Isolation of Moraxella catarrhalis in patients at King Khalid University Hospital, Riyadh

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ABSTRACT

Objectives: A retrospective study was carried out to assess the clinical significance of *Moraxella catarrhalis (M. catarrhalis)* isolated from 32 specimens received from patients seen during a 2 year period.

Methods: The identity of isolates was confirmed by DNAse production and reduction of nitrate to nitrite. Susceptibility testing and ß-lactamase production was carried out for each isolate.

Results: Twenty three of the patients were adults and 9 were children. Twelve (37%) of the isolates were from the sputum of patients aged more than 50 years with a clinical diagnosis of pneumonia, bronchitis or bronchiactesis. Six (18%) had *M. catarrhalis* isolated from sputum and had underlying cardiac, liver diseases or diabetes mellitus. The organism was isolated from the blood of one patient with pneumonia and one with leukaemia. It was also isolated from patients with sinusitis, conjunctivitis or otitis media. Twenty seven (84%) of the 32 strains produced ß-lactamase, resistance to erythromycin and clindamycin was detected in 13% of the isolates. All isolates were susceptible to ciprofloxacin, tetracycline, trimethoprim-sulfamethoxazle, gentamicin, chloramphenicol, polymyxin B and neomycin.

Conclusion: This study showed that *M. catarrhalis* can be an important respiratory tract pathogen in adults and children, able to invade the blood stream of patients with predisposing respiratory conditions and underlying systemic illnesses, as well as immunocompetent patients. Since most strains produce ß-lactamase, antibiotic therapy should be guided by *in-vitro* susceptibility tests.

Keywords: *Moraxella catarrhalis*, bronchopulmonary infection, extrapulmonary infections.

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Moraxella (Branhamella) catarrhalis is now accepted as the third most common respiratory tract pathogen after *Streptococcus pneumoniae (S. pneumoniae)* and *Hemophilus influenzae (H. influenzae).*[1,2] The bacterium colonizes the human respiratory tract and occasionally the genital tract.[2-4] Colonization of the upper respiratory tract is influenced by age and viral or mycoplasmal respiratory tract infections.[5] Nasopharyngeal colonization happens in 66% of children by the age of one year and in 77.5% by the age of 2 years.[6] In contrast, only 1 - 5% of healthy adults are colonized by *M. catarrhalis*.[2]

However in one study *M. catarrhalis* was recovered from the sputum of 83% of adult patients with chronic lung disease.[7] *M. catarrhalis* causes otitis media in infants and children, and bacterial tracheitis, sinusitis and atypical pseudocroup in preschool children.[5,8] It also causes conjunctivitis in infants and adults.[1] In adults *M. catarrhalis* has been associated with exacerbations of chronic obstructive pulmonary disease (COPD), pneumonia and nosocomial respiratory tract infections.[2,9] Invasive diseases due to *M. catarrhalis* are relatively

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uncommon but there is good evidence that the organism has been an occasional cause of bacteraemia, purulent pericarditis, endocarditis and meningitis.[1,8,10-12]

Since 1998, we noticed an increased rate of isolation of *M. catarrhalis* from different specimens submitted to the microbiology laboratory of King Khalid University Hospital (KKUH). Most of those specimens were from patients with underlying diseases like diabetes mellitus, cardiac, liver or renal impairment.

To assess the clinical significance of *M. catarrhalis*, we did a retrospective study of all *M. catarrhalis* positive specimens received at (KKUH) microbiology laboratory between July 1998 and December 1999.

Methods. The study included 32 specimens received from inpatients and outpatients from which *M. catarrhalis* was isolated during the above specified period. Relevant patient's clinical data were obtained from the patients' case notes. Gram stain results of sputum including abundance of leucocytes (>25 leucocytes/HPF) and the presence of large numbers of Gram negative diplococci were recorded. Suspected colonies of *M. catarrhalis* were grown on blood and chocolate agar plates and incubated overnight aerobically at 37°C with 5% CO_2 as well as at room temperature. *M. catarrhalis* isolates were identified according to colonial appearance, Gram staining, catalase, oxidase reactions, reduction of nitrate to nitrate, ability to grow on nutrient agar at room temperature and DNAse production. All isolates were tested for ß-lactamase production using chromogenic cephalosporin (Nitrocofin - Oxoid BR 66, England).

Susceptibility of *M. catarrhalis* to antimicrobial agents was determined by a disc diffusion method on chocolate agar. Antibiotics tested included: penicillin, ampicillin, augmentin, erythromycin, clindamycin, tetracycline, cefaclor, cefuroxime, ceftriaxone, ciprofloxacin and trimethoprim-sulfamethoxazole (Mast diagnostics. Bootle, Merseyside L20 IEA, UK). For isolates from eye and ear specimens topical agents including gentamicin, chloramphenicol, polymxin B and neomycin were also tested. (Mast diagnostics. Bootle, Merseyside L20 IEA, UK). Other pathogens isolated were also tested for their antibacterial susceptibility.

Results. During the two years of the study *M. catarrhalis* was isolated from 32 patients, 21 females and 11 males. Twenty three of the patients were adults with a mean age of 55 years (range 16-97 years). The rest were children (number = 9) with a mean age of 2 years (range 15 days - 6 years). Sixty three percent of the specimens were submitted between September and November. Nineteen organisms were from outpatients and 13 from inpatients.

Table 1 presents clinical data of patients with *M. catarrhalis* isolated. The majority were patients with upper respiratory tract infections. Table 2 presents laboratory finding in specimens from where *M. catarrhalis* was isolated. Gram staining of endotracheal tube specimens revealed neither leucocytes nor gram negative diplococci, but the cultures showed mixed growth of *M. catarrhalis* and *Pseudomonas aeruginosa*. All isolates of *M. catarrhalis* were oxidase, catalase and DNAse positive and all reduced nitrate to nitrite.

While only 27 (84%) of isolates produced ß-lactamase, all isolates were sensitive to augmentin, cefaclor, cefuroxime, ceftriaxone, ciprofloxacin, tetracycline and chloramphenicol, trimethoprim-sulfamethoxazole, gentamicin and polymxin B. While 5 (16%) of isolates were sensitive to penicillin

Table 1 - Clinical data of patients with *M. catarrhalis* isolated at KKUH (N=32).

Adults	Children	Culture site specimen	Clinical diagnosis	N (%)
6 (26)	-	Sputum	Pneumonia	6 (19)
3 (13)	-	Sputum	Chronic bronchitis	3 (9)
3 (13)	-	Sputum	Bronchiactesis	3 (9)
1 (4)	1 (11)	Sinuses fluid	Sinusitis	2 (6)
1 (4)	6 (67)	Eye swab	Conjunctivitis	7 (22)
-	1 (11)	Ear swab	Otitis media	1 (3)
1 (4)	-	Blood	Leukaemia	1 (3)
1 (4)	-	Blood	Pneumonia	1 (3)
3 (13)	-	Sputum	Heart disease	3 (9)
1 (4)	-	Sputum	Liver disease	1 (3)
2 (9)	-	Sputum	Diabetes mellitus	2 (6)
-	1 (11)	Endotracheal tube swab	Post trauma and epilepsy	1 (3)
1 (4)	-	Peritoneal dialysis fluid	Chronic renal failure	1 (3)
Total No (%), 23(100), 9 (100).				

Table 2 - Laboratory findings in specimens with *M. catarrhalis* isolates.

Specimen	N(%) with many leucocytes	N(%) with GNDC[b]	N (%) with pure culture	N (%) with mixed culture
Sputum	15 (83)	11 (61)	13 (72)	5[c] (28)
Sinuses fluid	ND[a]	ND[a]	2 (100)	-
Eye swab	6 (86)	7 (100)	7 (100)	-
Ear swab	1 (100)	1 (100)	1 (100)	-
Blood	Nil	2 (100)	2 (100)	-
Peritoneal dialysis fluid	1 (100)	1 (100)	1 (100)	-
Endotracheal tube swab	Nil	Nil	-	1[d] (100)

[a]-ND=Not Done, [b]-GNDC=Gram negaive diplococci, [c]-2 mixed with streptococcus pneumoniae, 3 mixed with Hemophilus influenzae, [d]=mixed with Pseudomonas aeruginosa

and ampicillin with no ß-lactamase production, 13% were resistant to erythromycin and clindamycin.

In addition to *M. catarrhalis* two sputum specimens grew *S. pneumoniae.* One of them was resistant to penicillin and three sputum specimens grew *H. influenzae* along with *M. catarrhalis*, one of which was resistant to ampicillin with ß-lactamase production. The other two isolates were sensitive to ampicillin and were non-ß-lactamase producers.

Discussion. The clinical significance of *M. catarrhalis* has been reported by previous investigators who recovered it from sputum of patients aged more than 50 years, having pneumonia, chronic bronchitis or bronchiactesis during the winter season.[2] As in our study, others reported that most patients affected had underlying diseases such as diabetes mellitus, heart, liver diseases or chronic renal failure. Our findings are in agreement with other studies which have related the clinical significance of *M. catarrhalis* to the abundance of leucocytes and the presence of Gram negative diplococci by Gram staining along with pure isolation from culture.[2,13]

Some of our patients who had *M. catarrhalis* pneumonia, had some underlying diseases, this is similar to other's findings which reported underlying diseases like cardiopulmonary disease, malignancy and steroid therapy in patients who had pneumonia due to *M. catarrhalis*.[13,14] Otitis media and pneumonia seen in our series of patients may be explained by the presence of *M. catarrhalis* in the oropharynx which leads to endogenous spread to the middle ear, and lung as previously reported.[15] There has been no good correlation between the presence of *M. catarrhalis* in the throat or nasopharynx and the development of sinusitis.[8] One reason for this could be the unstable colonization of the respiratory tract by *M. catarrhalis* where it occurs only as long as there is respiratory tract damage.[5] However, in this study in patients with clinical diagnosis of sinusitis we considered *M. catarrhalis* isolates from sinus fluid clinically significant as they were isolated in pure culture.

Although *M. catarrhalis* was isolated in pure culture from endotracheal tube swabs it was also considered significant since it was recovered from a two month old baby with trauma and epilepsy. This is in agreement with Aitken et al[16] who considered the isolation of *M. catarrhalis* from endotracheal tube swabs as significant. In our study two eye specimens from two neonates with conjunctivitis grew the organism. This in concordance with Ejlertsen et al[5] who stated that isolation of *M. catarrhalis* should command attention as it is not considered as part of the normal flora of neonates. Similarly Catlin[1] reported cases of pseudogonococcal conjunctivitis in neonates which was caused by *M. catarrhalis.* We isolated *M. catarrhalis* from the blood of an elderly patient with a clinical diagnosis of pneumonia. The significance of this is strengthened by the fact that after the age of 60 years there may be a reduction of immunoglobulin G and M titres, along with damage of the respiratory tract by viral infection that may promote invasion by *M. catarrhalis*.[1,17]

The paucity of positive blood culture in most patients with pneumonia has been attributed to the presence of circulating bacteriocidal antibodies in human serum.[7] The low virulence of the organism makes it unable to invade the blood although it causes lower respiratory tract infection.[7] The other case of *M. catarrhalis* bacteraemia in our study was in a leukaemic patient. This is in agreement with other studies in which patients with an impaired immune system, intravenous drug use or insulin dependent diabetes mellitus, or hematological

malignancies are prone to develop *M. catarrhalis* bacteraemia.[10] In contrast Domingo et al[11] reported that more than 40% of patients with *M. catarrhalis* bacteraemia have no identifiable immunosuppressive condition. In his study a case of *M. catarrhalis* bacteraemia was in an immunocompetent patient with bronchitis. This had been overlooked as the patient was treated as an outpatient and blood culture was not routinely carried out.[11]

Confirmation of the identity of *M. catarrhalis* in our study depended on the ability of the isolate to reduce nitrate to nitrite and the production of DNAse. This is similar to recommendation by Peiris et al[18] reported that DNAse production and nitrate reduction should be tested for the definitive identification of *M. catarrhalis*.

Among our strains 84% were ß-lactamase producer compared to a 97% ß-lactamase positivity in European isolates.[5] According to Enright et al[8] this increase could be due to greater prescription of ß-lactam antibiotics, and to the increase in the presence of ß-lactamase positive strains of other bacterial species such as *H. influenzae* and Neisseria gonorrhoeae.[8] Overall 82% of *M. catarrhalis* isolates, 18% of *H. influenzae* and up to 89% of *Staphylococcus aureus* strains produce ß-lactamase.[5] Many ß-lactamase positive strains show large zones of inhibition and also an inoculum-dependent susceptibility to ampicillin. This makes a disc diffusion test for susceptibility to ampicillin problematic.[8] Therefore, it is recommended in routine practice to test isolates that are susceptible to ampicillin by a disc diffusion test for ß-lactamase production.[8] All ß-lactamase producers should be considered resistant to ampicillin irrespective of this disc diffusion susceptibility to results.[8] A minimum inhibitory concentration (MIC) of ampicillin for *M. catarrhalis* should be carried out on isolates of bacteraemia patients treated with this drug. An MIC of <0.03 μg/ml is considered satisfactory as suggested by Domingo et al.[11]

We conclude that *M. catarrhalis* is now recognized as an important pathogen causing respiratory tract infections in adults and children. It can invade the blood stream to cause bacteraemia in patients with predisposing respiratory or systemic conditions, as well as in immunocompetent individuals. Clinicians should be alerted to its significance when isolated from various clinical specimens. Microbiology laboratories should be aware of the methods of isolation, identification and antibiotic susceptibility testing.

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