Moraxella catarrhalis – Pathogen or Commensal?

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Abstract *Moraxella catarrhalis* is an exclusively human commensal and mucosal pathogen. Its role as a disease-causing organism has long been questioned. Today, it is recognized as one of the major causes of acute otitis media in children, and its relative frequency of isolation from both the nasopharynx and the middle ear cavity has increased since the introduction of the heptavalent pneumococcal conjugate vaccine, which is associated with a shift in the composition of the nasopharyngeal flora in infants and young children. Although otitis media caused by *M. catarrhalis* is generally believed to be mild in comparison with pneumococcal disease, numerous putative virulence factors have now been identified and it has been shown that several surface components of *M. catarrhalis* induce mucosal inflammation. In adults with chronic obstructive pulmonary disease (COPD), *M. catarrhalis* is now a well-established trigger of approximately 10% of acute inflammatory exacerbations.

Although the so-called cold shock response is a well-described bacterial stress response in species such as *Escherichia coli*, *Bacillus subtilis* or – more recently – *Staphylococcus aureus*, *M. catarrhalis* is the only typical nasopharyngeal pathogen in which this response has been investigated. Indeed, a 3-h 26°C cold shock, which may occur physiologically, when humans inspire cold air for prolonged periods of time, increases epithelial cell adherence and enhances proinflammatory host responses and may thus contribute to the symptoms referred to as common cold, which typically are attributed to viral infections.

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N. Curtis et al. (eds.), *Hot Topics in Infection and Immunity in Children VII*, Advances in Experimental Medicine and Biology 697, DOI 10.1007/978-1-4419-7185-2_9,

1 Introduction

Moraxella catarrhalis is exclusively a human pathogen and commensal of the upper respiratory tract. All attempts to establish long-term respiratory tract colonization in non-human primates or other vertebrates have failed. *Moraxella catarrhalis* is a gram-negative, aerobic, variably piliated, nonmotile, strongly autoagglutinating and non-encapsulated diplococcus, which microscopically resembles *Neisseria meningitidis* and *Neisseria gonorrhoeae* but is more closely related genetically to *Acinetobacter spp.* and the Pseudomonadales [1]. All other members of the genus *Moraxella* are gram-negative rods. The molecular basis of the exquisite tropism of *M. catarrhalis* for humans has not entirely been elucidated. Some data indicate that the organisms' iron-uptake apparatus requires a specific ligand–receptor interaction between human iron transport proteins (e.g., lactoferrin and transferrin) and specific binding proteins on the bacterial surface (e.g., lactoferrin-binding protein B) [2].

Formerly known as *Micrococcus catarrhalis* (first described in 1896), and renamed *Neisseria catarrhalis* (1963) and *Branhamella catarrhalis* (1970), *M. catarrhalis* was moved to the Genus *Moraxella* in 1984 (Class 1 γ-proteobacteria, order *Pseudomonadales*, family *Moraxellaceae*, Genus *Moraxella*) and had long been considered a non-pathogenic commensal of the nasopharynx. Its role as disease-causing organism has only recently been recognized and its pathophysiological mechanisms are still poorly understood. Nonetheless, several lines of evidence now indicate that *M. catarrhalis* causes mucosal infections in immunocompetent children (mainly otitis media) [3] and adults (acute exacerbations of chronic obstructive pulmonary disease) [4]. Invasive disease (e.g., bacteraemia, endocarditis, meningitis, arthritis) is extremely rare and almost exclusively occurs in immunocompromised individuals [5].

2 Phylogenetic Evidence for Virulence

The species *M. catarrhalis* consists of two major phylogenetic lineages. This was first emphasized by Bootsma et al. [6] and was recently described in detail by Wirth et al. [7]. Differentiation can easily be made by DNA sequence analysis of the 16S rDNA gene [6]. The phylogenetically older subpopulation (type 2) is believed to have existed long before the emergence of *Homo sapiens*, while the phylogenetically younger subpopulation is believed to have emerged approximately 4 million years ago, together with *H. sapiens*. Interestingly, the latter subpopulation is associated with the expression of phenotypic traits characteristically associated with bacterial virulence. These include resistance to human complement and adherence to human epithelial cells. This subpopulation is also naturally transformation competent [8] leading to frequent homologous recombination and thus reduced genetic diversity as a result of clonal selection (Table 1) [7]. Thus, it appears that type 1 strains are closely adapted to the human host. Convincing clinical evidence supporting the notion that type 1 strains are more commonly associated with disease as opposed to

Table 1 Characteristics of the two major phylogenetic subpopulations of *M. catarrhalis*

	Subpopulation	
	Complement sensitive (16S rDNA type 2)	Complement resistant (16S rDNA type 1)
Age	~50 million years	~4 million years
Genetic diversity	Extensive	Low
Homologous recombination	Rare	Frequent
Virulence factor expression	Rare	Frequent

asymptomatic colonization is currently lacking, but there is circumstantial evidence described below.

3 Expression of Virulence Factors

3.1 Complement Resistance

Type 1 strains are typically complement resistant in vitro. Exposure to human complement does not affect the growth of these strains, while complement-sensitive strains (mainly type 2) are killed by human complement within 60–120 min of exposure. Complement resistance is believed to be mediated mainly by expression of the UspA2 outer membrane protein (OMP), although other OMPs (e.g., CopB [9]) are also essential for this phenotype. UspA2 is a trimeric autotransporter protein, expressed abundantly which binds the complement modulator serum protein vitronectin and which inhibits assembly of the C5–C9 membrane attack complex [10]. It has also been demonstrated that *M. catarrhalis* OM vesicles are capable of protecting complement-sensitive *Haemophilus influenzae* against the bactericidal effect of complement, mainly by binding/inactivation of vitronectin, C4bp, C3 and factor H [11]. Complement resistance is believed to be a virulence factor because several clinical studies have indicated that this phenotype is associated more commonly with disease-causing isolates than with colonizing isolates [12, 13].

3.2 Adherence to Human Epithelial Cells

Bacterial virulence of mucosal pathogens is generally believed to depend on the organisms' capacity to bind to the epithelial layer of the host's mucosal surface. Complement-resistant type 1 strains generally express UspA1, a major *M. catarrhalis* adhesin, which is closely related to UspA2 [14] and which binds human epithelial cells via cell-bound fibronectin or the CEACAM-1 [15, 16] adhesion molecule displayed on various respiratory epithelial cell types. Interestingly, specific domains of UspA1 function as binding sites for fibronectin or CEACAM-1 [15, 16]. Type 2 strains typically do not express UspA1 on their surface [17], although they carry the *uspA*1 gene on their chromosome. Thus, type 1 strains

Table 2 Currently known adhesins of *M. catarrhalis* and their respective cellular ligands

Ligand	Cell line	Host cell receptor	References
UspA1	HEp-2	Not determined	[19]
UspA1	Chang	Fibronectin/α5β1	[20]
UspA2H	Chang	Not determined	[21]
UspA1	A549	CEACAM1	[22]
Hag/MID	A549	Not determined	[23]
Hag/MID	Human middle ear cells	Not determined	[23]
McaP	A549	Not determined	[24]
McaP	Chang	Not determined	[24]
OMP CD	A549	Not determined	[25]
LOS	Chang	Not determined	[26]
LOS	HeLa	Not determined	[26]

express adhesins, which enable their close interaction with the respiratory tract epithelial surface. In addition, UspA1 has also been shown to be essential for the internalization of *M. catarrhalis* cells into nonprofessional phagocytes [18].

Moraxella catarrhalis is known to display various adhesins specific for receptors expressed by different respiratory tract cell lines (Table 2). This redundancy may allow the organism to colonize the middle ear, the oro- and nasopharynx, as well as the bronchial surface and alveolar space of the human respiratory tract.

3.3 Colonization and Immune Response

Up to 80% of children have been colonized at least once with *M. catarrhalis* by the time they reach the age of 2 years [27]. Thereafter colonization frequency drops continuously to <10% in older children and healthy adults. It increases again in the elderly [28]. Colonization is not prevented by the appearance of mucosal (salivary) IgA directed against the major surface immunogens [25, 29]. However, there is a strong correlation between the disappearance of mucosal *M. catarrhalis* and the appearance of bactericidal serum anti-*M. catarrhalis* IgG1 and IgG3, which are mainly directed against the UspA OMP [30–32]. These data indicate that systemic humoral immunity recognizes immunodominant *M. catarrhalis* surface antigens and eliminates surface carriage by the development of bactericidal IgG antibodies and, presumably, complement-mediated killing.

However more recent evidence indicates that *M. catarrhalis* may evade humoral immunity by (i) intracellular location in epithelial cells (Fig. 1) [18, 33] and by submucosal location in pharyngeal lymphoid tissue (Fig. 2) [34]. The clinical relevance of these observations has not been elucidated, but it appears possible that *M. catarrhalis*, similarly to *H. influenzae*, *Staphylococcus aureus* and *Streptococcus pyogenes*, may be capable of entering an intracellular niche which protects the organism against the host's immune system. Preliminary data suggest that neither

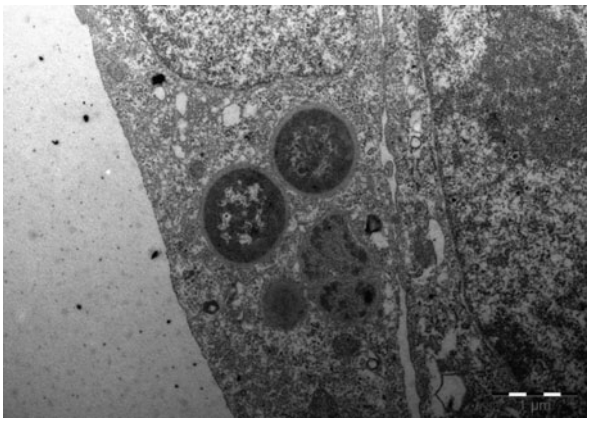

Fig. 1 Transmission electron micrograph of a Detroit 562 pharyngeal cell harbouring two *M. catarrhalis* cells 3 h after infection of a cellular monolayer with *M. catarrhalis* strain O35E and subsequent killing of all extracellular bacteria using 200 μg/ml of gentamicin

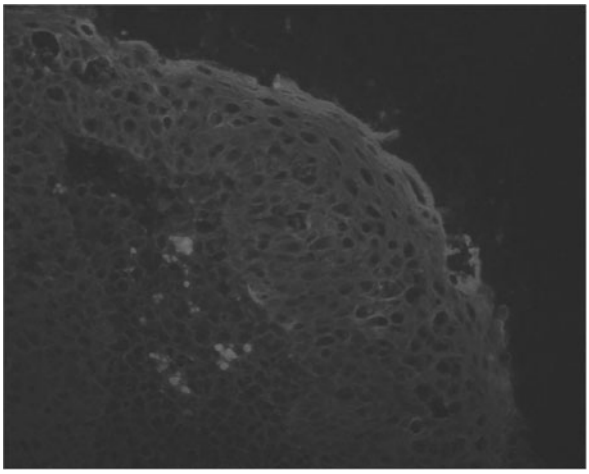

Fig. 2 Immunohistochemistry of a human tonsil showing subepithelial aggregates of *M. catarrhalis*. Squamous cell epithelium is stained with rabbit anti-human cytokeratin and goat anti-rabbit Cy3. *Moraxella catarrhalis* is stained with the monoclonal mouse monoclonal antibody 24B5 (a gift from Dr. E Hansen, Dallas, TX) and goat anti-mouse Alexa 488 and appears light

phase variation nor antigenic variation plays a substantial role in this phenomenon (author's unpublished data).

3.4 Biofilm Formation

A virulence mechanism employed by many bacterial species is the expression of biofilms, which protect individual bacteria from the detrimental effects of host immune mechanisms and antimicrobial substances. Biofilm formation in *M. catarrhalis* has not been investigated in detail, but some data clearly indicate that some clinical isolates produce biofilms in vitro [35, 36] and also in vivo on the middle ear mucosal surface [37].

3.5 *Cellular Invasion*

As stated above, recent evidence indicates that *M. catarrhalis* is capable of invading human respiratory tract cells in vitro and that this capacity is dependent on the expression of UspA1 and lipooligosaccharide [18, 33]. However, studies investigating long-term persistence have not been conducted to date. Thus, it remains unclear whether invasion is a means of evading the immune system as well as the effects of extracellular antimicrobial agents. Further studies will be needed to address these issues in detail.

3.6 *Proinflammatory Activity of* Moraxella catarrhalis

By contrast, it is now well established that both *M. catarrhalis* whole cells and outer membrane preparations induce strong proinflammatory stimulation in epithelial cells (interleukin-8) [38] and peripheral blood mononuclear cells (IL-1β, IL-6, TNF-α) [39]. These observations suggest that the presence of *M. catarrhalis* may cause inflammation and, consequently, clinical symptoms of respiratory tract disease in otherwise sterile locations.

4 Cold Shock Response of *Moraxella catarrhalis*

When humans inspire cold air (e.g., –1°C) for prolonged periods of time, their nasopharyngeal temperature drops to approximately 26°C within several minutes of the beginning of the exposure [40]. In an in vitro model, this "cold shock" affects the resident *M. catarrhalis* flora in several ways. Abundance of mRNA transcripts of the UspA1 adhesin increases with reduced temperature and reaches a maximum at 26°C [34], an effect possibly explained by a prolonged mRNA half-life at decreased temperature [41]. Consequently, more UspA1 adhesin is expressed on the bacterial surface at 26°C and adherence to epithelial cells such as Chang conjunctival cells [34], Detroit 562 pharyngeal cells and HEp2 laryngeal cells is enhanced (Fig. 3) [41]. Enhanced adherence translates into increased mucosal surface density, a phenomenon shown to be associated with an increased likelihood of acute otitis media in children [42]. On a molecular level, increased surface expression of

Fig. 3 Cold shock increases the outer membrane protein (OMP)-mediated release of proinflammatory cytokine IL-8 in Detroit 562 epithelial cells, which were incubated for 16 h with increasing doses of heat-inactivated strain O35E (**a**) and the strains 300,415, 420 (**b**) exposed to 26°C or to 37°C or stimulated with outer membrane vesicles isolated from *M. catarrhalis* exposed to 26°C or 37°C (**c**). IL-8 secretion in the supernatants was measured by ELISA. In each case, results from one representative experiment of either two or three replicates are shown. Results are expressed as mean ± 1 SD of duplicate wells. *, $P<0.05$ (two-way analysis of variance) at 26°C vs. 37°C. MOI stands for multiplicity of infections and indicates the ratio between bacteria inoculated and epithelial cells in a given well.

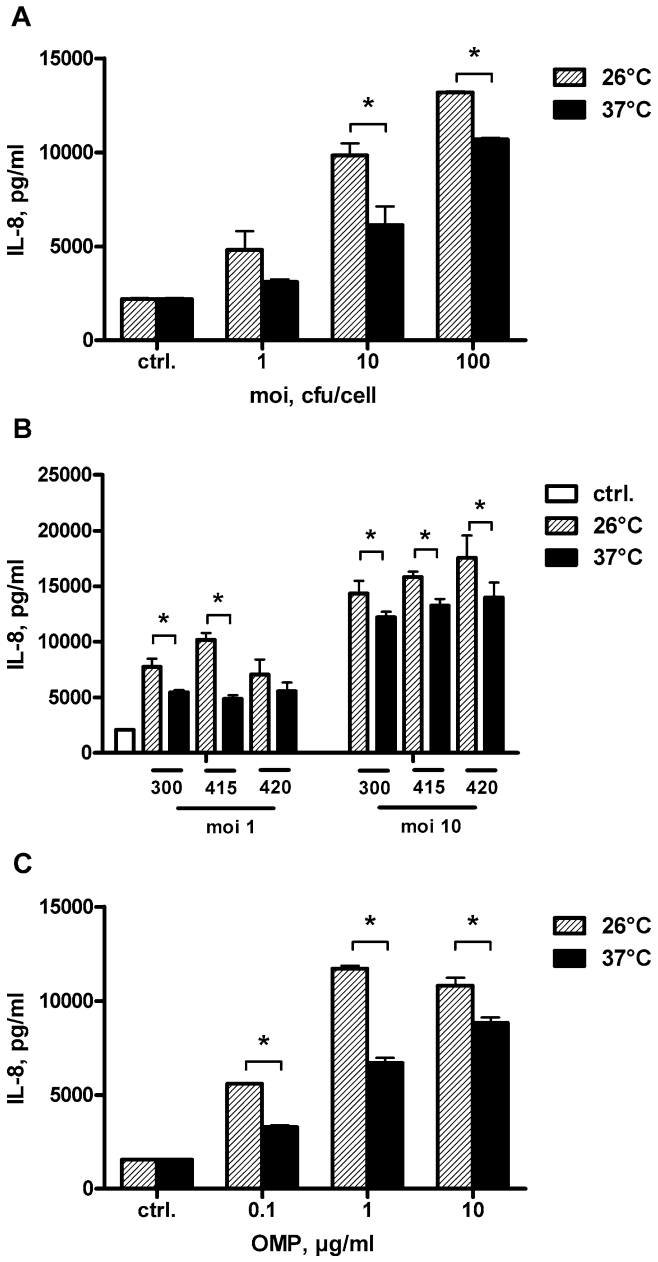

Fig. 3 (Continued)

UspA1 results in enhanced binding of fibronectin, which mediates binding to epithelial cells via α5β1 integrin [41]. The UspA1 cold shock phenomenon occurs in both phylogenetic lineages and is accompanied by increased expression of typical "cold

shock proteins" such as *recA* [34]. Genomic analyses of the cold shock response of *M. catarrhalis* have not been conducted to date. Unpublished data indicate that a limited number of OMPs are upregulated at 26°C and that others, e.g., haemagglutinin (which is an adhesin for A549 lung cells) or the m35 [43] outer membrane porin, are upregulated at 37°C.

Also of potential clinical relevance is the finding that *M. catarrhalis* undergoing a 3-h cold shock at 26°C induces a markedly enhanced proinflammatory immune response in comparison with bacteria held at 37°C. Both whole bacteria and outer membrane preparations induced a significantly enhanced release of IL-8 from Detroit 562 pharyngeal cells in one study (Fig. 3) [41].

5 Summary

The data summarized here emphasize that *M. catarrhalis* is a commensal and pathogen which is well adapted to the human respiratory tract niche, that it usually behaves as non-pathogenic commensal, but under certain circumstances it can become pathogenic. Such opportunities may include viral co-infections, augmented mucosal density of the organism (which might theoretically occur, for example, as a result of colonisation replacement phenomena secondary to pneumococcal vaccination) or – as specifically discussed in this article – exposure of the pharynx to cold air. More research is needed to understand the modes of transition from commensal to pathogen better. However, overall, the behaviour of *M. catarrhalis* resembles in many ways that of non-typable *H. influenzae*, with which it shares the nasopharyngeal habitat.

References

1. Murphy TF. *Branhamella catarrhalis*: epidemiology, surface antigenic structure, and immune response. Microbiol Rev. 1996;60:267–79.
2. Schryvers AB, Bonnah R, Yu RH, Wong H, Retzer M. Bacterial lactoferrin receptors. Adv Exp Med Biol. 1998;443:123–33.
3. Palmu AA, Herva E, Savolainen H, Karma P, Makela PH, Kilpi TM. Association of clinical signs and symptoms with bacterial findings in acute otitis media. Clin Infect Dis. 2004;38:234–42.
4. Murphy TF, Parameswaran GI. *Moraxella catarrhalis*, a human respiratory tract pathogen. Clin Infect Dis. 2009;49:124–31.
5. Ahmed A, Broides A, Givon-Lavi N, Peled N, Dagan R, Greenberg D. Clinical and laboratory aspects of *Moraxella catarrhalis* bacteremia in children. Pediatr Infect Dis J. 2008;27:459–61.
6. Bootsma HJ, van der Heide HG, van de Pas S, Schouls LM, Mooi FR. Analysis of *Moraxella catarrhalis* by DNA typing: evidence for a distinct subpopulation associated with virulence traits. J Infect Dis. 2000;181:1376–87.
7. Wirth T, Morelli G, Kusecek B, et al. The rise and spread of a new pathogen: seroresistant *Moraxella catarrhalis*. Genome Res. 2007;17:1647–56.
8. Meier PS, Troller R, Heiniger N, Hays JP, van Belkum A, Aebi C. Unveiling electrotransformation of *Moraxella catarrhalis* as a process of natural transformation. FEMS Microbiol Lett. 2006;262:72–6.

9. Helminen ME, Maciver I, Paris M, et al. A mutation affecting expression of a major outer membrane protein of *Moraxella catarrhali*s alters serum resistance and survival in vivo. J Infect Dis. 1993;168:1194–201.
10. Attia AS, Lafontaine ER, Latimer JL, Aebi C, Syrogiannopoulos GA, Hansen EJ. The UspA2 protein of *Moraxella catarrhalis* is directly involved in the expression of serum resistance. Infect Immun. 2005;73:2400–10.
11. Tan TT, Morgelin M, Forsgren A, Riesbeck K. H*aemophilus influenzae* survival during complement-mediated attacks is promoted by *Moraxella catarrhalis* outer membrane vesicles. J Infect Dis. 2007;195:1661–70.
12. Hol C, Verduin CM, van Dijke E, Verhoef J, van Dijk H. Complement resistance in *Branhamella (Moraxella) catarrhalis*. Lancet. 1993;341:1281.
13. Murphy S, Fitzgerald M, Mulcahy R, Keane C, Coakley D, Scott T. Studies on haemagglutination and serum resistance status of strains of *Moraxella catarrhalis* isolated from the elderly. Gerontology. 1997;43:277–82.
14. Aebi C, Maciver I, Latimer JL et al. A protective epitope of *Moraxella catarrhalis* is encoded by two different genes. Infect Immun. 1997;65:4367–77.
15. Brooks MJ, Sedillo JL, Wagner N, et al. Modular arrangement of allelic variants explains the divergence in *Moraxella catarrhalis* UspA protein function. Infect Immun. 2008;76:5330–40.
16. Brooks MJ, Sedillo JL, Wagner N, et al. *Moraxella catarrhalis* binding to host cellular receptors is mediated by sequence-specific determinants not conserved among all UspA1 protein variants. Infect Immun. 2008;76:5322–9.
17. Meier PS, Troller R, Heiniger N, Grivea IN, Syrogiannopoulos GA, Aebi C. *Moraxella catarrhalis* strains with reduced expression of the UspA outer membrane proteins belong to a distinct subpopulation. Vaccine. 2005;23:2000–8.
18. Spaniol V, Heiniger N, Troller R, Aebi C. Outer membrane protein UspA1 and lipooligosaccharide are involved in invasion of human epithelial cells by *Moraxella catarrhalis*. Microbes Infect. 2008;10:3–11.
19. Aebi C, Lafontaine ER, Cope LD, et al. Phenotypic effect of isogenic uspA1 and uspA2 mutations on *Moraxella catarrhalis* 035E. Infect Immun. 1998;66:3113–9.
20. Tan TT, Nordstrom T, Forsgren A, Riesbeck K. The respiratory pathogen *Moraxella catarrhalis* adheres to epithelial cells by interacting with fibronectin through ubiquitous surface proteins A1 and A2. J Infect Dis. 2005;192:1029–38.
21. Lafontaine ER, Cope LD, Aebi C, Latimer JL, McCracken GH Jr, Hansen EJ. The UspA1 protein and a second type of UspA2 protein mediate adherence of *Moraxella catarrhalis t*o human epithelial cells in vitro. J Bacteriol. 2000;182:1364–73.
22. Hill DJ, Virji M. A novel cell-binding mechanism of *Moraxella catarrhalis* ubiquitous surface protein UspA: specific targeting of the N-domain of carcinoembryonic antigen-related cell adhesion molecules by UspA1. Mol Microbiol. 2003;48:117–29.
23. Bullard B, Lipski SL, Lafontaine ER. Hag directly mediates the adherence of *Moraxella catarrhalis* to human middle ear cells. Infect Immun. 2005;73:5127–36.
24. Timpe JM, Holm MM, Vanlerberg SL, Basrur V, Lafontaine ER. Identification of a *Moraxella catarrhalis* outer membrane protein exhibiting both adhesin and lipolytic activities. Infect Immun. 2003;71:4341–50.
25. Meier PS, Freiburghaus S, Martin A, Heiniger N, Troller R, Aebi C. Mucosal immune response to specific outer membrane proteins of *Moraxella catarrhalis* in young children. Pediatr Infect Dis J. 2003;22:256–62.
26. Peng D, Hong W, Choudhury BP, Carlson RW, Gu XX. *Moraxella catarrhalis* bacterium without endotoxin, a potential vaccine candidate. Infect Immun. 2005;73:7569–77.
27. Faden H, Harabuchi Y, Hong JJ. Epidemiology of *Moraxella catarrhalis* in children during the first 2 years of life: relationship to otitis media. J Infect Dis. 1994;169:1312–7.
28. Vaneechoutte M, Verschraegen G, Claeys G, Weise B, Van den Abeele AM. Respiratory tract carrier rates of *Moraxella (Branhamella) catarrhalis* in adults and children and interpretation of the isolation of *M. catarrhalis* from sputum. J Clin Microbiol. 1990;28:2674–80.

29. Stutzmann Meier P, Heiniger N, Troller R, Aebi C. Salivary antibodies directed against outer membrane proteins of *Moraxella catarrhalis* in healthy adults. Infect Immun. 2003;71: 6793–8.
30. Goldblatt D, Turner MW, Levinsky RJ. *Branhamella catarrhalis*: antigenic determinants and the development of the IgG subclass response in childhood. J Infect Dis. 1990;162:1128–35.
31. Chen D, Barniak V, VanDerMeid KR, McMichael JC. The levels and bactericidal capacity of antibodies directed against the UspA1 and UspA2 outer membrane proteins of *Moraxella (Branhamella) catarrhalis* in adults and children. Infect Immun. 1999;67:1310–6.
32. Tan TT, Christensen JJ, Dziegiel MH, Forsgren A, Riesbeck K. Comparison of the serological responses to *Moraxella catarrhalis* immunoglobulin D-binding outer membrane protein and the ubiquitous surface proteins A1 and A2. Infect Immun. 2006;74:6377–86.
33. Slevogt H, Seybold J, Tiwari KN, et al. *Moraxella catarrhalis* is internalized in respiratory epithelial cells by a trigger-like mechanism and initiates a TLR2- and partly NOD1-dependent inflammatory immune response. Cell Microbiol. 2007;9:694–707.
34. Heiniger N, Spaniol V, Troller R, Vischer M, Aebi C. A reservoir of *Moraxella catarrhalis* in human pharyngeal lymphoid tissue. J Infect Dis. 2007;196:1080–7.
35. Luke NR, Jurcisek JA, Bakaletz LO, Campagnari AA. Contribution of *Moraxella catarrhalis* type IV pili to nasopharyngeal colonization and biofilm formation. Infect Immun. 2007;75:5559–64.
36. Pearson MM, Hansen EJ. Identification of gene products involved in biofilm production by *Moraxella catarrhalis* ETSU-9 in vitro. Infect Immun. 2007;75:4316–25.
37. Hall-Stoodley L, Hu FZ, Gieseke A, et al. Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. JAMA. 2006;296:202–11.
38. Slevogt H, Maqami L, Vardarowa K, et al. Differential regulation of *Moraxella catarrhalis*-induced interleukin-8 response by protein kinase C isoforms. Eur Respir J. 2008;31:725–35.
39. Fink J, Mathaba LT, Stewart GA, et al. *Moraxella catarrhalis* stimulates the release of proinflammatory cytokines and prostaglandin E from human respiratory epithelial cells and monocyte-derived macrophages. FEMS Immunol Med Microbiol. 2006;46:198–208.
40. Rouadi P, Baroody FM, Abbott D, Naureckas E, Solway J, Naclerio RM. A technique to measure the ability of the human nose to warm and humidify air. J Appl Physiol. 1999;87: 400–6.
41. Spaniol V, Troller R, Aebi C. Physiologic cold shock increases adherence of *Moraxella catarrhalis* to and secretion of interleukin 8 in human upper respiratory tract epithelial cells. J Infect Dis. 2009;200:1593–601.
42. Smith-Vaughan H, Byun R, Nadkarni M, et al. Measuring nasal bacterial load and its association with otitis media. BMC Ear Nose Throat Disord. 2006;6:10.
43. Jetter M, Heiniger N, Spaniol V, Troller R, Schaller A, Aebi C. Outer membrane porin M35 of *Moraxella catarrhalis* mediates susceptibility to aminopenicillins. BMC Microbiol. 2009;9:188.