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Moraxella catarrhalis: The virulence factor and pathogenesis strategy

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Abstract
Moraxella catarrhalis is a Gram-negative diplococcus bacterium, formerly known as *Neisseria catarrhalis* or *Branhamella catarrhalis*. The bacterium colonizes the nasopharynx as innocent commensal, the recent recognition of *M. catarrhalis* as an important pathogen in both the upper and lower respiratory tract. It is important human-restricted pathogen responsible for sinusitis and otitis media in children as well as infections of the lower respiratory tract, causing exacerbation of chronic obstructive pulmonary disease in adults. The mechanisms of colonization and pathogenesis of *M. catarrhalis* have been extensively studied and many virulence factors have been identified to date. The objective of this study summarized important virulence factor and pathogenesis strategy of *M. catarrhalis*.

Keywords: *Moraxella*, virulence factors, pathogenesis

Introduction

Moraxella catarrhalis

The genus *Moraxella* belongs to the family Moraxellaceae, the most significant species is *Moraxella catarrhalis* (*M. catarrhalis*), also known as *Branhamella catarrhalis*, *Micrococcus catarrhalis*, and *Neisseria catarrhalis*. *Moraxella catarrhalis* is a gram-negative, aerobic, unencapsulated, oxidase-positive diplococcus (de Vries, *et al.*, 2010; Shi *et al.*, 2018) [29, 89].

Bacterium was first isolated in 1896, it was considered to be a harmless commensal of the upper respiratory tract for a long period of time. The bacterium rapidly colonizes the nasopharynx soon after birth asymptomatically (Blakeway *et al.*, 2017) [16]. The bacterium has now firmly established its position as an etiological cause of human respiratory tract (de Vries, *et al.*, 2010) [29]. This institution is polymicrobial community with other pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenza* (Sethi and Murphy, 2008) [87]. However, it is now associated with a number of respiratory infections affecting both children and adults, including laryngitis, bronchitis and pneumonia (Suzanne *et al*., 2011; Bernhard *et al*., 2014) [14]. It is a causative agent of otitis media in children and lower respiratory tract infections in adults suffering from chronic obstructive pulmonary disease (COPD) (Schwingel *et al*., 2019; Tan *et al*., 2020) [87, 99]. Rarely, *M. catarrhalis* can also cause endocarditis, sepsis and meningitis (Aebi, 2011) [2]. Thus, idea has arisen that organism is not simply a commensal colonizer and important bacterial pathogen.

The strategies of pathogenesis and virulence factors of *M. catarrhalis* have been briefly studied. Usually, the virulence factors associated with whole bacteria or can be driven in part by the release of outer membrane vesicles (OMVs) (Augustyniak *et al*., 2018) [9]. It is not identified if virulence is linked with certain strains or subpopulations of *M. catarrhalis*, or if variances in clinical appearance can be attributed to the heterogeneous expression of specific *M. catarrhalis* virulence factors in the socializing population (Blakeway *et al*., 2017) [16].

Generally, some of these mechanisms which contain several virulence factors facilitating the transfer of periplasmic and outer membrane components to the host (Kaparakis-Liaskos, 2015) [44]. Moreover, OMVs can confer pathogen existence and colonization after their interaction with the other bacterial species (Tan *et al.*, 2007; Schaar *et al.*, 2011) [100, 85]. Recently, *Moraxella catarrhalis* is commonly found in several community of clinical isolates. This pathogen uses several virulence mechanisms to colonize and survive in its host (Masaki *et al*., 2011; Perez and Murphy, 2017) [58, 76].

In this review, we will summarize important virulence factor and pathogenesis strategy of this organism.

Virulence factors of *Moraxella catarrhalis*

Several virulence factors of *M. catarrhalis* have been identified and characterized, and many of these are raised through the plasma membrane and are either generalized to the outer membrane protein (OMPs) or secreted outside the cell. These molecules then mediate processes such as adherence to epithelial cells, complement resistance, biofilm formation, and nutrient acquisition in order to colonize and cause disease in the human host. Many of these abilities are multifactorial as showed in below.

A- External Structures

1. Lipooligosaccharides

Lipopolysaccharides (LPS) a major constituent of surface structures it has been shown that act as adhesions modules in several bacteria, which facilitate the attachment of a bacterium to host cells (Jacques, 1996) [42]. *Moraxella catarrhalis* has been shown to express a lipooligosaccharide (LOS) as important virulence factor and plays a crucial role in the initial stage of attachment and colonization of bacteria to human pharyngeal epithelial cells (Akgul *et al*., 2005) [5].

2. Outer Membrane Proteins

Gram-negative bacteria produce small round structures 20–250 nm in diameter are called outer membrane vesicles (OMVs). OMVs are made when small portions of OM swell disseminate far from the cell and release (Grenier *et al*., 1987) [32]. OMVs contain soluble proteins and as outside adherent material that perform various biological functions on the environment and on other cells, including playing a role in pathogenesis, bacterial stress response, nutrient acquirement, biofilm development, and horizontal gene transfer (Mashburn *et al.,* 2005) [59]. For example, *M. catarrhalis* expresses several adhesins that mediate adherence to human epithelial cells, including UspA1 (Lafontaine *et al*., 2000) [50], Hag/MID (Holm *et al*., 2003.) [40], McaP (Timpe *et al.,* 2003) [103], OMP CD (Holm *et al*., 2004) [41], and the FHA-like proteins MhaB1 and MhaB2 (Balder *et al*., 2007) [12].

OMVs act as delivery vehicles, and as contributors to bacterial survival and virulence (Furuta *et al*., 2009) [31]. OMVs enable bacteria to secrete complexes insoluble molecules in addition to soluble material used in bacterial envelope components. OMVs permit enzymes to reach reserved targets in a concerted, protected, and targeted formula (Kulp and Kuehn, 2010) [48]. Eight major proteins (A through H) designated OMPs ranging from 21 to 98 kDa were identified (Murphy, 1990) [68].

3. Pericellular Structures

Pili or fimbriae structures are used by bacteria to attached to mucosal epithelial cells of the host and initiated of disease by a wide range of pathogenic bacteria. Pili are composed of polymerized protein subunits called pilins, the pilin subunit of *M. catarrhalis* appears to be more highly conserved as there are no major pilin variants produced by a single strain and only two major PilA antigenic variants, termed clade 1 and clade 2, have been observed between strains (Luke-Marshall *et al*., 2011) [57].

Genes encoding Pil A (the major pilin subunit) and Pil Q (the outer membrane secretin through which the pilus filament is extruded) identified and cloned whereas Pil T (the NT Pase that mediates pilin) disassembly and retraction (Luke *et al.,* 2004) [56]. Accordingly, some strains may be pilus positive whereas others have been proven to lack pili (Ahmed *et al.,* 1991) [3]. Some studies have previously showed that bacteria with fimbriae bind more competently to lower bronchial epithelial cells than bacteria without fimbriae (Rikitomi *et al.,* 1997) [83], that may explain a role of these pili in the pathogenesis of pathogen bacteria.

4. Biofilm formation

Biofilm formation is an significant virulence factor for persistence of bacteria during the period in the host and severity of disease by many pathogenic species for example *M. catarrhalis* (Perez *et al*., 2014) [77]. Inside the biofilm, the bacteria are covered in a protective polymeric matrix, which expresses resistance to host immune defenses. And the bacteria in biofilms are in an abbreviated metabolic state and capability alternative gene expression shapes, contributing to enhanced resistance to antimicrobial action (Bakaletz *et al.,* 2012) [11]. The respiratory tract problems such as OM and COPD caused by three ecological bacteria *M. catarrhalis*, *Haemophilus influenza* and pneumococcus. From several studies, samples of respiratory tract patient and experimental models of disease show that *M. catarrhalis* forms polymicrobial biofilms with both *Streptococcus pneumoniae* and *Haemophilus influenzae* within the host (Perez *et al.,* 2014) [7]. Actually, polymicrobial biofilm formation with *M. catarrhalis* enhances survival *in vivo* of both species in contrast to single species biofilms. A formation of multispecies biofilms can consider a contributing factor to antimicrobial action failure, especially in combining with ß-lactamase producing bacteria like *M. catarrhalis*.

5. Capsule

In both G-positive and G-negative bacteria, the presence of capsules are considered an important virulence factor. Ahmed *et al.,* (1991) [3] has been previously suggested a polysaccharide capsule. A capsule is not detectable when colonies of *M. catarrhalis* are examined on agar plates resemble the site in many other bacterial pathogens. More research is necessary to definitely determine the presence of a capsule and to explain its role in virulence.

B. Producing of ß -Lactamase

Previously, *M. catarrhalis* that producing beta-lactamase was not described. But, with cumulative of investigations about antibiotic resistance in this organism all over the world was recorded (Beekmann *et al.,* 2005) [13]. The increase in occurrence of beta-lactamase strains especially in *M. catarrhalis* can be regarded as the fastest spreading of beta-lactamases (Khan *et al*., 2010) [45], approximately 95–99% of clinical isolates now appear to resist to penicillin and other classes of antibiotics (Khan *et al.*, 2010; Prashanth *et al*.,2011) [45, 79]. Abuse of antibiotics, including physicians over-suggesting and unfinished depletion of antimicrobial courses by patients, contribute to the fast and spread of antibiotic resistance genes in many pathogens, including *M. catarrhalis* (Pereza and Murphy, 2017) [76]. As well, traditional treatment of mixed infections caused by other airway pathogens associated with resistant problem (Prashanth *et al.,* 2011) [79]. Detection of antibiotic resistance genes in *M. catarrhalis* strains were recorded that more than

95% by two genes bro-1 and bro-2, respectively (Raveendran, *et al*., 2020) [80]. *M. catarrhalis* showed the rise of resistance to trimethoprim-sulfa methoxazole, nalidixic acid and ciprofloxacin (Hamze *et al*., 2019) [34]. And other study explained this bacteria may be resistant to macrolides and quinolones (Shaikh *et al*., 2015) [88].

Pathogenesis strategies

The dynamics inducing the pathogenesis of *M. catarrhalis* infection are not exactly understood. Generally, the pathogenicity of this bacterium, like other microorganisms, depends on the ability for binding to epithelial and mucus layer and escape from the host defense mechanisms (Liu *et al*., 2016). *M. catarrhalis* can attach to numerous types of cells, involving epithelial cells of bronchial, small airway, and type 2 alveolar cells (De Vries *et al.*, 2013) [28]. This review is planned to provide a detailed overview of understanding of the virulence features of *M. catarrhalis* pathogenesis and particularly underlines recent studies linking with adhesion, invasion, biofilm formation, evasion of the host immune system.

Adhesion to Host Epithelium

An essential step in the process of bacterial colonization and infection of respiratory tract epithelium is adherence to the respiratory mucosa (Siegel and Weiser, 2015) [90]. An important virulence trait of *M. catarrhalis* is an effective adhesion to epithelial cells (de Vries *et al*., 2013) [28].

The general mechanism of cellular adherence of *M. catarrhalis* to mucosal surfaces is mediated by binding of multifactorial incident macromolecules to surface receptors on eukaryotic target cells by numerous studies. These studies showed the presence or absence of fimbriae did not influence the capacity of the bacterium to adhere.

Molecules including fimbrial adhesins such as type IV pili (TFP) and LOS structures (Spaniol *et al.*, 2008) [97] and non-fimbrial adhesins like, the outer membrane proteins (OMPs) including the ubiquitous surface proteins A (UspAs) (Lafontaine *et al.*, 2000) [50], *M. catarrhalis* immunoglobulin D (IgD) binding protein/hemagglutinin (MID/Hag) (Forsgren, 2001) [30], *M. catarrhalis* adherence protein (McaP) (Timpe *et al.*, 2003) [103], OMP CD (Holm *et al*.,2004) [41], *M. catarrhalis* filamentous Hag (FHA)-like proteins (Mha proteins) (Plamondon and Campagnari, 2007) [78], have been identified.

Pili are important element for adhesion and colonization for *Moraxella catarrhalis* (Luke *et al*., 2007) [55], pili may initiate adhesion at long range, while outer membrane proteins (OMPs) may be involved during more close contact (Hill *et al.*, 2005) [39]. The different adhesin molecules bind to an array of receptors or fundamental molecules expressed on epithelial cells of respiratory tract: for instance the OMP CD of *M. catarrhalis* was shown to specifically attach to the mucin molecules from the nasopharynx and middle ear but not to mucin from the saliva and tracheobronchial mucin. Interactions such as these represent the first steps in the process of bacterial colonization and infection (Bernstein and Reddy, 2000) [15]. In addition to, the influence of charge on adherence and interaction between the negatively charged surface of *M. catarrhalis* cells and positively charged domains called microplicea on pharyngeal epithelial cells was found (Ahmed *et al.*, 2000) [4].

There is relationship between *M. catarrhalis* colonization of the human respiratory tract epithelium and increased risk of disease, specifically in children (Verhaegh, 2011) [104]. Therefore, it is reasonable to expect that an effective immune response raised proteins associated with OMVs of *M. catarrhalis* are involved in diverse biological functions. Many of them are important virulence factors permitting infection and colonization of host (Blakeway *et al*., 2017) [16]. The ubiquitous surface proteins (UspAs) are among the major virulence factors. UspAs are multifunctional proteins with important adhesion properties. They can be divided into three main groups: UspA1 (88-kDa), UspA2 (62-kDa), and UspA2H (92-kDa) proteins (Lafontaine, 2000) [50]. UspA1 mediating binding to epithelial cells and extracellular matrix (ECM) components (Brooks *et al.*, 2008) [22], and binding to carcino-embryonic antigen-related cellular adhesion molecule 1 (CEACAM1) (Hill and Virji, 2003) [38]. As well as, UspA2/UspA2H proteins predominantly playing a role in immune evasion (Attia *et al.*, 2005) [7]. Purpose of the modular structure of the predicted UspA1 and UspA2H proteins shown the presence of the VEEG-NINNY-VEEG amino acid sequence motif involved in binding to Chang conjunctival cells or fibronectin (Brooks *et al*., 2008) [25]. Given the fact that these variable domains appear to contribute to different functional characteristics of the UspA proteins, idea exchange could lead to getting of specific functional characteristics. HEp-2 laryngeal epithelial cells (McMichael, 1998) [60], and A549 type II alveolar epithelial cells (Brooks *et al.*, 2008) [24]. In addition to it binds to the ECM proteins fibronectin (Tan *et al*., 2005) and laminin (Tan *et al.*, 2006) [101]. Host receptors for other adhesin molecules of *M. catarrhalis* such as MID/Hag or McaP remain to be identified and is needed more research.

Risk factors associated with *M. catarrhalis* colonization have been previously studied in several countries and age groups, and the findings have indicated that crowding and contact with children are risk factors for colonization, not only for *M. catarrhalis*, but also for *S. pneumoniae* and *H. influenzae* (Labout *et al.*, 2008) [49]. Other reported risk factors are genetics (Yamanaka *et al.*, 2008) [110], smoking (Brook *et al*., 2008) [25], socio-economic status (Smith-Vaughan *et al.*, 2006) [96], synergy and interference with other micro-organisms (Brook and Gober, 2006) [23], frequency and location of sampling (Hendley *et al*., 2005) [37], season (Hendley *et al.*, 2005) [37], gender (Kilpi *et al.*, 2001) [46] and vaccination (Dagan, 2004) [27].

Invasion of the host epithelium

After *M. catarrhalis* has established itself at its colonization sites, a critical step of *M. catarrhalis* appears to be invasion of host cells, which would allow the bacterium has to survive both the host immunological response (cellular and humoral) and environmentally challenging conditions, such as iron limitation and effects of antibiotic treatment (de Vries *et al*., 2009) [29]. Adhesion between host epithelial cells and surface-exposed macromolecules, such as OMPs is an essential step for assisting pathogenesis of *M. catarrhalis* (de Vries *et al*., 2009) [29]. The aggressive capacity of *M. catarrhalis* to invade different epithelial cell types was demonstrated by several studies (Slevogt *et al.*, 2007) [94].

The actual mechanism of *M. catarrhalis* invasion into epithelial cells noticed by Spaniol *et al.*, (2008) [97], who found that the level of invasion of Chang cells by *M. catarrhalis* O35E was actually fivefold higher than the level of invasion of A549 cells by the same strain. These authors

also proven that the addition of a purified OMP preparation resulted in reductions of both adhesion and invasion, and a lack of UspA1 reduced both adhesion and invasion. Also, the invasion process was dependent on clathrin polymerization, which looked to oppose the earlier findings of Slevogt *et al*., (2007) [94]. Additionally, actin polymerization was also found to contribute to the invasion process, and antibodies directed to fibronectin and integrin 51 were able to inhibit invasion (Spaniol *et al*., 2008) [97]. In another publication, Hill *et al*., (2005) [39] indicated the importance of CEACAM1 binding for both adhesion and invasion by blocking a recombinant peptide (rD-7) that representing the CEACAM1-binding domain of UspA1 resulted in inhibition of cell invasion by *M. catarrhalis, H. influenzae*, and *N. meningitides*. Further, *M. catarrhalis* would be adept to interact with B lymphocytes through MID/Hag when existing within the sub epithelial barrier of lymphoid tissues (Heiniger *et al.*, 2007) [36].

At the present, the exact mechanism of epithelial cell invasion by *M. catarrhalis* is not completely understood, but it appears to be an active process involving several host cell and bacterial contrivances.

Biofilm Formation

Microbial biofilms has been include cells adhered to surfaces that are enclosed by a self-produced exopolymeric matrix that protects biofilm cells against different external worries (Melo and Azevedo, 2021) [62]. A colonization of host mucosal surfaces is a first and necessary step in the infectious process, biofilm formation has already been demonstrated to be an important manner involved in colonization and persistence of mixed bacterial etiology in the nasopharynx (Bair and Campagnari, 2019) [10].

These biofilm description of bacterial invasion of human cells leads to failure in antibiotic therapy, various studies have shown that pathogenic bacteria such as *Moraxella catarrhalis*, produce biofilm-like structures within the host cells and anti-bacterial agents cannot reach intracellular biofilm in normal concentrations (Mirzaei *et al*., 2020) [63].

A study by Bair and Campagnari, (2019) [10] characterized both monomicrobial and polymicrobial biofilms using an *in vitro* nasopharyngeal colonization model. Biofilm assays were designed to simulator the nasopharynx and bacterial persistence was measured over time. The information propose that colonization with *M. catarrhalis* stimulates stable polymicrobial biofilms with other otopathogens. Indeed, the capacity of *M. catarrhalis* to form biofilms has been confirmed using *in vivo* assay, and role of biofilms formed by otopathogens (*Streptococcus pneumoniae*, *Haemophilus influenza* and *Moraxella catarrhalis*) that contribute to otitis media repeated and established as chronic. The characters of biofilms formed by these bacteria and that factors influence them, and how these affect the host inflammatory response is important for the development of new strategies for the treatment of otitis media (Silva and Sillankorva, 2019) [91].

Previously, there were try to identify genes essential for biofilm formation by use of a transposon mutagenesis (Pearson *et al*., 2006) [74]. This study confirmed that the existence of UspA1 positively marks biofilm formation, while the presence of Hag has a negative influence. In addition to, UspA1 and UspA2H genes have played a role in biofilm formation (Pearson *et al.*, 2007) [74]. The study performed by Verhaegh *et al*., (2008) [105] suggested the isolates carrying UspA2 mutant gene have more efficient biofilm formation than isolates carrying UspA2H mutant gene, signifying that uspA2 could also play a role in biofilm formation.

The ability for formation of biofilms was related with frequency of UspA2 in the strains, a rate children isolates (5 years of age), and adults isolates (20 years of age) indicated reduced frequency of UspA2 (Verhaegh *et al.*, 2008) [105].

Although, the formation of micro colonies and biofilms formation was studied *In vitro* by using a pil A mutant and illuminated role of TFP micro colony and biofilm formation (Luke *et al*., 2007) [55].

M. catarrhalis O35E was exposed for mutation of Host factor 1 (Hfq), the result showed reducing in growth when it osmotic and oxidative stresses also an changed OMP composition, considered by slightly increased expressions of OMP J, OMP G1b, and Cop B (Attia *et al.*, 2008) [8]. Additionally, the hfq mutant strain showed a growth improvement in a biofilm system, which could be the (indirect) result of a changed outer membrane composition (Attia *et al*., 2008) [8].

Evasion of the host immune system

The first line of defense against bacterial pathogens (*M. catarrhalis*) was faces the tactics host's innate immune system in resisting a forging microbe. After colonization of mucosal surfaces and evasion the tissue by *M. catarrhalis*, the bacteria was exposed these tactics of immune system.

Usually, the innate immune system contains several main components, such as the complement system and pattern recognition receptors (PRRs), which include the Toll-like receptors (TLRs), NOD-like receptors, and mannose receptors of macrophages (Akira *et al*., 2006) [6]. The important strategies of microbe to avoid the innate immune system involve:

1. Avoidance of complement-mediated killing mainly via intervention with regulatory proteins (Liu *et al.*, 2016) [53].
2. Polyclonal, non-specific B cell activation and relaying of adaptive immunity (Vidakovics *et al*., 2010) [108].
3. Hiding inside lymphoid tissue, which is the main pool simplifying the host invasion (Heiniger *et al.*, 2007) [36].
4. Formation of bioflm (Perez *et al*., 2014) [77].
5. Participation in protease- anti protease imbalance (Parameswaran *et al*., 2009) [72].

Complement Resistance

The complement system is a fundamental component of the immune response that results in direct killed of pathogens or opsonization for increased phagocytosis. Therefore, complement resistance is an important virulence feature of various pathogens that subsequently increases the survival rate among bacteria within the human host (Blom *et al.*, 2009) [17]. All virulent bacterial species need to defeat the innate immune system in order to colonize and survive in their hosts. The human respiratory pathogens *Haemophilus influenzae* and *Moraxella catarrhalis* are have developed novel mechanisms to avoid complement-mediated killing (Riesbeck, 2020) [81].

Plamondon *et al*., (2007) [78] showed 89% strains of *M. catarrhalis* isolated from lower respiratory tract infections are resistant to complement-mediated killing, whereas strains from the upper respiratory tract of children are mostly sensitive (58%). *M. catarrhalis* does possess

mechanisms that have evolved to inhibit activation of complement pathways.

The activation of the complement system via 3 different routes, the classical pathway, alternative, and/or mannose-binding lectin pathway the, all of which leads to the terminal pathway resulting in formation of the membrane attack complex (MAC) and opsonization of bacteria for phagocytic killing. All pathways are tightly controlled by human fluid-phase or membrane-bound regulators (Bernhard *et al*., 2014) [14].

Previous reviews documented that *M. catarrhalis* may stimulate both the classical and alternative pathways (Riesbeck *et al.,* 2006) [82] and is a weak activator of the mannose-binding lectin pathway (Singh *et al*., 2010; Hallstrom *et al.,* 2011) [10, 33].

Moraxella catarrhalis is mainly dependent on outer membrane proteins, a family ubiquitous surface proteins (Usp) has been studied most thoroughly for its role in pathogenesis (Singh *et al.,* 2010) [92], Usp A1 and Usp A2 that interact with complement factor 3 (C3) and complement inhibitor C4b binding protein (C4BP) preventing the alternative and classical pathways of the complement system respectively (Singh *et al.*, 2010) [92].

Several of complement binding proteins are important adhesins and interactions with the host. Thus, some of the OMP are viable targets for new therapeutics, including vaccines aimed at preventing respiratory tract diseases such as otitis media in children and chronic obstructive pulmonary disease in elderly (Riesbeck, 2020) [81]. Although, the structural details facilitating these interactions are still unknown. Riesbeck, (2020) [81] showed lipooligosaccharides avoiding complement actions and consume host complement regulators C4b binding protein and factor H to impede the classical and alternative pathways of complement activation, respectively. In addition, the binding of human vitronectin, an inhibitor of the terminal pathway of complement, a bacterium motivated vitronectin to disrupt complement-mediated killing. Hybrid UspA2 (UspA2H) bind vitronectin at the highly diverse N-terminal head domain (Su *et al.,* 2013) [98]. UspA2 also has the capacity to attract vitronectin that in turn binds C9 and hereby inhibits membrane attack complex (MAC) formation. UspA2 as a major vitronectin binding protein and hence the UspA2/vitronectin interaction was studied in detail (Singh *et al.,* 2010; Hallstrom *et al*., 2011; Bernhard *et al.,* 2014) [92, 33, 14]. Indeed, most clinical isolates of *M. catarrhalis* are able to survive complement-mediated killing by normal human serum (Verhaegh *et al*., 2008) [105].

Recently, it was proven that *Moraxella catarrhalis* bacteria also bind plasminogen, which is converted to plasmin that degrades C3b and C5. (UspA2) and (UspA2H) were recognized as the plasminogen-binding factors in the outer membrane proteome of *Moraxella*. Moreover, expression of a series of truncated recombinant UspA2 and UspA2H proteins followed by a detailed analysis of protein-protein interactions suggested that the N-terminal head domains bound to the kinglet domains of plasminogen (Singh *et al*.,2015) [93].

C. Virulence-associated genes of *M. catarrhalis*

There are other a number of virulence factors and virulence-associated genes, have been identified in a number of respiratory pathogens, as *M. catarrhalis* isolates. Pathogenicity of some virulent strains contributes to possess a highly mutable genes (Blakeway *et al*., 2017) [16]. Although, the development of a vaccine against *M. catarrhalis* has been delayed by the absence of a suitable animal model. Increased knowledge of the organism's pathogenic properties and the host response to it may service to detect suitable vaccine targets or lead to other strategies to prevent infection. The characteristics of an active vaccine can be detected: surface epitopes, stimulate the immunity response, appearance *in vivo* at sites of pathogenesis, prevention, and stable state (Perez and Murphy, 2017) [76].

Moreover, many related genes have been identified in the genomes of a wide variety of bacterial species, suggesting the presence of the outer membrane protein genes and the potential for pathogenesis specially UspA1 and UspA2 antigens (Attia *et al*., 2006; Spaniol *et al.,* 2008) [97].

Meier *et al*., (2002) [61] evaluated the frequency of uspA1 and uspA2 genes as 99% and 77%, respectively. Also, study by Verhaegh *et al*., (2011) [106] were identified frequency of uspA1 gene (99%) compared to uspA2 (76%). Addition to UspA1 and UspA2, a number of different gene products of *M. catarrhalis* have been associated with colonization and complement evasion, including CopB (Sethi *et al*.,1997), lipooligosaccharide (LOS) (Akgul *et al*., 2005) [5], Hag (Pearson *et al*., 2006) [73], and OMPCD (Akimana *et al*., 2007) [52].

However, much of the evidence for the maintenance and manifestation of these factors e.g. G1a and G1b (Adlowitz *et al*., 2004) [1], *M. catarrhalis* adherence protein (Lipski *et al*., 2007) [52], Moraxella surface proteins 22, 75 and 78 (Ruckdeschel *et al*., 2008) [84], substrate-binding protein 2 (Otsuka *et al.,* 2014) [71], lactoferrin-binding protein A (Bonnah *et al.,* 1999) [18] and transferrin binding protein A (Myers *et al*., 1998) [70] is derived from studies that only examined a small number of isolates, or in the case of CysP (Murphy *et al.,* 2016) [16] and AfeA (Murphy *et al.,* 2017) [66], what can be gleamed from genomes available online.

All of these vaccine candidates have been associated with the production of antibody (Murphy *et al.* 2005) [69]. An antibody response in humans to various *M. catarrhalis* antigens, including highly conserved outer membrane proteins, has been demonstrated. As well as, their vaccine potential still is matter of current investigations.

In several studies performed by Verhaegh *et al.* (2008) [105] to define the frequency of virulence genes in *M. catarrhalis*. He was observed the prevalence of ompJ and ompCD genes in 100% and mcaP gene in 99% of the strains. Whereas in other study, he was confirmed the presence of MID/Hag gene in 85 strains, giving a frequency of 83% (Verhaegh *et al.,* 2011) [107]. But study by Mollenkvist *et al.* (2003) [65] showed the frequency of MID/Hag gene in all clinical isolated strains (100%). The molecular techniques reported about copB gene frequency in *M. catarrhalis* genes was 100% (Bootsma *et al.,* 2000; Verhaegh *et al*., 2008) [20, 5]. However, other studies was 50% to 55% (Mitov *et al.,* 2010; Liu *et al.,* 2017) [64, 54]. As well as, Zaleski *et al*., (2000) [111] showed role of Gal gene in serum-mediated killing and susceptible to complement attack.

Also, several virulence genes have been stimulated immune response of host, making them potential vaccine candidate genes. A genotyping variation of these genes are designed and studied of role with colonization (Perez *et al*., 2009).

Conclusion

M. catarrhalis was formerly believed a nonpathogenic member of the resident flora of the upper respiratory tract. But, with augmented investigations, the bacterium has delivered as a real pathogen, especially in patients with airway problems for both children and elderly persons. The first step of bacterium to be successful infected was adhere and colonize with their hosts. Bacterial infection induced by a wide virulence factors that can often be serious.

So, future studies were needed to compare *Moraxella* isolated as normal flora or pathogen and investigation some virulence factors other than those studied in this review.

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