**Nocardi a kruczakiae** sp. nov., a Pathogen in Immunocompromised Patients and a Member of the “N. nova Complex”

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Molecular methodologies have become useful techniques for the identification of pathogenic *Nocardi a* species and for the recognition of novel species that are capable of causing human disease. Two isolates recovered from immunocompromised patients were characterized as *Nocardi a nova* by biochemical and susceptibility testing results. The restriction fragment length polymorphism (RFLP) patterns obtained by restriction endonuclease analysis (REA) of an amplified portion of the heat shock protein gene were identical to those obtained with the type strain of *N. nova*. REA of an amplified portion of the 16S rRNA gene showed RFLP patterns that were unlike those obtained for the type strain of *N. nova* but that were similar to those obtained for the type strains of *N. africana* and *N. veterana*. Subsequent sequencing of a portion of the 16S rRNA gene produced identical results for the two patient isolates. Sequence analysis of 1,352-bp portions of the 16S rRNA gene indicated that these isolates were 99.8% similar to the recently described species *N. veterana* but were only 99.3, 98.1, and 98.1% similar to the type strains of *N. africana*, *N. nova*, and *N. vaccinii*, respectively. DNA-DNA hybridization studies confirmed that the two patient isolates belonged to the same species but were not closely related to *N. africana*, *N. nova*, *N. vaccinii*, or *N. veterana*. The patient isolates have been designated *N. kruczakiae* sp. nov. Because *N. africana*, *N. veterana*, and the new species are not readily differentiated from *N. nova* by phenotypic methods alone, the designation “*N. nova* complex” can be used to designate isolates such as these that phenotypically resemble *N. nova* but that have not been definitively characterized by 16S rRNA gene sequencing or DNA-DNA hybridization.

*Nocardi a* species have been implicated as agents of infectious diseases in both immunocompetent and immunocompromised patients. These organisms have been shown to be responsible for cutaneous, ocular, pulmonary, and disseminated infections. Immunocompromised patients are particularly at risk for developing pulmonary or disseminated disease; many different species of *Nocardi a* may be involved (4). Recent advances in molecular methodologies for the identification of nocardial isolates have made their identification (3, 10, 11) more rapid than that by standard phenotypic techniques; these molecular methodologies have also been important in the recognition of numerous new species of *Nocardi a* which can cause human disease (5, 6, 16).

Molecular methodologies have also begun to elucidate the genetic differences that exist among *Nocardi a* species and have provided data indicating that additional species remain to be described (9). Organisms comprising the “*Nocardi a asteroides* complex” have now been more completely characterized and have been shown to be genetically distinct species (9, 10, 17, 18). More recently, such methodologies have revealed that some isolates determined to belong to *Nocardi a nova* by phenotypic and heat shock protein (HSP) restriction fragment length polymorphisms (RFLPs) actually belong to the distinct species *Nocardi a veterana* (2, 8).

The molecular procedures used in our laboratory for the identification of *Nocardi a* species have allowed us to distinguish distinct species phenotypically similar to *N. nova*. Our procedure involves PCR amplification of portions of both the 16S rRNA gene and the 65-kDa HSP gene and subsequent restriction endonuclease analysis (REA) of the amplicons (3, 11). Our experience has shown that in most cases correct species assignment can be made when the identification obtained by REA of the 16S rRNA gene region is identical to that obtained by REA of the HSP gene region. However, when the identifications obtained by REAs of the two gene regions differ or when no identification can be obtained by REAs of one or both gene regions, subsequent sequencing of the 16S rRNA gene frequently suggests that the organism belongs to an unusual or undescribed species.

We describe here two isolates from immunocompromised patients which gave conflicting identifications by REA of the two gene regions; 16S rRNA gene sequencing of the two iso-
lates and DNA-DNA hybridization of the isolates showed that they belong to a distinct and heretofore undescribed species. These two isolates were responsible for serious pulmonary disease in two immunocompromised patients and represent members of the proposed species *Nocardia kruczakiae*.

(Some of the data pertaining to case 1 have been included both in a report describing infection with *N. asteroides* [2] and in a report summarizing the experience to date with *Nocardia* infections in chronic granulomatous disease [4].)

**CASE REPORTS**

**Case 1.** A 6-year-old white male with X-linked chronic granulomatous disease was admitted to the Warren G. Magnuson Clinical Center of the National Institutes of Health (NIH), Bethesda, Md., for treatment for what was presumed to be a fungal pneumonia. In October 1996, 20 days after he was discharged, he was found to have a new right upper lobe pulmonary infiltrate. A Gram stain of a biopsy specimen of the lesion showed a few beaded, branched gram-positive rods, and a modified acid-fast stain was positive for organisms morphologically consistent with *Nocardia* species. The cytology specimen contained branched gram-positive rods. Bacteriologic and fungal cultures grew a *Nocardia* species. He was treated with intravenous trimethoprim-sulfamethoxazole (TMP-SMX), ciprofloxacin, amphotericin B, and granulocyte transfusions. A computed tomographic (CT) scan of his chest showed improvement, and he was maintained on the same antibiotic regimen, except that itraconazole was substituted for amphotericin B. Because of progression of the infiltrate in his right upper lobe, a transthoracic needle biopsy was performed; it was negative for organisms by both microbiologic and cytologic studies. His antibiotic regimen was changed to imipenem, amphotericin B, and granulocyte transfusions. A chest radiograph obtained in January 1997 showed complete resolution of the pulmonary lesions.

**Case 2.** A 57-year-old Asian female underwent orthotopic liver transplantation in May 1996 for hepatitis B-related cirrhosis and received follow-up care at the Walter Reed Army Medical Center, Washington, D.C. Postoperatively she was treated with tacrolimus and prednisone to prevent graft rejection. A chest radiograph obtained in January 1997 showed multiple pulmonary mass lesions. A bronchoalveolar lavage (BAL) and a transbronchial biopsy were performed. Direct Gram-stained smears of both the BAL and the biopsy specimens showed filamentous gram-positive rods. Smears of both specimens were negative for fungi and acid-fast bacilli. Cultures of both specimens grew an *Aspergillus* species, not *Aspergillus fumigatus*, and a *Nocardia* species. Cytomegalovirus culture of the BAL specimen was positive. The *Nocardia* isolate was referred to the Microbiology Service at the NIH Clinical Center, where it was identified as a *Nocardia* species most closely resembling *N. asteroides* on the basis of its phenotypic reactions. The patient was treated with both amphotericin B and TMP-SMX. Follow-up chest films eventually showed complete resolution of the pulmonary lesions.

**MATERIALS AND METHODS**

**Organisms.** (i) **Reference strains.** The following reference strains were used for molecular, biochemical, and/or drug susceptibility studies (GenBank accession numbers for the sequences determined in the laboratory of the Microbiology Service at the NIH Clinical Center are given in parentheses): *Nocardia africana* DSM 44484T and ATCC BAA-280T (AY089701), *N. nova* ATCC 33726T (AY191250), *Nocardia verecundia* ATCC 11092T (AY191252), and *N. asteroides* DSM 44491T. Biochemical and susceptibility testing of *N. africana* at the Centers for Disease Control and Prevention was performed with *N. africana* ATCC BAA-280T. DMZ 44491T was deposited in the American Type Culture Collection and was given the designation ATCC BAA-280T.

(ii) **Patient isolates.** Two patient isolates were identified as *N. kruczakiae*. One patient (case 1, from whom isolate A was recovered from a lung biopsy specimen) was treated at the NIH. The second patient (case 2, from whom isolate B was recovered from a BAL specimen) was treated at the Walter Reed Army Medical Center.

**GenBank sequences.** For comparison of 16S rRNA gene sequences, sequences from the following organisms, which have been reported to be human pathogens, were used (GenBank accession numbers are given in parentheses): *Nocardia abscessus* ATCC 23824 (X84851), *N. asteroides* ATCC 19247T (Z36934), *N. asteroides* drug pattern type II (AF163818), *N. asteroides* drug pattern type IV ATCC 49872 (AY191251), *Nocardia brasiliensis* ATCC 19296T (Z36935), *Nocardia brevicatena* ATCC 15333T (X80600), *Nocardia cyriacigeorgica* DSM 44484T (AF282889), *Nocardia farrinica* ATCC 3316T (Z36936a), *Nocardia otiitidiscavi- narum* ATCC 14629T (X80599), *Nocardia paucivorans* DSM 44386T (AF179865), *Nocardia pseudobrasiliensis* ATCC 515127T (X84857), *Nocardia putida* DSM 44599T (AJ508748), *Nocardia transvalensis* ATCC 6865T (X80598), and *Rhodococcus equi* ATCC 6939T (X06036).

**Phenotypic identification.** Isolates were examined for aerial hyphae, acid-fast stain reaction, and biochemical characteristics, as described previously (2).

**Susceptibility testing.** Antimicrobial susceptibility testing was performed as described previously (2). MICs were determined according to NCCLS guidelines (7). The interpretive criteria used for all drugs except ampicillin, linezolid, and vancomycin were those recommended by the NCCLS (7); no interpretive criteria for ampicillin or vancomycin and no breakpoint for resistance to linezolid have yet been established for *Nocardia* species.

**Molecular analysis.** All molecular techniques were performed as described previously (2, 3, 10, 11), including DNA extraction, PCR, REA, of a 999-bp region of the 16S rRNA gene, REA of a 439-bp region of the 654-bp HSP gene, and 16S rRNA gene and HSP gene sequence determinations. The 16S rDNA and HSP gene sequences were assembled by using the SeqMan software (DNASTAR, Inc., Madison, Wis.); related sequences were identified by using the Basic Local Alignment Search Tool (BLAST; National Center for Biotechnology Information, NIH). For sequence comparison, all sequence lengths were adjusted to match the length of the shortest sequence (1,352 bp), and the sequences were aligned by using the ClustalV algorithm of MegAlign software (DNASTAR, Inc.). Percent similarity was determined by counting the number of base differences and relating the number of these differences to sequence length. Phylogenetic trees were constructed with the MegAlign software.

DNA-DNA hybridization was performed as described previously (2). The relative binding ratio (RBR; [percentage of heterologous DNA bound to hydronapate percentage of homologous DNA bound to hydronapate] X 100) was calculated by the method of Brenner et al. (1). The percent divergence (calculated to the nearest 0.5%) was determined by assuming that each degree of heteroduplex instability compared to the melting temperature of the homologous duplex was caused by 1% unpaired bases (1).

**Nucleotide sequence accession number.** The nucleotide sequence of the 16S rRNA gene of *N. kruczakiae* that we have determined has been submitted to GenBank and has been assigned accession number AY441974.
tol, i-erythritol, lactose, melibiose, and d-sorbitol; positive for acid production from the oxidation of D-glucose and maltose; shown in Table 1.

Results for the reference strains and the patient isolates are for the utilization of acetamide. All other biochemical test results for the reference strains and the patient isolates were essentially identical to those obtained for the type strains of N. vaccinii, N. veterana, and N. kruczakiae (Table 2). The rRNA genes of the type strains of N. vaccinii and N. veterana showed patterns identical to those obtained with the 16S rRNA genes of the two patient isolates (for HinP1I, bands of 420, 225, 175, and 55 bp; for DpnII, bands of 455, 250, 200, and 95 bp) showed patterns identical to those obtained with the 16S rRNA genes of the type strains of N. africana and N. veterana but unlike those obtained for the type strain of N. nova (data not shown). REA of the amplified region of the HSP genes of the two patient isolates (for MspI, bands of 130, 110, and 75 bp; for HinII, a band of 439 bp) showed patterns identical to those obtained with the HSP genes of N. africana, N. nova, and N. veterana (data not shown).

BLAST analysis and 16S rRNA and HSP sequence alignments. BLAST analysis showed that isolates A and B were the most similar to N. africana, N. nova, N. vaccinii, and N. veterana. Alignments of 1,352-bp regions of the 16S rRNA gene showed that isolates A and B were 100% similar to each other; the isolates showed 99.3, 98.1, 98.1, and 99.8% similarities to N. africana, N. nova, N. vaccinii, and N. veterana, respectively (Table 3). Sequence analysis of a 441-bp region of the HSP gene showed that isolates A and B were 99.8% similar to each other (1-bp difference) (data not shown). The HSP gene sequences of the two patient isolates showed the closest similarity to that of N. veterana (99.8 and 100%, respectively) and showed ≥99.3% similarity to those of N. africana, N. nova, and N. vaccinii (Table 3).

DNA-DNA hybridization. The DNA-DNA hybridization results showed that isolate A is highly related to isolate B (87% RBR with a divergence of 0.5% under optimum reassociation.
related type strains of late A has a lower level of relatedness to the phylogenetically conditions). In addition, hybridization studies showed that isolate A has a lower level of relatedness to the phylogenetically related type strains of \textit{N. africana} (58\% RBR with 4.5\% divergence), \textit{N. nova} (60\% RBR with 5.5\% divergence), and \textit{N. veterana} (35\% RBR with 8.5\% divergence).

### DISCUSSION

The proposed new species, \textit{N. kruczakiae}, has been shown to be a pathogen capable of causing pulmonary infections in immunocompromised patients. The results of our studies with the two patient isolates described here suggest that a few biochemical characteristics may differentiate \textit{N. kruczakiae} from the related species \textit{N. africana}, \textit{N. nova}, and \textit{N. veterana}. The critical tests necessary for the discrimination of these species include oxidation of glycerol, trehalose, and salicin; arylsulfatase production; and esculin hydrolysis (Table 1). Because our testing was performed with only two isolates, extended testing with more isolates in different laboratories is necessary to verify the usefulness and the reproducibilities of these tests for achieving identification. Variations in test media, inoculation techniques, and test interpretation among laboratories may affect the biochemical differences seen among species of \textit{Nocardia}; in our study, the results obtained for acid production from oxidation of i-myoinositol by \textit{N. veterana} and L-rhamnose by \textit{N. vaccinii} and \textit{N. veterana} differed from the carbohydrate assimilation results obtained for the same organisms by Gürtler et al. (5).

The susceptibility testing results for \textit{N. kruczakiae} were essentially identical to those obtained for \textit{N. africana}, \textit{N. nova}, and \textit{N. veterana}. The MICs of amoxicillin-clavulanate for all species were higher than those of ampicillin alone. This discrepancy has been noted previously for some \textit{Nocardia} isolates and has been attributed to the ability of clavulanic acid to act as a beta-lactamase inducer (8, 12). While susceptibility testing has been shown to assist in the identification of several of the more commonly isolated \textit{Nocardia} species (13–15), it appears that the use of susceptibility testing alone would be inadequate for the reliable identification of isolates belonging to this group. In fact, the basic phenotypic testing performed in most clinical laboratories (decomposition reactions and susceptibility testing) would, in most cases, characterize these organisms as \textit{N. nova}.

\textit{N. kruczakiae} shares some molecular characteristics with the previously described species \textit{N. africana} and \textit{N. veterana}; it is impossible to differentiate \textit{N. africana}, \textit{N. veterana}, and the proposed new species by REA techniques. By 16S rRNA gene

### TABLE 2. Susceptibility testing results for \textit{N. kruczakiae} patient isolates compared with those for the type strains of \textit{N. africana}, \textit{N. nova}, and \textit{N. veterana}

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Breakpoint for resistance (μg/ml)</th>
<th>MIC (μg/ml)</th>
<th>N. africana ATCC BAA-280T (µg/ml)</th>
<th>N. nova ATCC 33726T (µg/ml)</th>
<th>N. veterana DSM 44445T (µg/ml)</th>
<th>N. kruczakiae isolate A (ATCC BAA-948T) (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>≥16</td>
<td>≤0.25</td>
<td>≤0.25</td>
<td>≤0.25</td>
<td>≤0.25</td>
<td>≤0.25</td>
</tr>
<tr>
<td>AMOX-CLAV</td>
<td>≥32/16</td>
<td>32/16</td>
<td>32/16</td>
<td>16/8</td>
<td>64/32</td>
<td>64/32</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>≥8</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≥8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≥8</td>
<td>0.25</td>
<td>≤0.25</td>
<td>≤0.25</td>
<td>≤0.25</td>
<td>≤0.25</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>≥8</td>
<td>0.25</td>
<td>≤0.25</td>
<td>≤0.25</td>
<td>≤0.25</td>
<td>≤0.25</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≥16</td>
<td>0.25</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Linezolid</td>
<td>≥8</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>SMX</td>
<td>≥128</td>
<td>32</td>
<td>128</td>
<td>64</td>
<td>64</td>
<td>&gt;128</td>
</tr>
<tr>
<td>TMP-SMX</td>
<td>≥4/76</td>
<td>0.5/9.5</td>
<td>1/19</td>
<td>1/19</td>
<td>1/19</td>
<td>2/38</td>
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<tr>
<td>Vancomycin</td>
<td>≥8</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The MIC breakpoints used are those of the NCCLS (7). \textsuperscript{b} AMOX-CLAV, amoxicillin-clavulanate. \textsuperscript{c} No established breakpoints exist for this drug. \textsuperscript{d} Isolates for which MICs are ≤8 μg/ml are considered susceptible to linezolid (7).

### TABLE 3. Percent similarities of 16S rRNA and HSP gene sequences of \textit{N. kruczakiae} and type strains of \textit{N. africana}, \textit{N. nova}, \textit{N. vaccinii}, and \textit{N. veterana}

<table>
<thead>
<tr>
<th>Isolate</th>
<th>16S HSP</th>
<th>16S HSP</th>
<th>16S HSP</th>
<th>16S HSP</th>
<th>16S HSP</th>
<th>16S HSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{N. kruczakiae} isolate A (ATCC BAA-948T)</td>
<td>100</td>
<td>99.8</td>
<td>99.3</td>
<td>99.1</td>
<td>98.1</td>
<td>98.1</td>
</tr>
<tr>
<td>Isolate B</td>
<td>99.3</td>
<td>99.3</td>
<td>98.1</td>
<td>99.1</td>
<td>98.1</td>
<td>93.0</td>
</tr>
<tr>
<td>\textit{N. africana} DSM 44491T</td>
<td>99.3</td>
<td>99.3</td>
<td>98.5</td>
<td>92.3</td>
<td>99.3</td>
<td>99.3</td>
</tr>
<tr>
<td>\textit{N. nova} ATCC 33726T</td>
<td>98.1</td>
<td>92.1</td>
<td>98.0</td>
<td>99.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{N. vaccinii} ATCC 11092T</td>
<td>98.0</td>
<td>95.0</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} A 1,352-bp segment of the 16S rRNA gene (16S) and a 441-bp segment of the HSP gene (HSP) were evaluated.
REA, *N. kruczakiae* is indistinguishable from *N. africana* and *N. veterana* but can be differentiated from *N. nova*. *N. africana*, *N. kruczakiae*, *N. nova*, and *N. veterana* all show identical RFLP patterns by HSP gene REA. Sequence analysis of a 1,352-bp region of the 16S rRNA gene shows that *N. kruczakiae* is most similar to *N. veterana*, with differences at 3 bp (99.8% similarity) (Table 3). These base differences correspond to bases 103, 150, and 1397 of the sequence of *N. asteroides* ATCC 19247T (GenBank accession number X84850). Phylogenetic analysis of the 16S rRNA gene shows that *N. kruczakiae* exists in a clade with *N. veterana* (Fig. 1) and on a branch adjacent to *N. africana*. The sequence of *N. kruczakiae* differs from the sequences of *N. africana* and *N. nova* by 10 and 26 bp, respectively. Interestingly, DNA-DNA homology studies with genomic DNA suggest that *N. kruczakiae* may be more closely related to *N. africana* and *N. nova* than to *N. veterana*, implying that the genetic differences among these species may be more significant than those reflected in either the 16S rRNA or the HSP gene regions (Table 3; Fig. 1). However, *N. vaccinii* is biochemically distinct and shows REA patterns that are unique to this species. In addition, DNA-DNA hybridization results show that *N. vaccinii* is distantly related to *N. veterana* (16% RBR with 11.0% divergence [2]) and *N. nova* (21% RBR with 9.5% divergence [data not shown]). *N. vaccinii* has not been implicated as a human pathogen and, at least for clinical purposes, probably should not be considered a member of the “*N. nova* complex.”

We previously described a variable region of the 16S rRNA gene which is useful for the differentiation of many species of *Nocardia* (3). Sequence analysis of this region of *N. kruczakiae* shows a distinct base difference that distinguishes it from the other species in the “*N. nova* complex” (Table 4).

Sequence analysis of a 441-bp region of the HSP gene also showed that *N. kruczakiae* is most closely related to *N. veterana*. As expected from the HSP gene REA results, sequence analysis of the HSP gene did not show the same level of genetic diversity among isolates of the *N. nova* complex (*N. africana*, *N. kruczakiae*, *N. nova*, and *N. veterana*) as that of the 1,352-bp region of the 16S rRNA gene did. The HSP gene sequence of the type strain of *N. kruczakiae* differed from those of *N. africana* and *N. nova* by 4 and 5 bp, respectively (data not shown).

The incidence of *N. kruczakiae* as a pathogen is unknown. Because isolates of this species may have been misidentified as *N. nova* on the basis of phenotypic testing, it is quite likely that some isolates identified as *N. nova* may actually be represen-
tatives of the species *N. kruczakiae.* Further studies to determine the incidence of this species would also help to determine the extent of its involvement in infections at other anatomic sites, the spectrum of antibiotic susceptibilities of isolates of this species, and the geographic distribution of the species.

Analysis of phenotypic characteristics and susceptibility testing results will enable recognition of a group of organisms that may be evolutionarily rather closely related. Members of this group would give positive 14-day arylsulfatase results; would fail to decompose casein, tyrosine, and xanthine; and would be susceptible to erythromycin. Species with such phenotypic characteristics presently include *N. kruczakiae,* in addition to the three species (*N. africana,* *N. nova,* and *N. veterana*) that could be considered members of the “*N. nova complex,*” as suggested previously (2). Other as yet undescribed species are likely to share such characteristics as well. For definitive discrimination among these species, molecular methods must be used (at a minimum, a combination of 16S rRNA and HSP gene REAs to recognize members of this complex and to separate *N. nova* sensu stricto) and 16S rRNA gene sequencing for definitive identification of *N. africana,* *N. kruczakiae,* and *N. veterana.* Until it has been established that identification to the species level can be reliably performed by non-molecular biology-based methods and is clinically significant, the use of the designation “*N. nova complex,*” for such phenotypically indistinguishable organisms will help to avoid incorrect attribution of potentially significant clinical and microbiologic features to any of the four species included in the complex at present.

**Description of *Nocardia kruczakiae* sp. nov. *kruczakiae* (kruczak’e,i. M.L. gen. fem. *kruczakiae,* of Kruczak, in memory of Patricia Kruczak-Filipov, in honor of and recognition for her contributions to the Mycology and Mycobacteriology Sections of the Microbiology Service, Department of Laboratory Medicine, Warren G. Magnuson Clinical Center, NIH). The organism is an aerobic, modified acid-fast-positive, branched gram-positive rod. Colonies produce aerial hyphae. Acid is produced from the oxidation of glucose and maltose but not from adonitol, L-arabinose, cellobiose, dulcitol, D-erythritol, D-fructose, D-galactose, glycerol, D-myo-inositol, lactose, D-mannitol, mannose, melibiose, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, trehalose, or D-xylose. It grows at 25, 35, and 45°C, with the best growth at 45°C. It grows in the presence of lysozyme. It produces arylsulfatase at both 3 and 14 days and is able to hydrolyze esculin in the presence or absence of bile; but it is not able to decompose adonitol, casein, hypoxanthine, tyrosine, or xanthine. It does not use either acetamide or citrate as a sole source of carbon. It is susceptible to amikacin, ceftriaxone, clarithromycin, imipenem, linezolid, minocycline, and TMP-SMX; but it is resistant to amoxicillin-clavulanic acid, ciprofloxacin, and SMX. The organism is a respiratory pathogen of immunocompromised patients.

The type strain of the species is ATCC 19247T (DSM 44877), and the sequence of a 1,434-bp region of the 16S rRNA gene has been deposited in GenBank under accession number AY441974.

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The views expressed here are those of the authors and should not be construed as those of the Department of the Army, the U.S. Department of Defense, or the U.S. Department of Health and Human Services.

**REFERENCES**


**TABLE 4. Sequence of variable region of 16S rRNA gene**

<table>
<thead>
<tr>
<th>Species</th>
<th>Base at position:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>149</td>
</tr>
<tr>
<td><em>N. africana</em></td>
<td>A</td>
</tr>
<tr>
<td><em>N. kruczakiae</em></td>
<td>Gi</td>
</tr>
<tr>
<td><em>N. nova</em></td>
<td>A</td>
</tr>
<tr>
<td><em>N. veterana</em></td>
<td>A</td>
</tr>
</tbody>
</table>

* Compared to the sequence of *N. asteroides* ATCC 19247T (GenBank accession number X84850). ●, conserved bases.


