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# A comparison of culture and PCR methods for identifying *Moraxella catarrhalis* in patients with respiratory diseases

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ABSTRACT

*Moraxella catarrhalis* is considered as one of the most significant pathogens of the respiratory tract. This research aimed to isolate and identify *M. catarrhalis* in respiratory diseases patients from northern part of Iran using culture and PCR techniques. In this study, 280 samples including throat swab of the patients with pharyngitis (n=92), sinus secretions of the patients with sinusitis (N=85), ear secretions of patients with otitis media (n=43) and pulmonary secretions of the patients hospitalized in the intensive care unit (n=60) were collected. Culture technique and phenotyping tests were used to isolate and identify the bacterium. PCR technique was also used for its identification by usage of the specific primers. Of a total of 280 samples, 27 samples (9.64%), and 87 samples (31.07%), were reported to be positive in terms of presence of *M. catarrhalis* using culture and PCR techniques, respectively. The results obtained by this study show that *M. catarrhalis* is present in the human societies and hospital environments. Therefore, rapid identification and tracking of its strains can play a significant role in prevention from their development.

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## 1. Introduction

In the past decades, *Moraxella catarrhalis* was identified as a safe commensal flora in the upper respiration tract and could be identified in more than 50% of the healthy children. However, it has converted to a real pathogen by increasing the pathogenicity and developing the capability of β-lactamase enzyme. It is now listed as a nosocomial pathogen (Sharda et al., 2011; Spaniol et al., 2014; Sheikh et al., 2014; Bhattacharyya et al., 2015).

*M. catarrhalis* is a common cause of otitis media, sinusitis and conjunctivitis in the neonates and children covering 10-20% of their infections. Pneumonia resulted from it is not prevalent, but it has been reported in the elderly individuals, especially those with cardio-respiratory diseases. In the adults, *M. catarrhalis* is related to formation of chronic obstructive pulmonary disease (COPD) and pneumonia (Gupta et al., 2011; Ramana and Chaudhury, 2012; Krishna et al., 2016; Earl et al., 2016; Liu et al., 2017).

*M. catarrhalis* also plays a role in occurrence of mixed infections of respiratory tract with other pathogens such as *Streptococcus pneumonia* and *Haemophilus influenza*.

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Clinnically, infections originated from *M. catarrhalis* cannot be differentiated from the infections resulted from other bacteria (Timothy and Murphy, 2009)

Standard culture techniques and phenotypic tests can be used to detect this aerobic gram negative diplococcus. *M. catarrhalis* generates circular and opaque, gamma hemolysis colonies on the blood agar medium. Colonies of this bacterium are similar to those from oral *Neisseria*, which are a part of normal flora of the upper respiratory tract. This difficulty in differentiation between *M. catarrhalis* and oral *Neisseria* justifies has made *M. catarrhalis* not to receive enough attention as an opportunistic pathogen of respiratory tract. Since culture of secretions of pharynx, sinus and middle ear is not routinely enforced in the medical diagnosis laboratories, a full treatment is normally applied against the three main pathogens of this part of the body including *M. catarrhalis*, *H. influenza* and *S. pneumonia* (Bernharda and Aebi, 2012). Today, sensitive methods such as Polymerase Chain Reaction (PCR) have been developed to identify *M. catarrhalis* from secretions of the respiratory tract. Using these sensitive methods, new and significant information has been achieved concerning epidemiology and a model of disease caused by *M. catarrhalis* (Hall-Stoodley, 2009).

In this research, in addition to isolation and identification of the *M. catarrhalis* in the Northern part of Iran, frequency and the most significant reservoirs of this bacterium is introduced by the culture and PCR techniques.

## 2. Materials and Methods

This study was conducted from April, 2016 to March, 2018. Totally 280 samples including pharyngeal swab of the patients with pharyngitis (n=92), secretions of sinus of the patients with sinusitis (n=85), ear secretions of patients with otitis media (n=43) and pulmonary secretions of the patients hospitalized in the ICU (n=60) were collected. The patients ranged from 7 to 76 years old with an average of 48 years old. The demographic data of participants including gender, age and history of antibiotic consumption within the previous month was collected through a questionnaire. The informed consent was obtained from each patient prior participating in the study. A code was allocated to each patient and the samples were inoculated in the pipes containing BHI medium (Merck-Germany).

The samples were transferred to the laboratory shortly after collection and incubated in 37˚c for 48 hours. A loop of microbic suspension, on the sterile conditions, was taken from the liquid medium after incubation and cultured on the Blood agar (Merck-Germany) and incubated in 37˚C for 48 hours.

The plates cultured from each individual were investigated for the accurate colonies. Following the preparation of pure culture, a variety of diagnostic tests including catalase, oxidase, DNase, nitrate reduction and fermentation of glucose, sucrose and lactose were used to identify the considered bacterium.

For the purpose of molecular investigation, DNA extraction was performed on the samples collected from the BHI medium cultures using an extraction kit (Qiagen-Germany). PCR amplification was carried out using a pair of specific primers made by TAG Copenhagen Company (Denmark).

Each reaction was performed in a total volume of 25 µl containing 13 µl of molecular biology-grade water (Sigma Aldrich Company LTD., USA), 2.5 µl of 10× PCR buffer (Geneall, Korea), 1 µl of 10 pmol of each primers (table 1), 1 µl of 10 mM dNTPs (Geneall, Korea), 0.5 µl of smart taq DNA polymerase (Geneall, Korea), 1 µl of 50 mM MgCl2 (Geneall, Korea) and 5 µl of DNA template (Hays et al., 2007). The negative control tube contained the same PCR reagents as above but had 5 µl of water substituted for the DNA template.

PCR amplification conditions on a thermocycler (Biorad-Germany) were as follows: 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds, with a final extension at 72°C for 7 minutes.

An aliquot of all PCR products was run on a 1.5% (w/v) agarose gels with a 100-1500 bp DNA ladder (Fermntas-Russia) and electrophoresed at 75 V for 40 min. The bands were visualized using safe staining and photographed by a UV-transilluminator (UV doc, England).

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**Table 1.** Primers used for fragment amplification of the 16S rRNA gene of *M. catarrhalis*

| Primer name | Primers Sequence | Fragment size | Reference |
|---|---|---|---|
| 16SrRNA-F | 5´-TTGGCTTGTGCTAAAATATC-3´ | 140 bp | (Lu Xi et al., 2017) |
| 16SrRNA-R | 5´–GTCATCGCTATCATTCACCT-3´ | | |

## 3. Results

In this study, two methods including culture and PCR have been used to identify *M. catarrhalis* in the patients with respiratory infections. Of 280 studied samples, 27 (9.64%) samples were determined as positive for *M. catarrhalis* by culture method. 87 samples (31.07%) were also determined to be positive by PCR. The results achieved from two methods were analyzed on the basis of demographic information, including age, gender and history of antibiotic consumption.

As shown in table 2, two demographic variables including the history of antibiotic consumption and type of disease show, a significant relationship with the positive cases in PCR methods. With regard to frequency, positive cases in the culture and PCR methods are higher in the individuals who have any history of antibiotic consumption. Also, positive cases of the culture method and PCR technique in individuals with pneumonia were higher than other individuals. As specified, there is a noticeable difference between the results achieved from culture and PCR methods. Chi square test was used to compare the results achieved from the culture and PCR methods. The results related to this test are given in table 3. The results show that PCR is significantly more sensitive than culture in detection of *M. catarrhalis*.

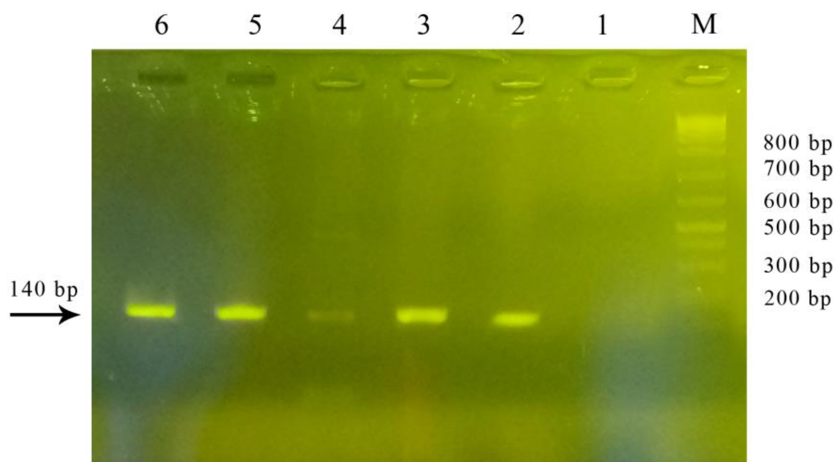


**Figure 1.** PCR amplification products analyses in a 2% agarose gel. The presence of a 140 bp band indicates the amplification of *16SrRNA* gene in the sample. Lane M: size marker 100-1500 bp, Lane 2-6: positive samples, lane 1: negative control

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**Table 2.** Demographic data of *M. catarrhalis* in patients with respiratory diseases detected by culture and PCR techniques

| Demographic variable | | No. Frequency | Percentage Frequency (%) | No of Positive culture | Frequency (%) | P-value | No of Positive PCR | Frequency (%) | P-value |
|---|---|---|---|---|---|---|---|---|---|
| Gender | female | 150 | 53.57 | 13 | 48.15 | 0.237 | 39 | 44.8 | 0.053 |
| | male | 130 | 46.43 | 14 | 51.85 | | 48 | 55.2 | |
| Age (year) | <20 | 26 | 9.29 | 4 | 14.8 | 0.369 | 13 | 15 | 0.077 |
| | 21-40 | 76 | 27.14 | 6 | 22.2 | | 23 | 26.5 | |
| | 41-60 | 92 | 32.86 | 8 | 29.6 | | 22 | 25.2 | |
| | >60 | 86 | 30.71 | 9 | 33.4 | | 29 | 33.3 | |
| history of Antibiotic consumption | yes | 187 | 66.79 | 18 | 66.6 | 0.179 | 65 | 74.7 | 0.001 |
| | No | 93 | 33.21 | 9 | 33.4 | | 22 | 25.3 | |
| Type of disease | Pharyngitis | 92 | 32.86 | 5 | 18.5 | 0.623 | 11 | 12.6 | 0.001 |
| | Sinusitis | 85 | 30.36 | 7 | 25.9 | | 18 | 20.7 | |
| | Otitis media | 43 | 15.36 | 6 | 22.2 | | 25 | 28.8 | |
| | Respiratory failure | 60 | 21.43 | 9 | 33.4 | | 33 | 37.9 | |

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**Table 3.** Comparison between culture and PCR techniques for detection of *M. catarrhalis*

| Methods | No. Samples | Frequency (%) | No of Positive | Frequency (%) | No of Negative | Frequency (%) | P- value |
|---|---|---|---|---|---|---|---|
| Culture | 280 | 100 | 27 | 9.64 | 253 | 90.36 | 0.001 |
| PCR | 280 | 100 | 87 | 31.07 | 193 | 68.93 | |

## 4. Discussion

M. *catarrhalis* is the fifth prevalent agent of respiratory infection after *H. influenza*, *S. pneumonia, Klebsiella pneumonia* and *Pseudomonas aeroginosa* (Tamang et al., 2005; Anita et al., 2011). In addition to capability of connection to cellular and mucous surfaces of respiratory system and biofilm formation, this bacterium is capable of intracellular replication in the lymphatic tissues of pharynx (Bullard and Lafontaine, 2005; Slevogt and Tiwari, 2007) and therefore the mucous membrane of the upper respiratory system can be a reservoir of this bacterium (Heiniger et al., 2007). Several factors, including age, living conditions, health, environmental agents, genetics and etc have been recognized to be effective on a difference between colonization rate of *M. catarrhalis* in the various societies.

Researchers conducted in recent years have reported different frequencies of *M. catarrhalis* in the various diseases of the upper respiratory tract. For instance, Tamang et al., khoramrooz et al., Osagie et al., Mazin et al., and Elrhman et al., isolated and identified this bacterium from the patients in 6.9%, 9.5%, 4.75%, 3.75% and 1%, respectively (Tamang et al., 2005; Khoramrooz et al., 2012; Osagie et al., 2013; Mazin et al., 2013; Elerhman et al., 2015).

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The results obtained from this research showed that *M. catarrhalis* existed in 9.64% (27 of 280) of the study population using culture technique, while its frequency was 31.07% (87 of 280) using PCR technique.

In a research conducted by Farajzadehe Shaiklh et al., in 2015, *M. catarrhalis*, was identified in 2.9% of the samples of otitis media by culture technique, while its detection rate reached 12% by PCR technique. The results obtained by this study are consistent with those obtained by farajzadeh et al., suggesting that the sensitivity of PCR is a much higher than the culture. *M. catarrhalis* is a slow growth bacterium and requires special culture media and cannot be identified routinely in the diagnostic laboratories. The increasing development of the molecular techniques for diagnosis of a variety of infectious agents and considering the difficulties related to the culturing *M. catarrhalis*, it is important to introduce the PCR technique as the standard method for detection of this bacterium in diagnostic laboratories.

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## Refereces

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