Occurrence of *Moraxella catarrhalis* isolated from respiratory tract Infection

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**Abstract**

During the period from November 2011 to February 2012, 96 (69.1%) isolates of *M. catarrhalis* were isolated from 139 outpatients of both sex (85 male and 54 female) with respiratory tract infection (either Tonsilities, Otitis media, Sinusitis, or Pneumonia) admitted to or presenting at two hospitals in Al-Najaf governorate. The *M.catarrhalis* appeared to be the most frequent microorganism isolated in this study, which had percentage 75.6% (96), followed by *Streptococcus pneumonia*, *Staphylococcus aureus*, and *Hemophilus influenzae* in 15.7% (20), 6.3% (8), and 2.4% (3), respectively. *M.catarrhalis* isolates had high frequency of isolate in throat swabs than other samples. In this study, only 14 (14.6%) isolates of *M. catarrhalis* were produced sidrophores by growing on M9 medium. In addition, there were 72 (75%) isolates of *M.catarrhalis* appeared to adhere with the epithelial cells and all isolates show resistance to complement. The phenotypic resistance of 40 *Moraxella catarrhalis* isolates to 11 commonly used antimicrobial agents by using Kirby-Bauer disk diffusion method. All isolate of *M. catarrhalis* were appeared to show highest rate of resistance (100%) to Penicillin and Ampicillin. Similarly, the isolate exhibited high rate of resistance to Amoxicillin (95%) and Cefotaxime (72.5%) and mild resistance to Ciprofloxacin and Cephalothin in 62.5% for each. Cloramphnicol (57.5%), 52.5% of isolate showed resistance to Gentamicin and Trimethoprim. Whereas no one of isolates show resistance to (Ampicillin + Cloxacillin) and Tetracycllin. In the present study 15 *M. catarrhalis* isolates show MIC of Penicillin at 512 μg/ml (ie. had highest concentration MIC) while 4 isolate show MIC of Ampicillin at 512 μg/ml. Phenotypic assay was performed to determine the presence of β-lactamase enzyme by using nitrocefin disk. While in genotypic β-lactamase assay, the *bro*-1 gene found in 25 (62.1%) isolates, while *bro*-2 gene was presented only in 3 (7.5%) isolates.

**Introduction**

*Moraxella catarrhalis* is a human-restricted, encapsulated, gram-negative mucosal pathogen. Further, though previously thought to be a commensal of the upper respiratory tract, the bacterium is now increasingly recognized as a true pathogen of both the upper respiratory tract and the lower respiratory tract of humans. It is the third most common bacterial cause of childhood otitis media (OM) after *Haemophilus influenzae* and *Streptococcus pneumoniae*, and it is responsible for up to 20% of cases (Hays, 2006; Vergison, 2008). Next to *H. influenzae, M. catarrhalis* is the second most common cause of exacerbations of chronic obstructive pulmonary disease (COPD), estimated to be responsible for 10 to 15% of these exacerbations, which accounts for 2 to 4 million episodes in the United States per year (Vrieset et al., 2009).

Rates of *M. catarrhalis* carriage in children and adults differ considerably. About two-thirds of all children are colonized within the first year of life, and it is expected that about half of these children will experience at least one period of OM during this year. In contrast, the rate of carriage in healthy adults is much lower (Fung et al., 1992).
M. catarrhalis exhibits an almost universal resistance to penicillin related antibiotics, with several studies indicating that world-wide, 90-100% of M. catarrhalis isolates produce β-lactamase (Abe et al., 2002). This is a striking statistic when one considers that before 1970 few isolates produced β-lactamase enzymes (Catlin, 1990). Research into M. catarrhalis β-lactamase production has shown that 3 different isotype groups may be identified, BRO-1, BRO-2 and BRO-3 (Christensen et al., 2010).

M. catarrhalis also appears to be able to invade host epithelial cells (Jordan et al., 2010), the intracellular survival of pathogens being an important aspect of host immune evasion (Bootsmaet et al., 2000). Moreover, once attached to the host mucosal surfaces, M. catarrhalis has the ability to interact and/or compete with the commensal flora and has the apparatus to survive and multiply under challenging nutrient-limiting conditions. Such conditions may result in the formation of microcolonies and biofilms (Christensen et al., 2010). Finally, M. catarrhalis has the ability to evade and survive host immune responses, a process particularly helped by its ability to withstand the effects of human serum (Bootsmaet al., 2000).

The present study is carried out to achieve the isolation and identification of M. catarrhalis from patients with RTI.

Materials and Methods

Patients and sample collection

This study was carried out in two hospitals in Najaf governorate (Al-Hakeem and Al-Zahra Maternity and Children) during the period between November 2011 to January 2012. A total of 139 samples (67 throat swabs, 32 ear swabs, 30 sputum, 10 nose swabs) were collected from outpatients suffering from upper respiratory tract infection (pharyngitis, otitis media, pneumonia, sinusitis respectively), they included both sex (85 male and 54 female), with different age groups.

Isolation and Identification of bacterial isolates

All samples were cultured on blood agar (HiMedia), chocolate agar (HiMedia), nutrient agar (HiMedia), brain heart agar media (HiMedia) using standard loop method. The media were incubated in a candle jar with CO₂ at 37°C for 24–48 hours depending on the morphological features of the colonies and microscopically examination with Gram’s stain, pure culture on chocolate agar plates were made from each single group of colonies (Gram-negative diplococci with a typical colony appearance). The pure cultures were prepared for biochemical tests to differentiate M. catarrhalis from other bacteria.

Microscopic properties: Gram’s stain was used to examine the isolated bacteria. Biochemical characterization were done according to (MaccFADIN, 2000). Moreover, the biochemical tests with APINH miniaturized diagnostic test were confirmed that all these isolate as M. catarrhalis. 

Sidrophore detection was performed on M9 media which is prepared as suggested by (Nassif et al., 1989). The media was inoculated with single colonies of overnight culture by streaking method and incubated for 24 hours at 37°C. The appearance of the growth of M. catarrhalis on M9 media indicated a positive result. Adherence to human oropharyngeal cells test was done according to Lomberg et al., (1986). The culture-and spot test (Verduin et al., 1995) was used to detection complement resistance of M. catarrhalis isolates. All M. catarrhalis isolates performed identification to susceptibility testing by modified Kirby-Bauer disk-diffusion method (Bauer et al., 1966). The selection of antibiotic was performed according to the guidelines recommended by the Clinical and Laboratory Standard Institute (CLSI, 2011).

Results and Discussion

Ninety six (69.1%) isolates of M. catarrhalis were isolated from a total of 139 outpatients of both sex (85 male and 54 female) with RTI. One of the bacterial isolates of
RTI samples, the remaining 43 (30.9%) were presented as other bacterial types. All these culture sterile isolates were identified on the basis of Microscopic examination, colonyed morphology and comparison of the biochemical characteristics with standard description in Macfaddin (2000) and Mims et al. (2008). In microscopic examination (Gram film), the organism was appeared as Gram negative diplococcus with flattened sides. Colonies of these isolate on blood agar and chocolate agar presented in large, grey, smooth, opaque and convex morphology figure (1).

![Figure 1](image1.png)

**Figure (1) M. catarrhalis on blood agar(A), chocolate agar(B).**

The identification of *M. catarrhalis* to the species level involved biochemical testing with production of Oxidase, Catalase, lack of acid production from the glucose, fructose, lactose, sucrose, and manose reduction of nitrate and non motile. Moreover, the biochemical tests with API NH miniaturized diagnostic test were confirmed that all these isolate as *M. catarrhalis* (figure 2).

![Figure 2](image2.png)

**Figure (2): The Identification Results of M. catarrhalis isolate by API NH.**

Figure (3) show the incidence of *M. catarrhalis* among other etiological agents isolated from patients with RTI in Najaf governorate, from which *M. catarrhalis* was appeared the most frequent microorganism isolated in this study, which had frequency of 75.6% (96), followed by *S. pneumoniae* with 15.7% (20), *Staphylococcus aureus* with 6.3% (8), and *Hemophilus influenzae* with 2.4% (3).
**Figure (3) Occurrence of** *M. catarrhalis* **among other etiological agent in RTI.**

*M. catarrhalis* was found to be the most common isolated respiratory pathogen. It was frequently isolated in pure culture or in combination with *H. influenzae* or *S. pneumoniae* and the seasonal recovery of *M. catarrhalis* was apparent for the November to May period, compared with June to October period (Mc Gillivray *et al.*, 2009).

Brockson *et al.* (2012) reported that the three most common bacterial causative agents of OM are *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae* (NTHI) and *Moraxella catarrhalis*, all of which are normal commensal species of the pediatric nasopharynx, and demonstrated that co-infection with RSV and NTHI predisposed to *M. catarrhalis*-induced ascending experimental OM. Since, Kennedy *et al.* (2000) showed that in children, viral infection predisposes to bacterial OM by facilitating ascension of select members of the colonizing NP flora into the middle ear space.

The high incidence of *M. catarrhalis* isolated in this study from patient with RTI among other bacterial isolates may be attributed to many reasons, firstly, *M. catarrhalis* are the most predominant pathogen in Nasopharynx of chiled and represent the common case in this study. Since, there is a strong relationship between age and colonization rates of *M. catarrhalis* (Brockson *et al.*, 2012). Secondly, the increase resistance of *M. catarrhalis* to antibiotic especially β-lactam antibiotic that predominates in the cure RTI by increase β-lactames enzyme production which may be due to increase predomance of this bacteria in hospital. And thirdly, the average length of stay in hospital is considerably longer for children colonized with *M. catarrhalis* compared to those not colonized and providing evidence that re-colonization with different *M. catarrhalis* types occurs.

Stenfors and Raisanen (2003) isolated *M. catarrhalis* in 16% from exudate of middle ear of patients with CSOM in Finland. Ad-Dahhan (2007) isolated *M. catarrhalis* in 17.4% from patient with URTI in Al-Najaf governorate. Al-Tememy (2004) found *M. catarrhalis* presented in 6.5% in patients with CSOM in Babylon city, while Al-Turphy (2000) referred that *M. catarrhalis* was isolated in 3.6% in patients with OM in Karbala city. In this study, *M. catarrhalis* was isolated in 75.6%.

![Image of bacterial distribution](image-url)
Occurrence of *M. catarrhalis* in clinical specimens:

Table (1) reveals that the high frequent of *M. catarrhalis* isolates was in throat swabs which had a frequency 58.3%, while the percent of isolates in specimens taken from ear, sputum and nose swabs presented in 31.3%, 17.7% and 3.3%, respectively.

Prevalence of *M. catarrhalis* from all specimens in this study was 69.1% and this result was higher than other previous study (Stenfors and Raisanen, 1990, Martinez et al., 1999, Ad-Dahhan, 2007).

### Table (1) Prevalence of *M. catarrhalis* in clinical specimens

<table>
<thead>
<tr>
<th>Type of specimens</th>
<th>No. of specimens</th>
<th><em>M. catarrhalis</em> isolates (No. of isolates)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat swab</td>
<td>67</td>
<td>56</td>
<td>58.3</td>
</tr>
<tr>
<td>Ear swab</td>
<td>32</td>
<td>30</td>
<td>31.3</td>
</tr>
<tr>
<td>Sputum</td>
<td>30</td>
<td>17</td>
<td>17.7</td>
</tr>
<tr>
<td>Nose swab</td>
<td>10</td>
<td>3</td>
<td>3.3</td>
</tr>
<tr>
<td>Total</td>
<td>139</td>
<td>96</td>
<td>69.1</td>
</tr>
</tbody>
</table>

*M. catarrhalis* may be the single cause of sinusitis, otitis media, tracheitis, bronchitis, pneumonia, and, less commonly, ocular infections in children. Where, nasopharyngeal colonization often precedes the development of *M. catarrhalis*-mediated disease (Broides et al., 2009). It has been suggested that there is a possible underestimation of isolation rates for *M. catarrhalis*, since the bacterium stops growing in environments with reduced oxygen concentrations, a condition frequently present during sinusitis and otitis media. This would indicate an even greater role for *M. catarrhalis* in the etiology of these infectious diseases (Verduin et al., 2002).

Several studies reveal that *M. catarrhalis* is the commonest bacterium isolated from specific clinical case. AL-Mazory (2002) isolated *M. catarrhalis* in 13.8% from patients with COPD as a commonest pathogens, while Boyle et al., (1991) reported that *M. catarrhalis* represent 26% from all sputum of patient with pneumonia. In this study the high percentage (58.3%) of isolates occur in throat swabs. In contrast with Ad-Dahhan (2007) who isolated bacteria in 17.4% from all cases.

### Distribution of *M. catarrhalis* according to the age and sex:

In this study the patients ages categorized into six groups (Table 2). The higher incidence of *M. catarrhalis* isolates were recorded at first age group (1-10) years with 27.1% (26), followed by the last age group (50-60) years and the second age group (11-20) years in 21.9% (21) and 19.8% (19), respectively. The low frequency of isolation was recorded at age group (31-40) years followed by age groups (21-30) years and (41-50) years in percentage 7.3%, 9.4% and 14.6%, respectively.
Table (2) Distribution of M. catarrhalis according to the age

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>No.of patient</th>
<th>M.catarrhalis isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>1-10</td>
<td>30</td>
<td>26</td>
</tr>
<tr>
<td>11-20</td>
<td>26</td>
<td>19</td>
</tr>
<tr>
<td>21-30</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>31-40</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>41-50</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>50≥ 60</td>
<td>28</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>139</td>
<td>96</td>
</tr>
</tbody>
</table>

In the present study the first age group (1-10) was the most dominance in infection (27%) compared with the age group (31-40) which recorded lowest percentage of infection (7.3%). This difference may be due to low immunity in this age group (children) and may be the children during the childhood are most exposed to infection during playing with infected material. Further, the extensive presence of M. catarrhalis in the age group (50≥ 60) years may be attributed to impaired immunity system and most elderly are infected with chronic diseases. The result of the current study agrees with some studies (Ad-Dahhan 2007; Vries et al. 2009) who stated that M. catarrhalis was the commonest pathogen in children less than 10 years.

Sidrophores production:

In this study, only 14 (14.6%) isolates of M. catarrhalis were produced sidrophores by growing on M9 medium containing 200μmol dipyridyl (Fig. 4). This result differs from that of Al-Tememy (2004) when she referred that all isolates of M. catarrhalis lack its ability to sidrophore production in her study.

M. catarrhalis expresses specific OMPs in response to iron-limited growth in vitro (Vries et al., 2009). Yu and Schryvers (1993) observed M. catarrhalis uses iron-saturated human transferrin or lactoferrin as the sole source of iron for growth in the absence of siderophores. These observations suggest that M. catarrhalis competes for iron bound to human transferrin and human lactoferrin in a manner similar to that used by Neisseria species. M. catarrhalis transferrin receptors show a strong preference for iron-saturated transferrin over apotransferrin, and in this regard they differ from Neisseria receptors. Labout et al. (2011) referred that two of the iron-repressible proteins are OMPs B1 and B2.
Adherance to epithelial cell:

In this study, there were 72 (75%) of *M.catarrhalis* isolate show it ability to adhere with epithelial cell.

Attachment to the epithelium of respiratory tract is likely to be an essential step in the pathogenesis of *M. catarrhalis* infection. The general mechanism of cellular adherence of *M. catarrhalis* to host cell surfaces has been studied previously by Rikitomi *et al.* (1991). Hemagglutination in gram-negative bacteria is often associated with expression of pili or nonpilus adhesive proteins that promote attachment to and colonization of host mucosal surfaces. Several studies (Ahmed *et al*., 1992; Balder *et al*., 2009; and Labout *et al*., 2011) have confirmed that nonfimbriated structure of *M. catarrhalis* are less adheret and therefore can escape phagocytosis but are more invasive, and there was no significant correlation between adherence and the number of fimbriae. Another study found no differences between the source of the isolate (blood or lungs) and hemagglutination (Luke *et al*., 2007).

Complement Resistance:

The complement resistant or sensitive phenotype of the 96 *M. catarrhalis* isolates used in this study had been previously determined using the “culture-and-spot” test by Verduin *et al.* (1994). This is a rapid and simple test for determining the complement resistance phenotype of *M. catarrhalis*, which exhibits a statistically significant concordance with the serum bactericidal assay and is based on the survival of bacteria on a blood agar plate after the application of a drop of 50% serum.

The pathogenesis of *M. catarrhalis* relies on its capacity to resist the human host defense, including complement. The complement system is very harmful for Gram-negative pathogens, including *M. catarrhalis*, and bacterial complement resistance is one of the most important virulence mechanisms (Nordstrom *et al*., 2005). *M. catarrhalis* has thus developed several efficient strategies to circumvent complement. It has been demonstrated that UspA1 and UspA2 interact and inhibit the alternative pathway of complement by noncovalently binding C3 (Hallstrom *et al*., 2008).
In this study, all isolates 100% (96/96) were found to be resistant to the effect of complement in human serum. The percentage of complement resistant *M. catarrhalis* isolated appears to be relatively high when compared to some studies (Hol et al., 1995) involving healthy children (100% versus 30-60%), other studies have yielded similar results (Hays, 2003).

**Resistance to Antimicrobial Agent:**

Table (3) shows the phenotypic resistance of 40 *Moraxella catarrhalis* isolates to 11 commonly used antimicrobial agents by using Kirby-Bauer disk diffusion method (Bauer et al., 1966).

All isolates exhibited resistance to penicillin and Ampicillin in 100%. Vast majority of isolate exhibited high rate of resistance to Amoxicillin 95% and Cefotaxime 72.5% and mild resistance to Ciprofloxacin and Cephalothin in 62.5% for each, to Cloramphenicol in 57.5%, 52.5% of isolate showed resistance to Gentamicin and Trimethoprim at else. Whereas all isolates were susceptible to these two antibiotics were the most potent and effective antibiotics against isolate in this study.

**Table (4-5) Antibiotic resistance of *Moraxella catarrhalis* isolate to Antibiotic**

<table>
<thead>
<tr>
<th>Type of Antibiotic</th>
<th>symbol</th>
<th>Resistant isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of isolate</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>AM</td>
<td>40</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>AX</td>
<td>38</td>
</tr>
<tr>
<td>(Ampicillin+cloxacillin)</td>
<td>APC</td>
<td>0</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>CTX</td>
<td>29</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>KF</td>
<td>25</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>C</td>
<td>23</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>CIP</td>
<td>25</td>
</tr>
<tr>
<td>Gentamycine</td>
<td>GM</td>
<td>21</td>
</tr>
<tr>
<td>Piillincillin</td>
<td>P</td>
<td>40</td>
</tr>
<tr>
<td>Trimethoprin</td>
<td>TMP</td>
<td>21</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>TE</td>
<td>0</td>
</tr>
</tbody>
</table>

In the present study MICs of Penicillin and Ampicillin for 40 *M. catarrhalis* isolates doing according to CLSI (2011) as showed in the table (4), in which 15 *M. catarrhalis* isolate show MIC of Penicillin at 512 μg/ml (i.e. had highest concentration MIC) while only 4 isolates show MIC of Ampicillin at 512 μg/ml.
M catarrhalis isolates that produced BRO-1 enzyme gave the highest MICs (512 µg/ml) to penicillin (15) and Ampicillin (4) than that the isolates produced BRO-2 enzyme as shown in table (5)

Table (4) MIC of penicillin and Ampicillin for 40 M. catarrhalis isolates.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>No. of isolate inhabited at MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;4 8 16 32 64 128 256 512 (Total)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>8 1 2 3 2 3 6 15 (40)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>7 5 3 3 6 8 2 4 (38)</td>
</tr>
</tbody>
</table>

Note: 38 are the total number of Ampicillin resistance strain

Table (5) Distribution of Penicillin and Ampicillin MICs among BRO1 and BRO2 producing M. catarrhalis isolates.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Enzyme type</th>
<th>Cumulative No. isolates inhabited at MICs (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of isolates</td>
<td>&lt;4 8 16 32 64 128 256 512</td>
</tr>
<tr>
<td>Penicillin</td>
<td>BRO1 (25)</td>
<td>0 0 0 0 0 1 3 6 15 0 0</td>
</tr>
<tr>
<td></td>
<td>BRO2 (3)</td>
<td>0 2 1 0 0 0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>BRO1 (25)</td>
<td>3 2 0 0 6 8 2 4</td>
</tr>
<tr>
<td></td>
<td>BRO2 (3)</td>
<td>0 1 1 1 0 0</td>
</tr>
</tbody>
</table>

Schmitz et al.(2002) reported that several antimicrobial remained very active against M.catarrhalis, including Amoxicillin-clavulanate ((MIC, <0.25 µg/ml), azithromycin (MIC, <0.12 µg/ml), ceftriaxone (MIC, 0.5 µg/ml), and levofloxacin (MIC, <0.03 to 0.06 µg/ml). Isolate producing the Bro-1 enzyme have been shown to be consistently more resistant to ampicillin than strains of the BRO-2 type (Verduin et al.,2002). This difference in MICs correlates well with the observation that BRO-1 is produced at a level two to three times higher than that of BRO-2 (Wallace et al.,1990). In conclusion M.catarrhalis

References


Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). 2011. Performance standards for antimicrobial susceptibility testing. Twentieth informational supplement. 30 (1)


