The Laryngoscope

Microbiology of Acute Maxillary Sinusitis in Children

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Objectives/Hypothesis: Acute rhinosinusitis is a frequent common cold-related complication in children. Despite the need for appropriate treatment, its underlying microbiology remains unclear. This study aimed to investigate the microbiology of acute rhinosinusitis in children.

Study Design: Prospective non controlled study.

Methods: Thirty-one pediatric acute maxillary sinusitis patients with severe symptoms were assessed. The subjects were 17 males and 14 females aged 5 to 14 years (mean age, 9.1 years). Maxillary sinus aspirates were collected and cultured, with subsequent viral and bacterial polymerase chain reaction (PCR) analysis. Bacteria were analyzed using culturing and PCR, and viruses were analyzed using PCR. The PCR kits used identify 18 types of respiratory viruses and 13 types of bacteria.

Results: At least one pathogen was detected in 30 of 31 aspirates (97%) using PCR, and none of the aspirates contained respiratory viruses alone. Ten aspirates (32%) contained both viruses and bacteria. The most common viruses detected were rhinovirus (13%) and influenza virus (10%). The most common bacteria were *Haemophilus influenzae* (45%), *Streptococcus pneumoniae* (32%), *Moraxella catarrhalis* (16%), and *Chlamydophila pneumoniae* (13%). Bacteria were found in 21 of 31 cases (68%) via bacterial culturing. Culturing revealed that *H influenzae* was the most common pathogen (42%).

Conclusions: In pediatric acute maxillary sinusitis, respiratory bacteria were detected in 65% of the sinus aspirates and both bacteria and viruses in 32%. The most common viruses were rhinovirus and influenza virus, and the most common bacteria were *H influenzae* and *S pneumoniae*. Viral and bacterial PCR is useful for accurately investigating the microbiology in pediatric sinusitis.

Key Words: Acute maxillary sinusitis, real-time PCR, respiratory viruses, bacteria, children.

Level of Evidence: 3

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INTRODUCTION

Upper respiratory tract illnesses are common during childhood and require frequent medical care. Acute rhinosinusitis frequently occurs as a complication of acute viral upper respiratory infection. Children with acute rhinosinusitis constitute approximately 6.5% to 13% of the patients with upper respiratory tract illnesses,[1–3] and some acute rhinosinusitis patients need medical care. Although acute rhinosinusitis is a common pediatric disease, its etiology remains unknown. A few studies have reported the presence of viral and bacterial pathogens through microbiological analysis of sinus aspirates obtained from children with acute sinusitis.[4–6] However, no study has comprehensively assessed viral and bacterial pathogens through polymerase chain reaction (PCR) using sinus aspirates obtained from children with acute sinusitis. To the best of our knowledge, this is the first study to investigate the microbiology of acute pediatric rhinosinusitis through molecular biological analysis.

MATERIALS AND METHODS

This cross-sectional study included 31 patients (17 males and 14 females aged 5–14 years (mean age, 9.1 years)) diagnosed with acute maxillary sinusitis from July 2017 to July 2019 at Matsubara ENT Clinic, 100 Ikedacho, Seki City, Gifu, Japan (all patients were diagnosed and treated by S. Matsubara). This study was approved by the Kanagawa Medical Practitioners Association (Yokohama, Japan, No. 16005). The inclusion criteria were as follows: acute maxillary sinusitis patients with prolonged severe symptoms (persistent buccal pain, massive purulent rhinorrhea, continuous low-grade fever, and/or productive cough) or no clinical improvement after antibiotics treatment (with antibiotics, nasal wash, and/or a nebulizer). In addition to the clinical symptoms, availability of sinus radiographs and endoscopic findings were prerequisites for inclusion in the study. We enrolled patients with sinus radiographs showing complete opacification or more than 50% air-fluid level in the maxillary sinus, and those with endoscopic findings showing middle meatus blockage and impaired drainage with sinusitis amelioration. In patients with these clinical symptoms, sinus puncture was indicated, based on their radiographic and endoscopic findings. Written informed consent was obtained from the parents of all

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children and informed assent was obtained from children over 7 years of age before enrollment.

Maxillary sinus aspirates were obtained under local anesthesia. The nose was cleaned and anesthetized with a 10% cocaine hydrochloride cotton swab for 5 min and subsequently with a 4% lidocaine with adrenaline 1:100000 tampon gauze for 5 min on the inferior nasal meatus. Thereafter, the inferior nasal meatus was cleaned and aspirated through the Schmidt method. All aspirates were cultured and subjected to multiplex viral and bacterial PCR.

For bacterial cultures, all samples were transported with BD culture swabs (Beckton Dickinson, Sparks, Maryland) to Miroku Medical Laboratory (Saku City, Nagano, Japan). Samples were plated on sheep blood agar and chocolate agar and incubated at 35°C for 24 hr in an atmosphere containing 5% CO_2. Pathogenic bacteria were identified using standard methods.

For viral and bacterial PCR, the maxillary aspirate was collected in transport media for viruses (Unitranz-RT Transport Medium, Puritan Medical Products, Guilford, Maine) and bacteria (Opti-Swab Liquid Amies Collection and Transport System, Puritan medical products). All media were transported at −20°C to Sawada Eye and Ear Clinic in Kochi City, Japan. All viral and bacterial PCRs were performed at the Sawada Eye and Ear Clinic the same day the samples were received. Nucleic acids were extracted from 200 μL aliquots of sinus aspirate samples using the QIAamp MinElute Virus spin kit and DNA mini kit (QIAGEN, Hilden, Germany) and assessed using the FTD respiratory pathogens 33 (Fast Track Diagnostics, Sliema, Malta) method with 10 μL of extract according to the manufacturer's instructions. The respiratory pathogens 33 kit encompasses 18 viruses, including respiratory syncytial virus (RSV), parainfluenza virus type 1 (PIV1), PIV2, PIV3, PIV4, coronavirus NL63 (hCoV-NL63), hCoV-229E, hCoV-OC43, hCov-HKU1, human metapneumovirus (hMPV), influenza virus type A, type B, and type C, adenovirus (AdV), human bocavirus (HBoV), enterovirus (EV), human parechovirus (hPeV), and rhinovirus, and 12 bacteria including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Haemophilus influenzae type B* (HiB), *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Legionella* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella* spp., *Pneumocystis jirovecii*, and *Bordetella pertussis*. Each PCR was performed using the CFX96 thermal cycler (Bio-Rad, Hercules, California). Multiplex real-time PCR conditions for the FTD kit were as follows: 42°C for 15 min, 94°C for 3 min, and 40 cycles at 94°C for 8 s and 60°C for 34 s. The sensitivity of this kit was 10^2 to 10^3 copies/mL.[7]

We used another bacterial PCR kit, the Cycleave PCR kit (Takara Bio, Shiga, Japan; catalog number CY214), to confirm the results of the FTD kit because the FTD kit does not include *Streptococcus pyogenes*. This kit can detect *S pneumoniae*, *H influenzae*, *M pneumoniae*, *C pneumoniae*, *Legionella pneumophila*, and *S pyogenes*, and it has a sensitivity of ∼10 colony-forming units (cfu) per well.[8,9] PCR was performed as previously described.[10]

RESULTS

Patient's Clinical Characteristics

All 31 patients had a history of acute respiratory tract illness with symptoms initially occurring at 5 to 20 days (mean, 11.6 days) before sinus aspiration (Table I). Eighteen of the 31 (58%) patients presented bilateral radiographic findings in the maxillary sinuses, and 13 (42%) patients had unilateral findings. Twelve of the 31 patients (39%) had prolonged fever, 5 (16%) had buccal pain or headaches, and 18 (58%) had a productive cough. Twenty of the 31 patients (65%) were unresponsive to antibiotic treatment (mainly amoxicillin). Seventeen of the 31 patients (55%) received a pneumococcal conjugate vaccine (PCV).

Microbiological Analysis Through Real-Time PCR

All the maxillary sinus aspirate samples obtained from 31 pediatric acute maxillary sinusitis patients were examined through viral and bacterial PCR. At least one microorganism was detected in the maxillary sinus aspirates from 30 patients (97%). Viral nucleic acids were detected in maxillary sinus aspirates in 10 patients (32%) (Fig. 1A). Furthermore, pathogenic bacteria were detected in all samples wherein viral nucleic acids were detected; none of the samples contained only viruses. The most common virus detected in the sinus aspirates was rhinovirus in four patients. Influenza virus was detected in three patients (type A, one case; type B, two cases), RSV in one patient, hBoV in one patient, and PIV3 in one patient (Table II). Bacterial PCR revealed the presence of 39 bacteria (Fig. 1B), among which *H influenzae* was the most common bacterium in 14 patients (36%), *S pneumoniae* in 10 patients (26%), *M catarrhalis* in 5 patients (13%), and *C pneumoniae* in 4 patients (10%); the age distribution of these patients is shown in Fig. 2. In patients aged ≥11 years, *S pneumoniae*, *H influenzae*, and *M catarrhalis* were detected. In patients ≤10 years, although three bacteria, *S pneumoniae*, *H influenzae*, and *M catarrhalis*, were found, various other bacteria, *C*

TABLE I.
Clinical Characteristics of Pediatric Patients With Acute Maxillary Sinusitis.

Variable	Value
Mean age	9.1 y (5–14 y)
Male	17/31 (55%)
Bilateral	18/31 (58%)
Days from onset to aspiration	11.6 d (5–20 d)
Prolonged fever	12/31 (39%)
Buccal pain/headache	5/31 (16%)
Productive cough	18/31 (58%)
Antibiotic pretreatment	20/31 (65%)
Pneumococcal vaccine (PCV)	17/31 (55%)

The table shows the clinical characteristics of 31 pediatric acute sinusitis patients who had severe or prolonged symptoms.

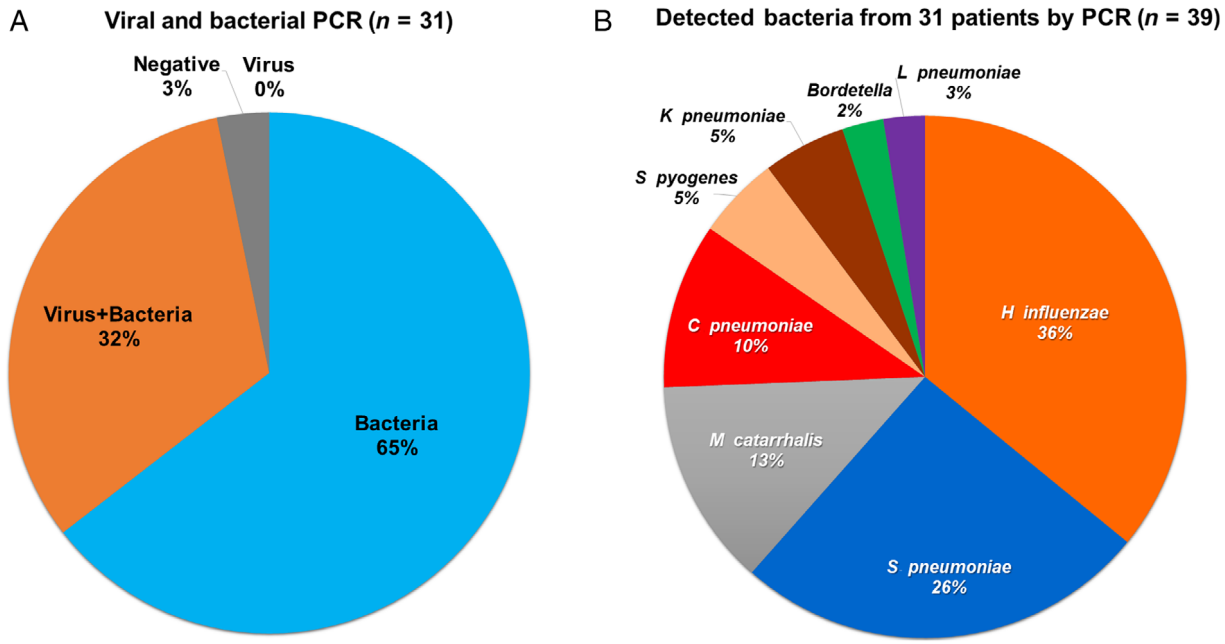

Fig 1. Proportions of microorganisms detected in the sinus aspirates of pediatric patients with acute maxillary sinusitis (A). The distribution of 39 bacteria detected through bacterial PCR from 31 patients (B).

pneumoniae, *S pyogenes*, and *L pneumophila*, were also detected. *C pneumoniae* was detected in children aged 8 to 10 years.

Comparison Between Bacterial Culture and PCR in Sinus Aspiration

We compared the results of bacterial culturing and PCR analysis of sinus aspirate samples in all the pediatric maxillary sinusitis patients (Fig. 3). At least one bacterial pathogen was detected on bacterial culturing in 21 patients (68%) and through bacterial PCR in 30 patients (97%). Bacterial culturing revealed that *H influenzae* was the most common pathogen and was detected in 13 patients (42%), including *H influenzae* alone in 12 patients and *H influenzae*, *S pneumoniae*, and *M catarrhalis* in 1 patient. Bacterial PCR revealed that *H influenzae* was the most common pathogen and was detected in 15 patients (48%), including *H influenzae* alone in 10 patients and *H influenzae* with other bacteria in 5 patients. *C pneumoniae* was detected in the sinus aspirates of 4 patients (3 with only *C pneumoniae*, 1 with *C pneumoniae* and *H influenzae*) using PCR. Bacterial PCR detected more bacteria than bacterial culturing did, including *C pneumoniae*.

The Effect of Pneumococcal Vaccine and Antibiotic Pretreatment on the Microbial Content

In the PCV vaccinated group (17 cases), the microbial content was as follows: *S pneumoniae*, 5 (36%); *H influenzae*, 8 (43%), whereas in the PCV unvaccinated group (14 cases) the microbial content was as follows: *S pneumoniae*, 5 (29%); *H influenzae*, 6 (47%). The rate of *S pneumoniae* in the PCV unvaccinated group was not significantly higher than that in the PCV vaccinated group (P = .71, chi-square test). In the antibiotic pretreatment group (20 cases), the microbial content was as follows: *S pneumoniae*, 6 (30%); *H influenzae*, 9 (45%). In the antibiotic non-pretreatment group (11 cases), the microbial content was: *S pneumoniae*, 4 (36%); *H influenzae*, 5 (45%). The detection rates of *S pneumoniae* (P = .72, chi-square test) and *H influenza* (P = .98, chi-square test) in the antibiotic pretreatment group were not significantly different from that in the nontreated group (Table III).

DISCUSSION

Few studies have performed bacteriological analyses of sinus aspirates from pediatric acute sinusitis patients.[4,5] Maxillary sinus bacteriology was only performed by Wald et al.,[4,5] and there have been no reports of bacteriology with combined bacterial culture and molecular methods. In 1981, Wald et al.[4] reported the bacterial composition of sinus aspirates in 30 children (47 sinuses) aged 1 to 16 years, wherein bacterial growth occurred in 34 of 47 sinus aspirate samples (72%), including *S pneumoniae* in 17 (36%), *H influenzae* in 11 (23%), and *M catarrhalis* in 9 (19%). In this study, bacterial cultures revealed at least one bacterium in 21/31 (68%) patients, including *H influenzae* in 13/31 (42%) patients and *S pneumoniae* in 6/31 (19%) patients. The total detection rate through bacterial culture was 68%, similar to that reported previously (68–72%).[4–6] The detection rate of *S pneumoniae* was lesser than that reported previously owing to the effectiveness of the PCV and previous antibiotic treatment because antibiotic treatment, especially amoxicillin, is effective for *S pneumoniae* while *H influenzae* is frequently resistant to amoxicillin. PCV and antibiotic pretreatment

TABLE II.
Viral and Bacterial PCR Analysis of Samples Obtained From Pediatric Patients With Acute Maxillary Sinusitis.

	Negative for Virus	Flu A	Flu B	RSV	hMPV	hBoV	AdV	RV	EV	HPeV	CoV NL63	CoV 229E	CoV OC43	CoV HKU1	PIV 1	PIV 2	PIV 3	PIV 4	Total
Streptococcus pneumoniae	**5**	**1**																	**6**
Haemophilus influenzae	**5**		**1**	**1**				**2**											**9**
M catarrhalis			**1**																**1**
S pneumoniae + H influenzae																	**1**		**1**
S pneumoniae + Moraxella catarrhalis	**1**							**1**											**2**
H influenzae + M catarrhalis	**1**																		**1**
S pneumoniae + H influenzae + M catarrhalis	**1**																		**1**
Chlamydia pneumoniae	**3**																		**3**
C pneumoniae + H influenzae	**1**																		**1**
Streptococcus pyogenes								**1**											**1**
S pyogenes + Legionella pneumophila	**1**																		**1**
L pneumophila																			**0**
Klebsiella pneumoniae	**1**																		**1**
K pneumoniae + H influenzae						**1**													**1**
Bordetella spp.	**1**																		**1**
M pneumoniae																			**0**
Salmonella spp.																			**0**
Pneumocystis jirovecci																			**0**
Staphylococcus aureus																			**0**
Negative for bacteria	**1**																		**1**
Total	21	1	2	1	0	1	0	4	0	0	0	0	0	0	0	0	1	0	31

AdV = adenovirus, CoV = coronavirus, EV = enterovirus, flu = influenza virus, hBoV = human bocavirus, hMPV = human metapneumovirus, PIV = parainfluenza virus, RSV = respiratory syncytial virus, RV = rhinovirus.

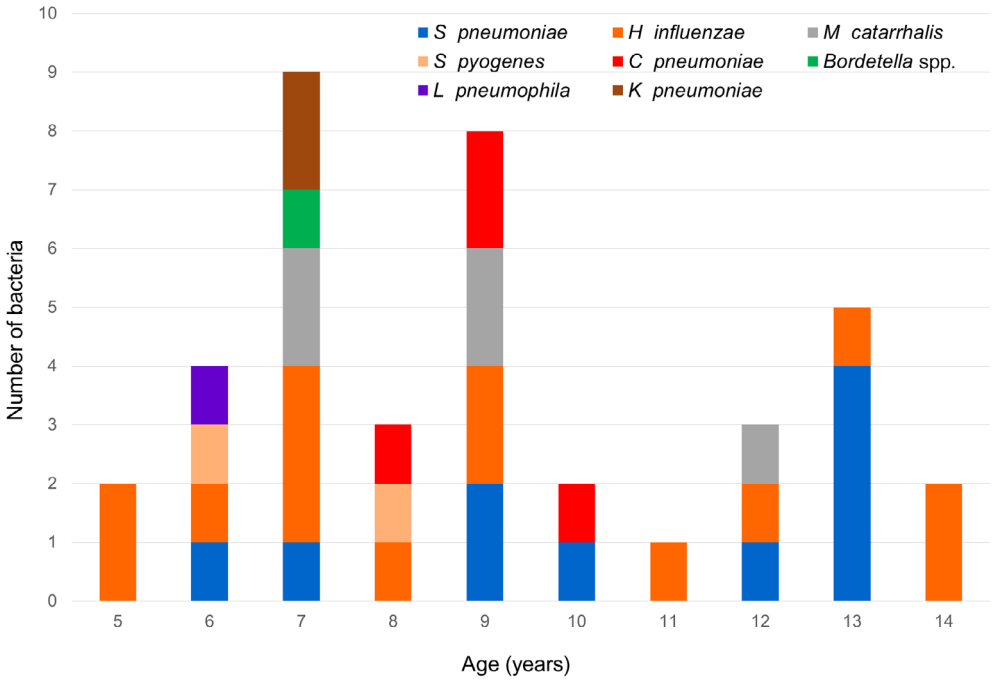

Fig 2. Age distribution of patients harboring 39 bacteria detected through bacterial PCR from 31 maxillary sinusitis patients.

did not influence the microbiology in acute sinusitis. PCV decreases pneumococcal infection, but the *S pneumoniae* recently detected in pediatric respiratory infections are often non-serotypes. In Japan, non-PCV serotypes were detected in 71% of pediatric pneumococcal infections in 2014.[11] We speculate that the herd immunity of PCV may suppress the

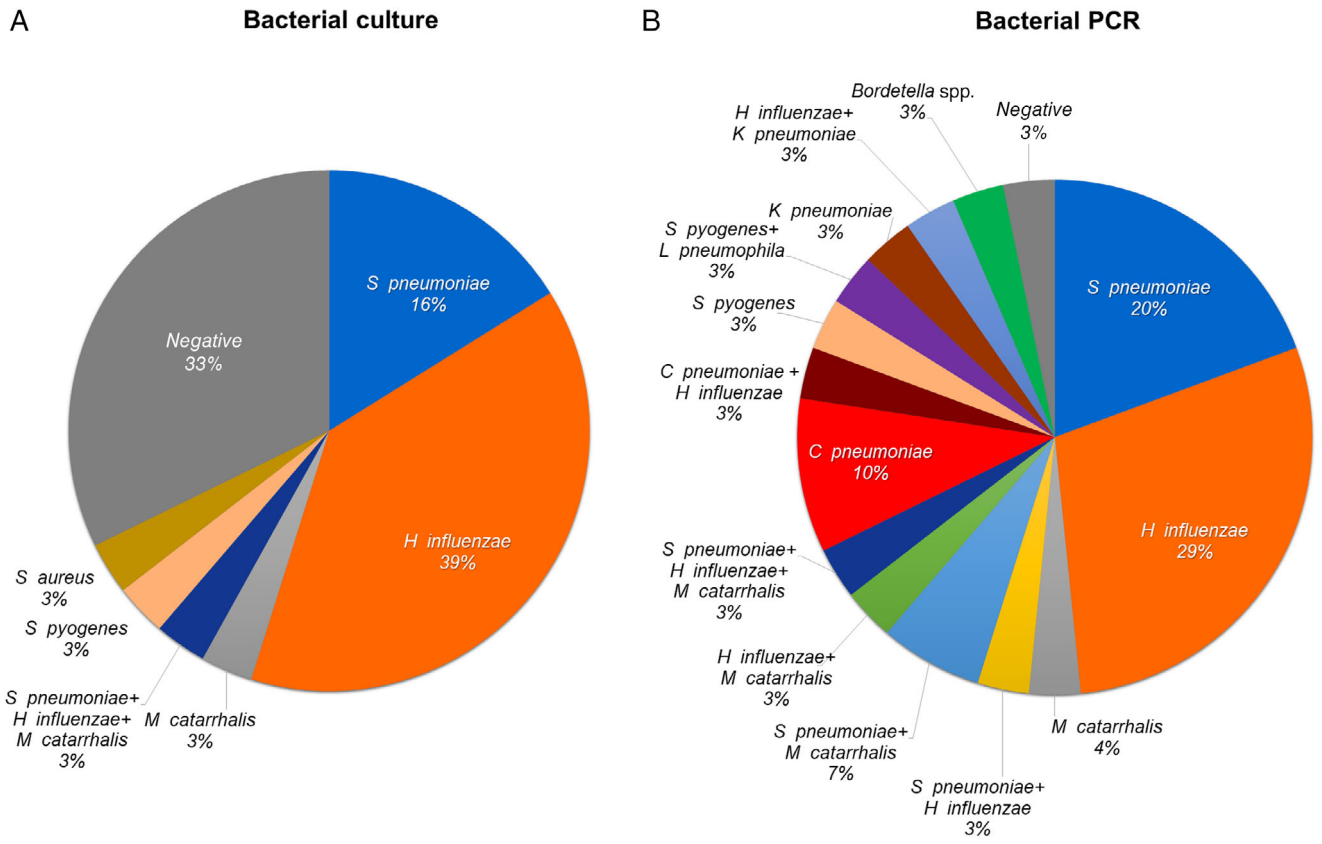

Fig 3. The distribution of isolated bacteria through bacterial culture (A) and bacterial PCR (B).

TABLE III.
Effect of Pneumococcal Vaccine and Antibiotic Pretreatment on the Microbiology in Pediatric Acute Sinusitis.

	PCV Vaccinated (n = 17)	PCV Unvaccinated (n = 14)	Antibiotic Pretreatment (+) (n = 20)	Antibiotic Pretreatment (–) (n = 11)
Streptococcus pneumoniae	5 (29%)	5 (36%)	6 (30%)	4 (36%)
Haemophilus influenzae	8 (47%)	6 (43%)	9 (45%)	5 (45%)
Moraxella catarrhalis	3 (18%)	1 (7%)	3 (15%)	2 (18%)
Chlamydophila pneumoniae	2 (12%)	2 (14%)	2 (10%)	2 (18%)
Streptococcus pyogenes	2 (12%)	0 (0%)	1 (5%)	1 (9%)
Klebsiella pneumoniae	1 (6%)	1 (7%)	2 (10%)	0 (0%)
Bordetella	0 (0%)	1 (7%)	1 (5%)	0 (0%)
Legionella pneumoniae	1 (6%)	0 (0%)	1 (5%)	0 (0%)

pneumococcal infection in the unvaccinated groups and that the non-serotype pneumococcal infections might occur at the same frequency in the vaccinated and unvaccinated groups. In 1992, Wald et al.[6] attempted to detect respiratory viruses through viral culture; however, adenovirus and parainfluenza virus were detected in only 1 of the 45 patients. Thus far, no study has reported PCR-based detection of respiratory viruses and bacteria from the sinus aspirates of pediatric sinusitis patients; this study, to our knowledge, is the first to elucidate the microbiology of acute pediatric maxillary sinusitis through molecular methods.

In this study, at least one microorganism was detected in 30 of 31 (97%) patients using viral and bacterial PCR. In 10 (32%) patients, one respiratory virus was detected, and more than one virus was not detected in any patient. Among these detected viruses, rhinovirus (13%) was the most common, followed by the influenza virus type A/B (10%), consistent with the findings of Marom et al.[2] that rhinovirus was most commonly detected, followed by the influenza virus, in viral PCR from nasopharyngeal swabs of children with acute rhinosinusitis. These data concur with our results, even though Marom et al. obtained their samples from nasopharyngeal swabs and not from sinus aspirates. In this study, all samples contained both respiratory viruses and bacteria, and none of them contained only respiratory viruses. It remains unclear whether viral sinusitis is predominant in adults or children. Several studies have reported the occurrence of viral pathogens in adult acute sinusitis. Pitkäranta et al.[12] reported the presence of rhinovirus or coronavirus in the sinus aspirates of 8 of 20 (40%) adult sinusitis patients. Puhakka et al.[13] reported viral infections in 81.6% of nasopharyngeal aspirates (not sinus aspirates) of the young adult patients with sinusitis studied. They believed that early-stage acute sinusitis has a viral etiology, but to our knowledge, no studies have reported viral pathogens in acute pediatric sinusitis. The present results show that severe sinusitis in children results from bacterial infections or viral and bacterial co-infections. However, the patients investigated in this study had severe and prolonged symptoms. Therefore, it is difficult to extrapolate the results to know whether early-stage sinusitis results from a viral or bacterial infection. We are planning to investigate the microbiology of mild acute sinusitis from the middle meatus sample in future studies.

In this study, bacterial PCR detected one bacterium in 22 of 31 (71%) patients, 2 bacteria in 7 (23%) patients, and 3 bacteria in 1 (3%) patient. The most frequently detected bacteria were *H influenzae* in 14 (45%) patients, *S pneumoniae* in 9 (29%), *M catarrhalis* in 4 (13%), and *C pneumoniae* in 4 (13%). *H influenzae*, *S pneumoniae*, and *M catarrhalis* are considered frequent causes of acute rhinosinusitis and acute otitis media in children. *H influenzae*, *S pneumoniae*, and *M catarrhalis* are common pathogens in sinusitis, while *C pneumoniae* has not been considered a common pathogen for acute rhinosinusitis in children. *C pneumoniae* is a well-established cause of lower respiratory tract infections; however, its role in upper respiratory tract illnesses is unclear.[14] Only one case has been reported wherein *C pneumoniae* was isolated from maxillary sinus aspirates using bacterial culture in one adult maxillary sinusitis patient,[15] and no studies have reported the presence of this bacterium in pediatric patients with sinusitis. Several studies have reported cases of chronic infections, otitis media with effusion,[16] and chronic sinusitis[17,18] in young adults (aged 18–28 years); for instance, Savolainen et al.[17] reported more than fourfold *M pneumoniae* antibody titer in 11 of the 245 (4.5%) patients with maxillary sinusitis, displaying no significant difference from 1 of 101 control (1.0%) subjects. *Chlamydia* spp. antibody titers were elevated in 2 of the 245 (0.8%) patients and 1 of 94 control (1.1%) subjects, with no significant difference. PCR is a useful tool to detect *Mycoplasma* or *Chlamydia* because it is difficult to discern infections from antibody titers and cultures. In this study, no patient with *M pneumoniae* was found, while 4 of the 31 (13%) patients with *C pneumoniae* infection were identified through PCR using maxillary sinus aspirates from pediatric maxillary sinusitis patients. We had previously reported that in pediatric patients with acute otitis media, *C pneumoniae* was detected in 0 of 122 children through real-time PCR using middle ear fluid as samples, and *M pneumoniae* was detected in one patient.[10] We speculate that *C pneumoniae* was not detected in pediatric acute otitis media but acute sinusitis because children with acute otitis media were

almost under 3 years old, which is not a susceptible age to *C pneumoniae*.

This study elucidates the microbiology of acute maxillary sinusitis in children. *C pneumoniae* infection was detected in 13% of the patients. This study is the first to report *C pneumoniae* as one of the pathogens in pediatric acute maxillary sinusitis. However, we do not speculate that *C pneumoniae* is a major pathogen in pediatric rhinosinusitis because the sinusitis patients included in this study all presented with prolonged symptoms or treatment failure. Nevertheless, if antimicrobial therapy is ineffective, the possibility that chlamydia may be the causative organism, for which penicillin and cephalosporins are not effective, should be considered. In this study, patients with acute sinusitis-associated *C pneumoniae* infections were aged 8–10 years. Antibodies against *C pneumoniae* have been detected in the serum of 5- to 14-year-old children in Japan.[19] The study showed that numerous children are affected by *C pneumoniae* infection after enrollment in elementary school. Careful attention should be paid to acute sinusitis patients with *C pneumoniae* infection at elementary school age because they do not present signs of pneumonia but only have sinusitis. The present results provide insights into the treatment of severe or prolonged acute pediatric rhinosinusitis.

This study has some limitations. The most important limitation is that patients enrolled in this study included severe cases who underwent sinus puncture; thus, the microbiology may not reflect common acute rhinosinusitis, including mild sinusitis cases. We intend to broaden the current understanding by investigating the microbiology of common acute rhinosinusitis from the middle meatus of children with acute rhinosinusitis. Furthermore, some of the present patients had received the PCV, while others had not. Hence, for ages under the post-PCV era, PCV administration decreases the detection rate of *S pneumoniae* infections.

CONCLUSION

We investigated the microbiology of 31 children with acute maxillary sinusitis using bacterial culture and viral/bacterial PCR. Respiratory viruses were detected in 32% of patients, using PCR; rhinovirus (4/31, 13%) was the most common, followed by influenza virus type A/B (3/31, 10%). Respiratory bacteria were detected in 65% of the patients, and bacteria and viruses in 32%, with *H influenzae* and *S pneumoniae* being the most common. *C pneumoniae* was detected in 13% of the patients via PCR. PCR is useful for investigating microbiology in pediatric sinusitis accurately.

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