vancomycin, β-lactams, aminoglycosides, doxycyclin, and rifampin. We detected no β-lactamase by the nitrocefin disk method. The MIC of ampicillin and amoxicillin-clavulanic acid was 0.032 μg/ml and the MIC of piperacillin was 0.25 μg/ml as estimated by the E-test (AB Biodisk, Solna, Sweden). The patient received antibiotic therapy with amoxicillin-clavulanate (1,000 and 125 mg, respectively, every 3 h). The neutrophil count was 4,900 cells per μl. A radiogram and clinical examination of the chest appeared normal, but a physical examination revealed extensive desquamative skin rashes due to the IL-2 treatment. One week later, after a second episode of fever, a second set of three blood cultures yielded the same organism. The patient was treated with a combination of amikacin (500 mg intravenously every 8 h) and piperacillin (3 g intravenously every 12 h). A rapid resolution of fever was obtained. No positive culture from oropharyngeal, skin, and stool specimens or from samples collected from catheters appeared for this bacterium.

The identification of this isolate (IBS T46944 [Institut de Bactériologie de Strasbourg, Strasbourg, France]; same as CCUG 35424 [Culture Collection, University of Göteborg, Göteborg, Sweden]) was based on both biochemical and genomic properties. The data are shown in Table 1. The bacterium was grown aerobically at 37°C on Mueller-Hinton agar (bio-Mérieux, Marcy L'Etoile, France). This isolate was gram-positive, pleomorphic, and bacillary shaped without extensive branching and formed rough beige colonies without mycelium which became salmon colored after several days of culture. The organism was weakly acid fast according to the Kinyoun acid-fast stain, modified for actinomycetes (4). The strain was urease and nitrate reductase positive and hydrolyzed esculin but did not exhibit β-galactosidase activity. Xanthine, adenine, tyrosine, and hypoxanthine were not hydrolyzed. As these results indicated a relationship with rhodococci or gordonae, we analyzed the *p*-bromophenacyl esters of mycolic acids of this isolate by using high-performance liquid chromatography (HPLC), and we obtained a number of peaks and retention times similar to those exhibited by *Gordona* species, especially *G. sputi* and *G. aichiensis*. The strain was able to produce acid from mannitol, glucose, and sucrose but not from rhamnose, mannose, and maltose. These biochemical characteristics fit those of *G. sputi* and *G. rubropertincta* following the identification scheme of McNeil and Brown (5).

As some authors demonstrated the usefulness of ribotyping as an aid in the identification of gordonae at the species level (1, 5), we determined the rRNA gene restriction pattern of this strain by using *Pvu*II endonuclease digestion. The pattern obtained from this isolate was different from those of *G. sputi* and other reference strains of *Gordona* species (Fig. 1). Therefore, in order to accurately identify this isolate we investigated the genomic similarities of this isolate with the type strains of *Gordona* species by using quantitative DNA-DNA hybridization and comparisons of small-subunit rRNA gene (rDNA) sequences. These experiments were carried out by using the procedures described in previous reports (7, 8). The comparison of nearly complete rDNA sequences showed that the sequence of the isolate differed from the sequence of the *G. sputi* type strain by only one nucleotide and from that of the *G. aichiensis* type strain by three nucleotides, whereas at least 41

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TABLE 1. Tests used to identify the clinical isolate within the genus *Gordona*

Strain	Colony pigmentation	Acid from[a]:		Retention time for HPLC of mycolic acids (min)	% 16S rDNA homology with T46944[b]	% DNA relatedness to T46944
		Mannitol	Rhamnose			
Clinical isolate T46944	Beige to pink	+	−	18.86–19.58	100	100
Reference strains						
G. sputi ATCC 29627^T	Beige to pink	+	−	18.99–19.69	99.9	60[c]
G. aichiensis ATCC 33611^T	Beige to pink	−	−	18.93–19.65	99.8	21
G. bronchialis ATCC 25592^T	Pink to orange	−	+	19.41–20.08	97.2	7
G. terrae ATCC 25594^T	Pink to orange	+	+	17.89–19.31	96.7	1
G. rubropertincta ATCC 25593	Pink to orange	+	−	18.03–19.29	96.8	1
G. hydrophobica DSM 44015^T	Tannish	ND[d]	+	ND	96.1	ND
G. amarae DSM 43392^T	White to tan	+	+	18.99–20.19	96.1	ND

[a] Strains were positive (+) or negative (−) for acid production from mannitol and rhamnose.
[b] For 1,311 nucleotides.
[c] Difference in melting temperature, 5°C.
[d] ND, not done.

differences with the other *Gordona* species were found. DNA-DNA similarity experiments confirmed the relationship with *G. sputi* but revealed significant genomic divergence between the isolate and the type strain of *G. sputi*, with only 60% similarity and a difference of 5°C in melting temperature. Less than 21% DNA relatedness was found with the type strains of other *Gordona* species, including *G. aichiensis*. According to all the data given above, this strain was classified as *G. sputi.*

The genus *Gordona* is a member of the mycolic acid-containing group consisting of the genera *Corynebacterium*, *Dietzia*, *Gordona*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, and *Tsukamurella*. It differs in producing dihydrogenated menaquinones with nine isoprene units and mycolic acids with 48 to 66 carbon atoms (3). Phylogenetic analyses based on small-subunit rDNA sequences confirmed that all *Gordona* species constitute a distinct and monophyletic group (8).

A proper identification of these organisms requires determination of the cell wall components or genomic similarities, determinations for which the methods are not available to most clinical laboratories. Nevertheless, both macroscopic and microscopic examinations coupled with a few biochemical tests can give a presumptive identification at the genus level (Table 2). Members of the genera *Dietzia* and *Corynebacterium* are acid-fast strain organisms, whereas the presence of macroscopic aerial hyphae in addition to a microscopic observation of branched filaments are typical of the genus *Nocardia*. Differentiation between rhodococci and gordonae can be achieved at the genus and species levels by using biochemical reactions such as acid production from sugars, assimilation tests, or enzymatic activities, but these tests are very time-consuming or fail to differentiate between some species (5). As a consequence, more sophisticated methods such as HPLC of mycolic acids or ribotyping have been used in separating these bacteria, but as shown in this report and as previously described (5), mycolic acid analysis by HPLC is unable to separate *G. sputi* from *G. aichiensis* as well as *G. terrae* from *G. rubropertincta*. Furthermore, a significant number of *Gordona* strains seem to be atypical within this genus (reference 1 and our observations) and therefore the use of reference methods in molecular taxonomy remains necessary for the accurate identification of some of these organisms. This report demonstrates that some *Gordona* strains can be borderline with regard to the species definition, exhibiting only 50 to 70% DNA relatedness with the type strain and associated with a unique ribotype pattern.

Species *G. terrae*, *G. amarae*, *G. hydrophobica*, and *G. rubropertincta* have been isolated from nature, whereas *G. bronchialis*, *G. sputi*, and *G. aichiensis* were originally associated with sputa of patients with pulmonary diseases (9). More recently, *G. bronchialis* and *G. terrae* were suspected as etiologic agents in sternal wound infections, catheter-associated

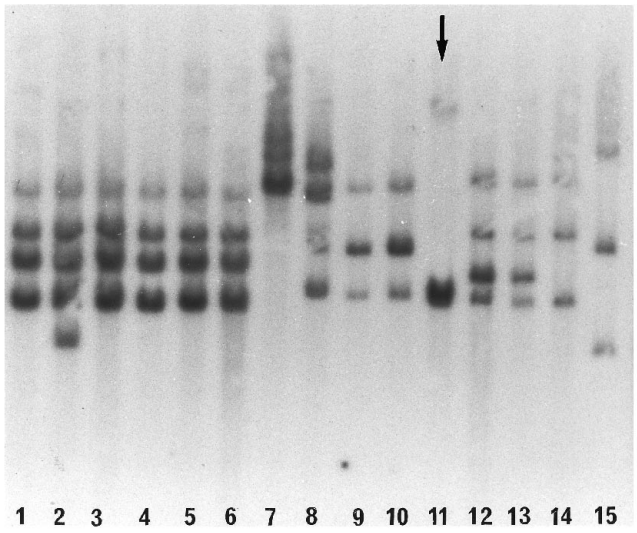

FIG. 1. rRNA gene restriction patterns after cleavage by *Pvu*II. Lanes 1 to 6, *G. terrae* strains (lane 3, ATCC 25594^T); lane 7, *G. rubropertincta* ATCC 25593; lanes 8 to 10, *G. bronchialis* strains (lane 9, ATCC 25592^T); lane 11, *G. sputi* isolate IBS T46944; lanes 12 to 14, *G. sputi*-like isolates; lane 15, *G. sputi* ATCC 29627^T. The arrow indicates the isolate recovered from the patient in this study.

TABLE 2. Diagnostic characteristics of acid-fast genera[a]

Genus	Macroscopic aerial hyphae	β-Galactosidase	Mycolic acid (no. of carbons)	Major menaquinone
Gordona	−	−	48–66	MK-9(H2)
Rhodococcus	−	V	34–52	MK-8(H2)
Tsukamurella	−	+	64–78	MK-9
Nocardia	+	+	44–60	MK-8(H4, −ω cycl)

[a] Data are from references 3 to 5. +, presence of hyphae or β-galactosidase activity; −, absence of hyphae or β-galactosidase activity; V, variable.

sepsis, and brain abcesses (5). Although *G. sputi* was initially isolated from the sputa of patients and caused mesenteric lymphadenitis of swine (10), it was not hitherto reported to be pathogenic for humans.

Herein, we report the case of a bacteremia caused by a strain identified as *G. sputi* in a patient with metastatic melanoma who received IL-2, treatment involving a neutrophil-phagocytic defect and impairment of epithelial barriers (6). The origin of the bacteremia remains unknown, but we can hypothesize that the bacterium gained access to the bloodstream via contamination of a catheter or via cutaneous ulcerations due to IL-2 treatment, although cultures from these specimens were negative. The isolation of *Gordona* strains requires 3 to 4 days of incubation, and therefore this delay can provide false-negative results and an underestimation of infections caused by these bacteria. Finally, our clinical case indicates that in addition to common bacteria such as *Staphylococcus aureus* or *Escherichia coli*, actinomycetes such as *G. sputi* should be considered as opportunistic pathogens responsible for bacteremia, the incidence of which may increase in patients who receive IL-2 (6). Previously reported cases of infections by *Gordona* strains were successfully managed with the administration of vancomycin (2). Our report suggests that a combination of penicillins and aminoglycosides can be a suitable therapy in cases of infection due to strains that are non-β-lactamase producing. An identification of these organisms requires appropriate methods, and the isolates should be sent to reference laboratories in particular since some strains display biochemical or genomic properties unlike those of characterized *Gordona* species.

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