Molecular epidemiological characterization of respiratory isolates of *Moraxella catarrhalis* in a pediatric intensive care unit

ANNE G MATLOW, MD, FRCPC, DONALD E LOW, MD, FRCPC, GIDEON PARET, MD, SCOTT JARRETT, DESMOND BOHN, MB, BCH, FRCPC, GEOFFREY BARKER, MB, BS, FFARACS, JEAN BOULANGER, MD, FRCPC, E LEE FORD-JONES, MD, FRCPC

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A perceived increase in the number of isolates of *Moraxella catarrhalis* from the respiratory secretions of patients intubated in the pediatric intensive care unit prompted a review of the clinical profiles of such patients and restriction enzyme analysis of the strains involved. Over two months, of 192 patients admitted to the unit, 154 were intubated. Of the 46 for whom endotracheal tube specimens were submitted to the laboratory, *M catarrhalis* was isolated in 12. *M catarrhalis* was not felt to be a significant respiratory pathogen by the attending medical staff in any of the patients from whom it was isolated. In only two patients (17%) could nosocomial acquisition be firmly invoked. Restriction enzyme analysis of the 12 strains ruled out the presence of an epidemic strain. Isolation of *M catarrhalis* from intubated children does not necessarily imply pathogenicity nor an outbreak situation.

Key Words: Epidemiology, *Moraxella catarrhalis*, Pediatrics, Respiratory infection

Caractérisation moléculaire épidémiologique d'isolats de *Moraxella catarrhalis* dans une unité de soins intensifs pédiatriques

RÉSUMÉ: L'observation d'une augmentation du nombre d'isolats de *Moraxella catarrhalis* dans les sécrétions de patients intubés à l'unité des soins intensifs a justifié une synthèse du profil clinique de ces patients et une analyse des enzymes de restriction en jeu. Sur une période de deux mois, des 192 patients admis à l'unité, 154 furent intubés. Des 46 patients dont des spécimens de sécrétions endotrachéales ont été envoyés au laboratoire, 12 contenaient *M catarrhalis*. Ce dernier ne fut pas considéré important comme pathogène respiratoire chez les patients porteurs par le personnel médical en devoir. Chez deux patients seulement, la transmission nosocomiale a pu être attestée. L'analyse des enzymes de restriction pour les douze souches a permis d'écarter la possibilité d'une souche épidémique. L'isolement de *M catarrhalis* chez des enfants intubés ne signifie donc pas automatiquement qu'il y ait pathogénicité ou épidémie.

Department of Microbiology and Pediatrics, The Hospital for Sick Children; and the Department of Microbiology, Mount Sinai Hospital, The University of Toronto, Toronto, Ontario

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Correspondence and reprints: Dr Anne Matlow, Department of Microbiology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8

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MORAXELLA CATARRHALIS IS A RESPIRATORY COMMENSAL in children (1) and can cause both upper and lower respiratory tract disease (2). Recent reports in both adult and pediatric literature have indicated that infection with this organism can be nosocomially acquired (3-5). A perception that there was an increased number of isolates of *M catarrhalis* from the respiratory secretions of patients intubated in the pediatric intensive care unit prompted a review of the clinical profile of patients from whom the organism was isolated, as well as restriction enzyme analysis of the strains involved.

PATIENTS AND METHODS

Patient group and bacteriological methods: The Hospital for Sick Children is a 550-bed tertiary care pediatric institution with a 19-bed pediatric intensive care unit. Between November 1989 and January 1990 all respiratory samples submitted to the microbiology laboratory from patients intubated in the pediatric intensive care unit were investigated for the presence of *M catarrhalis*. Gram stain was performed on all specimens, which were then plated on horse blood, chocolate and bile salts agar, and incubated at 37°C in 5% carbon dioxide for 24 h. Predominant flora was identified by routine microbiological methods (6). Colonies compatible with *M catarrhalis* were identified using an oxidase test and quad-Ferm (Analytab Products, New York). Beta-lactamase testing was performed by the chromogenic cephalosporin method (Cefinase; BBL Microbiology Systems, Maryland). Respiratory samples were also submitted for viral studies when clinically indicated.

After the designated two month study period, a chart review was done on all patients from whom *M catarrhalis* had been isolated, and clinical and demographic criteria were recorded. Patients were considered to have acquired *M catarrhalis* nosocomially if the first respiratory sample growing this organism was taken three days or more after admission and a previous negative culture had been obtained; community-acquired *M catarrhalis* was inferred if the culture was positive within three days of admission. Acquisition of *M catarrhalis* was said to be indeterminate if the first sample collected from the patient grew *M catarrhalis* yet was taken after three days of hospitalization.

VIROLOGICAL STUDIES

Patient specimens consisted of nasopharyngeal swabs placed in phosphate buffered saline containing 1.5% gelatin and gentamicin for transportation to the laboratory. The specimens were agitated by a vortex mixer, and pelleted by centrifugation, and the pellets were resuspended in approximately 25 to 50 µL of the residual solution and applied as 5 µL spots onto a masked glass slide (Shandon Inc, Pennsylvania). The remainder of the cell pellets was combined with the supernatant. Of this, 0.75 mL was used for the diag-

nosis of respiratory syncytial virus (RSV) by the RSV 'Testpack' (Abbott Laboratories, Illinois), and the remainder was inoculated into cell cultures of primary monkey kidney cells and a line of human kidney epithelioid cells (293 cells). The slide was air dried and fixed in cold acetone. The respective wells were stained with monoclonal antibodies to RSV, parainfluenza viruses 1, 2 and 3, and influenza viruses A and B. The slides were read by immunofluorescence microscopy.

Restriction endonuclease analysis: The strains of *M catarrhalis* isolated during the long term prevalence study were subjected to restriction enzyme analysis. Total genomic DNA was extracted using standard techniques (7). Restriction enzyme analysis of chromosomal fragments was performed by determining the digest patterns produced by *Hind* III, *Pst* I and *Hae* III according to the manufacturer's instructions (Boehringer-Mannheim Biochemicals, Indiana). Samples were electrophoresed on a horizontal 0.7% agarose gel in Tris-EDTA buffer at 30 V for 16 h. Gels were stained with 0.5 mg/mL ethidium bromide and photographed through a red filter on Polaroid 667 film (Polaroid, Massachusetts). A DNA marker consisting of a *Hind* III digest of lambda phage DNA was applied to the first well of each gel so that the different gels could be compared to each other. Two isolates were considered to be the same strain if discrete DNA restriction bands were identical. Because of the difficulty of comparing strains to each other when separated by too many isolates (usually more than four), especially when each has a different restriction endonuclease pattern, the authors ran only six isolates per gel. When it was not absolutely clear that two strains were not identical because they were separated by too many isolates or run on a different gel, they were then run side by side on the same gel.

RESULTS

During the two month study period, 192 patients were hospitalized in the pediatric intensive care unit and of these 154 were intubated and 136 ventilated. Endotracheal tube specimens were submitted to the laboratory as part of routine care for 46 patients; of these, *M catarrhalis* was isolated from 12 patients, for a period prevalence rate of 26% in intubated patients sampled. The details of the patients are outlined in Table 1. The mean age of the patients involved was 23.4 months. Seven isolates of *M catarrhalis* (58%) were considered community-acquired; two (17%) were considered nosocomial; and three (25%) were indeterminate. All patients from whom *M catarrhalis* was nosocomially-acquired or considered indeterminate had had prior surgery. Both patients with nosocomially-acquired *M catarrhalis*, and two of the three patients with indeterminate acquisition, had polymicrobial respiratory flora isolated from the same respiratory specimen. Eighty-six per cent of patients (six of seven) with com-

TABLE 1
Characteristics of patients with *Moraxella catarrhalis* in respiratory secretions

Patient	Age	Underlying diagnosis	Previous surgery	Hospital day of isolation (acquisition)	Chest x-ray	Gram stain	Other significant bacteria isolated from secretions
1	11.5 months	Subglottic stenosis	Yes	8 (nosocomial)	Right upper lobe atelectasis	PMN++ GPC	<i>Pseudomonas aeruginosa</i>
2	9.5 years	Trauma	Yes	2 (community)	Left upper lobe atelectasis	PMN+	None
3	2 months	Bronchiolitis, respiratory syncytial virus	No	1 (community)	Perihilar density	PMN+ GNC+++	None
4	4 months	Bronchiolitis	No	2 (community)	Right upper lobe density	PMN+ GNR+ GNC++ GPC++	None
5	27 months	Ventricular septal defect repair	Yes	5 (intermediate)	Bilateral atelectasis	PMN+++	None
6	13 months	Transposition of the great arteries, adenovirus	Yes	4 (intermediate)	Left upper lobe density	GNC+++	<i>Haemophilus influenzae</i> <i>Streptococcus pneumoniae</i>
7	5.1 years	Congenital heart disease	Yes	6 (intermediate)	Atelectasis	PMN+ GNB+++ GPC++	<i>Staphylococcus aureus</i>
8	13 weeks	Smoke inhalation	No	1 (community)	Edema	PMN+ GPC+++	<i>Staphylococcus aureus</i> <i>Streptococcus pneumoniae</i>
9	13 weeks	Croup	No	3 (community)	Edema	GNC+++ PMN+	<i>Haemophilus influenzae</i> <i>Staphylococcus aureus</i>
10	22 months	Croup, parainfluenza virus type 1	No	1 (community)	Normal	No PMN GNC++	None
11	5 weeks	Bronchiolitis, respiratory syncytial virus	No	6 (nosocomial)	Perihilar infiltrates	PMN++ GPC++	<i>Haemophilus influenzae</i>
12	29 weeks	Croup, parainfluenza type 1	No	3 (community)	Atelectasis	PMN++ GNC+ GNB+	<i>Streptococcus pneumoniae</i>

GNB Gram-negative bacilli, GNC Gram-negative cocci; GNR Gram-negative rods; GPC Gram-positive cocci; PMN Polymorphonuclear leukocytes

munity-acquired *M catarrhalis* had admitting diagnoses of croup or bronchiolitis, and 71% of isolates (five of seven) were unimicrobial. A viral diagnosis was made within a week of *M catarrhalis* isolation in five of 11 patients studied; in four of these *M catarrhalis* was classified as community-acquired. *M catarrhalis* was not felt to be a significant respiratory pathogen by the attending medical staff in any of the patients from whom it was isolated.

Bacteriological testing showed that all strains of *M catarrhalis* were beta-lactamase-producing. Restriction enzyme analysis demonstrated that no strains were identical. Each enzyme was able to demonstrate unrelatedness.

DISCUSSION

M catarrhalis is becoming increasingly recognized as a causal agent in various respiratory (including otitis media, sinusitis and pneumonia) and nonrespiratory (including bacteremia and meningitis) infections in

children (2). Despite reports which expand current knowledge of the pathogenic potential of this agent, limited information exists about the prevalence of this organism in the respiratory tract of normal children and adults in a community or hospital setting. In general, 10 to 36% of children are reported to harbour this organism (2).

Recent attention has focused on the role of *M catarrhalis* as a nosocomial pathogen. Although implicated previously (8), the most comprehensive study in this regard described eight cases of nosocomial *M catarrhalis* infection in adult patients in an intermediate care unit (3). Respiratory therapy, steroid use and location within the unit were found by case-control study to be risk factors for colonization or infection with this organism. Restriction enzyme analysis using three enzymes demonstrated that the strains of *M catarrhalis* carried by four of eight symptomatic patients, two symptomatic staff and one of 19 asymptomatic patients, were identical.

The role of *M catarrhalis* in nosocomial pediatric infections has been less certain. Haddad *et al* (9) reported two cases of bronchopulmonary infection in premature neonates hospitalized within the same cubicle of the neonatal care unit, and felt that the most likely source of infection was a culture positive nurse. No method of strain identification was performed. More recently, Cook *et al* (5) reported two cases of nosocomially-acquired pneumonia and nine cases of bronchitis associated with *M catarrhalis* in intubated patients in a pediatric intensive care unit. Plasmid profile analysis was not helpful in showing strain homogeneity.

The occurrence of bronchopulmonary infection due to *M catarrhalis* in children ventilated in a pediatric intensive care unit has previously been described (10, 11). Kasian *et al* (11) noted that *M catarrhalis* was more likely to be significant if cultured from respiratory secretions from ventilated pediatric intensive care unit patients when isolated in pure culture.

The finding in the present prospective surveillance study that a number of intubated patients had *M catarrhalis* isolated from their respiratory secretions raised the concern regarding nosocomial transmission of one or more strains. On review of the clinical characteristics of patients from whom the organism was isolated, it was apparent that in only two patients (17%) could nosocomial acquisition be firmly invoked. Furthermore, restriction enzyme analysis of the 12 strains ruled out the presence of an epidemic strain. Extreme genetic diversity within the genus was suggested by the finding of 12 different restriction patterns in all isolates studied.

This study confirms the usefulness of restriction enzyme analysis in the assessment of hospital strains of *M catarrhalis*. *M catarrhalis* may be a frequent respiratory isolate from intubated children (26% of samples tested using nonselective media). Isolation of *M catarrhalis* in this clinical setting does not necessarily imply pathogenicity, nor does it imply an outbreak situation; clinical assessment, as well as molecular studies when there are clusters of infection, may be necessary to clarify the implications of isolation of this organism.

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