ARTICLE ORIGINAL / ORIGINAL ARTICLE

DO BACTERIA HAVE A ROLE IN THE GENESIS OF OTITIS MEDIA WITH EFFUSION? The Lebanese Experience

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Helou D, Rassi S. Do bacteria have a role in the genesis of otitis media with effusion? The Lebanese experience. J Med Liban 2018 ; 66 (3) : 144-149.

ABSTRACT • Background : Otitis media with effusion (OME) is one of the most frequent diseases in the pediatric population. The ongoing incrimination of bacteria in the pathogenesis of OME may have therapeutic implications. **Objective** : To describe the pathogens cultured from the middle ear effusion of Lebanese children with persistent OME. **Methods** : Seventeen consecutive children (29 ears) undergoing tympanostomy tube insertion for persistent OME unresponsive to medical treatment were enrolled between January 2011 and January 2012. The middle ear effusion was sent for bacteriological analysis and Gram staining. **Results** : No bacteria were isolated. A small sample, lack of polymerase chain reaction (PCR) use, bacterial biofilms and other possible causes for this discordance between our results and the literature are discussed. **Conclusion** : Our study shows no bacterial origin in the genesis of OME. Further larger studies using PCR are needed to determine OME microbiology in Lebanon.

Keywords : otitis media with effusion; bacteria; microbiology; culture; tympanostomy tube

Helou D, Rassi S. Implication des bactéries dans la genèse de l'otite moyenne avec épanchement : une expérience libanaise. J Med Liban 2018 ; 66 (3) : 144-149.

RÉSUMÉ • Contexte : L'otite moyenne avec épanchement (OME) est une des maladies les plus fréquentes dans la population pédiatrique. L'incrimination continue des bactéries dans l'étiopathogénie de l'OME peut avoir des implications thérapeutiques. **Objectif** : Décrire les agents pathogènes isolés, par culture, de l'épanchement de l'oreille moyenne d'enfants libanais ayant une OME. **Méthodes** : Dix-sept enfants (29 oreilles), subissant une myringotomie avec mise d'un aérateur transtympanique pour OME rebelle au traitement médical, entre janvier 2011 et janvier 2012, ont été enrôlés dans l'étude. Le liquide d'épanchement recueilli par myringotomie a été envoyé pour analyse bactériologique, et traité par coloration de Gram et culture sur gélose au sang et gélose chocolat. **Résultats** : Aucune bactérie n'a été isolée des 29 prélèvements d'oreille moyenne. Un faible effectif, l'absence d'utilisation de PCR, les biofilms bactériens et autres causes possibles de cette discordance entre nos résultats et ceux rapportés dans la littérature sont discutés. **Conclusion** : Notre étude ne montre pas d'origine bactériologique dans la genèse de l'OME. Des études de plus grand effectif utilisant la PCR sont nécessaires pour déterminer la microbiologie de l'OME au Liban.

Mots-clés : otite moyenne avec épanchement; bactéries; microbiologie; culture; aérateur transtympanique

INTRODUCTION

Otitis media with effusion (OME) is defined by the presence, behind an intact tympanic membrane, of a middle ear effusion (MEE) lasting more than three weeks, without any sign or symptom of acute inflammation [1,2]. 2.2 million episodes of OME are annually diagnosed in the United States, yielding a cost estimate of 4 billion US dollars per year, and making OME one of the most frequent and costly diseases in children [2]: > 50% of children will experience OME during their first year of life, > 60% by two 2 years and > 90% at some time before school age [3].

The pathogenesis of OME, yet controversial, is multifactorial, resulting from the interaction of various factors: bacterial infection, Eustachian tube dysfunction, allergy, sinusitis, adenoid hypertrophy, gastro-esophageal reflux and immunological factors [4]. Although OME can occur at any age, it is particularly frequent and pronounced before the age of 10, given the serious consequences any resulting hearing loss can have on the child development, especially linguistic. This hearing loss can be aggravated by the potential complications of OME, including adhesive otitis, tympanosclerosis, retraction pocket and cholesteatoma [5,6].

OME was previously considered as a strictly inflammatory process and the effusion strictly sterile, resulting solely from Eustachian tube dysfunction, until Senturia *et al*. identified, in 1958, bacteria in MEE cultures, redefining previously accepted concepts, and placing bacterial infection at a leading position among factors involved in the pathogenesis of OME [7]. Since then, evidence pointing towards a bacterial etiology of OME continue to accumulate, and the same risk factors and pathogenic agents have been incriminated in both OME and AOM (acute otitis media), the most frequently isolated bacteria being *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis* [2,8,9].

In Lebanon, Matar *et al*. [10] and Nasser *et al*. [11] have reported positive cultures in 21.3% and 32% of cases respectively, with a clear predominance of *H. influ-*

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enzae in the samples. However, even the appropriate use of antibiotics is a source of adverse effects, economic burden and resistance. It is therefore crucial to identify the causes of OME to establish an adequate treatment, the only currently recommended being myringotomy with tympanostomy tube (TT) insertion [2], an invasive treatment that is not without anesthetic and surgical risks. To this purpose, changes in the nature, prevalence and proportions of pathogens held responsible for OME, during the last decades, geographical variations of these agents, as well as the increasing resistance to antibiotics, require a continuous update of the epidemiology and resistance profile of the bacteria implicated in OME.

The objective of our study is to describe the pathogens isolated, by culture, from the MEE of Lebanese children undergoing myringotomy with TT insertion for OME unresponsive to medical treatment.

MATERIALS AND METHOD

This is a prospective study conducted in an otolaryngology clinic at Hôtel-Dieu de France university hospital, from January 2010 to January 2011. Seventeen children (29 ears), undergoing myringotomy with TT insertion for OME, were enrolled in the study, and their parents' informed consent was obtained.

Included were children younger than 10 years old, having an OME diagnosed by pneumatic otoscopy (thickened or hypomobile tympanic membrane) and tympanometry (flat type B tympanogram) and persisting over four months, with no amelioration or resolution of the effusion despite medical treatment (intranasal corticosteroids and/or antibiotics), and meeting the indications for TT insertion [2].

Exclusion criteria comprised patients who, at the time of surgery or during the previous weeks, suffered from AOM or other upper respiratory tract infection, those who used antibiotics in the month preceding the surgery, those who had a prior ear surgery (other than TT insertion) and, finally, those with a history of chronic suppurated otitis media (CSOM).

A detailed medical history was obtained, with a special emphasis on age, gender, duration of OME, presence of hearing loss or language delay, history of upper respiratory tract infection, AOM or allergy, previous antibiotic use, and OME risk factors, including vaccination with Prevnar (pneumococcal heptavalent vaccine PVC7).

The surgery was performed under a microscope. A myringotomy for TT insertion was made in the anteroinferior quadrant of the tympanic membrane. The MEE was collected with a suction catheter connected to a mucus extractor, by an aseptic technique, then sent for direct culture within 30 minutes of its collection.

The microbiological analysis consisted of a routine culture of the effusions on two types of agar: blood agar under anaerobic conditions with optochin susceptibility test for *S. pneumoniae*, and cooked blood agar (chocolate agar) in a CO_2-rich environment for *H. influenzae*. After addition of a Sheadler flora and Gram staining application, the specimens were incubated for 24 h at 37ºC then submitted to the API test for a more specific identification of the retrieved species.

RESULTS

From January 2011 to January 2012, 17 children diagnosed with OME met the inclusion criteria: 12 boys (70.6%) and 5 girls (29.4%). The mean age ± SD was 4 ± 2.2 years, the majority (76%) being between 2 and 5 years old. Environmental risk factors, background and pathologies found among children with OME are summarized in figures 1 and 2. Note that 14 children (82.4%) had received the Prevnar (PVC7).

The mean duration of OME before surgery was 10.8 months, and there was at least a 3-month lapse between the last AOM episode and TT insertion. Also, more than a month had passed between the last antibiotic use and the time of surgery. Treatments used in the context of OME (outside acute infectious episodes) are summarized in figure 3.

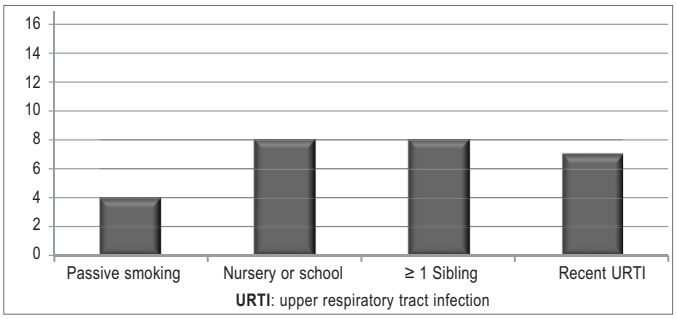

Figure 1. Environmental risk factors in OME children

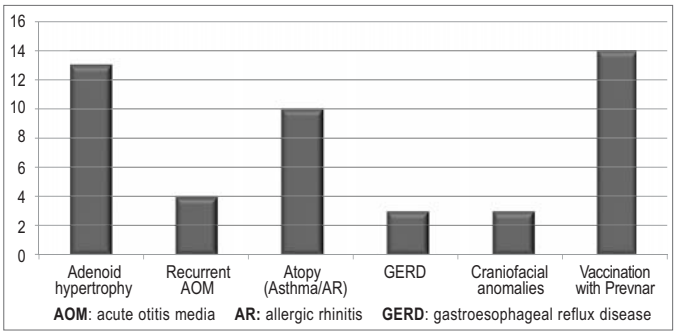

Figure 2. Background and pathologies among OME children

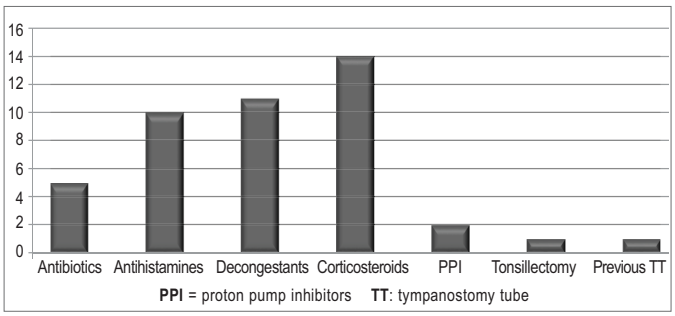

Figure 3. Treatments used in the context of OME

The diagnosis of OME was made following an episode of AOM in 30% of cases (n = 5), and symptoms suggestive of OME in 70% of cases (n = 12) (Figure 4). On otoscopy, the disease was bilateral in all 17 patients, invariably visualized as a tympanic membrane of decreased of absent mobility, but only 12 met the indications for TT insertion in both ears, the remaining five were operated on one ear only. They all had Jerjer type B tympanograms (i.e. flat with negative pressure). The only complication found was the presence of a retraction pocket in two children. During surgery, the effusion consistency was analyzed with the naked eye; it was serous in the vast majority of cases (13 children), and mucoid in four.

Of the 29 specimens collected from the 17 children, the culture was strictly negative in 23 specimens (79.3%), identified nonspecific organisms (polymorph) in five specimens (17.2%), and isolated a specific organism (rare colonies) in two specimens only (7%) (Figure 5). However, the two isolated organisms, *coagulase negative Staphylococcus* and *Candida albicans*, do not imply a positive culture, which will be discussed later.

In our 17 patients, in whom no bacteria were isolated, the absence of polymorphonuclear leukocytes and bacteria was confirmed by Gram staining of these negative samples.

DISCUSSION

Since Senturia *et al*. identified bacteria in MEE cultures in 1958 [7], various studies were conducted to update the bacteriology of OME, which was found to involve the same spectrum of microorganisms as acute effusions [2,6,12] with, however, a reversal of order between *Pneumococcus* and *Haemophilus* in most [5], *H. influenzae* being the most frequently isolated in OME [4,6,11]. The proportion of bacteria isolated by culture vary considerably depending on the time and geographical region, as summarized in table I. In particular, two similar Lebanese studies (1998 and 2011) found that *H. influenzae* was the most prevalent bacteria in the MEE [10,11,13]. Their results were concordant with those of the literature in term of global percentage of positive cultures.

Our study didn't identify any bacteria. The presence of *coagulase negative Staphylococcus* in a single sample would be due to intraoperative contamination, but we lack objective data to confirm it, given the absence of neutrophils in all samples and therefore the impossibility to compare the different results. We know, however, that the pathogenicity of such staphylococci is related to the presence of prosthetic material or foreign body, which wasn't the case in our study. Furthermore, at follow-up, this child did not experience any TT infec-

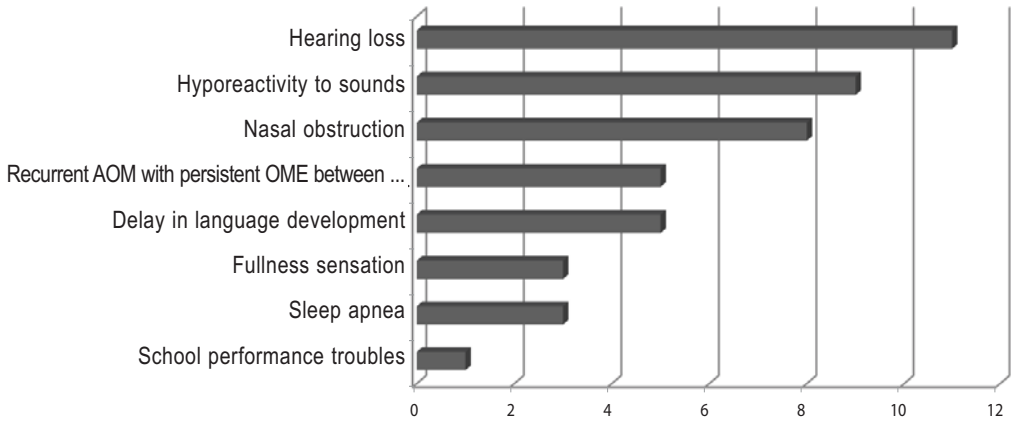

Figure 4

Circumstances of diagnosis Symptoms suggestive of otitis media with effusion (OME)

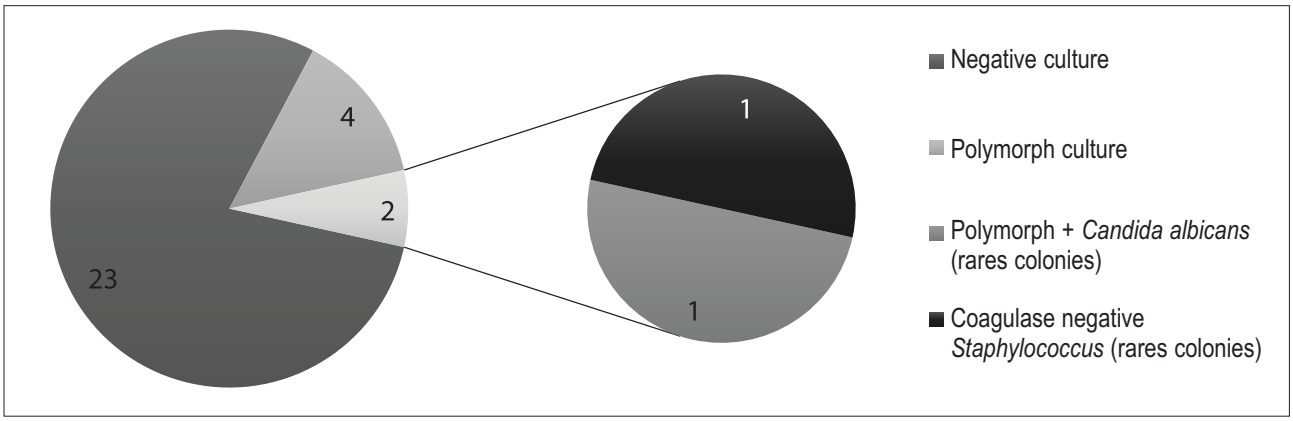

Figure 5 Results of middle ear effusion (MEE) bacterial analysis

TABLE I BACTERIAL PREVALENCE IN OTITIS MEDIA WITH EFFUSION – CULTURE OF SPECIMENS

Study		Culture + (%)	Bacteria (%)			
Author	Year		*H. influenzae*	*S. pneumoniae*	*M. catarrhalis*	Others
Hendolin *et al.* [35]	1999	NO	-	-	-	-
Sutton *et al.* [12]	2000	29.6	-	-	-	-
Leskinen *et al.* [22]	2002	33	15	11	7	-
Poetker *et al.* [6]	2004	37.8	16.2	4.1	10.1	7.4
Pereira *et al.* [34]	2004	25.1	10.2	6.3	3.9	5.5
Park *et al.* [36]	2004	14	7.9	1.4	0	4.7
Harimaya *et al.* [20]	2005	21.1	5.3	1.3	1.3	13.2
Martínez *et al.* [26]	2007	72.5	17.24	3.4	0	*
Ashhurst-Smith *et al.* [27]	2007	64	5	2.4	0	**
Jbara *et al.* [23]	2007	13.6	4.5	0	9	-
Güvenç *et al.* [21]	2008	NO	-	-	-	-
Jung *et al.* [37]	2009	34.6	0	0	0	***
Aydin *et al.* [18]	2012	NO	-	-	-	-
Matar *et al.* [10]	1998	21.3	90	0	20	-
Nasser *et al.* [11]	2011	32	62	26	12	-

* ***A. otitidis*** 48.27% ***S. aureus*** 7%. With use of a special culture for *A. otitidis*. ** ***A. otitidis*** 40% ***Corynebacterium ssp*** 30% ***S. aureus*** 5%.
*** **Coagulase negative *Staphylococcus*** 11.4% ***MRSA*** 4.1% ***P. aeruginosa*** 4.1% ***MSSA*** 3.8%

tion or other complication attributable to this kind of infection.

In Brazil, studies have reported various results, going from a negative culture in all specimens to 33.4% of positive results [14]. In fact, Saffer *et al*. growing the specimens of 94 ears of OME children on MacConkey media and blood agar, could not isolate any bacteria, except one *S. epidermidis* attributed to a contamination [14]. Moreover, the almost two times decrease in the percentage of cultures growing *S. pneumoniae* in most studies, compared to previous studies, may be due to the increasing prevalence of immunization against certain serotypes of *S. pneumoniae*. Indeed, the introduction of Prevnar (PVC7) was accompanied by a decrease in pneumococcus-associated AOM episodes [15,16], with a parallel increase in the prevalence of *H. influenzae* [17].

In our study, all children had received the Hib vaccine and 82.4% the PVC7. This high rate of vaccination in Lebanon could explain, if only in part, the negative cultures, by reducing colonization with capsulated *H. influenzae* as well as the involvement of *S. pneumoniae* in OME.

Moreover, the detection rate of the three main bacteria by *ordinary culture* is low in the literature, as depicted in table I, and up to nil in some studies [14,18].

Possible causes of these low rates and, by extension, of our negative cultures, are as follows:

1. Immunoglobulins and secretory lysozymes in the MEE would inhibit the proliferation of pathogenic bacteria, rendering the amount of bacteria lower than the culture detection limit (104 CFU/ml24h) and explaining why, compared to PCR, routine culture only provides a low bacterial identification rate (Tables I and II).

2. The presence of slow and hard to grow bacteria, such as *Alloiococcus otitidis*: Over the last decade, and since the pioneering publication by Faden and Dryja [19], a respectable number of studies have demonstrated the significant prevalence of a new pathogen, *Alloiococcus otitidis*, in OME, its frequent intracellular localization and the concomitant presence of inflammatory cells suggesting a pathogenic role for the bacteria [6,20-23,24,25-29]. However, as its slow growth requires special incubation conditions, its isolation by culture is very difficult, which explains why it wasn't described much earlier in the literature. In addition, its detection, by PCR, in external ear specimens of healthy individuals, questions its pathogenicity [25,30].

3. Pathogens in the MEE exist as biofilms, a kind of bacterial community where they are intertwined against each other, thereby reducing their metabolic activity and increasing their antibiotic resistance [31-33], possibly promoting the chronic inflammation and persistence of MEE. This explains, to some extent, the low or even negative isolation rates of bacteria by conventional culture. In fact, Hall Stoodley *et al*. have visualized for the first time in 2006, by electron microscopy, bacterial biofilms in 92% of middle ear mucosal specimens of children with OME or recurrent AOM [31].

4. A small sample size, which further decreases the probability of isolating bacteria: This is effectively the case in our study, being prospective, of predetermined duration (1 year), and limited to a single otolaryngology clinic.

5. Causes related to sampling and culture technique: a) Delay between the effusion collection, its arrival to the bacteriology laboratory and its incubation (lack of staff, slow relay; b) Imperfect culture technique (absence of a specific media for fastidious bacteria, lack of specific tests to confirm the suspected organism).

TABLE II BACTERIAL PREVALENCE IN OTITIS MEDIA WITH EFFUSION – PCR OF SPECIMENS

Study		PCR + (%)	Bacteria (%)			
Author	Year		*H. influenzae*	*S. pneumoniae*	*M. catarrhalis*	*A. otitidis*
Hendolin *et al.* [35]	1999	93.2	32.9	35.6	53.4	19.2
Sutton *et al.* [12]	2000	NO	-	-	-	-
Leskinen *et al.* [22]	2002	88	33	35	63	20
Poetker *et al.* [6]	2004	NO	-	-	-	-
Pereira *et al.* [34]	2004	57	39.1	12.5	10.2	X
Park *et al.* [36]	2004	36.7	29.1	4.7	10.8	X
Harimaya *et al.* [20]	2005	69.7	11.8	7.9	6.6	60.5
Martínez *et al.* [26]	2007	NO	-	-	-	-
Ashhurst-Smit *et al.* [27]	2007	NO	-	-	-	-
Jbara *et al.* [23]	2007	27.3	13.6	9	18.1	X
Güvenç *et al.* [21]	2008	45.6	15	2	2	26
Jung *et al.* [37]	2009	NO	-	-	-	-
Kim *et al.* [38]	2015	70.4	31.4	50	16.6	X
Aydin *et al.* [18]	2012	41.2	2.9	8.8	8.8	35
Matar *et al.* [10]	1998	74.5	94.3	8.6	28.6	5.7
Nasser *et al.* [11]	2011	NO	-	-	-	-

* **PCR**: polymerase chain reaction

Moreover, studies have shown much higher bacteria identification rates when using more sensitive techniques, particularly PCR; these rates ranging, in the case of OME, between 27% and 93%, with a mean of 61.5% (Table II). When comparing the results of direct culture and PCR, Pereira *et al*. have found that all positive culture samples were also positive by PCR, for the same pathogen; and the difference between positive MEE by culture (19.6%) and by PCR (57%) was statistically significant, PCR having increased by at least 192% the number of positive MEE for each bacteria incriminated in OME, when compared to direct culture [34]. Similarly, PCR detection rates ranging from 77.3% to 94.5% have been reported in cases where no bacteria was isolated by routine culture [18,23]. These results bring to light one of the biggest limitations of our study, that is the lack of PCR analysis of the MEE.

In summary, our results are largely different from those reported in the literature: they show no bacterial origin in the genesis of OME. These differences can be attributed to variations in inclusion criteria, as well as methodological, technical, geographical and microbiological variations. The main limitation of our study, besides the small sample size, is that it has relied solely on culture-based data of the MEE.

We shall then regard it as a preliminary study, with the intention to pursue it with a larger number of subjects and with the use of more specific techniques, particularly PCR, potentially leading to higher bacterial isolation rates. This would provide a more accurate representation of the role of bacteria in the pathogenesis of OME in order to develop appropriate preventive and therapeutic strategies, whose ultimate goal is to limit OME complications, mainly hearing loss and speech disorders, a very common problem with major economic burden.

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