

Isolation of *Moraxella catarrhalis* from sputum specimens of Malaysian patients

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Abstract

Moraxella catarrhalis has gained reputation as a pathogen in the lower respiratory tract especially in patients with underlying chronic lung diseases. It is considered significant when isolated from sputum specimens of adults with respiratory tract infections. A study was carried out to determine the prevalence of *Moraxella catarrhalis* isolated in sputum specimens and beta-lactamase production of these isolates.

Sputum specimens sent to the Bacteriology division, Institute for Medical Research from April 1990 until April 1993 were screened for *Moraxella catarrhalis*. A total of 1678 sputum specimens were processed and *Moraxella catarrhalis* was isolated from 15 (0.89%) of the sputum specimens. Six out of 15 (40%) were isolated from patients with chronic lung disease. Eight out of 15 (47%) were beta-lactamase producers.

Moraxella catarrhalis isolated in good-quality sputum must not be disregarded and should be looked for especially in patients with chronic obstructive pulmonary disease. Beta-lactamase production should be tested on all isolates so that appropriate treatment can be given. All the isolates in this study were sensitive to cotrimoxazole.

Key words: Chronic obstructive pulmonary disease, beta-lactamase producers, respiratory pathogen.

INTRODUCTION

Moraxella catarrhalis formerly known as *Micrococcus catarrhalis*, *Neisseria catarrhalis* and later *Branhamella catarrhalis* was first isolated in 1896 and was thought to be a harmless normal flora of human upper respiratory tract for the subsequent 50 years. However only in the past decade or so its role as a disease causing pathogen in humans has been reported and described.¹⁻³

It is the third most common pathogen isolated in childhood acute maxillary sinusitis and acute otitis media.¹ It has also been implicated as a cause of urethritis,² septicaemia,¹ conjunctivitis,⁴ empyema,⁵ meningitis and endocarditis.¹

In adults it is commonly associated with lower respiratory tract infections. Patients who are at particular risk of developing infections with this organism are those with underlying cardiopulmonary disease, chronic obstructive pulmonary disease, asthmatic and immunocompromised patients.^{6,7} Viral damage to respiratory tract epithelium may also promote invasion by *Moraxella catarrhalis*.

Moraxella catarrhalis may produce beta-lactamase especially in clinically significant isolates. Many treatment failures with ampicillin or amoxycillin are due to the production of this enzyme.

There has been no previous report on the isolation of *Moraxella catarrhalis* from clinical specimens in Malaysia. Its growth on primary plates of sputum cultures may have been regarded as part of normal flora because of its similar morphology to the *Neisseria* species and also the lack of awareness of its role as a pathogen of the lower respiratory tract.

The aim of this study is to determine the prevalence of *Moraxella catarrhalis* isolated in sputum specimens, the biochemical characteristics of the isolates, the beta-lactamase production and the antibiotic susceptibility of the isolates to some commonly used drugs.

METHODS

Sputum specimens sent to the diagnostic section of the Bacteriology Division, Institute for Medical Research from District hospitals of Bentong, Raub, Kuala Lipis, Kuala Kubu Bharu, National Leprosy Control Centre and Gombak from April 1990 until April 1993 were included in this study. Sputum specimens received from general practitioners involved in a community acquired infections project during the same duration of study were also included.

Gram-staining was carried out on sputum specimens collected within 24 hours in sterile containers. Only samples whose Gram stain

showed the specimen to be of good quality (pus cells greater than 25 and epithelial cells less than 10 per low power field) were screened for *Moraxella catarrhalis*.

Blood agar (Blood agar base no.2 supplemented with 10% ox blood), and chocolate agar (Blood agar base No.2 with heated blood) were used. The sputum specimens were inoculated and streaked on each agar plate and incubated in 5% carbon dioxide at 37°C for 18 to 24 hours.

The plates were then examined for growth and typical colonial morphology. Any associated growth were also noted down. The growth on the agar plate was semiquantitated according to its primary inoculum, second, third and fourth streak as I+ (scanty), 2+ (few), 3+ (moderate) and 4+ (heavy) respectively. Gram staining was carried out to determine the morphology of the isolates.

Biochemical tests were carried out for identification of *Moraxella catarrhalis*. Catalase and oxidase activity was determined following the method of Cowan and Steel.¹ Deoxyribonuclease production was tested by streaking a DNAase agar with the suspected organism including a positive and negative control. A clear zone around the colonies after flooding the plate with 1N hydrochloric acid was considered positive. The ability to reduce nitrate was tested by heavily inoculating the organisms into 1 ml of nutrient broth containing 0.1% potassium nitrate. Sulfanilic acid and alpha-naphthhlamine were added after incubating at 37°C for 2 hours. A red colour indicated positive reaction. Carbohydrate utilising tests were determined using conventional cystine – tryptic digest semisolid agar – base (CTA) medium containing 1% carbohydrate and a phenol red indicator. The 1% carbohydrates are glucose, maltose, sucrose and lactose. A carbohydrate free CTA control was included.

Beta lactamase production was detected using a chromogenic cephalosporin (nitrocefin) substrate.

Antibiotic susceptibility tests were conducted using agar disc diffusion test following the NCCLS method.¹ The discs used contained ampicillin 10 µg/l, tetracycline 30 pg/l, cotrimoxazole (1.25 pg trimethoprim, 23.75 pg sulfamethoxazole), erythromycin 15 pg/l, ciprofloxacin 5 µg/l, amoxicillin-clavulanic acid (20 µg amoxicillin, 10µg clavulanic acid) and sulbactam-ampicillin (sulbactam 10 pg/l, ampicillin 10 pg/l).

For every positive isolate, a questionnaire was filled to determine the clinical presentation,

relevant medical history and clinical findings of the patient.

Other respiratory pathogens like viruses, mycoplasma and chlamydia were not looked for.

RESULTS

A total of 1678 good quality sputum specimens were processed and screened for *Moraxella catarrhalis* in our diagnostic section during the three year period. *Moraxella catarrhalis* was isolated from 15 (0.89%) of the sputum specimens.

Moraxella catarrhalis was observed to grow well on blood agar and chocolate agar. Typical colonies were usually gray to white, opaque and smooth and slides across the surface of the agar when nudged with the end of a bacteriological loop (hockey puck on ice).¹ Growth of *Moraxella catarrhalis* on the agar plates was observed to be 4+ (5 cases), 3+ (6 cases) and 2+ (4 cases). The other isolates growing together were mainly normal flora and the growth was only few (2+). *Moraxella catarrhalis* was observed to be the sole pathogen except in one case where it was isolated with *Mycobacterium tuberculosis*. The biochemical tests results are summarized in Table 1.

The ages of the patients ranged from 3 years to 81 years. Out of the fifteen cases studied, only four were outpatients, the remaining eleven were inpatients. All patients had cough with purulent sputum as their major complaint. Not every patient presented with fever. Ten out of fifteen presented with fever while five did not complain of any febrile episodes. Nine patients were noted to have underlying illnesses. Out of these, 6 patients have chronic lung diseases. Among those

Table 1: Characteristics of *Moraxella catarrhalis* used in its identification.

Colonial morphology on	
ox blood agar	: white, opaque, smooth
Chocolate agar	: growth
Nutrient agar	: growth
Gram stain	: gram-negative diplococci
Oxidase	: positive
Catalase	: positive
Deoxyribonuclease	: positive
Reduction of nitrate	: positive
Utilisation of CTA sugars	
glucose	: negative
maltose	: negative
sucrose	: negative
lactose	: negative

with chronic lung diseases, one patient had acid fast bacilli (>50/length) seen in the sputum (*Mycobacterium tuberculosis* was later isolated). A 73-year-old patient had bronchiectasis with history of pulmonary tuberculosis 10 years ago. Three patients aged between 68 to 81 years old had underlying chronic obstructive airways disease and were admitted with acute exacerbation. One patient was admitted for acute exacerbation of bronchial asthma. His sputum yielded a heavy growth of *Moraxella catarrhalis* with few normal flora only. Two patients had ischaemic heart disease and one of them also had diabetes mellitus. A 74-year-old man who presented with fever and cough had *Moraxella catarrhalis* isolated from his sputum and was found to have carcinoma of the prostate during his stay in the ward.

Eight of the isolates were beta-lactamase producers and 7 were non beta-lactamase producers. Two of the beta-lactamase producers were isolated as pure growths from the sputum. Another two of the beta-lactamase producers were isolated from two patients who were treated with ampicillin.

All isolates were noted to be sensitive to cotrimoxazole. The isolates tested against tetracycline, erythromycin, ciprofloxacin, amoxicillin-clavulanic acid, sulbactam-ampicillin were found to be all sensitive to these drugs.

DISCUSSION

The pathogenic role of *Moraxella catarrhalis* in lower respiratory tract infections was first suggested by Ninane *et al.*" Its isolation from sites normally considered sterile support its pathogenic role. Transtracheal aspiration done to avoid contamination with oropharyngeal commensals is considered invasive, time consuming and inconvenient. Comparable results of isolating *Moraxella catarrhalis* from transtracheal and sputum specimen have been demonstrated by Aitken *et al* and Thornley *et al.*^{12,13} The method of following Gram stain directed culture can identify *Moraxella catarrhalis* with approximately 90% accuracy and has provided a more convenient method of looking for this species." In our patients we processed sputum specimens based on Gram stain findings which can be done in any routine diagnostic bacteriology laboratory.

The organism is more frequently found in specimens judged to represent lower respiratory tract secretions in adults with respiratory tract

disease than in those specimens determined to have large amounts of oropharyngeal contamination. Studies done by Vaneechoutte *et al* showed that *Moraxella catarrhalis* could only be isolated from 1 out of 193 poor quality sputum samples when non-selective medium was used." Schonheyder and Ejlertson also reported that the presence of *Moraxella catarrhalis* is significantly higher in sputum samples of good quality (8.1%) when compared with sputum samples of poor quality (2.7%).¹⁵

Moraxella catarrhalis was noted to grow easily on blood agar and chocolate agar. The biochemical tests are easy to perform and thus the isolation of this organism can be done easily in any bacteriology diagnostic laboratory. Because of its similar colonial appearance to *Neisseria* species, any suspected growth should be tested for deoxyribonuclease production. If the test is positive, we should proceed to do the other biochemical tests.

The majority of the isolates were recovered from hospital inpatients. Nine patients have underlying conditions of whom 6 had underlying chronic lung disease ranging from bronchial asthma, pulmonary tuberculosis, post pulmonary tuberculosis with bronchiectasis and chronic obstructive airways disease.' This supports the observation that patients with chronic lung disease are at risk of developing infection with *Moraxella catarrhalis*. Immunologic abnormalities due to underlying diseases such as diabetes or alcoholism are also important contributory factors. Only one of our patients had diabetes mellitus and none were alcoholics.

Three of our patients with chronic obstructive airways disease came with shortness of breath and productive cough a few days prior to presentation. *Moraxella catarrhalis* is an important cause of exacerbations of chronic obstructive airways disease and is generally accepted to be the third most common cause of exacerbations of COPD after *Haemophilus influenzae* and *Streptococcus pneumoniae*.¹⁶

All cases presented with symptoms of respiratory disease. All the isolates were considered aetiologically significant based on clinical presentation and gram stain screening. One 9-year-old boy had a pure growth of the organism isolated from his sputum. Even though studies have shown that *Moraxella catarrhalis* can be isolated from the upper respiratory tract of 50.8% of children,¹⁴ the isolation of a pure growth of *Moraxella catarrhalis* from the 9-year-old boy was considered significant because his clinical and chest x-ray findings were

suggestive of pneumonia which resolved after he was treated with cotrimoxazole.

Eight of the isolates were noted to be beta-lactamase producers. All the beta-lactamase producers were resistant to ampicillin. Two of the beta-lactamase producing isolates were recovered from patients initially treated with ampicillin. Their symptoms improved after treatment was changed to cotrimoxazole. It is important to test for beta-lactamase in all significant isolates as it will help the physicians to choose the right antibiotic treatment. Studies have shown that beta-lactamase producing *Moraxella catarrhalis* have increasingly been isolated as clinically significant isolates. Prior to 1977 only 4% of *Moraxella catarrhalis* were resistant to penicillin^{11,17} but in 1985 studies done by Alvarez *et al* showed that 86.7% of a sample of 53 strains elaborated beta-lactamase and this trend seems to be increasing."

All the isolates were found to be sensitive to cotrimoxazole. The beta-lactamase producers were all resistant to ampicillin. Our isolates tested against erythromycin and tetracycline were sensitive to these drugs. Resistance to erythromycin and tetracycline have been reported in the United States.¹⁹ Isolates tested against amoxicillin-clavulanic acid and sulbactam-ampicillin were also found to be sensitive. In-vitro, all beta-lactamases produced by *Moraxella catarrhalis* were found to be highly susceptible to clavulanic acid and its combination with amoxicillin helps to prevent the breakdown of the beta-lactam ring in amoxicillin." In Malaysia, ampicillin and amoxicillin being are extensively used as first line therapy for acute exacerbations of chronic bronchitis but with the increasing proportion of beta-lactamase producing strains of *Moraxella catarrhalis*, the clinical response to ampicillin therapy should be monitored carefully. If the symptoms do not improve, bacteriological examination of the sputum should ideally be done.

In this group of patients the bacteria isolated with *Moraxella catarrhalis* were normal flora. Mixed growths of this organism with other isolates such as *Haemophilus influenzae* and/or *Streptococcus pneumoniae* can occur. Beta-lactamase producing *Moraxella catarrhalis* can also act as an indirect pathogen by protecting the pathogenic bacteria with the release of beta-lactamase.²⁰ Beta-lactamase production has become extremely common and *Moraxella catarrhalis* has been recognised as a nosocomial pathogen capable of causing an outbreak of lower respiratory tract infections in a population of

hospitalised veterans.²¹

In conclusion, *Moraxella catarrhalis* is considered clinically significant when isolated from good quality sputum specimens collected from patients who present with clinical signs and symptoms suggestive of lower respiratory tract infection and whose microscopic examination of sputum show predominant intracellular gram-negative diplococci. *Moraxella catarrhalis* should be looked for especially in adults with chronic obstructive pulmonary disease. It can be isolated in any routine bacteriology division using non-selective media and basic biochemical tests. Beta-lactamase producers have been increasingly isolated from clinical infections. Therefore it is necessary to look for beta-lactamase production in all isolates as it will affect the type of antibiotic chosen for therapy.

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