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Bacteriology of Seromucous Otitis Effusion Fluid in Children

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Abstract

Introduction: The involvement of bacteria in seromucous otitis (SMO) has been widely reported, with various methods available to identify effusion pathogens, ranging from traditional culture to polymerase chain reaction (PCR). The objective of our study is to highlight the different bacteria present in the fluid from the middle ear of children with SMO and evaluate the sensitivity and resistance of bacterial strains found to commonly prescribed antibiotics.

Material and Methods: This is a prospective, descriptive study, targeting a group of 80 children (160 ears), aged between 04 and 14 years old and presenting confirmed SMO. This study spanned 24 months, from January 2020 to January 2022. In all our patients, the decision to install transtympanic aerators alone or in combination with an adenoidectomy was made. All of our patients (80) had middle ear effusion fluid sampled with direct examination and culture, while only 30 had PCR (PCR kits not available during this period) for Streptococcus pneumoniae, Haemophilus influenzae and Helicobacter pylori.

Results: The rate of positive cultures calculated based on 160 ears or samples taken bilaterally was 21.25%, with a majority of Staphylococcus aureus (35.29%). Streptococcus pneumoniae is only found in 8.82%, Haemophilus influenzae in 2.94% and Moraxella catarrhalis in 5.88%. PCR of the liquid found Haemophilus influenzae in 26.66% of cases, Streptococcus pneumoniae in 68.33% and Helicobacter pylori at 0%.

Discussion: Our positivity rate for these cultures was quite close to those in the literature, between 13 and 33%. However, for the agents found, we noted a preponderance of Staphylococcus aureus (35.3%), while in the other studies, Haemophilus influenzae was prevalent, 62.5% for Shareef et al., Streptococcus pneumoniae, 16.7% for Elmagd et al. We see that, in the majority of studies, PCR positivity rates were high. The detection rate of culture is multiplied by 2, or even by 3 in PCR, which confirms its strong superiority in detecting non-cultivable germs.

Conclusion: Our results clearly show the presence of multiple bacteria in the effusion fluid of seromucous otitis, but only a larger study in number and, if possible, with a control group, will be able to determine the microbiome of the ears of affected children. of this very common pathology which constitutes the starting point of all chronic ear pathologies.

Keywords: seromucosal otitis; effusion fluid; bacteria

Introduction

Seromucous otitis is a complex, multifactorial pathology where different processes are self-perpetuating to create the vicious circle of intra-tympanic effusion [1]. It is an extremely common pathology since almost all preschool children are affected by it at some point in their development. It is estimated that 80% of children have had an episode of SMO before the age of 10 (commonly before the age of 3), with 2 peaks, the first at 2 years and the second at 5 years [2,3]. It presents a public health problem in several countries and is a very common reason for taking antibiotics [4]. For a long time, the SMO effusion fluid was considered sterile, until 1958, when Senturia and his team discovered the presence of bacteria in this fluid [5].

Since then, the involvement of bacteria in SMO has been widely reported, with various methods available to identify effusion pathogens, ranging from traditional culture to polymerase chain reaction (PCR) [6,7]. Streptococcus pneumoniae and Haemophilus influenzae are the most commonly isolated pathogens. However, other germs have been highlighted in more recent studies [8,9]. The identification of the main risk factors involved and the main bacteria causing seromucous otitis in each population is essential and their knowledge will perhaps help us to establish a prevention strategy and appropriate treatments, which can reduce the incidence of this disease.

Material and Methods

This is a prospective, descriptive study targeting a group of 80 children (160 ears) with confirmed seromucous otitis, associated or not with hypertrophy of the adenoids. This study spanned 24 months, from

January 2020 to January 14, 2022. This study took place at the level of the ENT and CCF department of the Regional Military University Hospital of Constantine and the ENT and CCF department of the Benbadis University Hospital Center of Constantine. The bacteriological study (Cultures and PCR) was carried out at the microbiology unit of the central laboratory of the Regional Military University Hospital of Constantine. Its objectives were the study of the bacteriological profile of the SMO fluid and the evaluation of the sensitivity and resistance of the bacterial strains found to commonly prescribed antibiotics.

Inclusion Criteria

Children aged between 04 and 14 years, presenting persistent SMO after 03 months of monitoring, with or without hypertrophy of the adenoids and in whom the decision to install ATT alone or in combination with an adenoidectomy, was asked.

Exclusion Criteria

Children with craniofacial malformations, Down syndrome (trisomy 21), Crouzon syndrome or cleft palates, or a cavum tumor.

All ear samples (n=160) were sent urgently (< 15 min) to the microbiology laboratory and were studied by traditional culture, while only 60 samples (30 patients) were frozen for PCR study.

Results

The rate of positive cultures calculated in relation to 160 ears or samples taken bilaterally was 21.25% (34 positive samples out of 160 taken) (Table 1).

Table 1: Culture of middle ear fluid on both sides.

	Right Ear Culture		Left Ear Culture		Total	
	Frequency	%	Frequency	%	Frequency	%
-	62	77,5%	64	80%	126	78,75%
+	18	22,5%	16	20%	34	21,25%
Total	80	100,00%	80	100,00%	160	100,00%

Among these 34 positive samples, a majority of Staphylococcus aureus was found with 12 positive cases (35.29%), Streptococcus pneumoniae was found in only 3 cases (8.82%), Haemophilus influenzae in only 1 case (2 .94%) and Moraxella catarrhalis in 2 cases (5.88%). The Streptococcus genus was also predominant with 14 cases (32.35%) and Pseudomonas aeruginosa and Escherichia coli were detected in 1 case each (Table 2).

Table 2: Results of effusion fluid cultures.

	Right Eat		Left Ear		Total	
	Frequency	%	Frequency	%	frequency	%
Enterobacter Cloacae	1	5,55%	-	-	1	2,94%
Escherichia Coli	1	5,55%	1	6,25%	2	5,88%
Moraxella Catarrhalis	-	-	2	12,5%	2	5,88%
Haemophilus Influenzae	1	5,55%	-	-	1	2,94%
Pseudomonas Aeruginosa	1	5,55%	-	-	1	2,94%
Staphylococcus Aureus	5	27,77%	7	43,75%	12	35,29%
Staphylocoque A Coagulase Negative	-	-	1	6,25%	1	2,94%
Group C Streptococcus	-	-	2	12,5%	2	5,88%
Group G Streptococcus	1	5,55%	-	-	1	2,94%
Non-Groupable Streptococcus	5	27,77%	3	18,75	8	23,52%
Streptococcal Pneumonia	3	16,66%	-	-	3	8,82%
Total	**18**	100,00%	**16**	100,00%	**34**	100,00%

We also noticed that the species Alloiococcus otitidis and Helicobacter pylori were not detected at all in our samples, this may be due to a technical defect or an insufficient incubation period. For the PCR study of the liquid, the detection rate of Haemophilus influenzae in the 60 OM samples is calculated at 26.66%, while that of Streptococcus pneumoniae is estimated at 68.33%. Helicobacter pylori is also completely absent there (Table 3).

Table 3: PCR results of effusion fluid in 30 children (60 ears).

	Liquid PCR Right + Left Ear	
Haemophilus Influenzae	Frequency	%
-	44	73,33%
+	16	**26,66%**
Total	60	100,00%
Streptococcus Pneumoniae	Frequency	%
-	19	31,66%
+	41	**68,33%**
Total	60	100,00%
Helicobacter Pylori	Frequency	%
-	60	100,00%
+	00	**00%**
Total	60	100,00%

Table 4 summarizes the positivity or detection rates of the two main germs implicated in the etiopathogenesis of OSM by comparing culture versus PCR.

Table 4: comparison of culture versus PCR detection rates of the 3 main germs.

		Culture		PCR	
		Frequency	%	Frequency	%
Haemophilus Influenzae	**Middle Ear Fluid**	1/34	**2,94%**	16/30	**26,66%**
Streptococcus Pneumoniae		3/34	**8,82%**	41/30	**68,33%**

We note that PCR is more efficient in detecting the incriminated germs. Finally, we studied the sensitivity of these bacteria to amoxicillin and amoxicillin-clavulanic acid (Table 5) and we note that the majority of bacteria are sensitive to amoxicillin and even more to amoxicillin. -clavulanic acid, especially pneumococcus and Hib. The ineffectiveness of antibiotics suggests various theories, especially the non-adequate diffusion of the antibiotic inside the middle ear fluid.

Table 5: sensitivity of bacteria in effusion fluids to amoxicillin and amoxicillin-clavulanic acid.

Bacteria Found in Effusion Fluids	Penicillin, Amoxicillin, Ampicillin Resistance				Amoxicillin-Clavulanic Acid Resistance			
	Yes		No		Yes		No	
	Frequency	%	Frequency	%	Frequency	%	Frequency	%
Enterobacter Cloacae	1	6,66%	-	-	-	-	1	3,33%
Escherichia Coli	2	13,33%	-	-	2	50%	-	-
Moraxella Catarrhalis	2	13,33%	-	-	2	50%	-	-
Haemophilus Influenzae	1	6,66%	-	-	-	-	1	3,33%
Pseudomonas Aeruginosa	1	6,66%	-	-	-	-	1	3,33%
Staphylococcus Aureus	5	33,3%	7	36,8%	-	-	12	40%
Staphylocoque A Coagulase Negative	1	6,66%	-	-	-	-	1	3,33%
Group C Streptococcus	2	13,33%	-	-	-	-	2	6,66%
Group G Streptococcus	-	-	1	11,11%	-	-	1	3,33%
Non-Groupable Streptococcus	-	-	8	42,10%	-	-	8	26,66%
Streptococcus pneumonia	-	-	3	15,78%	-	-	3	10%
Total	15	100%	19	100%	4	100%	30	100%

Discussion

SMO is an extremely common condition, which in the vast majority of cases affects children under the age of 10. Its frequency is difficult to assess and differently described in world literature. It is the most common cause of deafness in children [10]. This

pathology was classically considered an aseptic disease of the middle ear until 1958, when Senturia et al discovered the presence of bacteria in the effusion fluid [5]. Many authors have demonstrated germs by simple direct examination, after culture of the effusion fluid and also by PCR. Thus, the study carried out by Uzeyir Gok [11] and al in 2001, made it possible to confirm the presence of bacterial agents by PCR, but also by culture of the liquid; on a sample of 37 samples taken from 20 patients aged 4 to 14 years, the aerobic culture was positive in 23.4% of cases. PCR found traces of DNA from ENT germs (Haemophilus influenzae, Moraxella catarrhalis and Streptococcus pneumoniae) in 94.5% of samples. Our culture positivity rate was enough close to those in the literature, between 13 and 33% (table 6), on the other hand for the agents found, we noted a preponderance of staphylococcus golden (35.3%), while in the other works Haemophilus influenzae was prevalent, 62.5% for Shareef and col [8], or Streptococcus pneumoniae, 16.7% for Elmagd and col [12].

Table 6: bacteriological study by culture of OSM fluid (Hi: Haemophilus influenzae, Sp: Streptococcus pneumoniae, Mc: Moraxella catarrhalis, Sau: Staphylococcus aureus, Sng: Non-groupable Streptococcus, Pa: Pseudomonas aeruginosa).

Culture	Ear Fluid	
	Rate Positivity	Main Germs Found
Yoo and col. 2018 [13]	13,3%	Hi:17,4%, Sp: 6,5%, Mc: 6,5%, Sau:2,17%, Scn: 17,4%, Pa: 2,17%
Shareef and col. 2018 [8]	33%	Hi:62,5%, Sp: 25%, Mc: 0, Sau: 12,5%
Elmagd and col. 2019 [12]	30%	Hi:6.7%, Sp: 16.7%, Mc: 6.7%
Our Series	21,25%	Hi:2,9%, Sp: 8,8%, Mc: 5,8%, Sau: 35,3%, Sng: 23,5%, Pa: 2,9%

SMO fluid culture positivity rates are low and many authors [11,14], explain this by the existence of bacteria in the form of biofilms, not detectable by traditional culture. These same authors confirm the superiority of PCR for the detection of pathogens in the effusion fluid. In our study as well as in the majority of international series, the detection rate of culture is multiplied by 2, or even by 3 in PCR, which confirms its strong superiority in detecting non-cultivable germs (Table 7).

	Ear Fluid	
	Culture	PCR
Gok and Col. 2001 [11]	24.3%	94,5%
Takada and Col. 2003 [15]	22,5%	44,5
Saki and Col. 2009 [14]	24.5%	36.7%
Aly and Col. 2012 [16]	34.9%	95.2%
Mills and Col. 2015 [17]	24%	92%
Sabz and Col. 2020 [18]	35%	65%
Our Series	21,25%	94,9%

Antibiotic resistance has been studied by so many authors [8,9,19,20] who tried to look for a cause of the ineffectiveness of antibiotic treatment in SMO. It is above all the presence of biofilms which would be responsible, which has led certain authors [21], in a fairly recent work (2021), to propose a combination of antibiotics; ceftazidime + amikacin and ceftazidime + ciprofloxacin, which according to the authors offer better synergistic action on the main otopathogens. In our study, the majority of amoxicillin-resistant bacteria are sensitive to the amoxicillin-clavulanic acid combination and our results are quite similar to those of Shareef and col [8] (Table 8).

Table 8: bacterial sensitivity and resistance to antibiotics (amoxicillin, amoxicillin-clavulanic acid).

	Bacteria	Sensitivity to Amoxicillin		Sensitivity to Amoxicillin-Clavulanic Acid	
		Yes	No	Yes	No
Shareef And Col. 2018 [8]	Sp	//	//	83,3%	16,7%
	Hi	//	//	93,3%	6,7%
	Sau	//	//	100%	0
Our Series	Sp	100%	0	**100%**	0
	Hi	100%	0	**100%**	0
	Mc	0	100%	0	100%
	Sau	**58,33%**	41,66%	**100%**	0

Conclusion

Sero-mucosal otitis (SMO) remains an extremely common pediatric condition, constituting the main cause of hearing loss in children and whose etiopathogenesis is chronic inflammation in the body maintained, among other things, by a chronic infection with low bacterial inoculum.

Our results clearly show the presence of bacteria in the fluid from the middle ear of children with SMO and only a larger study, possibly with a control group, will be able to determine with more precision the microbiome of the middle ear. middle ear and this may help to establish a preventive or even therapeutic plan.

Conflicts of Interest

The authors declare that they have no conflict of interest regarding the publication of this article.

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