

REVIEW ARTICLE

## ***Moraxella (Branhamella) catarrhalis* – clinical and molecular aspects of a rediscovered pathogen**

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Since its discovery at the end of the nineteenth century, *Moraxella (Branhamella) catarrhalis* has undergone several changes of nomenclature and periodic changes in its perceived status as either a commensal or a pathogen. Molecular analysis based on DNA hybridisation or 16S rDNA sequence comparisons has established its phylogenetic position as a member of the Moraxellaceae and shown that it is related more closely to *Acinetobacter* spp. than to the genus *Neisseria* in which it was placed formerly. However, confusion with phenotypically similar *Neisseria* spp. can occur in the routine diagnostic laboratory if appropriate identification tests are not performed. *M. catarrhalis* is now accepted as the third commonest pathogen of the respiratory tract after *Streptococcus pneumoniae* and *Haemophilus influenzae*. It is a significant cause of otitis media and sinusitis in children and of lower respiratory tract infections in adults, especially those with underlying chest disease. Nosocomial spread of infection, especially within respiratory wards, has been reported. Invasive infection is uncommon, but analysis of reports for England and Wales between 1992 and 1995 revealed 89 cases of *M. catarrhalis* bacteraemia, with the peak incidence in children aged 1–2 years. Carriage rates of *M. catarrhalis* are high in children and in the elderly, but its role as a commensal organism has probably been overstated in the past. Approximately 90% of strains are now  $\beta$ -lactamase positive and, given that the first such strain was reported in 1976, this represents a dramatic increase in frequency over the last 20 years which has not been paralleled in any other species. The BRO-1 and BRO-2  $\beta$ -lactamase enzymes of *M. catarrhalis* are found in other Moraxellaceae, but are not related to  $\beta$ -lactamases of any other species and their origin is therefore unknown. Molecular and typing studies have shown that the *M. catarrhalis* species is genetically heterogeneous and these methods have aided epidemiological investigation. Studies of factors that may be related to pathogenicity have shown the existence of three serotypes of lipooligosaccharide and the presence of fimbriae and a possible capsule. Some strains are serum-resistant, probably by virtue of interference with complement action, whilst transferrin- and lactoferrin-binding proteins enable the organism to obtain iron from its environment. An antibody response in humans to various *M. catarrhalis* antigens, including highly conserved outer-membrane proteins, has been demonstrated. Increased understanding of the organism's pathogenic properties and the host response to it may help to identify suitable vaccine targets or lead to other strategies to prevent infection. Whilst it remains, at present, the third most important respiratory pathogen, the impact of immunisation strategies for other organisms may change this position. The speed with which *M. catarrhalis* acquired  $\beta$ -lactamase demonstrates the capacity of this organism to surprise us.

### **Introduction**

*Moraxella catarrhalis* is an aerobic, oxidase-positive, gram-negative diplococcus which is now generally

accepted as a pathogen, although until relatively recently it was considered to be a harmless commensal of the upper respiratory tract. It is morphologically and phenotypically similar to *Neisseria* spp. and, in the past, this has resulted in the misidentification of *M. catarrhalis* as a species of the latter genus, most notably as *N. cinerea*, a commensal organism of the

adult pharynx. *M. catarrhalis* is now recognised as an important cause of respiratory tract infections in the elderly and in those with pre-existing respiratory disease, as a common cause of otitis media in children, and as an occasional cause of invasive disease.

Previous reviews [1, 2] documented that the organism was named *Mikrokokkus catarrhalis* by Pfeiffer [3] in 1896, although it had probably been discovered 14 years earlier in 1882 by Seifert [4]. In his fascinating review of the history of *M. catarrhalis*, Berk [2] described the evidence of Ghon and Pfeiffer [5] that this organism, along with *Streptococcus pneumoniae* and the organism now known as *Haemophilus influenzae*, is a respiratory pathogen. Early this century in 1905, Dunn and Gordon [6] investigated an apparent influenza epidemic in East Hertfordshire and demonstrated three different kinds of gram-negative cocci in respiratory secretions on the basis of cultural characteristics and carbohydrate fermentation tests. *M. catarrhalis* was the commonest isolate and was considered to be a significant pathogen. However, a study on gram-negative cocci in the common cold and influenza by Gordon in 1921 [7] described *M. catarrhalis* as a saprophyte of negligible virulence found in the throat of healthy adults. This view, that *M. catarrhalis* is a harmless commensal, has persisted for much of the remainder of this century and it has only recently regained prominence as a pathogenic organism.

The nomenclature of *M. catarrhalis* has changed several times and its current designation in the 1984 edition of *Bergey's Manual of Systematic Bacteriology*

as *Moraxella (Branhamella) catarrhalis* [8] is a compromise between two views. One suggests that *M. catarrhalis* is related so closely to other *Moraxella* spp. that it should be included in this genus, regardless of the fact that it would be a coccoid organism in a genus of bacillus-shaped species, as would the closely related 'false *Neisseria*' organism now known as *M. caviae*. The alternative view held by Catlin [9] proposes the formation of a new family – Branhamaceae – to include two genera, *Moraxella* and *Branhamella*. Genetic studies with DNA–DNA [10] and rRNA–DNA hybridisation techniques [11] suggest that *M. catarrhalis* is sufficiently similar to other *Moraxella* spp. to satisfy the usual criteria for inclusion in the same genus. More recently, we have compared 16S rDNA sequences from selected species of *Moraxella* and related genera [12]. The dendrogram (Fig. 1) is based on comparisons of these sequences and demonstrates that *M. catarrhalis* is closely related to *M. lacunata* subsp. *lacunata* and to the 'false *Neisseria*' *N. ovis*. There is no evidence to support the case for a separate *Branhamella* genus, and *M. catarrhalis* is related more closely to *Acinetobacter* spp. than to *Neisseria* spp. On these genetic grounds the organism will be referred to throughout this review as *M. catarrhalis*.

The 1984 edition of *Bergey's Manual of Systematic Bacteriology* [8] describes *M. catarrhalis* colonies of c. 2 mm diameter after incubation for 48 h on agar. Colonies are opaque, non-haemolytic and, characteristically, can be pushed along the surface of the agar with a loop, like a hockey puck [13]. *M. catarrhalis* does not utilise sucrose, glucose, maltose and lactose

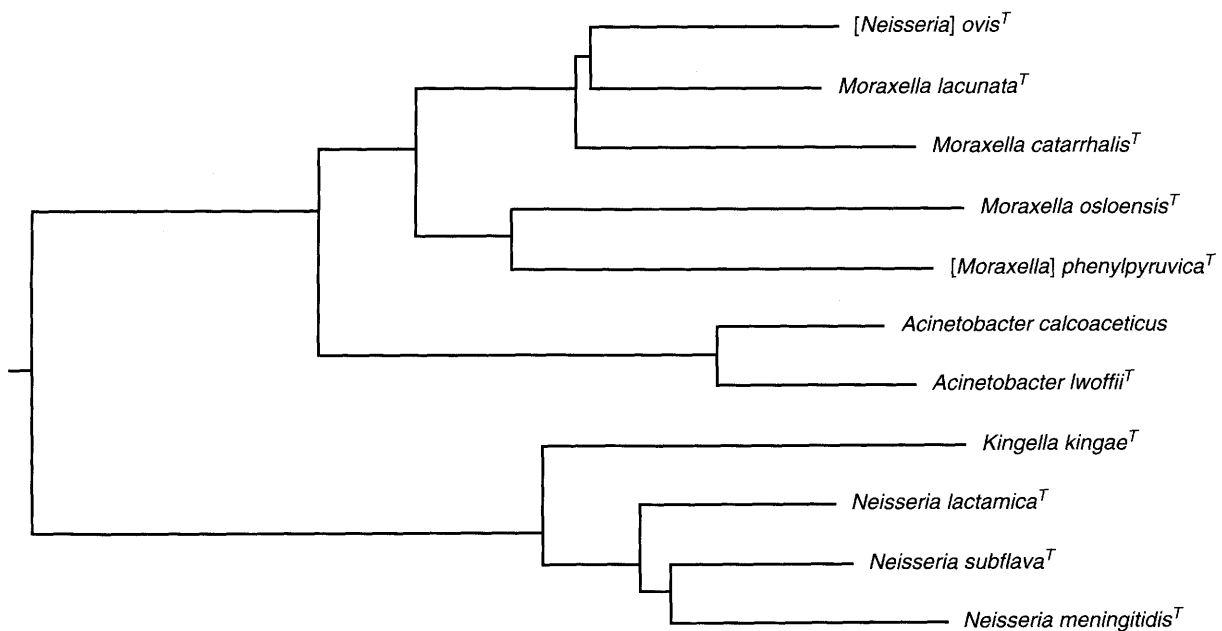


Fig. 1. Rooted phylogenetic tree representing the degree of similarity between 16S rDNA sequences in selected species of *Moraxella* and other related genera. Horizontal distances are equivalent to genetic distances; vertical distances have no meaning. Scale: — represents a genetic distance of 0.2%. <sup>T</sup>Type strain of species. (Reproduced from Enright *et al.* [12] with permission).

– the sugars used commonly for the identification of *Neisseria* spp. – but reliance on these criteria alone can lead to misidentification, as certain commensal neisserias, such as *N. cinerea*, have a similar negative reaction pattern. *M. catarrhalis* reduces both nitrate and nitrite and produces DNAase. A report on the identification of *M. catarrhalis* in the diagnostic laboratory [14] found that the tests listed above, with the addition of the superoxol test [15], were sufficient to differentiate between *M. catarrhalis* and morphologically similar *Neisseria* spp. A further useful additional test for identification of *M. catarrhalis* in the routine laboratory detects the presence of tributyrin esterase, which is produced by all strains [16]. Moraxellaceae other than *M. catarrhalis* may also be positive in the tributyrin test; therefore, DNAase or nitrate reduction tests should be performed for definitive identification [17]. Thus there are several potential pitfalls in the identification of *M. catarrhalis*, and these complicate the interpretation of clinical and microbiological studies on this organism, especially older studies performed before these difficulties became apparent.

### Clinical significance

The widely held view that *M. catarrhalis* is a common commensal of the nasopharynx is correct in children. A prospective study of 120 infants that examined nasopharyngeal carriage found that 66% of those tested were colonised with the organism at the age of 1 year, rising to 77.5% by the age of 2 years, with colonised infants showing a significantly greater risk of presenting with otitis media caused by *M. catarrhalis* [18]. Other studies have found carriage rates of 48.9% in children aged 3–12 years [19] and 54% in children aged < 4 years [20]. However, carriage rates are much lower in adults, with 1% carriage reported in 561 women admitted to hospital in labour [19] and 5.8% carriage found in another study of healthy adults, although this rose to 26.5% in people aged > 60 years [20]. The latter study critically examined 112 papers which stated that *M. catarrhalis* is a common commensal organism and found that none of these papers presented experimental evidence to support this view. The authors concluded that confusion of *M. catarrhalis* with *Neisseria* spp., especially the harmless *N. cinerea* (present in 90% of throats), was probably responsible for the original misconception that *M. catarrhalis* is predominantly a commensal organism.

Upper respiratory tract infections caused by *M. catarrhalis* present as otitis media or sinusitis and are most common in children [21]. *M. catarrhalis* is the third most common cause of either condition after *S. pneumoniae* and *H. influenzae* [21, 22], and can be cultured from up to 16% of middle ear effusions from children with otitis media [21]. Organisms from the nasopharynx can spread easily to the middle ear, and

strains of *M. catarrhalis* that are indistinguishable by restriction endonuclease analysis (REA) have been demonstrated in the nasopharynx and middle ear of seven of eight children with otitis media [23]. Otitis media is very common in children, with nearly 50% of all children estimated to have had at least one episode of the disease by the age of 1 year, rising to 70% by the age of 3 years [24]. Maxillary sinusitis is less well understood than otitis media, but is similar in its pathogenesis and microbiology. Many children suffer a degree of sinusitis during the course of an upper respiratory tract infection – although this may present as nothing more than a runny nose – and the necessity for antimicrobial treatment is uncertain [21]. Wald *et al.* [25] studied maxillary sinus aspirates from 30 children with upper respiratory tract symptoms and abnormal maxillary radiographs. The three common respiratory pathogens, i.e., *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*, were isolated from 34 of 47 sinus aspirates, often in combination. *M. catarrhalis* accounted for 22% of all isolates, but was more likely to be found in pure culture and in younger children. A poor correlation was found between the presence of a species in sinus aspirates and its presence in cultures of the nasopharynx or throat. In a different study, *M. catarrhalis* was isolated from aspirates taken from young adults with acute maxillary sinusitis at a much lower frequency of 2% [26].

The most common cause of morbidity due to *M. catarrhalis* in adults is exacerbation of chronic bronchitis in patients with pre-existing pulmonary disease [27]. *M. catarrhalis* is also the third most commonly isolated pathogen from the lower respiratory tract after *S. pneumoniae* and *H. influenzae*, and is often isolated in conjunction with one of these two pathogens. Slevin *et al.* [27] studied 101 patients with *M. catarrhalis* isolates from sputum, all judged to be clinically significant on the basis of sputum purulence, fever, chest X-ray appearance and peripheral leucocytosis. Of the 101 patients, 59 had reasons for general immunosuppression, while 94 had either chronic chest disease or a history of smoking. *M. catarrhalis* was isolated in pure culture from 71 patients, and in combination with other pathogens from 30 patients. As discussed previously, the pathogenic role of *M. catarrhalis* in such patients has not always been accepted and further validation of this has been required. Isolation of an organism from trans-tracheal aspirates provides good evidence that its presence in sputum is not a result of oropharyngeal contamination of lower respiratory tract secretions. Trans-tracheal aspirations were performed by Ninane *et al.* [28] on 193 miners with acute exacerbations of chronic bronchitis. These workers found *M. catarrhalis* to be the third commonest isolate after *H. influenzae* (44 isolates) and *S. pneumoniae* (22 isolates), with 14 pure cultures of *M. catarrhalis* and one mixed with *H. influenzae*. Thornley *et al.* [29] took trans-tracheal aspirates from 11 symptomatic patients with *M.*

*catarrhalis* in their sputum and confirmed the presence of the organism in the lower respiratory tract in 10 patients, of whom four yielded pure cultures of *M. catarrhalis* [29]. Lastly, a case control study that compared patients with *M. catarrhalis* isolated from expectorated sputum against controls with commensal *Neisseria* isolates confirmed an association between *M. catarrhalis* and the clinical features of bronchopulmonary infection [30]. Thus, there is good evidence to support the view that *M. catarrhalis* is a respiratory pathogen. Lower respiratory tract infection with *M. catarrhalis* is more likely to affect elderly patients and is more common in winter [31]. There is some evidence that, even in the absence of changes in laboratory technical or interpretive practices, the incidence of *M. catarrhalis* respiratory infection is increasing [32, 33].

Although exacerbation of chronic bronchitis is the commonest manifestation of *M. catarrhalis* infection, pneumonia may also occur. Wright *et al.* [34] described 42 cases of pneumonia diagnosed on the basis of a pure culture of *M. catarrhalis* from sputum together with the presence of a new infiltrate on chest X-ray or autopsy evidence of pneumonia. These patients were predominantly elderly with a serious underlying disease and, although the pneumonia itself was described as a mild illness, 45% of the patients died of their underlying disease within 3 months. *M. catarrhalis* pneumonia is therefore associated with a poor prognosis. Bacterial tracheitis is a further rare manifestation of *M. catarrhalis* infection which has been reported several times in children, sometimes in association with respiratory syncytial virus infection [35–37].

Invasive disease caused by *M. catarrhalis* is relatively uncommon, but is well-documented in the literature. Catlin [1] extensively reviewed the evidence that *M. catarrhalis* has caused meningitis, bacteraemia and endocarditis. It is difficult to assess the accuracy of strain identification in many older reports, but it seems likely that *M. catarrhalis* has been responsible for all of the above conditions at some time. In particular, there is good evidence that *M. catarrhalis* is an occasional cause of bacteraemia [38], with episodes reported in association with pneumonia [39], otitis media [40] and AIDS [41]. Analysis of *M. catarrhalis* bacteraemia reports made by laboratories in England and Wales to the Communicable Diseases Surveillance Centre, Colindale, London reveals a total of 89 cases over the 4 years from 1992 to 1995 inclusive (Table 1) [42]. The age distribution for 87 of these patients (Fig. 2a) shows that infection was commonest in children aged < 10 years, with 46% of cases in this age range. Further analysis of the latter group (Fig. 2b) shows that cases peak at the age of 1 year and tail off by the age of 5 years, although there were four cases in babies aged ≤ 10 days. Over the entire age range, there were similar numbers of cases in

**Table 1** Number of cases of *M. catarrhalis* bacteraemia reported to the Communicable Diseases Surveillance Centre, Colindale, London by laboratories in England and Wales, 1992–1995

Year	Number of reported cases
1992	21
1993	26
1994	20
1995	22

males (43) and females (45), with one case of unknown sex. Insufficient clinical details are available to categorise these patients with regard to underlying disease, but many had predisposing conditions, e.g., leukaemia, congenital heart disease, cystic fibrosis or prematurity. Isolates from these cases are not available to enable confirmation of bacteriological identity, but it is likely that any false-positive identifications would be balanced by under-reporting of such episodes, and several cases reported as bacteraemia associated with *Moraxella* or *Branhamella* spp. were not included in the survey. These figures demonstrate that *M. catarrhalis* bacteraemia is a well-recognised clinical entity. The predominance of this infection in young children is perhaps surprising, but may be related to the high carriage rates of *M. catarrhalis* in this age group [18–20]. Other possible contributory factors will be discussed later. Invasive disease other than bacteraemia is unusual, but a case of acute purulent pericarditis caused by *M. catarrhalis* has been recorded [43].

#### *Nosocomial infection*

There is now convincing evidence that the spread of *M. catarrhalis* within hospital wards can lead to significant outbreaks of nosocomial infection. Epidemiological evidence for this was presented by Ahmad *et al.* [44], but these authors were unable to confirm their suspicions because of the absence at that time of a suitable typing system. Patterson *et al.* [45] applied REA to isolates from an apparent outbreak in an intensive care setting and confirmed isolation of the same strain from five patients and two staff members, all of whom were symptomatic. We have also found good evidence of nosocomial spread of *M. catarrhalis*, with significant correlations between length of hospital stay and acquisition of *M. catarrhalis*. In these studies, SDS-PAGE and immunoblotting of *M. catarrhalis* whole cell proteins [46], and REA of bacterial DNA [46–48] demonstrated considerable genetic heterogeneity among strains of *M. catarrhalis*, but epidemiologically related isolates were more likely to be indistinguishable, and several clusters of apparent nosocomial infection in respiratory wards were identified. Carriage and spread of *M. catarrhalis* by ward staff is a possible route of infection, but it was not possible to demonstrate this directly, although indistinguishable *M. catarrhalis* strains were isolated from

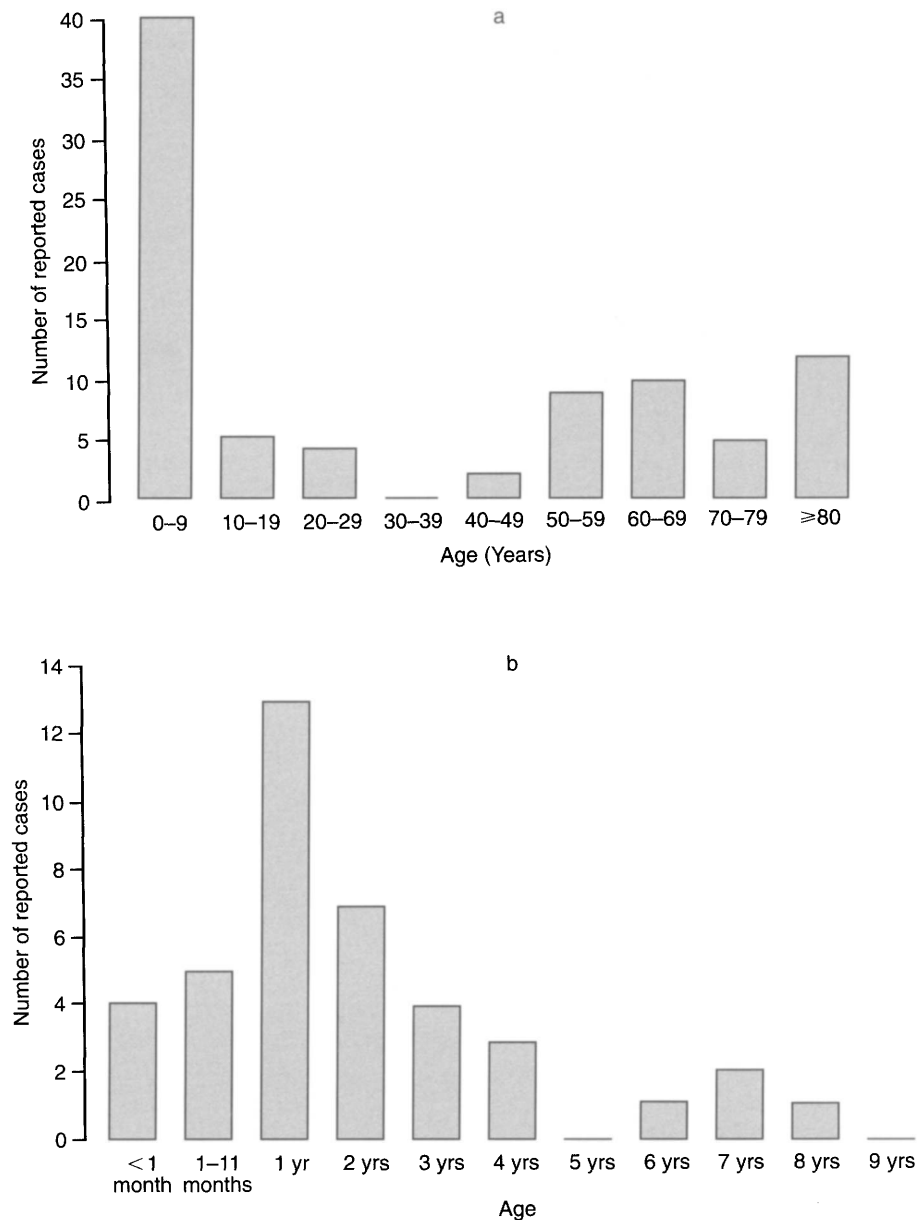


Fig. 2. a, Age distribution of 87 patients with *M. catarrhalis* bacteraemia; b, age distribution of 40 children under the age of 10 years with *M. catarrhalis* bacteraemia.

patients and settle plates placed in the same ward (personal unpublished data). Similar studies by Ikram *et al.* [49] also show persistence of the organism in the environment and an association between *M. catarrhalis* acquisition in hospital and length of stay.

### Antibiotic susceptibility

#### *Resistance to $\beta$ -lactam antibiotics*

A retrospective study in the USA of 18 *M. catarrhalis* isolates dating from 1952 to 1975 found no  $\beta$ -lactamase-producing strains before the first  $\beta$ -lactamase-positive isolates in 1976 [50]. Similarly, 46 French isolates dating from 1970 to 1976 were found to be  $\beta$ -lactamase-negative [51], with the first  $\beta$ -lactamase-positive French isolate reported in 1977 [52].

The subsequent increase in incidence of  $\beta$ -lactamase-positive *M. catarrhalis* isolates has been described as the fastest increase in prevalence of any known  $\beta$ -lactamase within a bacterial species [53]. Surveys of resistance in the UK in 1991, and in Denmark in 1994, showed that 91% and 84% of *M. catarrhalis* strains, respectively, were  $\beta$ -lactamase-positive [19, 54]. The reason for the dramatic emergence of  $\beta$ -lactamase-producing strains is not clear, and although it has been attributed to increased prescribing of  $\beta$ -lactam antibiotics, increases in the prevalence of  $\beta$ -lactamase-positive strains of other comparable species such as *N. gonorrhoeae* or *H. influenzae* have not been nearly so spectacular.

*M. catarrhalis*  $\beta$ -lactamases are of two types which can be distinguished by isoelectric focusing [52, 55]

and, as they are quite different from  $\beta$ -lactamase enzymes in any other genus, their original source is unknown [53]. The two enzymes are phenotypically similar and have been designated BRO-1 (previously called Ravasio type) and BRO-2 (previously called 1908-type), the name deriving from *Branhamella* and *Moraxella* [50]. BRO-1 is the most common and is present in *c.* 90% of  $\beta$ -lactamase-positive strains [51, 56, 57]. Strains with the BRO-1  $\beta$ -lactamase have 25-fold higher ampicillin MICs than  $\beta$ -lactamase-negative strains, while in those strains with the BRO-2 enzyme, ampicillin MICs are only four-fold higher than in  $\beta$ -lactamase negative strains [57]. It is thought that this difference is caused by higher levels of production of the BRO-1 enzyme (2–3-fold more than BRO-2) rather than any intrinsic difference in enzyme activity [53].

BRO  $\beta$ -lactamases are found only in *M. catarrhalis* and in two closely related species, *M. lacunata* [50] and *M. nonliquefaciens* [50, 58]. Conjugal transfer of BRO  $\beta$ -lactamase from one *M. catarrhalis* strain to another was demonstrated by Kamme *et al.* [59]. Transfer from *M. nonliquefaciens* to *M. catarrhalis* has also been demonstrated [59, 60]. The genetics of  $\beta$ -lactamase production in *M. catarrhalis* are poorly understood. Both BRO-1 and BRO-2 genes are thought to be chromosomal, although it has been proposed that both enzymes are the product of one gene and derive from a membrane-bound precursor enzyme [61]. Claims that the genes are plasmid-borne [62] have not been supported by other investigators, but there is no doubting the efficiency with which the enzyme has spread throughout the species population, leading to the suggestion that the genes are on a conjugal transposon [53]. The involvement of non-pathogenic *Moraxella* spp. in the gene pool may be significant in promoting spread, as widespread carriage of such organisms increases the opportunity for genetic exchange [63]. Other than the enzymes discussed above, there have been single reports of a further  $\beta$ -lactamase, designated BRO-3 [64], and of a strain with a plasmid-mediated TEM-1  $\beta$ -lactamase [65].

Antimicrobial susceptibility testing of *M. catarrhalis* reveals a bimodal distribution of MIC values of ampicillin among  $\beta$ -lactamase-positive strains, probably reflecting the presence of two populations with different  $\beta$ -lactamase enzymes [54]. However, disk testing for sensitivity to ampicillin is problematic, as many  $\beta$ -lactamase-positive strains show large zones of inhibition and would be classed as sensitive according to normal test procedures [53, 54]. The clinical significance of this is uncertain, but in the absence of extensive trials, it is probably better to err on the side of caution in routine practice and to test isolates that are apparently sensitive by standard disk sensitivity tests for the presence of  $\beta$ -lactamase by a chromogenic method. Both research groups that high-

lighted this problem have recommended that, whatever the results of disk sensitivity tests, all  $\beta$ -lactamase-positive isolates should be reported as ampicillin- or amoxicillin-resistant [53, 54].

There is evidence that *M. catarrhalis*  $\beta$ -lactamases can protect other respiratory pathogens from  $\beta$ -lactam antibiotics when present in mixed infections. Hol *et al.* [66] inoculated mice intranasally with *S. pneumoniae* and either  $\beta$ -lactamase-positive or -negative *M. catarrhalis*. Treatment with penicillin or amoxicillin was ineffective for those mice inoculated with  $\beta$ -lactamase-positive *M. catarrhalis*, leading to death resulting from pneumococcal pneumonia. Treatment with amoxicillin and clavulanic acid (a  $\beta$ -lactamase inhibitor) proved an effective treatment regimen for these mice. There is also clinical evidence that this so-called 'indirect pathogenicity' of *M. catarrhalis* can result in treatment failure when mixed infections of  $\beta$ -lactamase-positive *M. catarrhalis* and  $\beta$ -lactam-sensitive strains of *S. pneumoniae* or *H. influenzae* are treated with a  $\beta$ -lactamase-sensitive penicillin [67].

Several other pathogens, such as *S. pneumoniae*, *H. influenzae* and *N. gonorrhoeae*, have developed other mechanisms of resistance to  $\beta$ -lactams, most commonly by altering the structure of their penicillin-binding proteins. There is no evidence at present of any such clinically significant resistance mechanism in *M. catarrhalis*, but Catlin [1] has commented on the range of MICs of  $\beta$ -lactam agents found in  $\beta$ -lactamase-negative *M. catarrhalis* strains. It is important to continue to monitor the sensitivities of  $\beta$ -lactamase-negative strains in order to detect the emergence of any such phenomenon.

#### *Resistance to non- $\beta$ -lactam antibiotics*

Other than  $\beta$ -lactamase-mediated resistance to penicillins and inherent resistance to trimethoprim, *M. catarrhalis* remains generally sensitive to macrolide, cephalosporin and tetracycline antibiotics [54, 68]. It is also normally sensitive to co-amoxyclov. Erythromycin-resistant strains have been reported occasionally [27, 69] and co-trimoxazole is reported to be effective despite resistance to its trimethoprim component [69]. Fung *et al.* [54] reported that 6.5% of their strains were resistant to sulphamethoxazole. *M. catarrhalis* strains are almost universally susceptible to tetracycline, although two pairs of strains isolated from Texas and England [70] were tetracycline-resistant because of the presence of a non-transferable TetB determinant. Resistant strains have also been isolated in Spain [65] and China [71]. Roberts *et al.* [70] suggested that the TetB determinant may have transferred from *Haemophilus* spp. by conjugation or transformation. Aminoglycoside resistance has been observed, with one study reporting 17% streptomycin resistance in Spanish isolates and smaller numbers of kanamycin- and neomycin-resistant isolates [55].

## Typing methods

Until relatively recently there was no established, discriminatory typing system for *M. catarrhalis* strain differentiation. Phenotypic methods such as serotyping [72], isoelectric focusing of  $\beta$ -lactamase enzymes [56], bacteriocin typing [73], SDS-PAGE of outer-membrane proteins (OMPs) [74], esterase electrophoresis [75] and gel electrophoresis of soluble proteins [76] have all been used with varying degrees of success. A range of phenotypic characteristics, including serum resistance, haemagglutination,  $\beta$ -lactamase production and antimicrobial susceptibility pattern, was used by Soto-Hernandez *et al.* [77] to discriminate between *M. catarrhalis* strains. However, none of these phenotypic methods has provided a high level of discrimination between closely related strains.

The first genotypic method relied on analysis of plasmid DNA [78], but proved unsuccessful because of the small number of plasmids found. A chromosomal DNA-based typing method (REA) was used by Patterson *et al.* [45] to confirm a suspected outbreak of nosocomial infection. In this method, chromosomal DNA is purified and cleaved with restriction endonucleases to provide strain-specific fingerprints which are resolved on agarose gels. REA proved highly discriminatory for isolates of *M. catarrhalis* in this study although the method was not compared to other typing schemes. We have successfully used three methods for the investigation of suspected nosocomial infection: SDS-PAGE of whole cell polypeptides; immunoblots of these gels with normal human serum; and REA with the frequently cutting enzyme *Taq*I [46–48]. In subsequent studies (personal unpublished results), we have used pulsed-field gel electrophoresis (PFGE) with the rare-cutting enzymes *Not*I and *Sma*I. PFGE is similar to REA, except that fewer DNA fragments are obtained because of the use of restriction endonucleases that cut less frequently. The large fragments of DNA produced cannot be resolved by conventional gel electrophoresis, but separation can be achieved by switching the direction of the electric current continuously to allow the resolution of DNA fragments from *c.* 50 kb to several megabases in length. PFGE with *Not*I and *Sma*I has also been used by a Japanese group to type 38 hospital isolates of *M. catarrhalis* [79]. Their results suggest that this method is highly suitable for use in typing studies and the publication of recommended criteria for the more general application of PFGE to bacterial typing may lead to this becoming the molecular method of choice [80].

## Population genetics

To date there have been no published studies on the population genetics of *M. catarrhalis*. Studies on other organisms, such as *Escherichia coli* [81], have

attempted to assess whether an organism has a clonal population structure. In a clonal population, genetic recombination between strains or other species is undetectable and changes to the bacterial genome are thought to occur by mutation and localised DNA rearrangement [82]. Clonal populations are characterised by the existence of similar strains separated in time and space, e.g., strains isolated years apart from different countries. Non-clonal populations arise when recombination between strains or other species occurs frequently. This type of population is genetically heterogeneous with little identity between strains, even those from similar sources. The method chosen to examine the genetics of a population is very important, as the greater the discriminatory power of the technique used, the more accurate will be the results obtained. We have performed a limited study of *M. catarrhalis* population genetics with REA, PGFE and multi-locus enzyme electrophoresis (MLEE). MLEE, which is the most commonly used method in the study of bacterial population genetics, relies on comparisons of the electrophoretic mobility of known proteins, giving information (indirectly) about the strain at the genetic level. Preliminary results (personal unpublished results) suggest that most *M. catarrhalis* isolates are different from each other, but we have found occasional isolates obtained from different countries several years apart which are indistinguishable by all methods used. This leads us to believe that *M. catarrhalis* may have a similar population structure to that found in *N. meningitidis*, i.e., one with a non-clonal structure, but with the occasional emergence of a successful 'epidemic' clone.

## Pathogenicity and virulence

As *M. catarrhalis* has become accepted more widely as a pathogen, interest in potential virulence factors has increased. Endotoxin or lipopolysaccharide (LPS) plays a major role in the pathogenesis of gram-negative infection and therefore presents an obvious area for study. In *N. gonorrhoeae*, LPS is associated with membrane vesicles or blebs. Similar vesicles are found in *M. catarrhalis* broth culture supernates [83] and three major serotypes of *M. catarrhalis* LPS have been described by Vanechoutte *et al.* [72], accounting for 95% of all strains. The LPS of *M. catarrhalis*, like those of *N. meningitidis* and *N. gonorrhoeae*, does not have the long O-antigen side chains characteristic of the Enterobacteriaceae, and the oligosaccharide structure that determines the antigenic differences between the three serotypes has been studied extensively. The inner core contains branched polysaccharides with a common terminal  $\alpha$ -D-Gal-(1-4)- $\beta$ -D-Gal (1-4)- $\alpha$ -Glc epitope [84]. The terminal residues that determine the serotypes are  $\alpha$ -D-GlcNAc-(1-2)- $\beta$ -D-Glc for serotype A [84, 85],  $\beta$ -D-Gal-(1-4)- $\beta$ -D-Glc for serotype B [86], and  $\beta$ -D-Gal-(1-4)- $\alpha$ -D-GlcNAc for serotype C [87]. There is some

cross-reaction between serotypes, particularly between serotypes A and C [72, 86]. In a study with an animal model, formalin-killed isolates of *M. catarrhalis* were shown to produce effusions in the middle ear of chinchilla, indicating a possible role for endotoxin in the disease process [88]. Storm Fomsgaard *et al.* [89] also found that *M. catarrhalis* LPS had a similar effect to enterobacterial LPS in mice and in the *Limulus* amoebocyte lysate assay.

Pili or fimbriae are protein filaments that extend from the surface of bacteria and have a role in adherence to host mucosal epithelial cells [90]. Fimbriae have been found in some, but not all, strains of *M. catarrhalis* [91]. Adherence of the bacterium to respiratory mucosal cells is a primary step in the pathogenesis of infection and prevents the organism from being washed away by the mucociliary escalator. Hybridisation with a pilus gene from *M. bovis* showed the existence of class 4 pilus genes in four *M. catarrhalis* strains [91]. This class of pilus is present in other pathogenic bacteria, such as *N. gonorrhoeae* [92, 93], *N. meningitidis* [94], *Pseudomonas aeruginosa* [95] and *Vibrio cholerae* [96], as well as other pathogenic *Moraxella* spp. [94, 97, 98]. Electron microscopy by Marrs and Weir [91] indicated the presence of at least one other pilus class in *M. catarrhalis*, as is common in other bacterial species [99]. Other investigators have demonstrated the presence of non-type 4 fimbriae both *in vitro* [100, 101] and *in vivo* [102]. Recent studies of the nature of the receptor that mediates adherence of *M. catarrhalis* to the respiratory epithelium suggest that fimbrial adherence involves interaction with a specific glycosphingolipid receptor [103]. Identification of a specific receptor that mediates adherence opens up the possibility of inhibiting the binding of *M. catarrhalis* to the mucosal surface and thus preventing colonisation and subsequent infection.

Complement plays an important role in host defences against *N. meningitidis* and *N. gonorrhoeae*, as illustrated by two studies which showed that patients deficient with respect to complement were about 8000-fold more likely to acquire an infection by these organisms [104, 105]. Resistance to the action of complement is therefore an important virulence factor, especially in gram-negative pathogens [106]. There is good evidence that some *M. catarrhalis* strains produce a protein that interferes with the formation of the membrane attack complex of complement and thus renders the organism 'serum resistant' [107]. Helminen *et al.* [108] showed that a genetically manipulated *M. catarrhalis* strain which does not express the CopB OMP lacks the serum resistance of the parent strain, but that this property returns with restoration of gene expression. A survey of strains carried by healthy schoolchildren and isolated from adults with lower respiratory tract infections showed differences in the prevalence of serum resistance in

these different groups [109]. Serum-resistant strains were more likely to be found in younger children and in symptomatic adults than in older children. Hol *et al.* [109] did not study carriage in children aged < 4 years, but it is interesting to speculate that the peak incidence of *M. catarrhalis* bacteraemia in younger children discussed earlier may be associated with an increased incidence in the carriage of serum-resistant strains.

Human pathogens require iron to grow and differentiate, yet the blood and mucosae of man are iron-limited environments because of the binding of iron by human transferrin in serum and lactoferrin in mucus. Pathogenic bacteria have therefore evolved several mechanisms for iron-uptake, commonly involving the production of siderophores. These are secreted compounds that chelate iron in the environment and are then taken up by specific OMP receptor proteins [110, 111]. However, siderophores are not produced by *Neisseria* spp. or *M. catarrhalis*, and these organisms have developed different mechanisms for iron-uptake. *M. catarrhalis*, *N. gonorrhoeae* and *N. meningitidis* have transferrin and lactoferrin receptors present on their cell surfaces that remove bound iron from the human carrier protein before uptake into the bacterial cell [112, 113]. Campagnari *et al.* [114] showed that new OMPs, but not siderophores, are expressed if *M. catarrhalis* strains are grown in the absence of iron, and postulated that these proteins are important for the acquisition of iron *in vivo*.

Possession of a capsule is an important virulence mechanism for many organisms, usually preventing phagocytosis. There have been reports of a polysaccharide capsule in *M. catarrhalis* similar to that of *N. gonorrhoeae* [115–118], but this has not been well-defined. One further possible virulence mechanism is suggested by a study of five bacterial species, including *M. catarrhalis*, in infection of the lower respiratory tract. This showed that *M. catarrhalis* synthesises clinically significant amounts of histamine, as do *P. aeruginosa* and *H. influenzae* [119]. Histamine is involved in restriction of the airways resulting from smooth muscle contraction and slowing of ciliary beat *in vitro* [120], leading to a depressed rate of mucus clearance and increased colonisation of the respiratory tract.

#### Host responses

Serological evidence for infection by *M. catarrhalis* was presented by Eliasson [121], who found antibodies to P antigen in most healthy adult sera. Goldblatt *et al.* [122] demonstrated that the IgG response to *M. catarrhalis* in children increases after the age of 1 year, but the predominant response to OMPs is in the IgG3 subclass and does not appear until after the age of 4 years. The delayed appearance of antibody to



OMPs may be a further factor that contributes to the predominance of *M. catarrhalis* bacteraemia in children aged < 4 years. Although antibody to protein antigens is most commonly found in the IgG1 subclass, the IgG3 isotype is often found in responses against virus proteins and *H. influenzae* OMPs. The IgG3 response to *M. catarrhalis* OMPs has been confirmed by other investigators [123]. Unlike *H. influenzae*, OMPs in *M. catarrhalis* show little strain-to-strain variation [124]. The conserved nature of these proteins has raised interest in the possibility that one or more might form the basis of a protective vaccine. Helminen *et al.* [125] reported a monoclonal antibody (MAb) specific for a 300–400-kDa surface antigen (UspAP) that enhanced pulmonary clearance of the organism in passively immunised mice. Recombinant antigen was recognised in immunoblots by convalescent serum, but not acute phase serum, from patients with *M. catarrhalis* pneumonia. Antibody responses to the CopB antigen [126] and to the 84-kDa antigen OMP B1 have also been demonstrated in patients with *M. catarrhalis* infections [127]. A further possible vaccine candidate is the heat-modifiable OMP C/D, which is also expressed on the surface of the intact bacterium [128]. Antibodies to LPS have been measured in patients with *M. catarrhalis* lower respiratory infections, but do not appear to show a serotype-specific response [129].

## Conclusions

Since its initial recognition at the start of the century, and following a long intervening period of neglect, *M. catarrhalis* has now been rediscovered as a pathogen. In terms of clinical significance, it clearly comes third in the respiratory tract after *S. pneumoniae* and *H. influenzae*, but the impact of immunisation for both these species may disturb this balance in years to come. Lower respiratory tract infection in patients with underlying chest disease is the best known example of its clinical impact, but the potential of the organism to cause nosocomial infection is less well recognised. Otitis media in children caused by *M. catarrhalis* represents significant morbidity in simple numerical terms and may contribute to hearing problems in later life. The epidemiology of *M. catarrhalis* bacteraemia deserves further study in order to confirm the findings presented here of a peak in children aged between 1 and 2 years. Current work is now focusing on molecular aspects of the organism's epidemiology and pathogenicity. As it becomes more fully understood, the prevention of infection by *M. catarrhalis* may become possible. Vaccine targets include the highly conserved major OMPs, while interference with fimbrial adherence may also provide options for therapy or prevention. The speed with which this organism acquired the ability to produce  $\beta$ -lactamase warns us that this is a pathogen which should not be under-rated.

We are grateful to Dr S. Handysides, CDR Editor, for permission to report the analysis of *M. catarrhalis* bacteraemia.

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