

Evaluation of nasopharyngeal microbial flora and antibiogram and its relation to otitis media with effusion

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Abstract Acute otitis media and otitis media with effusion (OME) are the main causes of hearing impairment in children which require proper treatment, mainly antibiotic therapy. Patients whom were appropriate candidates for adenoidectomy were divided into two groups regarding the presence of middle ear effusion. Adenoid tissue specimens were cultured in both groups and the bacterial flora and anti-microbial resistance pattern were determined. 72 patients were studied, 42 % had OME while 58 % did not. The following bacteria were isolated and cultured from both groups with no meaningful difference in prevalence: *Streptococcus viridans* ($p = 0.265$), *Staphylococcus aureus* ($p = 0.72$), *H. influenza* ($p = 0.806$), *Enterococcus* spp (0.391), *Streptococcus pneumonia* ($p = 0.391$), non-hemolytic *Streptococcus* ($p = 0.230$). Bacterial sensitivity was similar for Amoxicillin–clavulanate ($p = 0.935$), Amoxicillin ($p = 0.935$), Cephalexin ($p = 0.806$), Cefixime ($p = 0.391$) and Azithromycin in both groups. The two groups showed no meaningful difference considering the bacterial flora of nasopharynx and their sensitivity. Bacteria in both groups were sensitive to Amoxicillin and Amoxicillin–clavulanate and resistant to Azithromycin, Cefixime and Cephalexin.

Keywords Microbial flora · Antibiogram · Otitis media with effusion

Introduction

Acute otitis media (AOM) and otitis media with effusion (OME) are the two most common diseases in young children who are visited by primary care physicians [1]. OME is defined as the presence of effusion (fluid) in the middle ear without any inflammation [2]. Nowadays, this problem is considered as an infectious disease which is commonly seen in 2 to 4-year-old children and is less common after 6 years of age. It occurs more frequently in winter rather than spring or summer [2]. Among the complicated and not fully known etiologies, Eustachian tube dysfunction and bacterial endotoxins are the most widely accepted hypotheses.

Antibiotics were introduced as an inseparable part of OME treatment from the late 1970s to the early 1980s. This was the time interval in which several studies proved the inefficiency of anti-histamines and decongestants in this regard [3]. Although it was initially believed that such effusions are sterile, recent studies revealed that the samples taken from asymptomatic children with MEE contain certain bacteria [4].

Of note, not all patients with adenoid hypertrophy develop OME and not all OME patients have adenoid hypertrophy.

As AOM and OME are now considered as infectious diseases and as the only pathway for the pathogens to reach the middle ear cavity in the presence of an intact tympanic membrane is through the Eustachian tube, we aimed to evaluate and compare the aerobic microbial flora and their resistance to the most common antibiotics used in treating

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OME in patients with adenoid hypertrophy, with and without OME.

Regarding environmental and demographic factors which affect the frequency of bacterial colonization in the nasopharynx, in particular the changes in the bacterial resistance pattern which is an invaluable guide in empirical therapy, it is essential to identify the nasopharyngeal microbial pattern in each geographical region at certain intervals.

Methods

In this survey, we studied the association between nasopharyngeal microbial flora and their antibiogram and the presence of OME. Seventy-two children who were appropriate candidates for adenoidectomy were selected and divided into two groups, based on the presence or absence of OME. The two groups were matched for age and sex.

All children with the following criteria were excluded from the study: fever or any type of upper respiratory tract infection during surgery or in the past 4 weeks, antibiotic consumption (due to any cause) in the previous 4 weeks, any craniofacial, genetic, hereditary, metabolic or immunologic abnormalities, previous adenoidectomy or otologic surgery and any chronic diseases of the ears except for OME.

Before disinfecting the oral and nasal cavities with antiseptic solutions, a sterile Crow–Davis mouth gag was placed in the mouth and the adenoid tissue was almost totally removed in a single move by a sterile adenoid curette; the specimen was then sent to the microbiology lab under sterile conditions.

The specimens were studied for aerobic bacteria. Two smears which were taken from the superficial and deep parts of the sample underwent microscopic examination after Gram staining.

Then the obtained specimens were cultured on chocolate agar and blood agar media with 5 % sheep blood. After 48 h at 35° of aerobic incubation, the presence of aerobic bacteria was investigated. In case of any growth, proper tests were accomplished to identify the organism. The pattern of resistance in isolated bacteria was assessed according to the CLSI (Clinical and Laboratory Standards Institute) standardized methods [5]. MIC (mean inhibitory concentration) was measured for each antibiotic and the resistance or sensitivity level of the bacteria was determined.

The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences and an

informed consent was signed by each participant or his/her parent prior to study entrance. Statistical analysis was performed using the SPSS software, version 19.1.

Results

Seventy-two patients were studied; 47 % were male and 53 % female. Thirty (42 %) individuals had OME whereas 42 (58 %) did not. Among the 30 patients with OME, 18 (60 %) were male and 12 (40 %) female.

Based on Chi-Square test with a 95 % confidence interval, no meaningful relationship was found between OME and the patient's sex ($p = 0.194$).

Among the 72 studied subject, 46 had the polymicrobial pattern of infection from which 20 (43 %) had OME and 26 (57 %) did not (Table 1).

Using Chi-Square test with a confidence interval of 95 %, no significant correlation was found between the presence of OME and the polymicrobial pattern ($p = 0.769$).

The most common bacteria in the patients with and without OME was *streptococcus viridans*; yet the statistical analysis showed no meaningful difference between the two groups regarding culture ($p = 0.265$).

Other bacteria isolated from the cultures in both groups are displayed in Table 2; no meaningful difference was diagnosed between two groups regarding the isolated pathogens.

In the OME group *E. coli* was isolated from six cultures which were significantly higher than the non-OME group (0.032); however, because it is considered as normal nasopharyngeal flora, it was also regarded as non-pathogen in our study.

The results obtained regarding the resistance and sensitivity of bacteria to the routine antibiotics used in managing OME, are summarized in Table 3.

According to Colmogrov–Smirnov test for MIC, *streptococcus viridans* did not have a normal distribution in the five antibiotic categories of the two groups; therefore

Table 1 Polymicrobial distribution pattern in patients with and without OME

	Polymicrobial		Non-polymicrobial	
	Number	Percent	Number	Percent
With OME	20	43	10	38.5
Without OME	26	57	16	61.5
Total	46	100	26	100

Table 2 Bacteria isolated from the cultures in the groups with and without OME

Isolated bacteria	With OME (%)	Without OME (%)	<i>p</i> value
<i>Streptococcus viridans</i>	53.3	71.5	0.265
<i>E. coli</i>	20	0	0.032
<i>Staphylococcus aureus</i>	13.5	14.4	0.720
<i>H. influenza</i>	6.6	4.7	0.806
Enterococcus.spp	0	4.7	0.391
<i>Streptococcus pneumonia</i>	0	4.7	0.391
Streptococcus non-hemolytic	6.6	0	0.230

Mann–Whitney test was applied showing no significant correlation between the two groups regarding MIC for this bacteria ($p > 0.05$ for all five antibiotics).

Discussion

OME is clinically defined as the presence of effusion in the middle ear with no inflammatory signs and symptoms [6]. The pathogenesis of OME is not completely understood. Classic literatures have shown that the effusions of OME result from chronic inflammation in response to residual bacterial degradation components. Nevertheless, the etiopathogenesis of OME appears to be much more complicated [7]. New increasing evidence suggests the major role of biofilms in the pathogenesis of OME [8–10]. Gastroesophageal reflux [11], poor clearance of middle ear fluids [12, 13], allergies [14–17] and finally genetic predisposition [18, 19] may also contribute.

A bacterial organism is identified in approximately one-third of middle ear effusions of children with OME who undergo myringotomy [20–24] and the pathogens are primarily those that are found in the nasopharyngeal biofilms.

Eustachian tube dysfunction seems an important factor in producing middle ear effusion; however, this problem could be completely mechanical or independent from respiratory tract infections [7]. Adenoids have been linked with the etiopathogenesis of acute, recurrent, and chronic infectious diseases of the sinonasal and respiratory tract.

They may cause mechanical obstruction of the nasopharynx and therefore Eustachian tube, or become a reservoir for resistant bacteria that can cause recurrent infections and permanent inflammation and greatly affect medical treatments [8].

We studied the relationship between OME and nasopharyngeal bacterial flora and the bacterial resistance pattern to antibiotics in 72 adenoidectomy candidates including 34 (47 %) males and 38 (53 %) females under the age of 15, out of which 42 % had OME. To the authors' knowledge, this is the first study investigating MIC and subsequent bacterial resistance according to CLSI standards.

Evaluating the bacteria in the planktonic but not the biofilm form was the main limitation of the current study. However, the most common bacteria isolated from the specimens of both groups was *Streptococcus viridans*, followed by *Staphylococcus aureus* and *H. influenza*, respectively. No meaningful difference was found between the two groups (with and without OME) regarding the isolated pathogens. Thus, the presence or absence of OME should not affect the antibiotic selection strategy.

In an almost similar study by Ma Clay [25], adenoidectomy candidates whom were divided into two groups based on middle ear involvement were investigated for resistant bacteria in the adenoid tissue culture. They found such pathogens to be significantly more prevalent in patients with middle ear effusion.

Marchisio et al. [26] showed that microbial colonization of the nasopharynx and their resistance patterns are different in patients with OME compared to non-OME cases, which is in contrast to our findings. This difference may be due to geographical varieties or their different antibiotic therapy policy.

In another study by Almac et al. [27] with a larger study population (180 patients), superficial and deep aerobic and anaerobic flora of the adenoid tissue were studied in children with and without middle ear effusion. The authors drew the same conclusion regarding the relationship between OME and nasopharyngeal bacterial flora. This can be attributed to the similar geographical areas and demographical characteristics of the subjects. Linder et al. [28] also reached to the same conclusion.

Table 3 The resistance and sensitivity level of bacteria towards the routine antibiotics used in managing otitis media with effusion

	With OME		Without OME		<i>p</i> value
	Sensitive	Resistant	Sensitive	Resistant	
Amoxicillin	18 (60 %)	18 (40 %)	32 (64 %)	18 (36 %)	0.298
Amoxicillin–clavulanate	4 (13.4 %)	26 (86.6 %)	6 (14.2 %)	36 (85.8 %)	0.935
Cephalexin	2 (6.6 %)	28 (93.4 %)	2 (4.7 %)	40 (95.3 %)	0.806
Azithromycin	0 %	30 (100 %)	0 %	42 (100 %)	–
Cefixime	0 %	30 (100 %)	2 (4.7 %)	40 (95.3 %)	0.391

Saafan et al. [29] conducted a study to determine the role of bacterial biofilm pathogenesis on developing OME. Using both culture and polymerase chain reaction (PCR) techniques, they found that adenoid samples from children with middle ear effusion had higher grade biofilm formation than those without effusion. However, interestingly the adenoid size was significantly lower in the OME group, indicating that bacterial colonization has a greater impact on OME pathogenesis than the size itself.

In general, OME resolves without medical intervention in the majority of patients [30–33]. Regarding the use of antibiotics in OME and based on a systematic review performed in 2012, it was stated that the available evidence does not support the routine use of antibiotics for children with OME [34]. A meta-analysis conducted in 1992 revealed that the clearance rate of OME could be improved during the first month of antibiotic therapy; however, frequent recurrences were observed while no further advantage was reported after the first month of receiving medication [35, 36].

The American Academy of Otolaryngology-Head Neck Surgery (AAOHN), American Academy of Pediatrics (AAP), and American Academy of Family Physicians (AAFP) clinical practice guideline suggest a single 10 to 14-day course of antibiotics (typically amoxicillin) in certain cases [37]. Hence, the 2008 National Institute for Clinical Excellence (NICE) guidelines does not recommend the administration of antibiotics in the treatment of OME [37].

In the current study we examined five routine first line antibiotics including Amoxicillin, Amoxicillin–clavulanate, Cephalexin, Cefixime and Azithromycin in both groups. The results indicated almost similar sensitivity of pathogens of both groups to the first two antibiotics whereas resistance was observed to the latter three.

Khoramrooz et al. [38] also found Amoxicillin–clavulanate to be effective against the bacterial flora causing OME.

Conclusion

The current study showed that there is no fundamental difference between the bacterial flora of individuals with and without OME. In addition, Amoxicillin and Amoxicillin–clavulanate are still the preferred first line medications in the management of middle ear effusions.

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