



Original Article

Detection of *Streptococcus pneumoniae* and *Moraxella catarrhalis* in patients with paranasal chronic sinusitis by polymerase chain reaction method

Ahmad Farajzadeh Sheikh ^a, Khadijeh Ahmadi ^{b,*}, Soheila Nikakhlagh ^c

^a Health Research Institute, Infectious and Tropical Diseases Research Center, Department of Microbiology, Medicine Faculty, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

^b Department of Microbiology, Medicine Faculty, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

^c Head and Neck Surgery, Hearing and Speech Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

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Abstract

Background: Sinusitis is a complex involvement of the upper respiratory system by bacteria, viruses, fungi, or other allergens. *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* are the dominant bacterial microorganisms involved in acute sinusitis, whereas in chronic sinusitis, *Staphylococcus aureus* and some anaerobic bacteria are the prevailing pathogens. Appropriate antibiotic treatment requires sinusitis bacteriology assessment. The aim of this study was to isolate bacteria in clinical samples from patients with chronic sinusitis.

Methods: A total of 55 samples were collected from patients with chronic sinusitis undergoing surgery at Imam Khomeini Hospital in Ahvaz, Iran. Samples were cultured in conventional medium, and for each culture, Gram staining, catalase, coagulase, oxidase, and DNase tests were performed and isolates were stored for polymerase chain reaction analysis.

Results: Twenty-three isolates were obtained from five patients, including *S. aureus* (23.6%), *Rhizomucor* (1.8%), and *Escherichia* (1.8%) by the culture method and *M. catarrhalis* (3.6%) and *S. Pneumoniae* (7.2%) by the polymerase chain reaction method. Compared with acute sinusitis, the microbiology of chronic sinusitis remains controversial. Results are affected by many factors, including diversity of molecular and culture methods, sterilization of sampling area, sample transfer to laboratory, use of antibiotics prior to surgery, and nasal polyps.

Conclusion: In Iran, the causative agents of chronic sinusitis are similar to those in other countries. Compared with other bacteria, *S. aureus* was observed more often in asthmatic patients with sinusitis.

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Keywords: *Moraxella (Branhamella) catarrhalis*; polymerase chain reaction; sinusitis; *Streptococcus pneumoniae*

1. Introduction

Sinusitis is a unique disease with a variety of manifestations. The sinuses are located in facial bones around the nose¹; the maxillary sinus is in the zygoma, the frontal sinus is near

the eyebrows, the ethmoid sinus is between the eyes, and the sphenoid is behind the ethmoid sinus.²

Sinusitis is the inflammation of the upper respiratory system. Infection is one of the causes of inflammation of sinuses. The symptoms of infection include fever, pain in the forehead, and green nasal discharges. Factors producing sinusitis may be classified as follows:

- (1) Inflammatory factors—including infections of the upper respiratory system, which are caused by cold, allergic rhinitis, dental manipulations, and swimming

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* Corresponding author. Dr. Khadijeh Ahmadi, Department of Microbiology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

E-mail address: kh.ahmadi53@gmail.com (K. Ahmadi).

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- (2) Systemic factors—including immune deficiency, immobile ciliary syndrome, cystic fibrosis, pregnancy rhinitis, and hypothyroidism
- (3) Mechanical factors—including canal atresia, nasal polyps, nasal septum damage, foreign body, trauma, nasal tumors, conical body hypertrophy, and adenoid hypertrophy
- (4) Medicinal factors—including breathing control pills, beta blockers, anxiety medication, aspirin, and cocaine²

Sinusitis is classified according to the duration of symptoms.³ Acute sinusitis lasts fewer than 30 days. Failure in treatment of acute sinusitis leads to chronic sinusitis, which lasts more than 90 days.^{1,4} Chronic sinusitis presents in either eosinophilic or neutrophilic form, but primarily in the eosinophilic form. In such cases, polyps are usually found, where *Staphylococcus aureus* is the dominant organism.⁵ Mechanical obstruction, lack of discharge from sinuses, nasal tumors, and nasal polyps are among the factors causing chronic sinusitis.² In a healthy person, the sinuses are sterile but may be colonized by some microorganisms such as viruses, fungi, and bacteria.³ In acute bacterial sinusitis, aerobic and anaerobic bacteria such as *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* are the dominant microorganisms.^{6,7} In chronic sinusitis, the percentage of microorganisms is reduced and replaced by *Staphylococcus* and anaerobic bacteria, including *Prevotella* and *Fusobacterium*.^{8,9} Fungal sinusitis is unusual, but the different involvements may be more complex in cases of chronic sinusitis. Strains of *Aspergillus* are the dominant organisms in fungal sinusitis, although *Mucorales* is also considered an important agent.^{8,9}

The aim of the present study was to determine the prevalence of aerobic bacteria *M. catarrhalis* and *S. pneumoniae* in clinical samples using culture and polymerase chain reaction (PCR) methods.

2. Methods

2.1. Clinical sample collection

In this study, samples were collected from 55 patients with chronic sinusitis undergoing surgery at Imam Khomeini Hospital in Ahwaz (southwest of Iran) between October 2012 and July 2013. The patients were selected according to physician diagnosis, and patients with acute sinusitis were excluded from the study. A questionnaire was completed for each patient including notation of some risk factors including use of antibiotics prior to the surgery, nasal polyps, dental manipulations, asthma, and allergies. Sinus samples were taken during the operation. After sterilization of the nasal vestibule and inferior meatus with ethanol, maxillary sinus samples were aspirated through a hollow needle using a sterile 18G Trocar needle, poured into phosphate buffered saline, and then sent to the microbiology laboratory of Ahwaz Medical School.

2.2. Bacteria isolation

Samples were cultured on blood agar, chocolate agar, and mannitol salt agar, and then incubated at 37°C in the presence of 5% CO₂ for 48 hours. For each culture, Gram staining, catalase, coagulase, oxidase, and DNase tests were performed.¹⁰ DNase, oxidase, and mannitol salt agar tests were performed for samples with suspected *M. catarrhalis* and *S. pneumoniae* infection, and isolates were stored for PCR analysis.

2.3. Molecular methods

DNA of each isolate was extracted using High Pure PCR Template Preparation kit (Roche Co., Monnheim, Germany). The concentrations of the components of PCR master mix to detect *M. catarrhalis* and *S. pneumoniae* are shown in Table 1, and the primers are shown in Table 2.

Normal saline was used as a negative control, and the chromosomal DNA of each bacterium (purchased from the Iran Pasteur Institute and after confirmation in biochemical tests) was used as a positive control.

The following program was used in amplification by DNA Taq polymerase: initial denaturation was performed at 95°C for 4 minutes, denaturation at 95°C for 45 seconds, annealing (for *S. pneumoniae* 66°C in 30 seconds, and for *M. catarrhalis* 55°C in 50 seconds), extension at 72°C for 40 seconds, and final extension at 72°C for 10 seconds.

The PCR products were detected by 1.5% agarose gel electrophoresis and after staining with ethidium bromide and observation under UV light.

2.4. Statistical analysis

Data were entered using the SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc. Descriptive statistics such as frequency and percentage were calculated for categorical data.

3. Results

In this study, 55 patients (48% male and 52% female) with chronic sinusitis undergoing surgery were examined. The following data were gleaned from the questionnaire.

Table 1

Concentration components of master mix for determination of *Moraxella catarrhalis* and *Streptococcus pneumoniae* in each reaction.

Master mix components	<i>M. catarrhalis</i> (μL)	<i>S. pneumoniae</i> (μL)
PCR buffer, 10×	5.0	5.0
dNTP, 10mM	1.25	1.0
Primer external sense, 10μM	2.0	1.0
Primer external antisense, 10μM	2.0	1.0
Taq DNA polymerase, 5U/μL	0.25	0.25
MgCl ₂ , 50mM	2.0	2.5
Template, 98 ng/μL	5.0	5.0
H ₂ O	32.5	34.25
Total	50	50

dNTP = deoxynucleotide triphosphate; PCR = polymerase chain reaction.

Table 2
Sequence of *Moraxella catarrhalis* and *Streptococcus pneumoniae* primers.

Bacteria	Name of primer	Primer sequences	Size of band (bp)	References
<i>S. pneumoniae</i>	STR1	5-GAT CCT CTA AAT GAT TCT CAG GTG G	80	Park et al ¹⁷
	DG74	3-ACT ATA GAA GAA AGG GAA GTT TCC A		
<i>M. catarrhalis</i>	MCA1M	5-TTG GCT TGT GCT AAA ATA TC-3	140	
	CAT2	3-GTC ATC GCT ATC ATT CAC CT-5		

Twelve (21.8%) of the patients had nasal polyps, 34 (61%) had used antibiotics prior to the surgery, 13 (23.6%) had dental infection, and 11 (20%) had asthma and allergies. The main objective was the isolation of *M. catarrhalis* and *S. pneumoniae*; in addition, we pursued microorganisms grown on the plate to obtain improved results. Thereafter, results of sinus culture showed positive bacterial culture in 17 cases (41.8%), which included 13 (23.6%) cases of *S. aureus* [10 (18.15%) asthmatic and 3 (5.45%) nonasthmatic], two (3.6%) cases of *Staphylococcus* coagulase negative [1 (1.8%) asthmatic, 1 (1.8%) nonasthmatic], one (1.8%) case of *Enterobacter* spp., and one (1.8%) case of *Rhizomucor*. We did not use PCR for these organisms, because it was not the aim of the present study. The two main bacteria of the study, *M. catarrhalis* and *S. pneumoniae*, were not isolated by the culture method.

The PCR method was used to detect *M. catarrhalis* and *S. pneumoniae*, which showed four (7.2%) cases of *S. pneumoniae* and two (3.6%) cases of *M. catarrhalis* (as shown in Figs. 1 and 2, respectively). The distribution of microorganisms is presented in Table 3.

4. Discussion

Paranasal inflammation of sinuses (sinusitis) is seen in all age groups and is mainly caused by viral infections of the respiratory system.³ Normally, the sinuses are sterile, but for many reasons, they may be colonized by some agents. Oral

infections such as dental inflammation, diabetes, and respiratory infections may have an underlying role in causing this disease.¹ The oral cavity may act as a reservoir for infection of the sinuses and the middle ear. Bacteria in the mouth may enter sinuses through the middle meatus or middle ear through a Eustachian tube and cause infection.⁸ Dental infections cause approximately 40% of chronic sinusitis cases. However, major resident nasal organisms such as *S. aureus* can also cause sinusitis.² Furthermore, sinus microflora is affected by antibiotic treatment prior to the operation.¹ The main pathogens associated with acute sinusitis include *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*. These pathogens also exist in chronic sinusitis, but at lower rates, and these are replaced by other microorganisms. In this study, we have undertaken to assess the frequency of *S. pneumoniae* and *M. catarrhalis* in patients with chronic sinusitis undergoing surgery in Ahwaz using the culture and PCR methods.

In a bacterial study by Karina et al⁹ in Brazil using the culture method, 40 bacterial strains were isolated from 68 otitis media patients, of which 10% were *S. pneumoniae*. In another study in California by Finegold et al¹⁰ on sinus

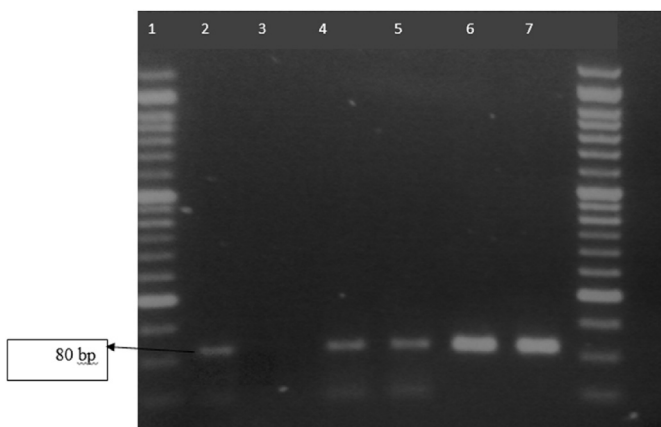


Fig. 1. Polymerase chain reaction amplification of *Streptococcus pneumoniae* on agarose gel electrophoresis. 1 = DNA ladder (50 bp); 2 = positive control; 3 = negative control; 4–7 = samples.

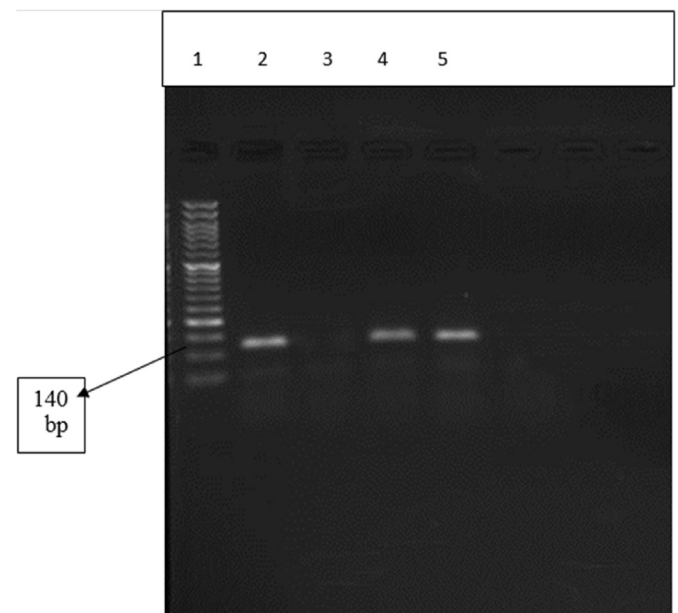


Fig. 2. Polymerase chain reaction amplification of *Moraxella catarrhalis* on agarose gel electrophoresis. 1 = DNA ladder (50 bp); 2 = positive control; 3 = negative control; 4, 5 = samples.

Table 3
Frequency of microorganisms isolated by culture and polymerase chain reaction (PCR) method.

Microorganism	N (%)
Culture	
<i>Staphylococcus aureus</i>	13 (23.6)
<i>Staphylococcus coagulase negative</i>	2 (3.6)
<i>Enterobacter</i> spp.	1 (1.8)
<i>Rhizomucor</i>	1 (1.8)
PCR	
<i>Streptococcus pneumoniae</i>	4 (7.2)
<i>Moraxella catarrhalis</i>	2 (3.6)

discharge using culture from 70 isolates involving patients with maxillary sinusitis, 15% were *S. pneumoniae*. In another study in Washington by Brook⁷ using culture on chronic sinusitis, from 33 bacterial isolates, 8% were *S. pneumoniae* and 2% were *M. catarrhalis*. However, in the present study, these two bacteria were not isolated in culture. Factors causing the difference in results are use of antibiotics prior to the operation over a specific period, normal flora in the sampling site, and sensitivity of the main bacteria. In a study conducted by Brook⁸ in the United States on sinuses using culture and PCR methods, 48 clinical samples were taken from patients with sinusitis; of this total, 39 were isolated, of which 10% were *S. pneumoniae*. In a study on chronic sinus infection by Brook et al¹¹ in the United States, 12% *S. pneumoniae* and 1% *M. catarrhalis* were isolated from clinical samples by determining the beta-lactamase activity.

The present study results showed 7.2% *S. pneumoniae* and 3.6% *M. catarrhalis*, using PCR method, which is in agreement with the results in the studies just cited. In many studies, including the present one, isolation of these two bacteria is widely different in culture and PCR methods. Factors affecting this difference including high sensitivity of bacteria, sampling method, and use of antibiotics prior to the surgery can lead to negative culture results. However, the effects of these factors are reduced in the molecular method and include both living and dead bacteria. These differences indicate the high sensitivity of molecular methods compared with culture.¹ Molecular methods do not differentiate between dead or living bacteria, and only DNA is important, which may be attributable to previous infections and not the present one, and can also affect the difference in results.

A study by Zurak et al¹² in Britain showed that bacterial colonization may affect neutrophilic and eosinophilic activities in atopic people with chronic sinusitis. Colonization by *S. aureus* and *Pseudomonas aeruginosa* increase granulocyte activity in patients with asthma and allergy. The effect of bacterial colonization on granulocytic activity depends specifically on the interaction between bacteria and the host antigens. Asthma, a host-dependent factor, causes severe sinus mucosal response to bacterial antigen and toxins.¹² In the present study, the dominant organism isolated from people with allergies was *S. aureus* (26%; based on completed questionnaires and the fact that most samples were from patients with allergies).

In a study on sinus discharge by Hashemi et al¹³ in Iran, 52 strains of bacteria were isolated from patients with chronic

resistant rhino sinusitis with or without polyps by culture, including *S. pneumoniae* (10%), *S. aureus* (12%), coagulase negative staphylococci (25%), *Enterobacter* (3%), *Klebsiella* (9%), and *Citrobacter* (2%). In another study by Nour et al¹⁴ in Iran involving 50 clinical samples from patients with chronic sinusitis, *S. aureus* (2%), coagulase negative staphylococci (16%), *K. pneumoniae* (8%), *Escherichia coli* (10%), *S. pneumoniae* (4%), *Enterobacter aeruginosa* (16%), and *Candida* (1%) were isolated. In addition, they compared nasal sinuses and nasopharynx cultures and found that nasopharynx culture was a reliable marker for sinus bacteriology.

In chronic sinusitis, enterobacteriaceae can also be considered a causing agent. In the present study, *Enterobacter* (1.8%) was isolated, which nearly agrees with the above-mentioned studies.⁷ Fungal agents can also cause sinus inflammation. Common fungi include *Aspergillus*, *Candida albicans*, and *Rhizomucor*. In the present study, *Rhizomucor* (1.8%) was isolated from discharges, which is consistent with several prior studies.⁹

In a study by Tayyar et al,¹⁵ the bacteriology of chronic maxillary sinusitis and normal maxillary sinuses was evaluated using culture and multiplex PCR. The most isolated bacteria were *S. aureus*, α -hemolytic streptococci, *S. pneumoniae*, *H. influenzae*, coagulase-negative staphylococci, and anaerobes. PCR was used to investigate *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *Alloiococcus otitidis* in the study and control groups. According to their result, *A. otitidis* may be one of the pathogens causing sinusitis.¹⁶ In another study, Kim et al¹⁶ compared PCR and fungal culture for the detection of fungi in patients with chronic sinusitis and normal controls. Their study revealed that PCR is a more sensitive method for fungus detection than fungus culture, both in patients with chronic sinusitis and in normal controls.¹⁷

Given the preceding discussion, nasal polyps are a risk factor for sinusitis. Nasal polyps form after inflammation of mucosal membrane, obstruct the sinuses, and disrupt proper discharge, which consequently cause microorganism growth. As expected, in the present study, 21.8% of patients with sinusitis had nasal polyps.

In conclusion, the frequency of *S. pneumoniae* and *M. catarrhalis* was assessed in samples from patients with chronic sinusitis who were seen at our hospital in Ahvaz, Iran. The results obtained showed that *S. aureus* and *S. pneumoniae* were the most common bacteria in these samples, followed by *M. catarrhalis* and *Enterobacter*, and—to a lesser extent—*Rhizomucor*, identified as a cause of infection. Most of the *S. aureus* strains were isolated from patients with allergic asthma, which was exacerbated by the release of superantigens and the body's reaction to them. Furthermore, nasal polyps, allergy, and dental infections can also be considered factors influencing this disease.

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