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Bacteriological and Molecular Study of *Moraxella catarrhalis* Isolated from Respiratory Tract and Middle Ear Infections in Children

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Abstract

Introduction: *Moraxella catarrhalis* is increasingly recognized as an important pathogen causing respiratory tract and middle ear infections in children. This study aimed to investigate the prevalence, antibiotic resistance patterns, lipooligosaccharide (LOS) serotypes, molecular characteristics, and virulence gene profiles of *M. catarrhalis* isolated from pediatric patients in Baghdad.

Methods: A total of 200 nasopharyngeal and middle ear swab specimens were collected from children aged 6 months to 5 years presenting with acute respiratory tract infections or otitis media in Baghdad hospitals between September 2024 and February 2025. The study included 120 respiratory tract swabs and 80 middle ear swabs. Bacterial isolates were identified using conventional microbiological methods and confirmed by PCR amplification of the copB gene. LOS serotyping was performed using monoclonal antibodies against LOS serotypes A, B, and C. Genetic diversity was assessed by multilocus sequence typing (MLST), and the virulence genes ompE, ompB2, and ompCD were detected by PCR. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method according to CLSI guidelines, and β-lactamase production was determined using the nitrocefin disk test.

Results: The overall prevalence of *M. catarrhalis* was 24.0%, with a higher isolation rate from respiratory tract specimens (29.2%) than middle ear specimens (16.3%). LOS serotype A was the predominant serotype (62.5%). MLST analysis revealed substantial genetic diversity, with ST449 being the most common sequence type (31.3%). The ompE gene was the most frequently detected virulence gene (85%), followed by ompB2 (70%) and ompCD (65%). High resistance was observed to ampicillin (75%) and erythromycin (50%), whereas all isolates were susceptible to ciprofloxacin and ceftriaxone.

Conclusion: *Moraxella catarrhalis* is an important cause of respiratory tract and middle ear infections among children in Baghdad. The predominance of LOS serotype A, the high prevalence of virulence-associated genes, the genetic diversity of circulating strains, and increasing resistance to commonly used antibiotics underscore the need for continuous molecular surveillance and rational antimicrobial use to improve clinical management.

Keywords: Moraxella catarrhalis, Respiratory infections, Otitis media, Serotyping, MLST, Virulence genes

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Introduction

Moraxella catarrhalis (*M. catarrhalis*) is a Gram-negative aerobic diplococcus that colonizes the upper respiratory tract of humans, especially children and considered a harmless commensal bacterium, it is now considered an important pathogen of various respiratory diseases, including acute otitis media (AOM), sinusitis, bronchitis, and pneumonia (1). It plays an important role especially in children and adolescents, and is one of the major cause's of otitis media and lower respiratory track tract infections (2). The virulence of *M. catarrhalis* is associated with several virulence factors, including outer membrane proteins such as ompE, ompB2, and ompCD, which promote adhesion to host cells, immune evasion, and biofilm formation (3). In addition, lipooligosaccharide (LOS) structures play an important role in immune regulation and contribute to strain variation, which has important implications for vaccine development (4). Antimicrobial resistance in *M. catarrhalis* strains is an increasing issue, specifically because of prevalent production of β-lactamases, which make many β-lactam medications useless. Resistance to macrolides and other frequently used antibiotics has likewise been documented, hindering treatment choices (5-7). Although clinically significant, *M. catarrhalis* has received less research attention compared to other respiratory pathogens like *Streptococcus pneumoniae* and *Haemophilus influenzae* (4). Molecular typing techniques, like multilocus sequence typing (MLST), have enhanced our knowledge of genetic diversity and epidemiology, playing a vital role in tracking. Data on the prevalence, molecular traits, and antibiotic resistance patterns of *M. catarrhalis* isolated from pediatric patients in Iraq, especially in Baghdad, remain scarce. This study sought to address this gap by conducting an extensive bacteriological and molecular analysis of *M. catarrhalis*

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strains isolated from children with respiratory conditions.

Materials and Methods

Sampling

A total of 200 clinical swab samples were collected from the nasopharynx and middle ear of children aged 6 months to 5 years, who exhibited symptoms of upper respiratory tract infection or otitis media. The samples were transported to the microbiology laboratory using Amies transport medium and processed within two hours of collection.

Isolation and Identification of Moraxella catarrhalis

Clinical specimens were cultivated on chocolate agar plates and incubated at 37°C in a 5% CO_2 atmosphere for 24 to 48 hours. Colonies displaying the characteristic morphology of *M. catarrhalis*—specifically gray-white, smooth, opaque colonies—were chosen for additional analysis.

Preliminary identification was based on

- Gram staining
- Oxidase test
- Catalase test
- DNase test

Molecular confirmation was subsequently performed by PCR amplification of the copB gene, which is highly conserved in *M. catarrhalis*. Only PCR-confirmed isolates were included in subsequent analyses (8).

LOS Serotyping

LOS serotypes were identified using the slide agglutination method, employing monoclonal antibodies specific to serotypes A, B, and C. Isolates that did not exhibit any agglutination reaction with any antiserum were classified as non-typeable.

Multilocus Sequence Typing (MLST)

Genetic characterization was carried out through Multilocus Sequence Typing (MLST). For this, seven housekeeping genes were amplified and sequenced following the protocol for *Moraxella catarrhalis* MLST. The allele numbers and sequence types (STs) were determined using the PubMLST database (9-12).

Detection of Virulence Genes

Genomic DNA was isolated from verified isolates utilizing a commercial bacterial DNA extraction kit, following the guidelines provided by the manufacturer. PCR assays were conducted to identify three virulence-associated genes: ompE, ompB2, and ompCD. The amplification process took place in a thermal cycler, employing previously published primer sequences and optimized amplification conditions. The resulting PCR products were then separated using electrophoresis on a 1.5% agarose gel, stained with ethidium bromide, and observed under UV light. These were then compared against a 100 bp DNA ladder(Table 1).

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was conducted using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar enhanced with 5% defibrinated sheep blood.

The bacterial inoculum was adjusted to 0.5 McFarland standard before inoculation.

The following antimicrobial discs were tested

- Ampicillin (10 µg)
- Erythromycin (15 µg)
- Trimethoprim–Sulfamethoxazole (1.25/23.75 µg)
- Ciprofloxacin (5 µg)
- Ceftriaxone (30 µg)

Following incubation at 35–37°C with 5% CO_2 for 20 to 24 hours, the diameters of the inhibition zones were measured and interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI M100, 2024). The production of β-lactamase was assessed using the nitrocefin disk test. Each susceptibility test was conducted in triplicate to guarantee reproducibility.

Results

Isolation and Identification of Moraxella catarrhalis

Out of the 200 collected specimens, 48 isolates, which account for 24.0%, were identified as *Moraxella catarrhalis*. The isolation rate was notably higher in respiratory tract specimens at 29.2%, compared to 16.3% in middle ear specimens. Each isolate displayed the typical morphological and biochemical characteristics of *M. catarrhalis*, such as gray opaque smooth colonies on chocolate agar, Gram-negative diplococci morphology, positive reactions for oxidase and catalase, and molecular confirmation through PCR. (Table 2).

Distribution of LOS Serotypes

The serological characterization of LOS antigens revealed that serotype A accounted for the highest percentage, making up 62.5%. This was followed by serotype B at 20.8%, and serotype C at 4.2%. Additionally, 12.5% of the isolates could not be typed, as shown in(Table 3).

Multilocus Sequence Typing (MLST)

The MLST analysis identified significant genetic diversity among the isolates. Sequence type ST449 was the most common at 31.3%, with ST64 and ST215 following at 18.8% and 12.5%, respectively. The other isolates were distributed

Table1. PCR Conditions for Detection of Virulence Genes

Gene	Expected Product	Annealing Temp
ompE	420 bp	56°C
ompB2	540 bp	55°C
ompCD	390 bp	54°C

Table 2. Distribution of *M. catarrhalis* isolates

Sample type	Samples	Positive Isolates	(%)
Respiratory Tract	120	35	29.2
Middle Ear	80	13	16.3
Total	200	48	24.0

across various sequence types, as detailed in(Table 4).

Detection of Virulence Genes

PCR analysis revealed a significant presence of virulence-associated genes among the M. catarrhalis isolates, as shown in Figure 1. The ompE gene was identified in 85% of the isolates, with ompB2 and ompCD following at 70% and 65%, respectively, as detailed in(Table 5).

Antibiotic Resistance Patterns

Antimicrobial susceptibility testing revealed a significant resistance to ampicillin, with 75% of isolates affected, while 50% showed resistance to erythromycin and 37.5% to trimethoprim-sulfamethoxazole, as highlighted in(Table 6).In contrast, all isolates were completely susceptible to both ciprofloxacin and ceftriaxone, as illustrated in (Figure 2).

Discussion

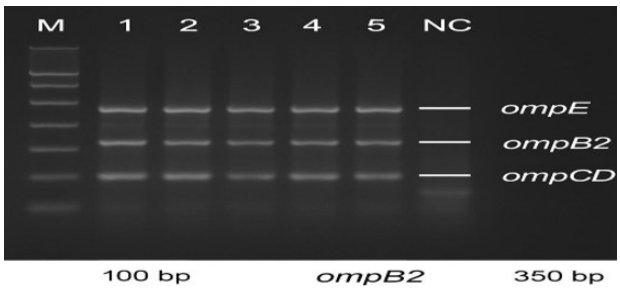
The study showed that *Moraxella catarrhalis* was found in 24% of pediatric respiratory and middle ear samples, with a notably higher presence in respiratory tract samples compared to middle ear specimens. This reinforces the recognized role of the nasopharynx as the main habitat for *M. catarrhalis*, from where it can spread to nearby areas and lead to upper respiratory tract infections and otitis media. Comparable prevalence rates have been observed in pediatric populations across various countries, reaffirming that *M. catarrhalis* continues to be a significant opportunistic respiratory pathogen (13-16).

The study's finding of the predominance of LOS serotype A aligns with earlier research showing that serotype A is the most prevalent clinical serotype globally. This particular serotype is linked to improved bacterial survival, heightened inflammatory responses, and a stronger potential for causing disease. The identification of non-typeable isolates indicates continuous genetic variation in the genes responsible for LOS biosynthesis, potentially aiding in immune evasion and adaptation to various host environments (17-18).

MLST analysis demonstrated significant genetic diversity among Iraqi isolates, with ST449 being the most common clone identified. International surveillance studies have similarly highlighted the prevalence of ST449 and ST64, indicating that these sequence types may have

Table 3. Distribution of LOS Serotypes

Serotype	Number of Isolates	Percentage (%)
A	30	62.5
B	10	20.8
C	2	4.2
Non-typeable	6	12.5



Figures 1. Prevalence of Virulence-Associated Genes

Table 4. Distribution of MLST Sequence Types

Sequence Type (ST)	Number of Isolates	Percentage (%)
ST449	15	31.3
ST64	9	18.8
ST215	6	12.5
Others	18	37.5

Table 5. Prevalence of Virulence-Associated Genes

Gene	Positive Isolates	Prevalence (%)
ompE	41	85
ompB2	34	70
ompCD	31	65

Table 6. Antibiotic resistance profile

Antibiotic	Resistance (%)	Sensitivity (%)
Ampicillin	75	25
Erythromycin	50	50
Trimethoprim- sulfamethoxazole	37.5	62.5
Ciprofloxacin	0	100
Ceftriaxone	0	100

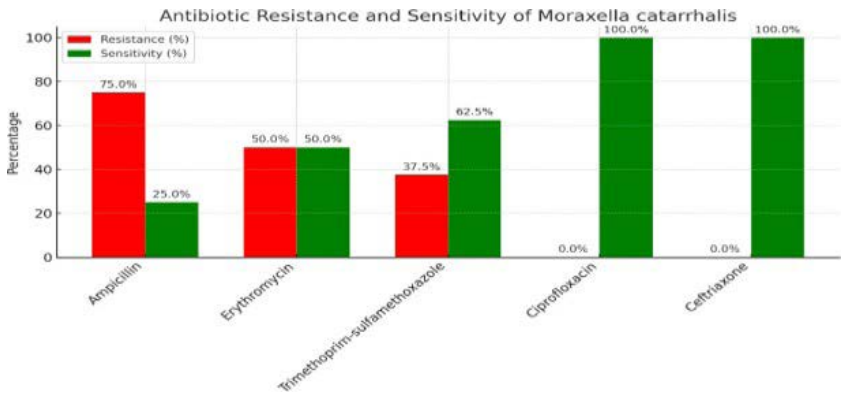


Figure 2. Antibiotic Resistance and Sensitivity of M. catarrhalis

advantageous traits that support their persistence and spread. The detection of various sequence types suggests that *M. catarrhalis* infections result from a genetically diverse array of strains rather than a single widespread clone (19-20).

PCR analysis revealed a significant prevalence of the virulence-associated genes ompE, ompB2, and ompCD. These outer membrane proteins are crucial for bacterial adhesion, nutrient acquisition, complement resistance, and the colonization of respiratory mucosa. Their extensive distribution among clinical isolates suggests these genes are highly conserved and likely play a vital role in bacterial pathogenicity. Recent molecular epidemiological studies have reported similar frequencies, underscoring their potential as vaccine candidates and diagnostic markers (21).

Antimicrobial susceptibility testing revealed significant resistance to ampicillin and erythromycin, while ciprofloxacin and ceftriaxone remained completely effective. This high resistance level to ampicillin is likely due to β-lactamase production, specifically the BRO-1 and BRO-2 enzymes, which are known as the main mechanisms of β-lactam resistance in

M. catarrhalis. These results align with global surveillance data indicating rising resistance to commonly used antibiotics, yet demonstrating excellent efficacy of third-generation cephalosporins and fluoroquinolones. However, since fluoroquinolones are generally not recommended as a first-choice treatment for young children, ceftriaxone continues to be a more suitable therapeutic option for severe infections (22-27).

Conclusion

The findings from this study offer refreshed epidemiological and molecular insights into *M. catarrhalis* among Iraqi children. The dominance of LOS serotype A, the prevalence of virulence genes, the variety of MLST sequence types, and the growing resistance to β-lactam antibiotics highlight the necessity for ongoing molecular surveillance and antimicrobial resistance monitoring. This is crucial for supporting effective clinical management and the development of future vaccines.

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Authors' Contribution

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Competing Interests

The authors declare that they have no conflicts of interest.

Ethical Approval

Ethics Committee (CSEC/1155/2166) was approved by the College of Medicine, University of Ibn Sina University of medical and pharmaceutical sciences.

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