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Role of adenoid biofilm in chronic otitis media with effusion in children

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Abstract To study the extent of surface adenoid biofilm and to evaluate its role in the pathogenesis of chronic otitis media with effusion (COME) in children. The study was carried out on 100 children between 3 and 14 years of age, who were divided into two groups. The first group (50 children) had otitis media with effusion associated with adenoid hypertrophy, whereas the second group (50 children) had adenoid hypertrophy without middle ear effusion. Adenoidectomy with ventilation tube insertion was done for group 1 cases, whereas, only Adenoidectomy was done for group 2 cases. Microbiological study, Scanning electron microscope and multiplex- PCR were done for suspected adenoid biofilms and specimens from middle ear effusion. Adenoids removed from children with COME had higher grade biofilm formation (74 %) than the second group (42 %). No correlation was found between adenoid size and biofilm formation. Culture of adenoid tissue in group 1 patients was positive in 52 % of cases compared to 96 % by PCR, while in group 2 culture of adenoid tissue was positive in 38 % compared to 48 % by PCR. Culture of middle ear fluid was positive in 32 % of cases only compared to 80 % by PCR. A positive correlation was found between results of bacterial biofilm visualized by SEM and bacteria detected and identified by PCR technique. On the other hand, no correlation was found between results of bacterial biofilm visualized by SEM and bacteria detected by culture. The size of the adenoid is not the main determinant factor in OME pathogenesis but the degree of bacterial colonization is much more important. Adenoids in COME may act as a reservoir of chronic infection rather than causing mechanical Eustachian obstruction. Higher grade biofilm formation was found in cases with middle ear effusion than those with adenoid hypertrophy only. These findings support the hypothesis that there would be an association between adenoidal biofilm formation and COME. This study focused on the value of PCR in detecting pathogens in the adenoid and middle ear specimens although the bacterial culture would be negative.

Keywords Biofilm · Adenoid · Otitis media · Effusion · Scanning · PCR · Culture

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Introduction

Chronic otitis media with effusion (COME) is the most common chronic ear disorder in children. COME is defined as the persistence of middle ear fluid beyond 12 weeks and has multiple causes including environmental and host factors [1]. An early childhood history of COM can result in auditory and verbal disabilities that exert influence into late childhood, rendering treatment of recurrent otitis media (ROM) and otitis media with effusion (OME) desirable [2].

Middle ear effusion in otitis media was at one time considered sterile. In studies of middle ear effusions that produced "sterile" cultures, polymerase chain reaction (PCR) confirmed the presence of bacteria (typically *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis*), in apparently sterile middle ear fluid [3].

Repeated cycles of antibiotics for treatment of COME make it the leading reason for antibiotic usage in children. Treatment may also entail surgery for the placement of tympanostomy tubes (TT) to reduce the incidence of ROM and alleviate middle ear fluid associated with OME. Adenoidectomy has been shown to be an effective treatment for COME. Adenoidectomy removes a physical obstruction of the Eustachian tubes, thereby, restoring mucus drainage and normal pressure in the middle ear affecting the ability of pathogens to invade and reside within the middle ear space. However, adenoidectomy is effective for reducing the recurrence of COM regardless of the size of adenoids suggesting that physical obstruction of the airway may not be the principal risk factor in COM [2]. Biofilm-colonized adenoids may be a potential source of planktonic bacteria that have the ability to migrate, it may be reasonable to hypothesize that nasopharyngeal biofilms can disseminate and disperse to form biofilms in the middle ear, thereby, causing COME [4].

A biofilm is a structured community of cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface. It may be made up of fungal and bacterial cells that communicate with one another in a cooperative manner. The self-produced matrix, which is slime like, may include polysaccharides, nucleic acids, and proteins [5]. Biofilms are initiated when free-floating, planktonic bacteria anchor to biologic or inert surfaces. The attached bacteria multiply and progress from a state of monolayer to a micro-colony and then to a critical mass. Biofilm formation is thought to provide a mechanism for enhanced bacterial survival. Bacteria in biofilms lack the antibiotic susceptibility of planktonic bacteria. Extracellular matrix that makes up most of the biofilm serves to protect the bacteria against antibodies, immune-system phagocytosis, antibiotic penetration, and complement binding. There is also a decreased need for oxygen and nutrients when bacteria exist in the biofilm state, in addition, biofilms are environments where bacteria can share their DNA by transfer of genetic information via plasmids to encourage variability and adaptive mutations such as antibiotic resistance [6].

In this prospective study, we have tried to find if there is a correlation between possible adenoid biofilms and middle ear effusion in cases of COME.

Patients and methods

This study included 100 children between 3 and 14 years of age. Fifty of them had adenoid hypertrophy with OME (group 1) and the other 50 children had only adenoid hypertrophy (without OME) with obstructive sleep apnea (OSA) (group 2). Adenoidectomy with ventilation tubes insertion was done for group 1 patients, whereas adenoidectomy alone was done in patients of group 2. Surgeries were done in the Otolaryngology Department Tanta University Hospital between May 2009 and October 2010. The study was approved by the local ethics committee. The exclusion criteria were the presence of a previous adenoid and/or middle ear operation.

Patients were subjected to history taking, complete ENT examination and tympanometry to evaluate middle ear pressure and detect the presence of middle ear effusion. The size of the adenoid was assessed with a flexible fiberoptic endoscope with the patient under general anesthesia at the beginning of the operation. The adenoids were graded according to its anatomic relationship with the vomer, soft palate, and torus tubarius [7]. The enlarged adenoid may not come in contact with any of these anatomic structures (grade 1), whereas, sometimes it may touch the torus tubarius (grade 2), torus tubarius and vomer (grade 3), and torus tubarius, vomer and soft palate at rest (grade 4).

The adenoid tissuespecimen was divided into three parts, the first part for bacterial culture and identification and the second part for detection of bacterial pathogens in adenoid tissues using multiplex polymerase chain reaction (multiplex-PCR) technique. The third part was cleaned with isotonic sodium chloride solution to remove blood and secretions, fixed in 2.5 % glutaraldehyde and sent to electron microscope unit for scanning electron microscopic examination (SEM).

The middle ear effusions (MEE) were obtained by tympanocentesis performed in the inferior portion of the tympanic membrane using an Alden-Senturia trap (Storz Instrument Co, St. Louis, MO, USA) with a needle attached. A tuberculin syringe with an 18-gauge needle attached was an alternative. The middle ear aspirates were sent to the Clinical Pathology Department for bacterial culture and identification and bacterial pathogens detection using multiplex-PCR.

All patients and control groups were informed of the operations, and they all signed informed consent forms.

Evaluation of adenoid and middle ear pathogens by bacterial culture and identification

The adenoid specimens for aerobic bacterial culture were inoculated on 5 % sheep blood, chocolate, MacConkey agar plates, and haemophilus test medium (HTM) plates (Oxoid, UK). They were incubated at 37 °C at 5 % CO_2 for 24–48 h and observed for growth of colonies. Anaerobic cultures were incubated at 35 °C at 5 % CO_2 for 48 h and then observed. While, middle ear effusion (MEE) specimens were inoculated into pediatric blood bottles culture (Oxoid, UK) and incubated at 37 °C for 5 days and at the end of 5th day, inoculated onto blood and chocolate agar plates before discarded as negative. When culture bottles

were positive, the bottles were pulled, Gram staining and subculturing were completed. Bacterial identification was performed using gram staining and microbact identification kits (Oxoid, UK).

Evaluation of adenoid and middle ear pathogens by multiplex-PCR

PCR used in this study is a method for simultaneous detection (multiplex-PCR) of *S. pneumoniae*, *H. influenza*, *M. catarrhalis* and *S. aureus* [8]. The 16S rRNA gene, which contains both variable and constant sequences, was chosen as the target of PCR amplification. Constant sequences are common to several bacteria, and variable sequences are specific to each species.

Streptococcus pneumoniae ATCC 49619, *H. influenzae* ATCC 49247, *M. catarrhalis* ATCC 43627 and *S. aureus* ATCC 13565 [Manassas (VA), USA] were used as control strains. DNA was extracted from middle ear effusions, adenoid specimens and control strains using QIAamp DNA Minikit (Qiagen, Hilden, Germany), according to the appropriate protocols in the manufacturer's instructions. Twenty-five-microliter PCRs were performed containing 1.0 U of Taq DNA polymerase; 5 mM each of the four deoxyribonucleotide triphosphates (Qiagen, Hilden, Germany); 1.5 mM MgCl2; 0.1 μM of each primer and 2 μL of DNA of samples and control strains.

The following primers were used: *H. influenza* (forward) 5′-GGAGTGGGTTGTACCAGAAGTAGAT-3′ and *S. pneumoniae* (forward) 5′ AGTCGGTGAGGTAACCGTAAG-3′, both with a universal reverse 5′ AGGAGGTGATCCAACC GCA-3′ primer. *M. catarrhalis primer* (forward) *was* 5′-TTG GCTTGTGCTAAAATATC-3′ and (reverse) 5′-GTCATC GCTATCATTCACCT-3′. *S. aureus* primers were (forward) 5′-CCTATAAGACTGGGATAACTTCGGG-3′ and (reverse) 5′-CTTTGAGTTTCAACCTTGCGGTCG-3′. The specificity of these primers has been previously established [9].

The PCR profile was performed using the following conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min, followed by an extension at 72 °C for 5 min. The PCR products were separated in 2 % agarose gel in Tris–acetate-EDTA buffer and DNA bands were visualized with ethidium bromide by UV light illumination.

Evaluation of adenoid biofilm by scanning electron microscope (SEM)

The fresh specimens were immediately fixed in 2.5 % glutaraldehyde and sent to electron microscope unit for scanning electron microscope examination (SEM). The images were taken with the JEOL SEM ASID-10 and the LEO 4.3 HVP SEM (Carl Zeiss AG, Oberkochen, Germany) electron microscopes. To examine the areas of interest on a specimen, SEM images were captured within a voltage range of 5–80 kV and within a magnification range of 50–5,000 times. The biofilms were defined as the areas where multilayered remnants of tissue and microorganisms exist.

With respect to the average biofilm extension, grade 1 with less than 25 % of sample surfaces was involved, grade 2 with 25–50 % of sample surfaces was involved, grade 3 with 50–75 % of sample surfaces was involved, and grade 4 with more than 75 % of sample surfaces was involved.

Statistical tests were conducted using SPSS version 13.0 (SPSS Inc, Chicago, IL, USA). The nonparametric Mann–Whitney test was used in the comparison of biofilm formation and adenoid size among groups. In all analyses, P values <0.05 was considered statistically significant.

Results

There were 59 male and 41 female patients in the study; their ages ranged from 3 to 14 years (mean, 5.72 years).

Biofilm visualization by SEM

The biofilm formation was detected in 74 % of samples of group (1) and only in 42 % of samples of group (2) with grades from 1 to 4 (Table 1; Fig. 1). Adenoid samples removed from the children with COME had higher grade biofilm formation than the group (2) who were operated on for adenoid hypertrophy alone. The difference between the two groups was statistically significant. Biofilm formation did not change with respect to sex, age and adenoid size. Regarding adenoid tissue size, there is a statistically significant larger adenoid size in group 2 than group 1 (Table 1).

Table 1 Adenoid size and biofilm formation in both groups

	Group 1 ($n = 50$)		Group 2 ($n = 50$)		P
	n	%	n	%	
Adenoid tissue size					
Grade I	5	10	0	0	0.2
Grade II	6	10	0	0	0.3
Grade III	21	42	7	14	0.05
Grade IV	18	36	43	86	0.01
Biofilm formation					
Negative	13	26	29	58	0.3
Grade 1	0	0	12	24	0.02
Grade 2	12	24	7	14	0.05
Grade 3	19	38	2	4	0.01
Grade 4	6	12	0	0	0.03

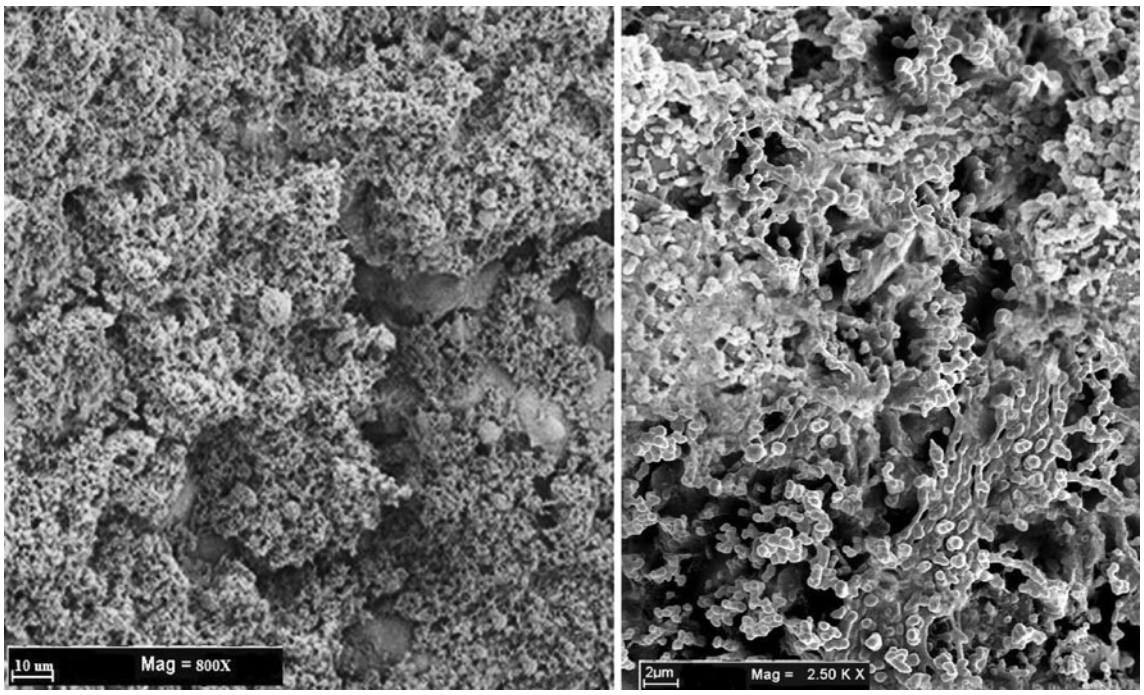

Fig. 1 Shows the electro-micrographs of bacterial cells colonization forming biofilm on the adenoid surface by scanning electron microscope. ×800 (*left*) and ×2,500 (*right*)

Pathogen identification

In group 1 patients, culture and bacterial identification of adenoid tissue was positive in 26 samples (52 %), while in group 2 patients, it was positive in 19 samples (38 %) only (Table 2). Culture of middle ear fluid was positive in 16 samples (32 %) (Table 2; Fig. 2).

PCR was utilized to identify the bacterial pathogens. In group 1, 96 % of adenoid samples and 80 % of middle ear specimens were positive, compared to only 48 % of adenoid samples in group 2 (Table 2).

Table 3 shows whether the bacteria identified by both culture and PCR in the studied groups were single or multiple. *S. pneumoniae*, *H. influenza*, *S. aureus*, and *M. catarrhalis* either alone or in combinations were the most common pathogens detected in both adenoid and middle ear samples by both culture and PCR (Tables 4, 5). *H. influenza* was the most common pathogen in adenoid specimens of group 1 patients both by culture (58 %) and by PCR 83 %. Both *S. pneumoniae* (42 %) and *H. influenza* (42 %) were the most common pathogens detected in adenoid samples of group 2 patients by culture, while by PCR *H. influenza* was the most common (71 %). *S. pneumoniae* (44 %) was the most common pathogen detected in middle ear effusions of group 1 patients by culture, while by PCR *H. influenza* was the most common (82 %) (Table 5).

Table 2 Results of identified bacterial pathogens by cultures and PCR in the studied groups

Result	Culture				PCR			
	Positive		Negative		Positive		Negative	
	No	%	No	%	No	%	No	%
Adenoid pathogens in Group I	26	52	24	48	48	96	2	4
Adenoid pathogens in Group II	19	38	31	62	24	48	26	52
Pathogens identified in MEE	16	32	34	68	40	80	10	20

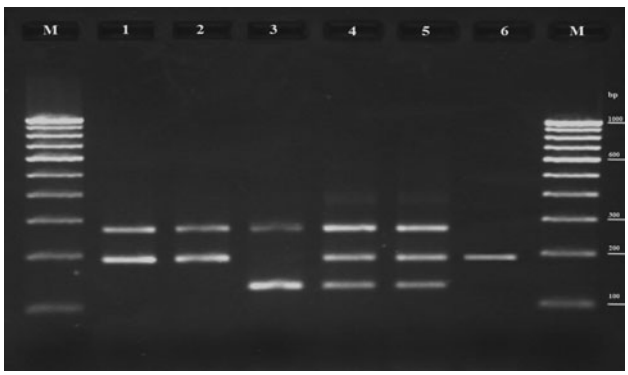

Fig. 2 DNA products from multiplex-PCR detection and identification of bacterial pathogens from adenoid specimens. *LanesM* molecular marker GeneRuler 100-bp DNA ladder; *lanes1*, *2* show both *S. aureus* and *H. influenza*, *lane3* shows both *S. aureus* and *M. catarrhalis*, *lanes4*, *5* show *S. aureus*, *H. influenza* and *M. catarrhalis*, *lane6* shows *H. influenza*

Table 3 Results of bacteria identified by both culture and PCR in the studied groups

Result	Adenoid pathogens identified in group I		Adenoid pathogens in group II		Pathogens identified in MEE	
	Culture (%)	PCR (%)	Culture (%)	PCR (%)	Culture (%)	PCR (%)
Single pathogenic bacteria	34.6	21	28	22	25	14
Multiple pathogenic bacteria	17.4	75	10	26	7	66
Negative	48	4	62	52	68	20
Total	100	100	100	100	100	100

Table 4 Pathogenic bacteria identified by both culture and PCR in the studied groups

Result	Adenoid pathogens in Group I		Adenoid pathogens identified in Group II		Pathogens identified in MEE	
	Culture	PCR	Culture	PCR	Culture	PCR
S. pneumoniae						
No.	4	3	4	2	4	1
%	8	6	8	4	8	2
H. influenzae						
No.	6	3	3	4	1	0
%	12	6	6	8	2	0
S. aureus						
No.	5	3	5	4	4	3
%	10	6	10	8	8	6
M. catarrhalis						
No.	2	2	2	1	3	3
%	4	4	4	2	6	6
Mixed *S. pneumoniae* and *H. influenzae*						
No.	6	11	4	8	3	10
%	12	22	8	16	6	20
Mixed *H. influenzae* and *M. catarrhalis*						
No.	3	9	1	4	1	12
%	6	18	2	8	2	24
S. pneumoniae, *H. influenzae* and *M. catarrhalis*						
No.	0	10	0	1	0	5
%	0	20	0	2	0	10
S. pneumoniae, *H. influenzae* and *S. aureus*						
No.	0	7	0	0	0	6
%	0	14	0	0	0	12
Negative						
No.	24	2	31	26	34	10
%	48	4	62	52	68	20

A summary of results of bacterial biofilms visualized by SEM and bacteria detected by culture and PCR techniques in adenoid samples of both groups was shown in Table 6. Table 7 and Fig. 3 show a positive correlation between bacterial biofilm visualized by SEM and bacteria detected by PCR technique in adenoid samples of both groups. On the other hand, no correlation was found between the results of bacterial biofilm visualized by SEM and bacteria detected by culture.

Discussion

Otitis media with effusion (OME) is defined as the presence of fluid in the middle ear without signs or symptoms of acute ear infection [10]. It usually follows one or more episodes of AOM, and it is characterized by the persistence of fluid in the middle ear, even after resolution of the acute phase of the disease; however, OME can even occur independently, being associated with other causes that may

Table 5 Incidence of pathogenic bacteria identified by both culture and PCR in the studied groups

Result	Adenoid pathogens in Group I		Adenoid pathogens in Group II		Pathogens identified in MEE	
	Culture ($n = 26$)	PCR ($n = 48$)	Culture ($n = 19$)	PCR ($n = 24$)	Culture ($n = 16$)	PCR ($n = 40$)
S. pneumoniae						
No.	10	31	8	11	7	22
%	38	65	42	46	44	55
H. influenzae						
No.	15	40	8	17	5	33
%	58	83	42	71	31	82
S. aureus						
No.	5	10	5	4	4	9
%	19	21	26	17	25	22
M. catarrhalis						
No.	5	21	3	6	4	20
%	19	44	16	25	25	50

Table 6 Results of bacterial biofilm visualized by SEM and positive bacterial results of culture and PCR techniques in adenoid samples of both groups

	Biofilm formation by SEM		Positive bacterial culture		Positive bacteria detected by PCR	
	No.	%	No.	%	No.	%
Adenoid pathogens identified in Group I	37	74	26	52	48	96
Adenoid pathogens identified in Group II	21	42	19	38	24	48

Table 7 Correlation between bacterial biofilm visualized by SEM and bacteria detected by culture and PCR techniques in adenoid samples of both groups

Parameters	Bacterial biofilm by SEM			
	Group I		Group II	
	r	*P* value	*r*	*P* value
Positive bacterial culture	0.155	0.423	0.012	0.962
Positive PCR results	0.796**	<0.001*	0.839**	<0.001*

* P value < 0.05 is significant

** Positive correlation

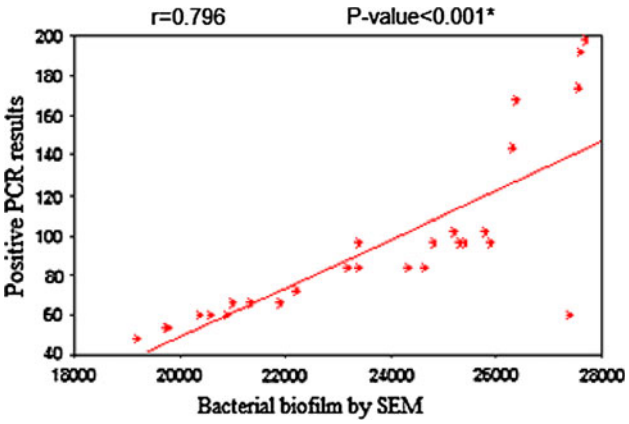

Fig. 3 Correlation between bacterial biofilm visualized by SEM and bacteria detected and identified by PCR technique in group 1

decrease the Eustachian tube function [11]. During the last decade, many researchers tried to explain pathogenetic mechanisms of OME to determine effective methods of prevention and treatment of this disease. Except from mechanical or functional Eustachian tube dysfunction, there are also others pathophysiological factors and mechanisms contributing in development of OME: bacterial colonization and biofilm formation, viral infections, allergy and immunological factors [12].

Adenoids have been associated with the pathogenesis of acute, recurrent, and chronic infectious diseases of the upper respiratory system. The adenoids may cause mechanical obstruction of the nasopharynx, play an important role in the pathogenesis of otitis media or become a reservoir for pathogenic bacteria especially resistant bacteria that can cause recurrent infections and greatly affect medical treatments [13].

Biofilms are complex structures that confer unique protective attributes to its inhabitants; the persistence of bacteria in a biofilm happens with mechanisms other than efflux pumps, modifying enzymes, and target mutations. There are three main hypotheses to explain this phenomenon. The first hypothesis is the possibility of slow or incomplete penetration of the antibiotics into the biofilm.

The second hypothesis depends on the altered chemical microenvironment within the biofilm. A third and still speculative hypothesis is that a subpopulation of microorganisms in a biofilm forms a unique and highly protected phenotypic state, a cell differentiation similar to spore formation [14, 15].

In recent years, the role of biofilms has gained popularity as a component in the explanation of chronicity of infections and resistance to antibiotic chemotherapy [14]. Bacterial biofilm can cause several diseases in otolaryngology among those diseases; the role of biofilms in the pathogenesis of COME has gained momentum. It is reasonable to investigate the role of adenoid biofilms as a factor that may contribute to the development of COME [4]. So, the present study aimed to study the extent of surface adenoid biofilm and its role in the pathogenesis of COME in children.

In the present study, the role of adenoid surface biofilm formation was investigated in children with COME. The patients were divided into two groups; group 1 had COME, adenoidectomy and ventilation tube insertion was done, and group 2 had healthy ears, only adenoidectomy was done to treat OSA. The biofilm formation was detected in 74 % of adenoid samples of group 1 (OME) and only 42 % of samples of group 2. In group 1: 12 % of children has grade 4, 38 % grade 3 and 24 % grade 2, compared to group 2 where none of the children has grade 4, 4 % grade 3, 14 % grade 2 and 24 % grade 1. It is concluded that adenoid samples from children with middle ear effusion had higher grade biofilm formation than those without effusion. The difference between the two groups was statistically significant. Biofilm formation did not change with respect to sex, age and adenoid size. Regarding adenoid tissue size, there is a statistically significant larger adenoid size in group 2 than group 1 which indicates that the size of the adenoid is not the main determinant factor in OME pathogenesis but the degree of bacterial colonization is much more important. Adenoids in COME may act as a reservoir of chronic infection rather than causing mechanical Eustachian obstruction. These results would support the role of adenoidectomy in the management of COME resistant to medical therapy to eradicate the reservoir of chronic infection.

This is in agreement with Saylam et al. [14] who reported that adenoid tissues of children with COME had denser surface biofilms compared with those without COME. These findings support the hypothesis that there may be an association between adenoidal biofilm formation and COME. Also Hoa et al. [4] sought to document nasopharyngeal biofilm density in children with COME and reported that patients with recurrent acute otitis media demonstrated nasopharyngeal (NP) surface biofilm in 99 % of cases and patients diagnosed with COME had NP surface biofilm involvement in 27.7 % of cases. The significance of such an observation leads us to suggest that middle ear disease may occur along a spectrum with COME existing somewhere on the continuum. Also, Coticchia et al. [16] reported that adenoids removed from children with chronic rhinosinusitis have, on average, 94.9 % of the mucosal surface area covered with biofilms, compared with 1.9 % of the surface area removed from children with obstructive sleep apnea.

In the present work, *H. influenza* was the most common pathogen in adenoid specimens of group 1 patients both by culture (58 %) and by PCR (83 %). Both *S. pneumoniae* (42 %) and *H. influenza* (42 %) were the most common pathogens detected in adenoid samples of group 2 patients by culture, while by PCR *H. influenza* was the most common (71 %).

We analyzed the adenoid biofilms for the most common middle ear pathogens (*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *S. aureus*) employing multiplex-PCR in parallel with SEM to help clarify the relationship and give credence to what has been previously inferred from SEM data on the presence of these pathogens within the biofilm matrix. Ninety-six percent of adenoid samples had middle ear pathogens present, with majority (75 %) showing polymicrobial colonization. Eighty percent of middle ear fluid samples showed at least one of the following middle ear pathogens: *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *S. aureus*. Micro-organisms found in the adenoid biofilm detected by PCR are the same of the common middle ear pathogens. These findings support the hypothesis that there may be an association between adenoidal biofilm formation and COME. A positive correlation was found between results of bacterial biofilm visualized by SEM and bacteria detected and identified by PCR technique. On the other hand, no correlation was found between results of bacterial biofilm visualized by SEM and bacteria detected by culture.

Hoa et al. [15] found that dense adenoid biofilms may act as a reservoir for reinfection of the tubotympanum. They reported that all otitis prone children demonstrated biofilm with surface area greater than 85 % by SEM. All biofilms contained middle ear pathogens and were frequent in poly-microbial distributions: 4 of 6, 4 of 6 and 3 of 6 samples contained *H. influenzae*, *S. pneumoniae* and *M. catarrhalis*, respectively. Giebink [17] also confirmed that the three most commonly encountered pathogens playing a role in etio-pathogenesis of OME are *H. influenzae*, *S. pneumoniae* and *M. catarrhalis*.

In the present study, bacterial cultures of middle ear fluid were positive only in 32 % of samples, while PCR detected pathogens in 80 % of samples which were predominantly poly-microbial, with a total of 66 % of samples having multiple concurrent pathogens. *S. pneumoniae*

(44 %) was the most common pathogen detected in middle ear effusions of group 1 patients by culture, while by PCR *H. influenza* was the most common (82 %).

Microbiological culturing of potential pathogenic aerobic upper respiratory tract infection bacteria after sampling may not show growth, and in COME the bacteria are only cultured from middle ear effusions in approximately 25–40 % of the cases indicating sterile effusions in 60–75 % [17, 18]. Using the polymerase chain reaction (PCR) for potential pathogenic bacterial DNA, it has been demonstrated that DNA products from these bacteria are present in the effusions in, approximately, 80 % of cases despite the fact that the bacteria cannot be cultured [18–20]. Rayner et al. [21] performed a study involving 93 middle ear effusions from children with a median age of 17 months who underwent myringotomy or had ventilation tubes inserted for COME lasting longer than 3 months. They found that 31 % of the effusions were PCR-positive for *H. influenzae* but negative by culture for *H. influenzae*. All PCR positive samples were also positive for *H. influenzae* GAPDH-specific mRNA by RT-PCR. This suggested recent presence of metabolically active *H. influenza*.

The findings of bacterial culture in the present study regarding the type and frequency of bacteria in effusions from COME patients are close to those reported by Pereira et al. [22] where *H. influenza* was the most common, followed by *S. pneumoniae* and *M. catarrhalis*. Pereira et al. reported a higher prevalence of *H. influenzae* (39.1 %), followed by *S. pneumonia* (12.5 %) and *M. catarrhalis* (10.2 %).

These findings, together with the recurrent and chronic clinical nature of COME and other URTIs, have given rise to the theory that bacterial biofilm may be involved in the pathogenesis of these diseases.

Conclusion

The size of the adenoid is not the main determinant factor in OME pathogenesis but the degree of bacterial colonization is much more important. In other words, adenoids in COME may act as a reservoir of chronic infection rather than causing mechanical Eustachian obstruction. Higher grade biofilm formation was found in cases with middle ear effusion than those with adenoid hypertrophy only. These findings support the hypothesis that there would be an association between adenoidal biofilm formation and COME.

This study focused on the value of PCR in detecting pathogens in the adenoid and middle ear specimens although the bacterial culture would be negative. A positive correlation was found between results of bacterial biofilm visualized by SEM and bacteria detected and identified by PCR technique. On the other hand, no correlation was found between results of bacterial biofilm visualized by SEM and bacteria detected by culture.

Conflict of interest The authors have no conflict of interest, financial or otherwise with any organization

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