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ORIGINAL ARTICLE

Microbiology of Middle Ear Effusions in 60 Children Undergoing Tympanostomy Tube Placement*

Isabel de Miguel Martínez, Ángel Ramos Macías, Silvia Borkosky Barreiros, Juan L. Muñoz-Bellido

Servicio de Microbiología. Complejo Hospitalario Universitario Insular - MaternoInfantil de Gran Canaria. Las Palmas de Gran Canaria. Universidad de Las Palmas. (IIM)
Servicio de Otorrinolaringología y Patología Cérvico Facial. Complejo Hospitalario Universitario Insular - MaternoInfantil de Gran Canaria. Las Palmas de Gran Canaria. Universidad de Las Palmas. (ARM; SBB)
Departamento de Medicina Preventiva, Salud Pública y Microbiología Médica. Universidad de Salamanca. Salamanca and Departamento de Microbiologia, Hospital Universitario de Salamanca. Salamanca. (SLMB) SPAIN.

Objective: To analize the bacterial involvement in otitis media with effusion (OME).

Materials and Methods: We obtained middle ear effusion samples, under surgical control, from 60 children with OME. Samples underwent microbiological study by using microbiologic culture of the middle ear effusion (MEE)

Results: Bacteriological cultures were positive in 75% of OME. Alloiococcus otitidis was the bacteria most frequently isolated (36%) as well as Haemophilus influenzae non-serotype B (23,3%%)

Conclusions: Though OME is usually considered a non-infectious disease, bacteria are found from a number of OME samples, and his role as etiological agent should be considered.

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Otitis media with effusion (OME) is defined as the presence of a liquid collection into the middle ear, frequently after an acute otitis media episode (AOM), with no signs or symptoms of infection. OME is very frequent in children. Sixty percent of children have suffered at least one episode by two years of age[1, 2]. The etiology of OME remains unclear, but the evidence of involvement of some microorganisms is growing. Microorganisms usually involved in AOM, such as Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis, group A streptococci and Staphylococcus aureus, are also isolated in OME, though less frequently than in AOM, and some studies describe Alloiococcus otitidis as the most prevalent microorganism in OME effusion [3-5].

The aim of this study was to investigate bacterial involvement in OME, and to demonstrate the possibility of finding Alloiococcus otitidis with a high frequency when adequate sampling and culture methods are used.

Materials and Methods

The study included 60 children with ages between 1 and 10 years with OME, underlying tympanostomy tube insertion at the Department of Otolaryngology, between January 2004 and May 2007.

Only patients with non-purulent middle ear effusion evaluated weekly for >1 month, in the otology section of our department, with intact tympanic membrane, and no symptoms or signs of AOM or upper respiratory tract infection before surgery were included. All these cases were diagnosis of OME in our external consultant area, and had all the criteria established before for a control period of more than 6 months, before they were sent to be included in this study.

Corresponding address:
Isabel de Miguel Martínez
Servicio de Microbiología. Hospital Universitario Insular de Gran Canaria.
Avda/ Marítima s/n CP: 35016. Las Palmas de Gran Canaria. SPAIN.
E-mail: imigmar@gobiernodecanarias.org

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Middle ear effusion samples were obtained by aspiration during the tube insertion surgery. Parents were previously informed, and the surgery was performed once they had consent it. When OME was presented bilaterally (31 patients), samples were obtained from both ears.

Data were recorded concerning gender, age, date of beginning of AOM, history of allergies, upper respiratory tract infections, other otitis episodes, unilateral or bilateral.hearing loss.

Samples were obtained from the middle ear during the surgical procedure by needle aspiration. Specimens were placed into a sterile syringe without transport medium, and kept at room temperature. Samples were inoculated onto appropriate culture media as soon as possible and always by 90 minutes since they were obtained.

Gram staining was done to all samples, which were cultured within 90 minutes of sampling onto blood agar, chocolate agar, McConkey agar, Sabouraud agar and Brucella agar plates and in BHI broth (bioMérieux, Le Balme Les Grottes, France). All the culture media were incubated at 35ºC. Sabouraud and McConkey agar plates and BHI broth were incubated under aerobic atmosphere, blood and chocolate agar plates under 5% CO_2 and Brucella agar plates under anaerobic atmosphere.

Colonies growing were identified by using conventional microbiological methods: API 20 Strep (bioMerieux, Le Balme Les Grottes, France), HNID panel Microscan (Dade Behring, Deerfield Illinois, USA), Neisseria 4H (Bio Rad, Hercules, CA, USA), Wider (Francisco Soria Melguizo, Madrid, Spain). Antibiotic susceptibility was determined by agar diffusion, microdilution (Wider, Francisco Soria Melguizo, Madrid, Spain) or E-test (AB biodisk, Solna, Sweden) according manufacturers instructions and according CLSI guidelines when available.

Results

Among 60 children with OME, 45 (75%) were male and 15 (25%) female. The mean age was 2,6 years (range: 1-10 years). The most frequent clinical antecedents were adenoidal hypertrophy (96%) and AOM (90%). Fifteen percent of patients had suffered a upper respiratory tract cold recently when OME symptoms began. Ten percent of children presented allergy to acarus. Concerning hearing levels, 50% presented a regular audiometry at surgery, and 50% presented a mild to moderate transmission hearing loss. In all cases a Jerger type C or D was found in impedansmetry.

In whole, we obtained 91 samples (62 corresponding to 31 patients from which we obtained bilateral samples, and 29 from patients with unilateral disease). Bacteria were cultured in 45 patients (75%) (Table 1). In all the 31 patients with bilateral OME, the results obtained in both ears were identical (12 negative in both ears, 19 positive, with the same microorganism in both ears).

No mixed cultures were obtained in any case.

Gram staining did not show leukocytes in any case, whatever the result of culture was, but showed microorganisms in 95% of culture positive samples, and in no negative samples.

The most frequently isolated microorganism isolated was *A. otitidis*, found in 22 patients (36,6%), followed by *H. influenzae* (14 cases, 23,3%) and *S. aureus* (6 patients, 10%), Table 1.

Table 1. Frequency of isolation of bacteria from OME

	Patients (%)
A.otitidis	22 (36.7)
H.influenzae	14 (23.3)
S.aureus	6 (10)
S.pneumoniae	2 (3.3)
R.picketti	1 (1.7)
Negative	15 (25)

Initial growth of A. otitidis isolates occurred after 72-96 hours at 37 ºC degrees on BHI broth, blood agar, chocolate agar and brucella agar. There was no growth at 45 ºC. The isolates were gram-positive cocci and catalase test positive. Results for most important identification biochemical tests are presented in Table 2.

Table 2. Characteristics of Alloioicoccus otitidis

Reactions/enzymes	Alloiococcus otitidis
Pyrrolidonyl arylamidase hydrolysis	positive (100%)
Hippurate hydrolysis	positive (100%)
Nitrate reduction	negative (100%)
β-galactosidase	positive(100%)
Alkaline phosphatase	positive(100%)
Leucinamine arylamidase	positive (90%)
Fermentation of carbohydrates	negative (100%)
Growth at 45ºC	negative (100%)

The antibiotic susceptibility of the microorganisms isolated appears in Table 3. *A. otitidis* demonstrated susceptibility to beta-lactam, and were resistant to trimethoprim-sulfamethoxazole and erythromycin. *S. aureus* strains were penicilinase producers and all of them methicillin susceptible. Thirty-six percent of the *Hemophilus influenza* isolates were beta-lactamase producers. *S. pneumoniae* presented an intermediate resistance against penicillin CMI=0.125 μg/ml, and resisted to erythromycin and clindamycin (constitutive MLS resistance phenotype)

Discussion

Microbiological studies are not performed usually in OME, because of difficulties for obtaining good samples and because antibiotic treatment is not indicated.[2] Nevertheless, previous studies show that bacteria are detected by culture or by PCR, in 85-88% of middle ear effusions[5,6], and the isolation of some microorganisms such as A. otitidis correlates with the persistence of effusion. [6, 7]

The presence of antibodies countering these bacteria in the middle ear has also been described [8].

Previous studies have shown that molecular methods are more sensitive in these cases, since detect microorganisms in up to 88% of samples, vs. 45% of conventional culture[6]. This difference is more pronounced for A. otitidis, since these authors find it in 20-45% of samples by molecular methods[5,6], but do not isolate it in any case when they use culture methods[6].

In this study, and using only conventional culture methods, we have found microorganisms in a proportion of MEEs moderately lower than other studies have found by molecular methods (75% vs. 88%). As a matter of fact, we have cultured *A. otitidis* in 36% of MEEs, while other studies do not find it by culture methods and find it by molecular methods in 20-45%[5,6]. Nevertheless, our study shows that, if we have a good sample, and process it soon by correct methods, *A. otitidis* can be found in a high number of MEEs even with conventional culture if we do not have molecular methodology available.

As other authors have also found, *A. otitidis* was the microorgansim most frequently isolated (36.7%), followed by *H. influenzae* (23.3%). In other studies, *H. influenzae* has been found in 15% by culture and in 33-37% by PCR6. *S. pneumoniae* and *M. catarrhalis*, are frequently found alone or associated to other microorganisms in OEE (7-11% by culture, 21-35% for pneumococci and 18-63% for *M. catarrhalis* by PCR). In this study, pneumococci were found in a very low proportion (3.3%) and *M. catarrhalis* were not found at all.

Results obtained suggest that the culture of surgical samples from middle ear can be useful for the

Table 3. Antibiotic susceptibility (% susceptible) of bacteria isolated from OME.

	Pen	Amp	Amc	Cfx	Cft	Er	Clarit	Cda	Sxt
S.aureus	0	0	100	100	100	66	100	60	100
H.influenzae	—	64	100	100	100	—	100	—	100
S.pneumoniae	0*	—	—	100	100	0	0	0	0
A. otitidis	100	100	–	–	100	0	0	–	0
R.picketti	—	0	0	0	0	0	0	0	100

Pen: penicillin; Amp: ampicillin; Cfx: Cefotaxime; Cft: Ceftriaxone; Er: erythromycin; Carit: Clarithromycin; Cda: Clindamycin; Sxt: Co-trimoxazole; Cip: Ciprofloxacin; R: resistant; S: susceptible. I:intermediate

**Both isolates were intermediate to penicillin*

diagnosis of bacteria involved in OME when adequate sample and processing are used. Though molecular techniques will be always more sensitive, they are not commercially available for these microorganisms, and reproducibility of in home PCR can be low. [9]

Moreover, the possibility of recovery of the whole microorganisms by conventional methods allows to perform susceptibility tests to control the possibility of emergence and diffusion of antibiotic resistance among these microorganisms.

Though molecular methods are probable the future for the diagnosis of the involvement of these microorganisms in OME, conventional culture procedures can lead to good results, mainly in the case of A. otitidis, when samples are correctly obtained and processed. Therefore, these procedures should not be undervalued, because they can give valuable information concerning etiology of OME, mainly when molecular technology is not available.[10]

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