



Identification and Detection of Pathogenic Bacteria in Adenoid Tissue of Adenoidectomized Children: Emergence of *Staphylococcus aureus* as the Most Prevalent Pathogen

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Abstract

Background: Adenoids act as reservoirs for pathogenic bacteria and chronic adenoid infection leads to its hypertrophy.

Objectives: The present study aimed to determine the frequency of *Staphylococcus aureus*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae* in children by polymerase chain reaction (PCR) and culture and to determine the antimicrobial resistance patterns of the isolates.

Methods: In this cross-sectional study, 200 adenoid tissues were taken from children with adenoid hypertrophy in the operating room under general anesthesia conditions. The isolation and detection of bacteria were performed by culture and PCR methods. The antibiotic susceptibility patterns of the isolates were determined by the disc agar diffusion method according to the CLSI guidelines.

Results: *Staphylococcus aureus* was the most common bacterium (102; 51%) isolated by the culture method, followed by *S. pneumoniae* (44; 22%), *M. catarrhalis* (33; 16.5%), and *H. influenzae* (6; 3%). By multiplex PCR, the detection rate of microorganisms was as follows: *S. aureus* 40.5% (n = 81), *S. pneumoniae* 15.5% (n = 31), *M. catarrhalis* 11% (n = 22), and *H. influenzae* 4% (n = 8). *Haemophilus influenzae* strains were sensitive to all of the tested antibiotics. Seven *S. aureus* isolates were resistant to ceftioxin and identified as MRSA.

Conclusions: This study showed the importance of *S. aureus* in the etiology of adenoid hypertrophy as the most frequent isolate. It may be due to the long-term duration of adenoid hypertrophy in children that helps the colonization of *S. aureus* in adenoid tissue and leads to chronic infection.

Keywords: Adenoidectomy, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*

1. Background

Adenoid hypertrophy is a common childhood disease, with a 2 - 3% prevalence (1). The adenoid tissue as part of the Waldeyer ring belongs to the lymphatic system in the posterior nasopharynx (2). Adenoids and tonsils include the first-line defense against various microorganisms entering the body through the mouth or nose (3). The etiology of adenoid hypertrophy has not been fully understood although there is a relationship between adenoid enlargement and microbial agents, allergies, genetic predisposition, external stimuli such as smoking, and rarely neoplastic proliferation (4, 5). Adenoid hypertrophy can lead to the nasopharynx obstruction and cause symptoms including snoring, Rhinorrhea, nasal congestion, mouth breathing, halitosis, hyponasal speech, restless sleep, hypersomnolence, and enuresis. It also plays an important role in

chronic rhinosinusitis and middle ear infection with effusion (6). In most cases, the removal of a local reservoir of pathogenic microbiota such as adenoid and tonsils decreases the clinical signs and symptoms of affected patients (7).

The treatment of adenoid hypertrophy is determined based on the degree of airway obstruction and related morbidity although a nonsurgical approach is used in less serious cases (8). Identifying microbial pathogens has an important role in choosing appropriate antibiotics for the treatment of adenoid inflammation (9). Studies showed that *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* are major causes of otitis media isolated from adenoid tissue in patients with adenoid hypertrophy (10, 11). *Staphylococcus aureus* is another pathogen detected in the adenoid of 17.8% to 47.4% of patients with

adenoid hypertrophy (12-15). These pathogens have different mechanisms for long-term persistence in adenoid tissue and are strongly associated with adenoidal colonization in patients with recurrent otitis media, recurrent adenoiditis and tonsillitis, and obstructive adenoid hypertrophy (13). Although some studies from Iran reported various frequency of *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *S. aureus* in children with adenoid hypertrophy, there is no report from the southwest of Iran.

2. Objectives

Considering the adverse effects and complications of adenoid hypertrophy in children and the importance of these bacteria, the present study aimed to determine the frequency of *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *S. aureus* in children by PCR and culture and to determine the antimicrobial resistance pattern of the isolates.

3. Methods

3.1. Sample Collection and Isolation of Bacteria

In this cross-sectional study, a total of 200 children were diagnosed with adenoid hypertrophy and referred to the Department of Otolaryngology of Imam Sajjad Hospital, affiliated to Yasuj University of Medical Sciences, during January 2017 and February 2018. Children with immunological defects, anatomic abnormality, respiratory infection, antibiotic therapy two weeks before and at the time of the surgery were excluded. Adenoidectomy was performed in the operating room under general anesthesia conditions. The adenoid tissue was removed under an aseptic condition and placed into a sterile container to sent to a microbiology laboratory.

The adenoid tissue of each patient was homogenized with tissue grinder, and then inoculated in specific culture media for the primary isolation of bacteria. Mueller-Hinton agar with 5% sheep blood was used for the primary isolation of *S. pneumoniae* and *S. aureus*. For *S. pneumoniae*, plates were incubated at 37°C in the presence of CO₂ for 24 to 48 hours and then identified by conventional biochemical tests such as alpha hemolysis, catalase test, susceptibility to optochin (5 µg) and bile solubility. *Staphylococcus aureus* was identified with the catalase test, tube coagulase, DNase test, and mannitol fermentation (16) and finally confirmed by the detection of the *nucA* gene (17).

Chocolate agar containing vancomycin (5 mg/mL), clindamycin (1 mg/mL), and bacitracin (300 µg/mL) was used for the primary isolation of *H. influenzae* in candle jar with 5% carbon dioxide. Biochemical tests such as catalase,

oxidase, requirements for X and V growth factors, and fermentation of sucrose and glucose were used for the identification of this bacterium (18). Chocolate agar with vancomycin (5 µg/mL), clindamycin (1 µg/mL), bacitracin (300 µg/mL), and acetazolamide was used for *M. catarrhalis* (19). Before adenoidectomy, past medical histories and demographic data of patients were collected from their medical records. Besides, clinical data including snoring, mouth breathing, recurrent pharyngitis, and ear infections were recorded.

3.2. Multiplex Polymerase Chain Reaction

The DNA was extracted from adenoid tissue according to Bartlett et al. guidelines (20). Multiplex polymerase chain reaction (PCR) was used for the detection of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* according to the 16SrRNA gene-specific primer designed before by Hendolin et al. (21). The primer sequences used in this study are shown in Table 1. The PCR assay was carried out in a 25 µL total volume containing 12.5 µL master mix (Amplicon, Denmark), 25 pmol of each primer, and 5 µL of template DNA. The PCR conditions were as follows: initial denaturing at 94°C for 5 minutes, followed by 35 cycles of denaturing at 94°C for 30 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 40 seconds. The final extension continued at 72°C for 5 minutes using a thermocycler (Bio-Rad, T100, USA). The PCR products were separated by electrophoresis in the 1.5% agarose gel stained with DNA safe stain, and then visualized under UV light using the gel documentation system (Major Science, Taiwan).

3.3. Antimicrobial Sensitivity

The antibiotic susceptibility patterns of the isolates were determined by the disc agar diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (22). Mueller-Hinton agar with 5% sheep blood was used for *S. pneumoniae*, chocolate agar with Mueller-Hinton agar base was used for *H. influenzae* and *M. catarrhalis*, and Mueller-Hinton agar medium was used for *S. aureus*. We used *S. aureus* ATCC 25923 as a quality control strain. The antibiotics used in this study are shown in Table 2.

4. Results

Totally 200 children with adenoid hypertrophy were included in this study. The patients' age was between 1 and 14 years with a mean age of seven years. Of these, 119 children were male and 81 children were female. There were 124 patients under seven-years-old. Of 200 patients, 186, 181, 192, and 65 had mouth breathing, snoring, recurrent

Table 1. Primer Sequences Used for Detection of *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*

| Primer Name | Primer Sequence (5' to 3') | Product Size (bp) |
|---------------------------------|---|-------------------|
| Common lower primer | 5'-CTA CGC ATT TCA CCG CTA CAC-3' | |
| <i>Alloicoccus otitidis</i> | 5'-GGG GAA GAA CAC GGA TAG GA-3' | 264 |
| <i>Haemophilus influenzae</i> | 5'-CGT ATT ATC GGA AGA TGA AAG TGC-3' | 525 |
| <i>Moraxella catarrhalis</i> | 5'-CCC ATA AGC CCT GAC GTT AC-3' | 237 |
| <i>Streptococcus pneumoniae</i> | 5'-AAG GTG CAC TTG CAT CAC TAC C-3' | 484 |
| <i>Staphylococcus aureus</i> | Forward: 5'- CTG GCA TAT GTA TGG CAA TTG TT-3' | 670 |
| | Reverse: 5'- TAT TGA CCT GAA TCA GCG TTG TCT-3' | |

Table 2. Clinical Characteristics of Study Subjects (n = 200)

| Clinical Characteristics | Values |
|--------------------------|--------|
| Age | |
| ≤ 7 | 124 |
| > 7 | 76 |
| Mean | 7 |
| Gender | |
| Male | 119 |
| Female | 81 |
| Mouth breathing | 186 |
| Snoring | 181 |
| Recurrent pharyngitis | 192 |
| Recurrent otitis | 65 |

pharyngitis, and recurrent otitis, respectively. The clinical characteristics of patients are shown in [Table 3](#).

Using the culture method, *S. aureus* was the most prevalent pathogen isolated from 102 (51%) patients, followed by *S. pneumoniae* in 44 (22%), *M. catarrhalis* in 33 (16.5%), and *H. influenzae* in six (3%). By multiplex-PCR, the detection rates of the microorganisms were as follows: *S. aureus* 40.5% (n = 81), *S. pneumoniae* 15.5% (n = 31), *M. catarrhalis* 11% (n = 22), and *H. influenzae* 4% (n = 8). Nine different patterns of the isolated bacteria were observed among patients, of which *S. aureus* alone (n = 57 cases), *S. aureus* + *S. pneumoniae* (n = 21), and *S. aureus* + *M. catarrhalis* (n = 17) were the most prevalent patterns. These data are summarized in [Table 4](#).

There were no statistically significant sex-related differences in the isolated bacteria using both culture and PCR methods. Also, according to the clinical findings, there was no significant relationship between the presence of each bacterium and mouth breathing, snoring, recurrent pharyngitis, and recurrent otitis. All of the 102 *S. aureus* isolates were susceptible to gentamicin, trimethoprim-sulfamethoxazole, amikacin, rifampin, and linezolid antibiotics. Also, all of the *S. aureus* isolates were resistant to penicillin. Totally seven isolates were resistant to cefoxitin

and identified as MRSA isolates. All of the *S. pneumoniae* isolates had most sensitivity to linezolid, ciprofloxacin, levofloxacin, and vancomycin antibiotics (100%) and a high percentage of the isolates were resistant to cotrimoxazole (95.5%), ceftriaxone (88%), and clindamycin (38.6%). All the isolates of *H. influenzae* were sensitive to ciprofloxacin, levofloxacin, erythromycin, cotrimoxazole, and rifampicin. A high rate of sensitivity was observed among *M. catarrhalis* against ciprofloxacin (100%), levofloxacin (100%), and erythromycin (91%), although 94% of the isolates were resistant to cotrimoxazole.

5. Discussion

In the present study, *S. aureus* was the most prevalent isolate identified in 51% of children with adenoid hypertrophy, which was more than earlier reports from other countries: Brazil (2.7%), Turkey (22.2%), Poland (34%), India (34%), The United States (33.3% and 8.8%), Hungary (15%), and Iraq (23.6%) (7, 13, 23-28). This study showed the importance of *S. aureus* in the etiology of adenoid hypertrophy. The variation in the frequency of this bacterium among different countries may be due to the different health care programs in various geographical locations. *Staphylococcus aureus* has many factors, including the presence of various compounds in the cell wall, such as teichoic acid and microbial surface component recognizing adhesive matrix molecules (MSCRAMM), which cause the long-term colonization of this bacterium in body tissues.

Other mechanisms, such as intracellular survival and localization of bacteria within the biofilm, protect bacteria from the effects of host immunity and antibiotics and increase bacterial survival and colonization in the adenoid tissue (7). Besides, the long-term duration of adenoid hypertrophy can help the colonization of *S. aureus* in adenoid tissue and lead to chronic infection. In our region, it seems that children with adenoid hypertrophy referred to otorhinolaryngologists at the end stage of the disease and most adenoidectomy procedures were performed with a delay during summer vacation. This lateness can cause the colonization of *S. aureus* and the gradual emergence of this bac-

Table 3. Antibiotic Resistance Patterns of Bacteria Isolated from Adenoidectomized Children^a

| Antibiotics | <i>Staphylococcus aureus</i> (n = 102) | <i>Streptococcus pneumoniae</i> (n = 44) | <i>Moraxella catarrhalis</i> (n = 33) | <i>Haemophilus influenzae</i> (n = 6) |
|---------------|--|--|---------------------------------------|---------------------------------------|
| Penicillin | 102 (100) | 16 (36.4) | - | - |
| Vancomycin | - | 0 (0) | - | - |
| Linezolid | 0 (0) | 0 (0) | - | - |
| Erythromycin | - | - | 3 (9) | 0 (0) |
| Clindamycin | 5 (4.9) | 17 (38.6) | - | - |
| Cotrimoxazole | - | 42 (95.5) | 31 (94) | 0 (0) |
| Ciprofloxacin | 4 (3.9) | 2 (0.5) | 0 (0) | 0 (0) |
| Levofloxacin | - | 0 (0) | 0 (0) | 0 (0) |
| Rifampicin | - | - | 12 (36.4) | 0 (0) |
| Ceftriaxone | - | 8 (18.21) | - | - |
| Gentamicin | 0 (0) | - | - | - |
| SXT | 1 (1) | - | - | - |
| Amikacin | 0 (0) | - | - | - |
| Tetracycline | 32 (30.39) | - | - | - |

^aValues are expressed as No. (%).

Table 4. Isolation and Detection of Bacterial Pathogens by Polymerase Chain Reaction and Culture in 200 Children with Adenoid Hypertrophy^a

| Culture/PCR Bacteria | Culture+ PCR+ | Culture- PCR+ | Culture+ PCR- | Total |
|---------------------------------|---------------|---------------|---------------|-----------|
| <i>Staphylococcus aureus</i> | 78 (39) | 2 (1) | 22 (11) | 102 (51) |
| <i>Streptococcus pneumoniae</i> | 31 (15.4) | 0 (0) | 13 (6.5) | 44 (22) |
| <i>Moraxella catarrhalis</i> | 17 (8.4) | 5 (2.5) | 16 (8) | 38 (18.9) |
| <i>Haemophilus influenzae</i> | 6 (3) | 2 (1) | 0 (0) | 8 (4) |

^aValues are expressed as No. (%).

terium as a prevalent organism. Besides, 6.8% of *S. aureus* isolates were identified as MRSA. Lin et al. reported that MRSA adenoiditis may be associated with adenoid hyperplasia and biofilm formation (29).

Streptococcus pneumoniae was the second prevalent organism in this study isolated from 22% of patients, which is less than the reported values from Turkey (60.9%), Poland (64%), United States (50%), and India (39%) but higher than previous reports from the United States (16.6%) and Hungary (15%) (11, 23-27). One of the reasons for differences in the frequency of *S. pneumoniae* among different studies is related to the use of pneumococcal vaccines in children. The isolation rate of *M. catarrhalis* in this study was 16.5%, which is lower than that reported in similar studies from Turkey (19.5%), Poland (18.5%), and United States (38.3% and 8.3%) but higher than a report from India (13%) (11, 23-26). In contrast to the results of this study indicating *H. influenzae* in only 3% of patients, many studies reported that *H. influenzae* was isolated in higher percentages in Poland, United States (two studies), Hungary, and Iraq with isolation rates of 73%, 60%, 66.7%, 50%, and 11.6%, respectively (23, 25-28). Local health care programs, diversity in geographical regions, and national vaccination programs for chil-

dren can affect the prevalence of bacteria in the respiratory tract and adenoid tissue.

Few studies have investigated the role of bacteria in adenoid tissue by the PCR method. The detection rates for *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae* in this study were 15.5%, 11%, and 4%, respectively, which were much lower than the rates in a study from Brazil (58.5%, 2%, 37%, and 49%, respectively). Also, *S. aureus* was detected as 40.5% in this study, which is much higher than that in Brazil (5.8%) (7). Antimicrobial susceptibility testing of bacteria in the present study showed that *S. aureus*, *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae* had the highest sensitivity to fluoroquinolones (ciprofloxacin and levofloxacin). This may be due to the limited use of this group of antibiotics in children because of their potential arthropathy in juvenile animals (30). In total, *S. pneumoniae* and *M. catarrhalis* had high rates of resistance to cotrimoxazole. While all of the *H. influenzae* isolates were sensitive to all of the tested antibiotics, some strains of *S. pneumoniae* were resistant to clindamycin and penicillin and *M. catarrhalis* strains to rifampicin. *Staphylococcus aureus* strains isolated in this study were also highly susceptible to gentamicin, trimethoprim-sulfamethoxazole, clindamycin, amikacin,

rifampin, and linezolid, while all of the isolates were resistant to penicillin and 31% to tetracycline. Because of the diversity of bacterial pathogens in adenoid tissue and predominance of *S. aureus* in this study, antibiotic treatment needs bacterial evaluation, culture, and antibiograms.

5.1. Conclusions

In conclusion, in addition to the three most common pathogens of adenoid hypertrophy (*S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*), *S. aureus* was the most important pathogenic bacterium in the etiology of adenoid hypertrophy. The dominance of *S. aureus* may be due to the long-term duration of adenoid hypertrophy that helps the colonization of *S. aureus* in adenoid tissue and leads to chronic infection.

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Footnotes

Authors' Contribution: Conception and design of the study: Seyed Sajjad Khoramrooz and Gholamabas Sabz; acquisition of data and laboratory techniques: Sedighe Moradi, Marzieh Taheripoor, and Marjan Salehi; analysis and interpretation of data: Seyed Sajjad Khoramrooz, and Asghar Sharifi; drafting the article: Seyed Sajjad Khoramrooz, Gholamabas Sabz, and Sedighe Moradi; final approval of the version to be submitted: Seyed Sajjad Khoramrooz and Mohsen Naghmachi.

Conflict of Interests: The authors declare that there is no conflict of interest regarding the publication of this article.

Ethical Approval: This study was approved by the Yasuj University of Medical Sciences Ethics Committee (Ethical approval ID: IR.YUMS.REC.1396.69).

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Patient Consent: Before specimen collection, written informed consent was obtained from the parents of each individual.

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