Mucoid Nitrate-Negative *Moraxella nonliquefaciens* from Three Patients with Chronic Lung Disease

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Received 26 February 2004/Returned for modification 2 April 2004/Accepted 5 May 2004

Mucoid strains of *Moraxella nonliquefaciens* were recovered from the sputa of three indigenous Australians with chronic lung disease. These atypical strains failed to reduce nitrate, and one strain produced β-lactamase. While the mucoid phenotype of *M. nonliquefaciens* has rarely been reported, the mucoid nitrate-negative biovar has never been previously reported.

**CASE REPORTS**

**Case 1.** A 17-year-old Aboriginal male presented with a productive cough and a history of recurrent chest infections as a young child. His chest radiograph was abnormal.

Microscopic examination of a Gram-stained smear of his sputum showed moderate to numerous polymorphonuclear leukocytes and short, plump, encapsulated, gram-negative, rod-shaped bacteria. Bacteriologic culture of the specimen on tryptone soya agar (Oxoid, Basingstoke, United Kingdom) containing 5% sheep blood and on chocolate agar (Oxoid) in air supplemented with 5% CO₂ at 35°C yielded a predominance of highly mucoid, coalescing colonies of gram-negative rods, along with *Haemophilus influenzae* and *Streptococcus pneumoniae*. The mucoid gram-negative rods were positive for oxidase and catalase, negative for indole production and urease activity, and inactive in the GC Sugar Set (Oxoid) for glucose, lactose, maltose, and sucrose. The isolate was referred to the Microbiological Diagnostic Unit Public Health Laboratory (MDU) for identification (isolate MDU1744).

**Case 2.** A 54-year-old Aboriginal woman was admitted to the hospital with pneumonia. She had a history of chronic cough and multiple chest infections, including earlier episodes of pneumonia and bronchiectasis, as well as a lung abscess treated 6 years previously. She also had type 1 diabetes mellitus, which was poorly controlled, and a history of alcohol abuse. Six months after this admission, the patient died with lung cancer.

Two specimens of sputa were submitted for bacteriologic investigations. Gram-stained smears of the sputa showed numerous polymorphonuclear leukocytes as well as abundant short, plump, gram-negative rods that showed a tendency to resist decolorization and to stain as gram-positive rods. Moderately to abundant, highly mucoid colonies of gram-negative rods grew on culture of the sputa on Columbia agar (Becton Dickinson, Sparks, Md.) containing 5% horse blood. Growth was promoted by incubation in 5% CO₂. No growth occurred anaerobically or on MacConkey agar with crystal violet (Becton Dickinson). The isolate was nonhemolytic and oxidase positive and did not produce acid from carbohydrates. Antimicrobial susceptibility testing by the Kirby-Bauer disk diffusion method and the National Committee for Clinical Laboratory Standards breakpoint criteria (6) showed that the mucoid gram-negative rod was susceptible to cefalexin, erythromycin, penicillin, tetracycline, and vancomycin. Because the patient was allergic to penicillin and had developed skin sores, she was treated with cefalexin (250 mg, four times per day) for 7 days.

The isolate’s susceptibility to vancomycin, together with the observation of gram-positive rods in the Gram-stained smear of the sputum, suggested an identification of *Bacillus* sp. However, the isolate could not be definitively identified, so it was referred to MDU (isolate MDU7683).

**Case 3.** A 46-year-old Aboriginal man presented with a cough that was associated with the production of copious amounts of purulent sputum. He was a smoker with bronchiectasis who had suffered multiple chest infections through childhood. A computed tomography scan of his lungs showed evidence of bronchiectasis in the bilateral lower lobes and in the right middle lobe.

The Gram-stained smear of his sputum showed moderate to numerous polymorphonuclear leukocytes and encapsulated gram-negative cocobacilli, mainly arranged in pairs. Abundant, highly mucoid colonies were grown in pure culture. The isolate was aerobic, catalase positive, oxidase positive, and indole negative. Antimicrobial susceptibility testing by the Kirby-Bauer method and National Committee for Clinical Laboratory Standards criteria (6) showed susceptibility to ceftazidime, ciprofloxacin, gentamicin, imipenem, piperacillin, and ticarcillin-clavulanic acid. An invalid result was obtained when identification was attempted with the API 20NE system (bioMérieux, Marcy-l’Étoile, France), and the
isolate was forwarded to MDU for identification (isolate MDU4845). On receipt of the isolates at MDU, subcultures were made to plates of Columbia agar (Oxoid) containing 5% horse blood (HBA), nutrient agar (Oxoid nutrient broth no. 2 with 0.3% yeast extract and 1% agar), and MacConkey agar (CM 507; Oxoid). The HBA plates were incubated in air at 35 and 42°C, as well as anaerobically, and in 5% CO₂ at 35°C. All plates were examined after incubation for 24 and 48 h. Motility was determined by the hanging drop method. Conventional tests were used to determine the biochemical reactions of the isolates (17). Acid production from carbohydrates was tested in semisolid cystine trypticase agar (CTA) medium containing a phenol red indicator (Becton Dickinson) with inoculation confined to the upper 0.5-cm layer. DNase activity was tested by floating the cultures grown for 48 h on DNase test agar (Oxoid) with 1 M HCl and watching for clearing of the opaque medium surrounding the colonies as evidence of DNase activity. The production of β-lactamase was detected by the development of a pink color in the chromogenic cephalosporin compound Nitrocefin (Oxoid).

Optimal growth was observed on HBA incubated in 5% carbon dioxide. The highly mucoid, coalescing colonies measured about 2 mm in diameter after 24 h and about 4 mm in diameter after 48 h. No hemolysis was present. Growth on nutrient agar was poor. The isolates did not grow under anaerobic conditions or on MacConkey agar. Gram-stained smears of the colonies showed plump, gram-negative rods and coccobacilli, often arranged in pairs and occasionally in short chains. Some of the rods seen in the Gram-stained smears of the isolate MDU1744 stained as gram-positive rods. The rods were nonmotile. The biochemical characteristics of the three isolates were identical and are given in Table 1. The microscopic morphology, growth characteristics, and biochemical reactions of the three isolates strongly indicated that they were all nitrate-negative biovars of the mucoid phenotype of Moraxella nonliquefaciens (14, 15, 17). Identification of the isolates was confirmed by 16S rRNA gene sequencing. A BLAST database search (1) of the 1,451 nucleotides sequenced found ≥99.6% identity to 12 published M. nonliquefaciens sequences. The sequences of the three isolates were found to be identical to one another, except for a base ambiguity at one position in the isolate MDU1744. This is probably due to the presence of two alleles for this gene (9). The isolate MDU4845 produced β-lactamase.

No reports on the isolation of mucoid M. nonliquefaciens have been published in recent decades. In 1951, the isolation of a mucoid strain of M. nonliquefaciens was reported from the sputum of a 55-year-old man with bronchitis and bronchopneumonia (5). Subsequently, a mucoid strain was isolated from a 76-year-old man with chronic bronchitis (10); and in 1968, the year of the last reports, two mucoid strains were isolated from an 84-year-old man and a 61-year-old man, both of whom suffered from chronic bronchitis and emphysema (3).

The isolation of two mucoid strains from asymptomatic, healthy carriers was reported in 1955, along with that of a non-mucoid strain of M. nonliquefaciens from a 73-year-old woman with bronchopneumonia and chronic bronchitis (7). Nonmucoid M. nonliquefaciens was later reported from both the tracheal aspirate and the sputum from a 78-year-old man with pneumonitis and pulmonary abscess (13). Several of the early investigators showed that when tested in mice, only the mucoid strains of M. nonliquefaciens caused death of the mice (3, 5, 7). It is apparent that M. nonliquefaciens, whether mucoid or non-mucoid, has only rarely been implicated as the causative agent of lower respiratory tract disease, usually chronic disease.

Our mucoid strains differed from those reported earlier in that our strains did not reduce nitrate. Only 5% of the 243 isolates of M. nonliquefaciens sent to the Centers for Disease Control and Prevention for identification were found to be negative for nitrate reduction (17). Susceptibility to vancomycin and a tendency to resist decolorization in the Gram stain, noted for isolate MDU7683, have both been reported for Moraxella spp. (2, 17). The isolate MDU4845 showed a further unusual characteristic in its ability to produce β-lactamase.

All three strains of mucoid, nitrate-negative M. nonliquefaciens were isolated from Australian Aborigines living in the northern, tropical areas of Australia in the Northern Territory (two strains) and North Queensland (one strain). That these pulmonary infections were detected only in indigenous Australians from restricted geographic areas probably simply reflects the population distribution of Aboriginal and Torres Strait Islander Australians in the states and territories and the heavy burden of respiratory illnesses borne by indigenous communities. According to the Australian Bureau of Statistics (http://abs.gov.au), analyses of the census data collected on 7 August 2001 indicate that indigenous Australians comprise only 2.2% of the total Australian population but 3.1% of the Queensland population and as high as 25.1% of the population of the Northern Territory. Bronchiectasis was diagnosed in two of our three patients. The morbidity rate for bronchiectasis in Aboriginal children (under 15 years of age) in remote communities has been described recently as “unacceptably high,” with 1 to 2% of the children showing symptoms of this progressive, chronic disease (4). Furthermore, respiratory illnesses are the second-highest cause of death among the adult indigenous population (4). Despite a significant fall in death rates from pneumonia between 1996 and 2002 attributable to the implementation of pneumococcal and influenza vaccination pro-
grams, the mortality rate from respiratory illnesses overall in indigenous Australians is still four times as high as it is for the rest of the Australian population (12).

Prominent among the mucoid microorganisms associated with infections of the lower respiratory tract is mucoid Pseudomonas aeruginosa, the causative agent of a chronic lung disease that is characteristic of, but not restricted to, cystic fibrosis. The extracellular polysaccharide produced by the mucoid phenotype is an alginate that has been shown to protect against phagocytosis. Moreover, the host’s immune response to the alginate antigens results in the production of nonopsonic antibodies that cannot mediate phagocytosis (8). Although the extracellular polysaccharide produced by mucoid M. nonliquefaciens differs in chemical composition from the alginate of M. nonliquefaciens, it presumably also serves to protect against phagocytosis. More recent studies indicate that mucoid P. aeruginosa uses extracellular quorum-sensing signals to coordinate the formation of biofilms on lung surfaces (16). Biofilms offer further protection to alginate-embedded bacteria from the host defense mechanisms as well as significant protection from the action of antibiotics (8). Whether mucoid M. nonliquefaciens forms a biofilm on lung surfaces is obviously unknown, but the ability to form such biofilms might be a common characteristic of the mucoid microorganisms that are associated with chronic lung disease.

The phenotypic identification of these mucoid strains of M. nonliquefaciens was complicated by their failure to reduce nitrate, and definitive identification included confirmation by 16S rRNA gene sequencing. This is apparently the first report of the isolation of the mucoid phenotype of M. nonliquefaciens from patients having chronic lung disease for more than three decades and the first report ever of the isolation of its nitrate-negative biovar from such patients.

**Nucleotide sequence accession numbers.** The 16S rRNA gene sequences of the isolates have been deposited in the GenBank sequence database under the accession numbers AY526574 (MDU1744), AY526575 (MDU7683), and AY526576 (MDU4845).

**REFERENCES**