

Mobile genetic elements in *Moraxella catarrhalis*

John Hays

Department of Medical Microbiology and Infectious Disease; Erasmus MC; Rotterdam, The Netherlands

Moraxella catarrhalis is a Gram-negative diplococcus that is a strict human pathogen, which for a long period of time was regarded as a simple commensal. Research now shows that this organism is a pathogen its own right and is associated with both upper and lower respiratory tract infections. Further, there appears to be a dichotomy in the pathogenic potential of *M. catarrhalis* with upper respiratory tract infections mainly occurring in children, and lower respiratory tract infections mainly occurring in adults with predisposing pulmonary complications e.g., chronic obstructive pulmonary disease (COPD).

Until recently, very little was known about the number and types of mobile genetic elements present within this species, largely due to the fact that infections by competing bacterial respiratory pathogens e.g., *Streptococcus pneumoniae*, *Haemophilus influenzae*, group A streptococci etc tend to generate more serious disease complications. In two recent articles however, whole genome sequencing has been utilized to characterize the type and number of mobile genetic elements present in 12 clinically relevant isolates of *M. catarrhalis*.^{1,2}

With respect to plasmid mobile elements, only 1 out of 12 isolates sequenced appeared to carry a plasmid, this plasmid comprising 12 ORFs and representing 0.6% of the total number of ORFs predicted for this particular bacterial isolate. Sequence analysis showed that this as yet uncharacterized plasmid possessed homologues of genes found in plasmid pLQ510, but that these homologues were also associated with: (1) an incomplete VirB-family type four secretion system (T4SS), (2) multiple transposases and (3) resolvases; genes not previously reported in plasmid

pLQ510. In fact, it is possible that the presence of the extra genes indicate the discovery of a new integrated and conjugative element (ICE) rather than the presence of a plasmid per se (Fig. 1). However, at this moment, no experimental data is available to confirm or refute this hypothesis. In fact, the lack of plasmids found during our sequencing of the 12 isolates is to be expected, as there have only been a few previous reports of plasmids being isolated from *M. catarrhalis*, and only 2 plasmids (pLQ510 and pEMCJH03) currently having been characterized.^{3,4} The most intensively studied of the 2 well-characterized *M. catarrhalis* plasmids is pLQ510, a plasmid first characterized by Beaulieu et al. in 1988, who showed that this was a 12.2 kb plasmid that tended to be species specific.³ Structural analysis of this plasmid was published 10 years later, when Liu and Hansen showed that the plasmid comprises 12,082 bp with a 38% GC content.⁵ Interestingly, further research in 2009, showed that this plasmid carried putative bacteriocin production and secretion genes via a transcriptionally linked *mcbABCI* locus.⁶ It was further shown that the presence of the *mcbABCI* locus provided a distinct growth advantage upon co-culture of an *mcbABCI* locus-containing strain with a non-*mcbABCI* locus-containing strain. Importantly, the *mcbABCI* locus was also shown to be present in the chromosome of several other *M. catarrhalis* strains and was therefore not restricted to the pLQ510 mobile genetic element. At the present moment in time, whether other bacteriocin-carrying plasmids exist within the *M. catarrhalis* species remains to be determined, as does the exact mechanism by which the *mcbABCI* locus is transferred.

Key words: *Moraxella catarrhalis*, plasmid, transposon, phage, beta-lactamase

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*Correspondence to: J.P. Hays;
Email: j.hays@erasmusmc.nl

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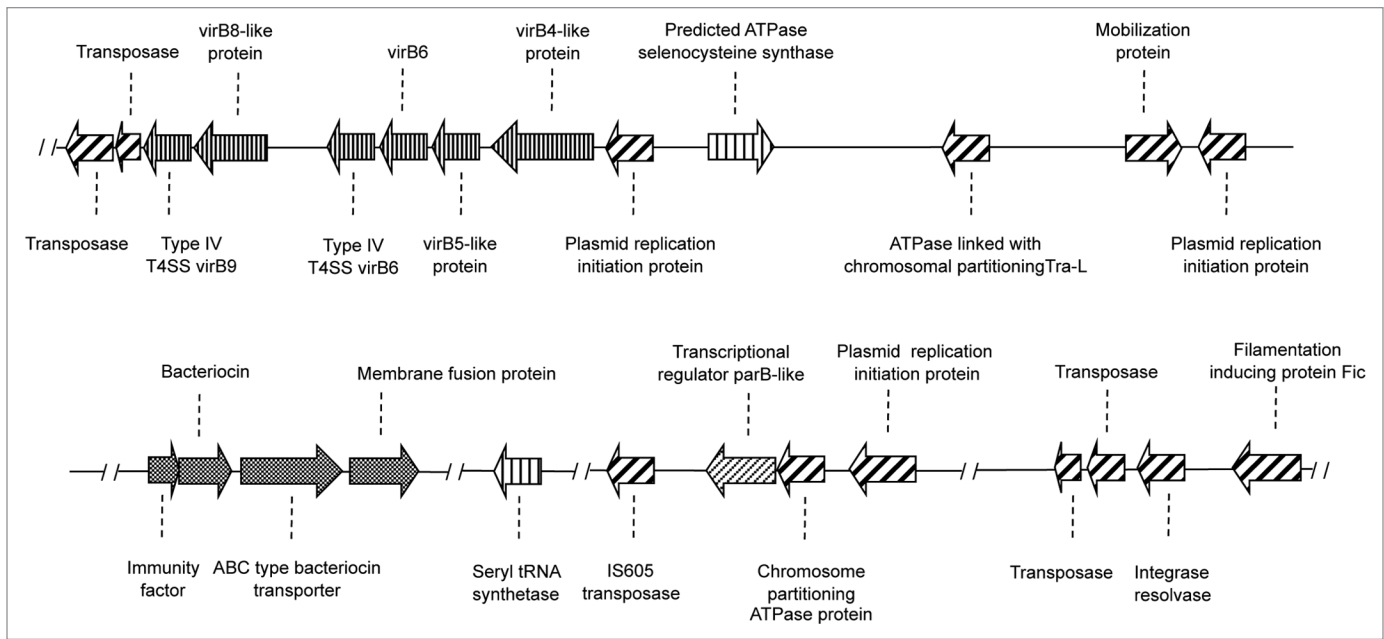


Figure 1. Schematic diagram of the putative integrated and conjugative element associated with the bacteriocin expressing phenotype in *M. catarrhalis* isolate Bc7.

In 2005, the author published an article characterising a small 3.5 kb mobilizable plasmid called pEMCJH03, which contained mobilizable genes only (no virulence). Natural transformation of this plasmid was only successful in 25% of strains tested, and the plasmid showed a low transformation efficiency (615 CFU/ μ g, range 60–1,040 CFU/ μ g). As with plasmid pLQ510, attempts were subsequently made to convert pEMCJH03 into a suitable cloning an expression vector, though this procedure encountered unexpected obstacles. For example, the author successfully cloned an enhanced green fluorescent protein gene and a kanamycin resistance cassette into pEMCJH03, only to find that within one generation the majority of fluorescent cells decreased by approximately 95% (unpublished data), even in the presence of kanamycin selection pressure. Further, similar attempts using a *lux* gene have also failed (Personal Communication: R. Harris, Clafin University, USA). Interestingly, Wang and Hansen described plasmid pWW115 (a spontaneous plasmid deletion mutant of an artificial construct of pLQ510 and an unrelated plasmid pACYC184), which was used to clone a *uspA2* gene (associated with complement resistance) into a serum sensitive isolate of *M. catarrhalis*.⁷

The result was a change in phenotype from serum sensitive to serum resistant. However, similar to the use of antibiotics, serum treatment generates selective pressure on bacteria, such that individual bacteria possessing deleterious mutations are not selected. Taken together, these results most likely indicate that plasmid expression of cloned genes in *M. catarrhalis* may be hampered by an as yet uncharacterized mechanism, especially for genes that do not provide the host with a phenotypic advantage. Further, this observation could provide an explanation as to why there appears to be very few (virulence) plasmids associated with this particular bacterial species, there being natural limits on the acceptance/correct functioning of non-host plasmids and plasmid-borne genes within *M. catarrhalis* isolates and between co-colonizing species (*H. influenzae*, *S. pneumoniae*, *S. pyogenes* etc.). These mechanisms could operate via restrictions/modification systems,⁸ or possibly via a high gene mutation frequency. Importantly, this issue could affect the ability to clone and express, for example, putative vaccine candidate genes in *M. catarrhalis*, suggesting that cloned genes should be regularly re-sequenced in order to establish that no unintended mutations have become established in the putative

vaccine candidate gene being expressed. Alternatively, a different bacterial species may have to be chosen for future vaccine gene expression in order to maintain genetic stability.

Phage ORFs were found in all 12 isolates sequenced ranging from 3–49 ORFs per genome, and representing 0.2–2.5% of the total ORFs per genome. However, precious little phage research has been performed on *M. catarrhalis*, and indeed there is a lack of understanding of the impact of phages on all species of *Moraxella*.^{9,10}

Similar to the paucity of plasmids and phages observed during *M. catarrhalis* whole genome sequencing, the number of transposon open reading frames (ORFs) found in this bacterial species also appears to be very low, with a range of between 3–8 transposon ORFs per genome. In fact, fewer than 10 transposon ORFs were detected in the 12 clinical isolates (isolated from four different countries) that were sequenced, with transposons accounting for only 0.15%–0.44% of all ORFs per genome. This low percentage indicates that transposition may play only a minor role in *M. catarrhalis* evolution and pathogenicity. In fact, the best-known “mobile genetic element” associated with *M. catarrhalis* is the *bro* beta-lactamase gene. This gene was first described in a few

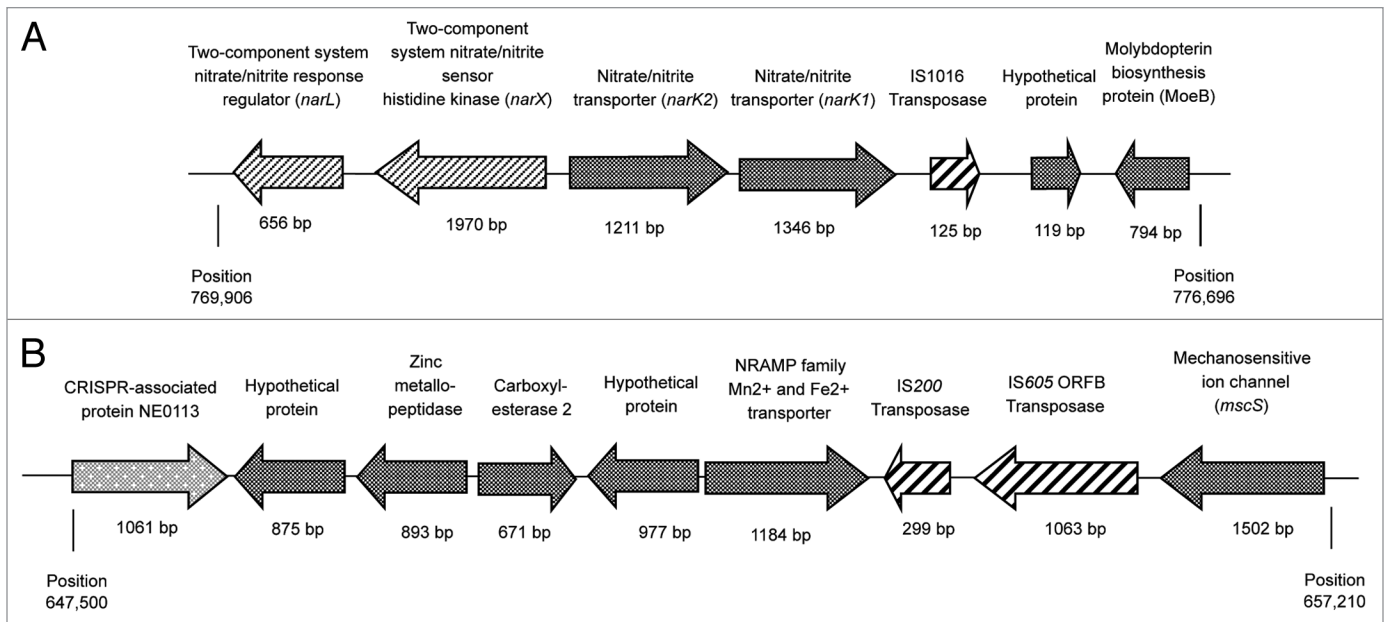


Figure 2. Schematic representation of the position of transposon IS1016 (A), and IS200 and IS605 (B), in the genome of *M. catarrhalis* isolate RH4. Isolate RH4 was the first *M. catarrhalis* isolate to be fully sequenced and annotated as a complete, contiguous, chromosome.

clinical *M. catarrhalis* species in the 1970s, but is now present in approximately 95% of clinical isolates. Further, this spread is not related to clonal expansion as *M. catarrhalis* isolates show a distinct lack of clonality. Bootsma et al. showed that the acquisition of this gene probably occurred via a homologous recombination event, though the actual origin of the gene itself is still a mystery (BRO beta-lactamase being unrelated to any known beta-lactamase so far described).¹¹⁻¹³ However, natural competence has been found to be a feature of *M. catarrhalis* biology, showing that the bacterium is able to naturally acquire foreign DNA.¹⁴ The most likely ‘donor’ candidates for the *bro* gene are the related *Psychrobacter*, *Acinetobacter* and *Neisseria* genera, and these genera could also possibly act as donors for mobile genetic elements, though the different environmental niches that these genera currently occupy in relation to *M. catarrhalis* may mean that mobile genetic element transfer between these bacterial genera are rare events.

Of the transposon ORFs identified, homologues to IS1016, IS200 and IS4 transposons (sub)families were identified in all 12 genomes sequenced, with five genomes also containing an IS605 family transposon. As mentioned in our

sequencing publication, for all 12 isolates, the IS1016 element was found adjacent to a nitrate uptake locus (Fig. 2A), and the IS200 element was found in a locus containing the CRISPR-associated protein (NE0113) and a mechanosensitive ion channel (*mscS*; Fig. 2B). The exact effect of these insertions (if any) is as yet unknown, though no flanking genes appear to be disrupted. One possible explanation being that deleterious transposition events have been selected-out of the *M. catarrhalis* population by selection pressure. For example, *narK1* and *narK2* are involved in the capability of bacteria to grow under anaerobic denitrifying, microaerophilic and occasionally aerobic growth conditions.¹⁵ Any disruption of these genes could potentially seriously affect the growth (and hence colonization and virulence) potential of the affected bacterium.

Finally, it should be stressed that most of the scientific research performed to date, and all of the 12 *M. catarrhalis* isolates sequenced so far, tend to belong to one of the two major genotypic lineages that have been described in *M. catarrhalis* (rather unimaginatively described as ‘Lineage 1’), which is much more genotypically conserved (as determined using pulsed field gel electrophoresis)

than ‘Lineage 2’ isolates.¹⁶ Currently, more sequencing work is being performed in order to better characterize Lineage 2 genotype isolates. However, Lineage 1 genotype isolates tend to be more frequently associated with clinical infections, meaning that an increased prevalence of pathogenic gene carriage or increased virulence gene expression in Lineage 2 isolates facilitated by mobile genetic elements is unlikely.

From current research, mobile genetic elements per se appear to play only a minor role in influencing *M. catarrhalis* evolution, with occasional homologous recombination events having a much more profound, and clinically relevant, effect. However, research is continuing with the current emphasis focussing on whole genome sequencing and comparative genomics of Lineage 2 versus Lineage 1 isolates. Nevertheless, comparative genomics indicates that *M. catarrhalis* isolates tend to be refractory to the uptake of mobile genetic elements, though the exact mechanism facilitating this phenomenon has yet to be understood.

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