Bacteriology of Otitis Media with Effusion in Children and Their Sensitivity to Antibi - otics in Erbil City

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Abstract

Background and objectives: Otitis media with effusion is considered one of the major causes of hearing loss in children. The pathogenesis remains unclear, an interaction between bacterial infection, Eustachian tube dysfunction, allergy and immunologic factors is proposed. Newer theories suggest a bacterial induced inflammatory reaction is the main aetiology. The aim is to analyse the bacterial involvement in otitis media with effusion and to determine the bacterial susceptibility to antimicrobial agents used in Erbil. **Methods:** Middle ear effusion fluid was collected from 40 children aged 2-12 years with secretory otitis media and scheduled to undergo tympanostomy tube placement. The samples were inoculated into culture media and stained by a gram stain. The bacterial susceptibility to (amoxicillin clavulanic acid, cefixime, and azithromycin) was tested. **Results:** Bacterial culture was positive in 24 out of 72 ears (33.3%). The most frequent isolated bacteria were Haemophilus influenzae (62.5%), while Streptococcus pneumonia and Staphylococcus aureus constituted (25%) and (12.5%) respectively. Sensitivity to amoxicillin clavulanic acid was 93.3% for H. influenzae, 83.3% for S. pneumoniae, and none for S. aureus, While 66.7% of S. aureus, 16.7% of S. pneumoniae, and none of the H. influenza were sensitive to azithromycin. Most of the cultured bacteria were resistant to cefixime.

Conclusions: The prevalence of bacteria in otitis media with effusion in our study is comparable to what is reported in the literature, with Haemophilus. influenzae being the most frequent microorganism cultured. The finding of the high percentage of bacterial resistance to Cefixime and azithromycin suggests that the injudicious overuse of antibiotic is leading to the increase in bacterial resistance to antibiotics.

Keywords: Tympanic membrane; Middle ear effusion.

Introduction

Otitis media with effusion (OME) is defined as the presence of fluid (serous or mucoid) in the middle ear cleft without the symptoms and signs of infection like otalgia and pyrexia. Otitis media with effusion is the leading cause of hearing loss in children. It's estimated that 80% of children have had an episode of OME by the age of 10 years (usually before the age of 3 years)[1], with 2 peaks, first at the age of 2 years and the second at the age of 5. Children diagnosed in winter are susceptible as twice as children diagnosed in summer[2]. In children with an episode of acute otitis media (AOM), it has been found that about 45% have persistent effusion after 1 month, and 10% after 3 months[3].

Two main theories exist for the pathogenesis of OME; the classic theory proposes that Eustachian tube dysfunction plays a key role, the "ex-vacuo" theory postulated by Politzer states that the chronic middle ear negative pressure leads to the accumulation of middle ear fluid[1,3,4]. Newer theories describe a bacterial-induced inflammatory reaction of the middle ear mucosa, this leads to the release of inflammatory mediators, which cause secretion of a mucin-rich effusion by up-regulating mucin genes[5]. Prolonged stimulation of the inflammatory response and poor mucociliary clearance lead to persistence of the middle ear fluid, giving rise to the clinical presentation of OME. Until 1985, when Senturia et al. found bacteria in the middle ear effusion fluid, OME was considered an inflammatory condition, the effusion fluid was presumed sterile[6], in about 40% of middle ear effusion, cultures were positive for bacteria including Haemophilus Influenza, Streptococcus Pneumoniae, Moraxella Catarrhalis, group A streptococci, Staphylococcus aureus, and others[1,2,5,7].In other studies, bacterial DNA was found in approximately 80% of middle ear effusion(MEE) by polymerase chain reaction (PCR) studies[1,4,5,7]. This discrepancy is explained by the presence of biofilms. Biofilms as defined by Hall-Stoodley, are "surface-associated microbial communities surrounded by an extracellular polymeric substance matrix" which are notoriously resistant to host immune responses and antimicrobial therapy[4,7]. Also, bacteria in biofilms are difficult to culture.

In 1989 Faden and Dryja recovered Alloiococcus Otitidis from MEE of children with recurrent OME[8]. This bacterium is a gram-positive slow growing organism that requires special media for growth[8], and its detection rate was more frequent than the other middle ear pathogens by multiplex PCR[9,10]. It was proposed that the above bacteria are part of the normal flora of the middle ear cleft. However, many previous studies have dem¬onstrated that these bacteria have immune-stimulatory ability thus could not be part of the normal flora of the middle ear[10,11].

Most episodes of OME resolve spontaneously within 3 months, but approximately 30% - 40% of children have repeated OME attacks, and 5% - 10% of episodes last 1year[12]. Middle ear fluid from OME results in decreased tympanic membrane mobility and serves as a barrier to sound conduction leading to hearing loss, and may be associated with balance problems, poor school performance, behavioural problems, ear discomfort, recurrent

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AOM, reduced quality of life QOL, or may cause structural damage to the tympanic membrane and middle ear requiring surgical intervention[1,12]. Diagnosis of OME starts with microscopic examination of the tympanic membrane that shows a retracted intact eardrum with varying colours (clear, yellowish, or bluish) depending on the viscosity[1,2]. The use of pneumatic otoscopy for the diagnosis of OME is strongly recommended by the American Academy of Otolaryngology-Head and Neck Surgery[12], which showed a decrease or absence of the tympanic membrane movement. The sensitivity of pneumatic otoscopy performed by trained specialists ranges from 85 -93 %[2]. The most common reference standard test used for the diagnosis of OME is tympanometry, with type B (flat) tympanogram most frequently associated with OME having the sensitivity between 56-75% and specificity of 50-98%[2,12,13]. An age appropriate hearing test is recommended for children with bilateral OME that persist for more than 3 months[12,13]. A pure tone audiometry is possible to perform for children older than 4 years, the observation of a conductive hearing loss between 10-50 dB is expected, but most children present with hearing loss between20-30 dB[1,2]. The natural history of OME is spontaneous resolution; the resolution rate reaches approximately 90% by 9 months[2]. So, for children who are not at risk of speech, language, and learning difficulties watchful waiting is appropriate[1,2,7,12]. Antibiotic use in cases of OME is controversial, balancing the potential benefit against the side effect and cost is challenging, A 2012 Cochrane review of 23 studies on the use of antibiotics, either for short- or long-term use for the treatment of OME, showed a small benefit for complete resolution of the effusion. In contrast, antibiotic therapy did not have any significant impact on hearing level or the rate of subsequent tympanostomy tube insertion[12]. Another meta-analysis of controlled studies showed a 14% increase in the resolution rate when antibiotics were given[13]. Topical and systemic steroid, nasal and systemic decongestant and antihistamine are not recommended for the treatment of OME. If the effusion persists for more than 4-6 months or associates with hearing loss, then tympanostomy tube placement is recommended[7,12,13].

Identification of the main bacteria causing otitis media with effusion (OME) in each population is essential. Knowledge of the most prevalent bacteria would initiate the search for the mode of acquisition of such bacteria and may aid in establishing appropriate control and treatment programs, which may decrease the incidence of OME. So, the aim is to examine the bacterial involvement in otitis media with effusion and to determine the bacterial susceptibility to commonly prescribed antimicrobial agents used in Erbil.

Materials and methods

Forty children between the ages of 2-12 years, who were diagnosed with bilateral or unilateral OME for more than three months and scheduled to undergo tympanostomy tube insertion at Rizgary teaching hospital-ENT, Head & Neck surgery HNS department in Erbil were included. The diagnosis was made on the basis of history, examination (intact tympanic membrane TM with retraction, color change or decreased mobility), and tympanometry types B and C. Children presented with acute otitis media AOM, recent upper respiratory tract infection craniofacial abnormality (cleft palate), or antibiotic use within 7 days of surgery were excluded. Consents were taken from the parents for both the surgery and participation in the study. The study was approved by Kurdistan board for medical specialty. Middle ear effusion were collected from patients under general anesthesia, the external auditory canal was cleansed using 10% povidone iodine, myringotomy was performed with a radial incision in the anterior inferior quadrant of the tympanic membrane, the middle ear fluid was collected using needle aspiration into a sterile syringe, each collected sample was added to Stuart transport medium and transferred to the microbiology lab within 90 minutes of aspiration. All samples were inoculated onto blood agar media, McConkey agar media and chocolate agar media then incubated for at least 48 hours under 35°C, chocolate and blood agar were under microaerophilic atmosphere. The positive bacterial cultures were identified by gram staining and biochemical study. The antibiotic sensitivity of 3 commonly used antibiotics in our hospital was tested (amoxicillin-clavulanic acid, cefixime, and azithromycin) using disc diffusion method, following the guidelines of the British society for antimicrobial chemotherapy (BSAC) methods for antimicrobial susceptibility testing[14].The antibiotic concentration in the discs was 20/10 Mg for amoxicillin/clavulanic acid ,15mg for azithromycin and 5Mg for cefixime. The cut value for calculating the sensitivity and resistance for each of these antibiotics is mentioned in Table 1. All the microbial studies were performed by the same microbiologist in the same lab.

Table (1): Cut-off value for antibiotic sensitivity and resistance

Antibiotic	Resistance	Sensitive
Amoxicillin-clavulanic acid	<13mm	>18mm
Azithromycin	<18mm	>19mm
Cefixime	<19mm	>20mm

Data were analysed using the Statistical Package for Social Sciences (SPSS, version 22). Fisher's exact test was used (rather than Chi square test) because the expected count of more than 20% of the cells of the tables was less than 5. A p value of $\leq$ 0.05 was considered statistically significant.

Results

Forty patients were included in the study. Their mean age + SD were 5.3 + 2.6 years, ranging from 2 to 12 years. The median was 4.5 years. Table 2 shows that more than half (55%) of the patients aged less than 5 years, and 60% were males. The male: female ratio was 1.5: 1.

Table (2): Age and gender distribution of the studied sam

Age (years)	No.	(%)
1-4	22	(55.0)
5-8	13	(32.5)
9-12	5	(12.5)
Gender		
Male	24	(60.0)
Female	16	(40.0)
Total	**40**	**(100.0)**

The total number of affected ears was 72 ears, 35 (48.6%) on the right side, and 37 (51.4%) on the left side.
Bacterial culture was positive in 24 out of 72 ears (33.3%) as presented in Figure 1.

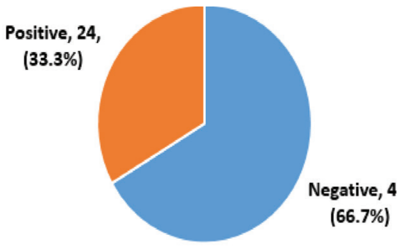

Figure (1): Results of bacterial culture

Figure 2 shows that the most common type of bacteria was Haemophilus. influenzae (62.5%), while Streptococcus pneumonia and Staphylococcus aureus constituted 25% and 12.5% of the positive cultures respectively.

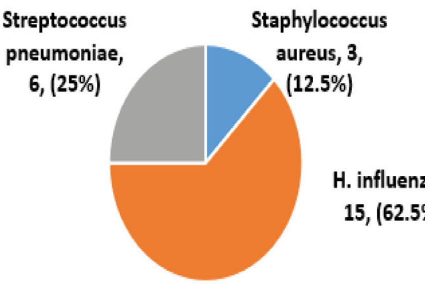

Figure (2): Types of bacteria detected by culture

Table 3 shows that 93.3% of Haemophilus. influenzae cultures were sensitive to Amoxicillin/clavulanic acid, and 83.3% of Streptococcus pneumoniae were also sensitive, while none of the Staphylococcus aureus bacteria was sensitive to the mentioned antibiotic (p = 0.001).

Table (3): Sensitivity of bacteria to Amoxicillin/ clavulanic acid

Result of culture & sensitivity	Staphylococcus aureus No.	(%)	H. influenzae No.	(%)	Streptococcus pneumoniae No.	(%)	p
Sensitive	0	(0.0)	14	(93.3)	5	(83.3)	
Resistant	0	(0.0)	1	(6.7)	1	(16.7)	0.001*
Moderate	3	(100.0)	0	(0.0)	0	(0.0)	
Total	3	(100.0)	15	(100.0)	6	(100.0)	

Regarding sensitivity to azithromycin, 66.7% of S. aureus and 16.7% of S. pneumoniae were sensitive to it, while none of the H. influenza were sensitive to azithromycin (p = 0.006) as presented in table 4.

Table (4): Sensitivity of bacteria to azithromycin

Result of culture & sensitivity	Staphylococcus aureus No.	(%)	H. influenzae No.	(%)	Streptococcus pneumoniae No.	(%)	p
Sensitive	2	(66.7)	0	(0.0)	1	(16.7)	
Resistant	0	(0.0)	7	(46.7)	0	(0.0)	0.006*
Moderate	1	(33.3)	8	(53.3)	5	(83.3)	
Total	3	(100.0)	15	(100.0)	6	(100.0)	

Table 5 shows no significant differences in the proportions of antibiotic sensitivities (p = 0.615). None of the bacteria was sensitive to cifixime. On the contrary, all the Staphylococcus aureus cultures were resistant to cifixime, 80% of H. influenza and 66.7% of Streptococcus pneumonia were resistant to the mentioned antibiotic.

Table (5): Sensitivity of bacteria to cifixime

Result of culture and sensitivity	Staphylococcus aureus No.	(%)	H. influenzae No.	(%)	Streptococcus pneumoniae No.	(%)	p
Resistant	3	(100.0)	12	(80.0)	4	(66.7)	0.615*
Moderate	0	(0.0)	3	(20.0)	2	(33.3)	
Total	3	(100.0)	15	(100.0	6	(100.0)	

Discussion

In this study, the percentage of positive cultures was 33.3% which is similar to what is reported in the literature (20%-40%)[2,10,15]. We found similar results in the study of S. J. Kim et al. were they found that (33.6%) of the middle ear effusion samples had positive culture results[16].
In two studies first by Farajzadah sheikh et al in Iran and the second by Park et al 2004 in Korea, bacterial cultures were positive in (13.4%) and (14%) respectively using ordinary bacterial culture[11,17].
While in tow studies performed by de Miguel Martínez I et al. in Spain bacteriological cultures were positive in (75%) and (72.5%) respectively[10,18]. The discrepancies between our results and those studies might be explained by the difference in inclusion criteria, sampling methods, culturing technique, and geographical location. Also, the ability of these micro-organisms to form biofilms, as a biofilm might be located in a small area of the effusion and might be missed unless the entire MEE fluid is sampled and analysed.
Single bacteria were isolated from each of the positive cultures. The most common bacteria isolated in our study

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is H. influenzae (62.5%), followed by Streptococcus pneumonia 25% and Staphylococcus aureus 12.5% and no Moraxella catarrhalis were cultured. In review of the literature, in the past 15 years, various bacteria were cultured with different percentages.

In Park et al study H. influenzae was detected in (7.9%), Streptococcus pneumoniae in (1.4%) while Moraxella catarrhalis was not detected at all. Other bacteria such as Staphylococcus aureus or Streptococcus pyogens were detected in (4.7%)[17].

In a study done by Pereira et al, the direct culture revealed pathogens in (25.1%) of MEE, and the majority of the isolated bacteria were S. pneumoniae (12.5%), H. influenzae (39.1%), and M. catarrhalis (10.2%)[15].

In Farajzadah Sheikh A et al. and de Miguel Martínez I et al. the most frequently isolated micro-organism was A. otitidis, followed by (M. catarrhalis and S. pneumoniae) for the first study and (H. influenzae and S. aureus) for the second[11,18].

While in 2 studies done by Kim SH, et al. in 2017 and S. J. Kim et al. in 2013 both in Korea, the most frequently isolated Gram-positive bacterial species were coagulase negative Staphylococcus aureus CNS followed by methicillin-susceptible S. aureus (MSSA)[16,19].

Differences in bacterial strains from different studies may reflect a change in the bacterial popu¬lation in each community including ours toward more resistant strains partly because of patterns of antibiotic use and partly due to the introduction of vaccines against H. influenza type b and S. pneumonia.

In our community, there are no clearly followed guidelines for antibiotic prescription and the witnessed practice of indiscriminate and overuse of antibiotics could be the cause of the antibiotic resistance observed in our finding. As demonstrated in the results, none of the bacteria found were sensitive to cefixime, and again none of the H. influenza were sensitive to azithromycin, 66.7% of S. aureus, and only 16.7% of S. pneumoniae were sensitive to azithromycin. Regarding Amoxicillin/clavulanic acid, 93.3% of H. influenzae cultures were sensitive to it, and 83.3% of S. pneumoniae were also sensitive, while none of the S. aureus bacteria were sensitive to the mentioned antibiotic. Since there were different bacterial strains cultured in different geographical areas, and the variety in antibiotic prescription guidelines, we expect that the antibiotic resistance will be diverse too. Pereira et al. investigated the sensitivity to penicillin in 26 bacterial isolates of effusion samples, the sensitivity of H. Influenza was found to be (77%) and that of S. pneumonia was (37.5%)[15]. While in a study by de Miguel Martínez I et al it was detected that both S. aureus and H. influenzae were resistant to ampicillin10. From the aforementioned we emphasize that each community is unique in its most prevalent bacteria cultured in OME, and their antibiotic susceptibility.

Conclusions

Knowing the bacterial involvement in OME is indispensable, especially from a clinical point of view since these microorganisms may constitute a fundamental factor in the aetiology, prognosis, and treatment of OME.

Due to the high percentage of antibiotic resistance of the isolated bacteria we recommend establishing a guideline for the antibiotic use in the treatment of upper respiratory tract infection and acute otitis media. Implementing control on over the counter purchase of antibiotics might be a good first step.

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