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Exploring the Phenotypic and Genetic Characteristics of Respiratory Infections Caused by *Moraxella Catarrhalis*

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Annotation: Investigating the virulence genes of Moraxella catarrhalis in the respiratory system was the goal of this investigation. Baghdad Medical City received sixteen (60) swabs that were taken from individuals with respiratory tract disorders. Three (5%) out of 60 (100%) Moraxella catarrhalis-caused respiratory tract infections in patients and tested positive for the bacteria using the USP A1, cop B, and omp CD. According to the research's findings, Moraxella catarrhalis is harmful to humans If the respiratory system is separated from it. Additionally, all isolates of Moraxella catarrhalis share the characteristics of Usp A1, Cop B, and Omp CD. Future molecular investigations were therefore required to compare Moraxella isolated as a pathogen or normal flora, as well as to examine certain virulence factors and the antibiogram profile at the molecular level.

Keywords: Moraxella catarrhalis, PCR, Virulence Genes.

Introduction

Originally isolated in 1896, Moraxella catarrhalis was long believed a benign normal human respiratory tract flora. However, over the past 20 years, it has been linked to numerous respiratory illnesses that affect both adults and children, such as pneumonia, bronchitis, and laryngitis. As a result, it has become a major bacterial infection rather than just a commensal colonizer[1].The bacterium quickly colonises the nasopharynx shortly after birth, and a number of factors, such as the existence of siblings, attendance at nursery, and respiratory conditions, influence nasopharyngeal carriage of this disease peculiar to humans[2]. In early childhood, Otitis media (OM) is a condition that is especially significant respiratory condition. A physician notes that the most common bacteria that are isolated found in children's nasopharynx experiencing otitis media (OM) are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*, which can appear as single pathogens or in combinations. Recent studies have shed light on the biological processes that enable *M. catarrhalis* to colonize and contribute to disease progression, with many findings highlighting the role adhesion of bacteria as a crucial factor in this process. Additionally, *M. catarrhalis* is the second most frequent reason for chronic obstructive pulmonary disease (COPD). M. catarrhalis is the most frequently identified bacteria in the sinuses, throat, middle ear, sputum, and mouth samples[3,4]. Historically regarded as a harmless commensal organism, there is limited understanding of its pathogenic traits and virulence factors. Like other microorganisms, its pathogenicity relies on its capacity to evade defence mechanisms of the host and adhere to cellular surfaces and mucus layers[5]. *M. catarrhalis* has the ability to attach to Type 2 alveolar cells, small airway epithelial cells, and bronchial epithelial cells are among the different cell kinds. [6]. The adhesion factors of this bacterium that contribute to M. catarrhalis Adherence Protein (McaP), Hemagglutinin/Moraxella catarrhalis Immunoglobulin D-Binding Protein (MID/Hag), ubiquitous surface protein A (UspA), and outer membrane proteins (OMPs) are all involved in the formation of biofilms. Furthermore, to remove iron from host cells, *M. catarrhalis* uses its outer membrane protein B (copB). [7]. Research has identified two distinct genetic lineages associated with three different 16S rRNA types in *M. catarrhalis*, which exhibit phenotypic differences in their resistance to serum from humans (sero-sensitive in contrast to sero -resistant) and their capacity to stick to the human epithelial cells[8]. Notably, it has been discovered that the sero-resistant lineage (16S rRNA type 1) is more virulent than the other lineages (types 2 and 3). [9,10].

Materials and Methods

A total of sixty Swabs from patients were obtained diagnosed with the upper respiratory system infections and submitted to Baghdad Medical City between October 2022 and January 2023. The ages of the patients varied from 6 months to 78 years. As detailed in Table 1, only three isolates of *Moraxella catarrhalis* were identified.

Collection of Specimens:

Specimens are typically obtained from the patients with respiratory, tract issues using disposable swabs. These collections are conducted with guidance to prevent contamination. The respiratory tract provides the swabs., and those intended for It is best to use tubes for culturing. with normal saline to keep them moist until they reach the laboratory. Alternatively, they can be immediately sealed in their covers and transported to the lab within half an hour of collection. Each specimen is promptly inoculated onto MacConkey's plates, nutritional agar, blood agar, and chocolate agar plates. After that, the swabs are inoculated onto the culture mix and cultured for 24 hours at 37°C in an aerobic environment.

Moraxella Catarrhalis Isolation and Identification Laboratory Diagnosis

From each primary positive culture, one colony was isolated in accordance with the diagnostic protocols advised by 9,10, and its recognition was based on its morphological characteristics (Colony size, shape, colour, characteristics of the pigments, gradient, elevation, edge, and texture).

To examine the morphological characteristics of bacterial cells, a bacterial smear stained with Gramme stain was employed.

Genetic study:

1 The extraction of DNA from Gram Negative Bacteria:

This technique was developed using the purification kit for genomic DNA that was enhanced by Promega, USA.

1. A 1.5 ml sterile microcentrifuge tube was filled with an overnight culture that had been extracted.
2. The tube containing the collected cells was filled with 600µl of Nuclei Lysis Solution. To lyse the bacterial cells, the fluid was pipetted five to six times. It became a highly viscous fluid.
3. Lyse the cells by incubating them at 80°C for five minutes, then allowing them to cool to room temperature.
4. After mixing the nuclear lysate with 3µl of RNase Solution, the tube was inverted two to five times to mix the sample. The mixture was allowed to cool to room temperature after being incubated for 15 to 60 minutes at 37°C.
5. After adding 200µl of Protein Precipitation Solution to the nuclear lysate, it was rapidly For ten to twenty seconds, vortex. After vortexing, the sample may show little protein clumps. It was then incubated on ice for five minutes.
6. At ambient temperature, centrifuge at 13,000–16,000 × g for three minutes. There should be a visible Protein that is dark brown particle.
7. The supernatant was moved to a sterile 1.5 ml microcentrifuge tube that held 600 µl of isopropanol at room temperature.
8. The white thread-like DNA strands were slowly combined by inversion until they formed a discernible mass.
9. Centrifugation at ambient temperature for two minutes at 13,000–16,000 × g. The DNA would show up as a little white pellet.
10. Following the decantation of the supernatant, 600 µl of 70% ethanol at room temperature was added to the DNA. To clean the margins of the microcentrifuge tube and the DNA pellet, the Several times, the tube was gently inverted. centrifugation, similar to Step 9.
11. Using a sequencing pipette tip or a dawn Pasteur pipette, the ethanol was carefully aspirated. Since the DNA pellet is now somewhat loose, caution must be used to prevent aspirating it into the pipette. To allow the pellet to air-dry for ten to fifteen minutes, the tube was inverted on sanitised absorbent paper.
12. There were 100 µl of DNA in the tube. Rehydration solution, then incubating the DNA helped to rehydrate it.for an hour at 65°C. The tube is gently tapped to mix it periodically. The solution can also be incubated at 4°C or room temperature for a whole night to rehydrate the DNA.
13. The temperature range for the DNA storage was 2–8°C.

Thermal Cycling Conditions:

The process of making several Polymerase Chain Reaction, or PCR, is the process of making duplicates of a gene. Otherwise, there would not be enough DNA to be employed in additional tests techniques. PCR is performed using an automated cycler. that quickly the reaction mixture is heated and cooled in the tubes. Eleven states that PCR is carried out in three main phases over 30–40 cycles: annealing, extension, and denaturation.

1-Denaturation:

The denaturation process stops all enzyme activity when the double strand of DNA melts open to reveal DNA with a single strand.

2-Annealing:

The annealing of prime process starts as soon as the mixture of reactions cools down and the oligonucleotide primers that flank the region that has intensified combine beside template DNA molecule's single strands. The DNA synthesis reaction cannot begin or be primed without the oligonucleotide primers.

The primer and template are pieces of double-stranded DNA that the polymerase binds to and begins copying. The ionic link between the template and primer becomes so strong after a few bases are put in that it no longer breaks.

3-Extension:

The primers, which naturally contain a few bases, already possess higher attraction between ions and the template at 72°C, which is optimal for the polymerase, compared to the forces that break these points of interest.

The polymerase reads the template from the 3' to 5' side and adds dNTPs from 5' to 3'. Attached to the primer on the 3' side, the bases complement the template. The final product of a PCR needs to be examined before being used in subsequent processes. This is to verify the formation of a product.

Finding the Primers in This Investigation:

In certain PCRs, extracted from bacterial cells, DNA was utilised as a template to identify some of genes that cause pathogenicity of *Moraxella catarrhalis*. To purify the DNA The Promega DNA extraction kit is used to extract DNA from bacteria. was utilised. The primers described in **Table (1)** were used to amplify a fragmented gene.

Table 1: Primers sequences and PCR conditions

Genes	Primer sequence (5´- 3´)	Size bp))	PCR conditions	Reference
mapA F mapA R	ATAGGATCCGCACCAGCCTC ATCAAAT AATGGATCCTTGTGCCAGTG CCATTT	140	94ºC 4min 1x 94ºC 2min 55ºC 1min 30x72ºC 1min 72ºC 8min 1x	Hoopmanet al.,2008
mcaP F mcaP R	CGCAATAAAGATCACCATGC TTG CGGGATCCCGCTGACACATT GCATTGATAAA	220	94ºC 4min 1x 94ºC 1min 57ºC 1min 30x72ºC 1min 72ºC 7min 1x	Verhaeghet al.,2008
uspA1 F uspA1 R	CGTTATGCACTAAAAGAGCA GGTC GCATCTGACCAGCTTAGACC AATC	260	94ºC 4min 1x 94ºC 2min 55ºC 1min 30x72ºC 1min 72ºC 7min 1x	Verhaegh et al.,2008

Table (2): Primers sequences

hag Fhag R	GTCAGCATGTATCATTTTTAAGG TGAGCGGTAAATGGTTTAAGTG	175	94ºC 4min 1x 94ºC 2min 56ºC 1min 30x 72ºC 1min 72ºC 7min 1x	Verhaegh et al.,2008
copB F copB R	GGCGTGCGTGTTGACCGTTTTG GTTTGGCAGGCGATAGGCGACAT	590	94ºC 4min 1x 94ºC 2min 55ºC 1min 30x 72ºC 1min 72ºC 8min 1x	Verhaegh et al.,2008
ompCDF ompCDR	ACGCACTGGCAAGAAGCTAGA GACCTGCACCAACCAAGACAT	300	94ºC 4min 1x 94ºC 2min 55ºC 1min 30x 72ºC 1min 72ºC 8min 1x	Verhaegh et al.,2008

Preparation of Primers

The downstream and upstream lyophilised oligonucleotide primers were synthesised in accordance with the manufacturing company's instructions (Bioneer, USA) and stored at -20ºC.

Results

DNA amplification was performed in a final 20 µl volume that contained the following information, as indicated in **Table (3).**

Table 3: Reaction mixture's contents

No.	Contents of reaction mixture	Volume
1	Master mix	5 µl
2	Upstream primer	3 µl
3	Downstream primer	3 µl
4	DNA template	5 µl
5	Nuclease free water	4 µl
Total volume		**20 µl**

Detection of Amplified Products by Agarose Gel Electrophoresis:

Agarose gel electrophoresis verified the successful PCR amplification.2 grammes of agarose powder were dissolved in 100 After allowing millilitres of TBE buffer (pH:8) to cool to 50 degrees Celsius in a boiling water bath, ethidium bromide was added at a concentration of 5µ/ml. to create agarose gel. At one end of the tray, a comb was fastened to create wells for loading DNA samples. After being carefully powered into the tray, the agarose was left to solidify for 30 minutes in the room temperature[11].

The comb was then carefully taken from the tray.5µl of the DNA sample was moved into the marked wells of the agarose gel, and the 5µl DNA ladder was put in one well. combined with 1µl of loading solution. The tray was the gel was preserved in an electrophoresis chamber with the surface covered with TBE buffer. The electricity was permitted to run at 70 volts for 60 minutes. A digital camera was used to take pictures of the gel, and a UV transilluminator was utilized to view the DNA bands.

Isolation of *Moraxella catarrhalis*

During the period from October 2022 to January 2023, 60 (sixteen) swabs were taken from patients with respiratory tract infections sent to Baghdad Medical City. The age range of the patients was 10 months to 72 years. As indicated in **Table (4),** only three isolates of M. catarrhalis

were isolated.

Table 4: The prevalence of *M. catarrhalis* in relation to other respiratory tract etiological agents

NO. swabs	Number of isolates		
	M .catarrhalisisolates	Other bacteria	Negative culture
60	3(5%)	40(66.7%)	17(28.3%)

Five percent people are isolated. This result is roughly comparable to the In a study published, the respiratory tract's isolation rate of *M. catarrhalis* by [12] that found a rate of 9.8%, while a study published by [13] indicated The rate of isolation is approximately 3.6% of patients, while that the majority of children have been colonized by this bacteria within the first year of their lives[14,15].

Results of PCR product gel electrophoresis

The PCR products gel electrophoresis result was displayed in Figure 1.

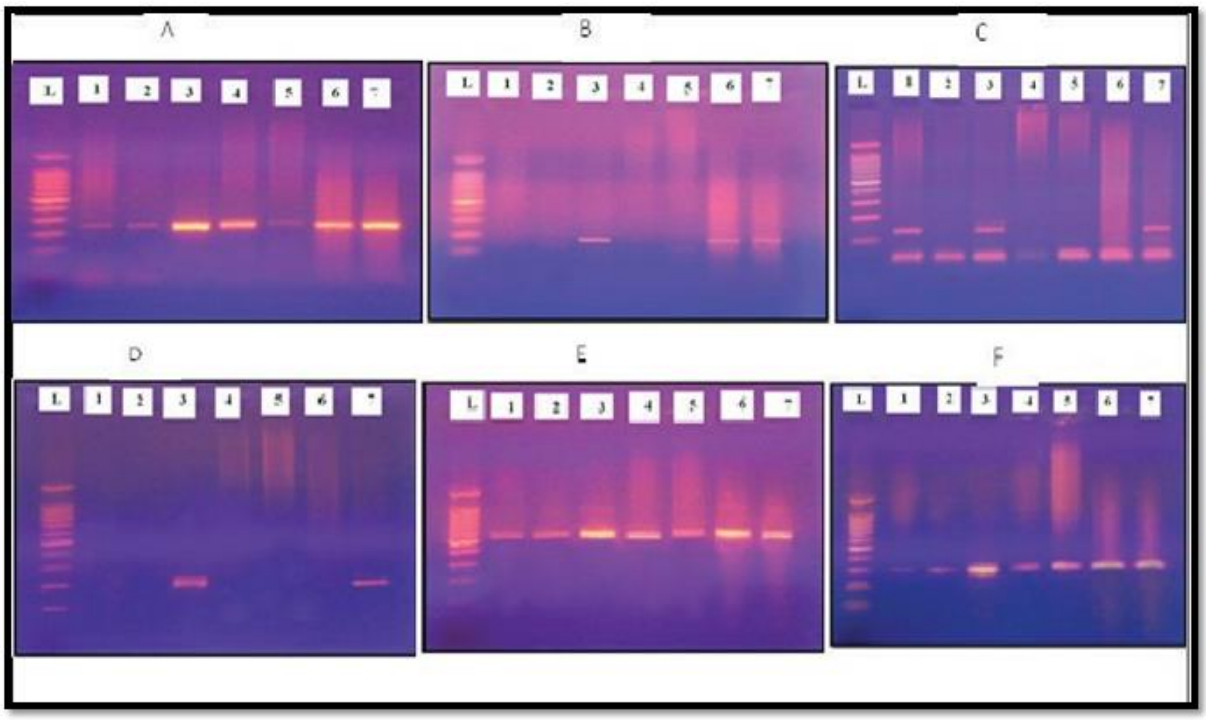

Figure 1: UspA1 PCR product gel electrophoresis (Figure 1: A) (1, 2, 3, 4, 5, 6,7) Results for M. catarrhalis isolates that are positive for USP A1 B: Hag PCR product gel electrophoresis (3,6,7) Positive M. catarrhalis isolates (1, 2, 4, and 5)Isolates of *M. catarrhalis* giving negative resultsL is Ladder. C: MapA(1,3,7) isolates with positive PCR product gel electrophoresis, while MapA(2,4,5,6) isolates with negative resultsL is a ladder, D: Gel electrophoresis of the PCR product of isolates of MCA P (3 and 7) that showed positive results from isolates of MCA P (1, 2, 5, and 6) that showed negative results.L is a ladder,

E: PCR product of copB (1, 2, 3, 4, 5, 6, and 7) *M. catarrhalis* isolates with cop B positive results by gel electrophoresis L is the ladder. F: M. catarrhalis isolates having positive omp CD results on gel electrophoresis of the PCR product of omp CD (1, 2, 3, 4, 5, 6, and 7).L is the ladder.

Discussion

***Moraxella catarrhalis* UspA1 Molecular Detection**

Ubiquitous surface protein UspA1 was molecularly detected using a particular PCR primer. UspA1 was discovered to be present in every bacterial isolate, as seen in Figure (1:A). Adhesion and invasion, and defence resistance to the human complement system are examples of the

multifunctional properties of M. catarrhalis UspA proteins[16].

On the other hand, if this marker is positive, the bacteria will adhere to the surfaces of the host mucosal, which is a crucial stage in colonization[17]. The protein UspA1 that is required for *M. catarrhalis* to adhere to certain cell lines in vitro, such as Change the alveolar, laryngeal, and conjunctival epithelial cells. It has been demonstrated that the host cellular receptor carcinoembryonic Cell adhesion molecule 1 (CEACAM1) associated to antigens mediates adherence; therefore, when uspA1 mutants were tested for adherence, they discovered a decrease in their capacity to attach themselves to human epithelial cells obtained[18].

This study's results are also the same as those reported by [19,20], who discovered that 99 percent of isolates had the uspA1 gene.UspA1 was found to contribute to the development of biofilms through *M. catarrhalis*.*M. catarrhalis.* UspA1 and A2 attach to the third complement components (C3) in a noncovalent manner [20]. "It is noteworthy that out of all the bacteria examined, only M. catarrhalish possesses the special capacity to disrupt the complement system's innate immune system by binding C3 [21]. Additionally, C4BP of the complement component system 22 is bound by the Usp family, which inhibits both the traditional and unconventional component routes. The detection of epithelial cell invasion is another function of UspA1, and it the interaction between UspA1 and fibronectin and α-5-β 1 integrin appears to be the primary determinant. [23].

Hag detection in isolates of *Moraxella catarrhalis*

The unique marker was utilised to perform molecular detection of the Hag protein. It was discovered that, as seen in Figure (1: B), only three isolates produced good results using this primer. The cell surface of *M. catarrhalis* contains the *Moraxella catarrhalis* protein, also known as the *Moraxella catarrhalis* Ig D binding protein (MID)24, along with the UspA1 and UspA2 proteins25. MID/Hag is a Multipurpose plays a significant part in M. catarrhalis6 pathogenesis. The feature of Hag that has been investigated the most is its capacity to bind IgD, or immunoglobin D. [24]. Additionally, this adhesin facilitates M. *catarrhalis* to human erythrocyte binding. This characteristic is known as hemagglutination.[25,26]. Hag acts as a direct mediator. adhesion to collagen and lung cells, but it is insufficient to confer binding to conjunctival monolayers, according to Bullard et al. [27,28]. In contrast to the findings of [19] who discovered a90% of isolates of M. catarrhalis tested positive for hag. this investigation revealed that 5% is positive, while Forsgrenet al.,[29] demonstrate a 53.5% identity with MID.

Detection of mapA Marker

The chart One of the key genes that codes for the synthesis of acid phosphatase is the gene. This gene was found using a particular PCR primer. It was discovered three *M. catarrhalis* isolates possessed the map A gene, while the remaining isolates lacked it (Figure 1:C). According to a prior work by [30], the enzyme synthesis of acid phosphatase in M. catarrhalis strains is significantly influenced by the mapA gene. However, the extracellular production of acid phosphatase by the *Moraxella catarrhalis* isolates was found to be phenotypically significant. This suggests that the Many genetic loci may encode the same enzyme. or possess multiple isoenzymes. The ability of *M. catarrhalis* to manufacture this enzyme whether it is present or not of the map A gene may also suggest that another gene in the Moraxella genome encodes this enzyme. Compared to other *M. catarrhalis* the expression for autotransporters (UspA2), MapA, is a comparatively low level. This could be because it’s the catalytic nature of enzyme action eliminates the need for substantial protein expression[30].

Detection of mcaP Gene in *Moraxella catarrhalis*

McaP was found in isolates of *Moraxella catarrhalis.* (as in Figure 1: D). It was discovered this gene only produced positive results in two isolates. Additionally, Bacterial cell surface expression of the autotransporter protein family includes the *Moraxella catarrhalis* adherence protein (McaP) [31]. *Moraxella catarrhalis* was found to express McaP, an adhesin that exhibits phospholipase B and esterase activity. Despite the fact that Moraxella has a highly conserved mca P gene, as

indicated via19. But as 28 points out, the lack of this signal for five isolates could be explained by a difference in the gene sequence due to the change in the amino acid sequences. Furthermore, because Mca P contains both phospholipase and esterase activity, as shown by [32], it is also regarded as an autotransporter[28].

Detection of cop B Gene

One of the proteins whose expression is stimulated in iron-limiting conditions is CopB, sometimes referred to as Omp B2 27. Cop B was detected in M. catarrhalis isolates by molecular detection employing a PCR primer, as illustrated in Figure 1:E. This finding is consistent with the one made by [19] who discovered that the copB gene was present in every isolate that was examined. The OMP composition changed specifically when growth occurred in iron-limiting conditions, and at least four proteins—including CopB—were only present in these settings. The uptake of iron from the environment is significantly influenced by this gene product[33].

Human transferrin and the protein lactoferrin were both elevated In reaction to iron deficiency, and the copB mutant significantly reduced their ability to bind to these proteins, suggesting that the CopB protein uses iron. As stated via27, the copB mutants exhibited increased susceptibility to human serum, suggesting that Cop B protein plays a significant role in Moraxella catarrhalis serum resistance. Thus, at different environmental iron levels, this bacterial gene can control the growth of the organism[27].

Molecular Detection of omp CD in *Moraxella Catarrhalis*

The highly conserved, heat-modifiable porin-like protein known as Omp CD most likely functions as an adhesin to human lung epithelial cells[34]. To detect the ompCD gene, a particular PCR primer was employed. All of the isolates of Moraxella catarrhalis were determined to have ompCD, as seen in Figure 1:F. Nevertheless, OmpCD's biological roles have not yet been identified. Sequence research revealed that it is related to toporins, with Pseudomonas aeruginosa porin OprF being the most closely related gene product.

Additionally, ompCDmutant was more susceptible to serum effects even though it expressed normal amounts of Hag, UspA1, and UspA2 as well as the mechanism enabling Omp serum resistance mediated by CD seems to be exclusive to *M. catarrha* Lis itself, according to de Vrieset al., [6]. The omp CD mutants developed more slowly, became more sensitive to serum, and bound to A549 lung cells ten times less, according to 30 findings. This study's findings are consistent with those of [6] who found that this protein is revealed and expressed on the surface by almost all *M. catarrhalis* isolates that have been investigated thus far[34].

Conclusion

This investigation found that *Moraxella catarrhalis* is harmful humans If it has been divided from the the respiratory system and that all isolates of the bacteria share the characteristics of usp A1, cop B, and omp CD. Future molecular investigations were therefore required to compare Moraxella isolated as a pathogen or normal flora, as well as to examine the antibiogram profile at the molecular level and other virulence variables that were not examined.

Source of Funding: Self

Conflict of Interest: Non

Ethical Clearance: Swabs from patients were those who had upper respiratory tract infections obtained for the study under the direction of the Ethical Committee for Human Subjects, and they were sent to Baghdad Medical City.

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