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Research

The Prevalence of Bacterial Etiologies Causing Otitis Media with Effusion among Omani Children

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ABSTRACT

Objectives

Our main objective was to determine the prevalence of bacterial etiologies of Otitis Media with Effusion (OME) among Omani children as well as to evaluate the antibiotic susceptibility to penicillins.

Methods

A prospective cross-sectional study was conducted on 28 Omani children under 5 years of age with OME. The Middle Ear Effusion (MEE) was sent for direct culture and polymerase chain reaction (PCR).

Results

Out of 38 MEE, 8(21.1%) showed growth on direct culture. The majority of bacteria were sensitive to penicillin. 17 (44.7%) samples were positive using PCR with 5 (29.4%) of them had more than one positive pathogen. The detection rates for H. Influenzae, M. catarrhalis and S. pnemoniae were 31.6%, 18.4% and 10.5% respectively.

Conclusion

The prevalence of bacteria causing otitis media with effusion among Omani children was not different than the rates reported worldwide however the order of the most common ones differs slightly.

Keywords: *Otitis media with effusion; Bacteria; Polymerase Chain Reaction.*

INTRODUCTION

Otitis Media with Effusion (OME) is defined classically as the presence of Middle Ear Effusion (MEE) in the absence of acute infection signs.[1] There are many theories to explain the pathogenesis of OME. They include recurrent acute otitis media, biofilm formation, allergy, eustachian tube dysfunction and gastroesophageal reflux.[2,3] However, there is enough evidence that OME follows acute otitis media that can be either recognized or unrecognized.[4] The point prevalence of OME is estimated to be up to 40% for the children under 5 years.[5] OME is one of the main causes of hearing impairment in childhood.[6] In an Omani community-based cross-sectional study, OME was found to be the cause of 2.15% and 3.14 % of bilateral hearing loss and bilateral disabling hearing loss respectively.[7] As only the efficacy of the acute phase management of OME with both antibiotics and corticoids has been

proven, the understanding of microbiology and sensitivity of causative organisms of OME will help in reducing both the complications and the need for surgical intervention.[8,9]

In 1950s, Senturia et al. has opened the door for microbiological studies of MEE.[10] Since then, the evidence of bacterial infection as an important factor in the pathogenesis of OME has been supported by multiple methods. The main method was the use of Polymerase Chain Reaction (PCR) which has dramatically improved the detection rate of bacteria in the MEE. Those studies agreed that the most common bacteria in the MEE includes Hemophilus influenzae, Streptococcus pneumoniae, and Moraxella catarrhalis with variation in the order and detection rates.[3,6,9,11,12]

The main objective of this study was to determine the prevalence of bacterial etiologies of OME among Omani children using both direct culture and PCR. We also aim to evaluate the antibiotic susceptibility of bacteria to penicillins and the serotype of isolated Streptococcus pneumoniae.

METHODS

Ethical Approval

This cross-sectional study was approved by our hospital's ethical committee and conducted between Jan 2018 and Nov 2019 in Alnahda Hospital (a tertiary governmental hospital) in collaboration with the Central Public Health Laboratories (CPHL). Informed consents were taken from the parents/legal guardians of the included patients.

Subjects

The study includes patients with the following: 1) An Omani child under five years with complete vaccination history; 2) diagnosed with OME for at least three months with failed medical treatment and planned for surgical intervention with myringotomy and ventilation tube insertion; 3) signs of fluids in the middle ear on clinical examination by using otomicroscope and a type B curve that persists on the tympanometry study at time of diagnosis and prior to operation; 4) no history of treatment with antibiotics for the last 6 weeks prior to surgical intervention.

Intraoperatively, the external ear, including the external ear canal, was cleaned with povidone-iodine and then irrigated with normal saline. After myringotomy, a sample of the middle ear effusion was aspirated under aseptic technique and sent for direct culture, antimicrobial sensitivity and PCR.

Culture Identification

The MEE was cultured routinely in the following media; sheep blood agar, chocolate agar, Mackonkey agar, Sabouraud agar and neomaycin anaerobic media (BioOman, United Labs L.L.C, Sultanate of Oman). Sheep blood agar and chocolate agars were incubated aerobically with 5% Co2 at 37.0°C, Mackonkey agar was incubated aerobically at 37.0°C, Sabouraud agar at room temperature (23-26°C) and neomycin anaerobically. Plates were read at 24hrs except neomycin at 48hrs of incubation. Sabouraud agar was incubated for 7 days. Automated bacterial identification system was used (Vitek, bioMerieux, France).

Serotyping of S. Pneumoniae

ImmuLexTM Pool Antisera (SSI Diagnostica, Hillerd, Denmark) kit was used for pneumococcal sero-grouping/serotyping. The latex agglutination method and interpretation was performed as per the manufacturer's instructions.

Antimicrobial Susceptibility Testing (AMST)

AMST done following CLSI (Clinical Laboratory Standards Institute) guideline. Penicillin E-test (E-test, bioMerieux, France) was used to test the susceptibility of S. Pneumoniae to penicillin. Beta-lactamase production in H. Influenzae and M. Catarrhalis tested using intralactam strips (MAST ID, Mast group, United Kingdom).

Genomic DNA Extraction

Part of the MEE was sent to CPHL on refrigerant gel packs for the PCR study. The samples were stored at 2°C to 8°C for a maximum of 48 hours. Genomic DNA of bacteria was extracted using QIAamp® DNA Mini kit (Qiagen Cat No. 51304). The MEE was centrifuged at 8000 rpm for 10 minutes. The supernatant was discarded, and pellet was suspended in 500 µL nuclease free water by pulse vertexing for 10 seconds. The suspension was centrifuged at 8000 rpm for 5 minutes. The pellet was used for further processing as per the manufacturer's instructions. DNA was stored at -200° C for further analysis.

Real Time PCR

Regrading PCR, FTD Bacterial Pneumonia CAP (Fast Track Diagnostics Limited, Malta, Cat No. FTD-29.1-64) was used to identify 7 bacterial pathogens in the samples. The bacterial targets included in the kit were Streptococcus pneumoniae, Haemophilus influenzae, Moraxellacatarrhalis, Staphylococcusaureus, Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila and internal control. In Brief, 10 µL of genomic DNA is added in 15 µL of primer-probe mastermix (in two tubes) as per manufacturers recommendations. The PCR conditions used were: initial holding at 50°C for 15 minutes, second holding cycle at 94°C for 1 minute, 40 cycles of 94°C for 1 minute and 60°C for 60 seconds. PCR runs were performed on ABI Fast 7500 real time PCR machine.

Statistical Analysis

To compare the association between direct cultures and PCR, McNemar's chi-square test was used. A P-value of less than 0.05 was considered significant.

RESULTS

Total of 38 MEE samples were collected from 28 patients. In 10 patients, the samples were collected separately from both ears. There were 16(57.1%) males and 12(42.9%) females. The age ranged between 1 and 5 years with mean age of 3.5 years.

The direct culture was positive in 8 (21.1%) MEE samples (Table 1). H. influenza was identified in 4 (10.5%) of them. Only one (2.6%) culture was identified as S. Pneumoniae which was found to be serogroup 33. NoM. catarrhalis was isolated by this method. The remaining 3 (13%) positive cultures were of no clinical significance in the MEE. Of interest, the only positive patient among those who provided samples from bilateral ears had both samples positive for H. influenzae. In one sample, H. parainfluenza was reported in the culture while PCR was positive for H. influenza. The isolate was not available to reevaluate this conflict when identified. The only strain of S. pneumoniae was sensitive to penicillin. All H. influenzae isolates were sensitive to Ampicillin except for one isolate which was beta lactamase positive and reported as sensitive to Amoxicillin-clavulanate.

Table 1. The results of direct culture and PCR

Bacteria	No (%) of positive culture	No (%) of positive PCR
H. Influenzae	4 (10.5)	12 (31.6)
S. pnemoniae	1(2.6)	4 (10.5)
M. catarrhalis	0	7 (18.4)
Others	3 (7.9%)	2 (5.3)
Total of positive samples	8/38 (21.1)	17/38 (44.7)

There were 17(44.7%) MEE samples positive in PCR analysis, 5 (29.4%) of them were positive for more than one pathogen making the total number of pathogens detected by PCR 25 (Table 1). The individual detection rates for *H. Influenzae, M.catarrhalis, S.pnemoniae and S.aureuswere* 12 (31.6%), 7 (18.4%), 4 (10.5%) and 2 (5.3%) respectively (Table 1). Mycoplasma pneumoniae, Chlamydophila pneumoniae and Legionella pneumophila were not detected in any of the MEE samples. All significantly positive direct cultures were positive by PCR with the same pathogen. Over all, the sensitivity of PCR in detecting pathogens was significantly higher than direct culture ($p<0.05$). It was also individually significant for the detection of H. influenzae ($p<0.05$) but not for the other bacteria. Regarding the samples that were sent from bilateral ears of same patients, same pathogen was detected in both ears in 5(50%) patients, no pathogen was detected in both ears in 3(30%) and 2(20%) patient had different results.

DISCUSSION

To our knowledge, this is the first study in Oman and the gulf countries to focus on this topic. Alshammari et al. and Al-Mazrou et al. evaluated the microbiological causes of otitis media in Saudi Arabia, however, their focus was on acute otitis media rather than OME.[13,14]

Our detection rate using direct culture was comparable to Park et al. and Pereira et al. results as they have reported positive cultures of 14% and 19% respectively.[6,9] Both papers reported the same order of our detected bacteria with *H. influenzae* being the most common one. However, Daneil et al. reported a higher percentage of positive cultures of 45%.[11] The culturing methods which were used in that study (wide range of media and prolonged culturing time) can explain their higher positivity rate especially with the fact that the commonest three positive cultures in their study were coagulase negative *staphylococci, Veillonella spp. and S. aureus* which are not among the known commonest pathogens in the MEE.

The PCR results reported by Pereira et al. and Park et al. were comparable to our findings as they reported 57% and 36.7% positive PCR respectively.[6,9] However, Gok et al. reported much higher rate of positive PCR (94.5%).[3] The high detection rate of Gok et al. study as compared to our and the other studies can be explained in different ways. One of them is related to the sampling as the bacterial DNA might present in one part of the MEE and it requires to sample the entire effusion to ensure the result of PCR. The other explanation is related to the inclusion criteria of patients with recent acute otitis media where the chance of getting positive bacterial results is much higher even with use of antibiotics. Gok et al. didn't specify if they have evaluated their subjects for recent upper respiratory tract infection or acute otitis media in particular prior to the operation and samples collection.

The detection rates of the individual bacteria in positive PCR in our study was comparable to most of the studies.[3,6,9,15] The variability of detected pathogens was also comparable to published data. In a systematic review of 17 papers by Ngo et al. concerning the microbiology of otitis media including OME, they found that *H. influenzae, S. pneumoniae, and M. catarrhalisare* the most common three pathogens which is going with our results. Moreover, they found that *H. influenzae* was the commonest isolated pathogen globally.[15] However, the order of the second and third most common bacteria in our study is different compared to the findings of Pereira et al., Park et al. and Gok et al. as all of them reported *S. pneumoniae* as the second and M. catarrhalis as the third most common bacteria in PCR.[3,6,9] This minor discrepancy can be attributed to the small sample size leading to relatively low detection rates of these two bacteria in our study.

We have demonstrated significant difference in the detection rate of bacteria between direct culture and PCR in general. The significant difference between culture and PCR was shown individually with H. influenzae alone. Pereira et al. found significant difference in the detection rate of bacteria between direct culture and PCR in general and individually with the three common isolates that were similar to the ones detected in our study.[9] Our insignificant statistical correlation with the other 2 bacteria can be explained again by our small sample size and hence the small rate of those two bacteria.

Regarding the significance of the PCR findings, it is important to mention that there are three possible scenarios for any evidence of bacteria in the MEE. The first one is having viable and culturable pathogen, in this situation the bacteria will be detected by direct culture method. The second scenario is when there is viable but not culturable bacteriai.e. in low metabolic activity and do not divide usually post antibiotic intake. The importance of PCR appears in this setting where it will be able to detect the evidence of previous presence of the pathological bacteria.[6] The logic question here will be either the positive PCR is representing unviable bacteria since it detects only the presence of fragments of DNA which is the third scenario. Post et al. eliminated this possibility when they demonstrated that the DNA of heat-killed bacteria will not be detectable using PCR after day 3, while it was detectable for the samples treated by antibiotics despite remaining unculturable.[16] Another evidence about the viability of the unculturable bacteria in the MEE was shown by Daniel et al. after using bacterial viability stain and confocal laser scanning microscopy. They have detected viable bacteria using this technique in 51 (82.3%) samples as compared to 28 (45.2%) positive direct cultures. On the other hand, Martinez et al. found no positive cultures from samples collected from healthy ears of 30 children. This finding emphasis on the pathological nature of the detected bacteria in the MEE.[12]

Part of our aim was to evaluate the sensitivity of bacteria to antibiotics, mainly penicillins as first line treatment, and to identify the serotypes of *S. pneumoniae* isolates. The purpose of this is to guide antimicrobial therapy and to know if any of the vaccine serotypes is involved in the pathogenesis of OME. Pumarola et al. found that S. pneumoniaesero type 19A has the highest antimicrobial resistance with rate of 78% (7/9 samples).[17] However, due to the low detection rate of this bacteria in the direct cultures of our study (one positive S. pneumoniae of serotype 33), it was difficult to draw any conclusion concerning our population. On the other hand, Pereira et al. found 23% penicillin-resistant H. Influenzae and Pumarola et al. found that H. influenzae was sensitive to ampicillin in 81% of the culture positive samples and 11% were beta lactamase positive.[9,17] These findings are comparable to our results.

CONCLUSION

Our study provides an evidence that the detection rates of bacteria in both direct cultures and PCR are not different from the reported studies in this filed worldwide. This finding emphasis on the importance of bacteria in the pathogenesis of OME. More studies with larger sample size are needed to generalize the conclusions of antibiotic sensitivity and types of bacteria in the MEE.

CONFLICTS OF INTEREST

None.

REFERENCES

1. Rosenfeld RM, Shin JJ, Schwartz SR, et al. Clinical Practice Guideline: Otitis Media with Effusion Executive Summary (Update). *Otolaryngol Head Neck Surg*. 2016; 154(2): 201-14. doi: 10.1177/0194599815624407

2. Otitis Media with Effusion (serous otitis media) in Children: Clinical Features and Diagnosis - UpToDate . 2020.

3. Gok U, Bulut Y, Keles E, Yalcin S, Doymaz MZ. Bacteriological and PCR Analysis of Clinical Material Aspirated from Otitis Media with Effusions. *Int J Pediatr Otorhinolaryngol*. 2001; 60(1): 49-54. doi: 10.1016/s0165-5876(01)00510-9.

4. Teele DW, Klein JO, Chase C, Menyuk P, Rosner BA. Otitis Media in Infancy and Intellectual Ability, School Achievement, Speech, and Language at age 7 years. Greater Boston Otitis Media Study Group. *J Infect Dis*. 1990; 162(3): 685-694. doi: 10.1093/infdis/162.3.685

5. Otitis Media With Effusion: Practice Essentials, Pathophysiology, Etiology. 2020.

6. Park C-W, Han J-H, Jeong J-H, et al. Detection Rates of Bacteria in Chronic Otitis Media with Effusion in Children. *J Korean Med Sci*. 2004; 19(5): 735-738. doi: 10.3346/jkms.2004.19.5.735

7. Al Khabori M, Khandekar R. The Prevalence and Causes of Hearing Impairment in Oman: A Community-Based Cross-Sectional Study. *Int J Audiol*. 2004; 43(8): 486-492. doi: 10.1080/14992020400050062

8. Rosenfeld RM, Culpepper L, Doyle KJ, et al. Clinical Practice Guideline: Otitis Media with Effusion. *Otolaryngol Head Neck Surg* . 2004; 130(5 Suppl): S95-S118. doi: 10.1016/j.otohns.2004.02.002

9. Pereira MBR, Pereira MR, Cantarelli V, Costa SS. Prevalência De Bactérias Em Crianças Com Otite Média Com Efusão. *J Pediatr (Rio J)*. 2004; 80(1): 41-48.

10. Bh S, Cf G, Cd C, Es B. Studies Concerned with Tubotympanitis. *Trans Am Otol Soc*. 1958; 46: 89-121.

11. Daniel M, Imtiaz-Umer S, Fergie N, Birchall JP, Bayston R. Bacterial Involvement in Otitis Media with Effusion. *Int J Pediatr Otorhinolaryngol*. 2012; 76(10): 1416-1422. doi: 10.1016/j.ijporl.2012.06.013

12. Miguel Martínez I de, Ramos Macías Á, Masgoret Palau E. Bacterial Implication in Otitis Media with Effusion in the Childhood. *Acta Otorrinolaringol Engl Ed*. 2007; 58(9): 408-412.

13. Alshammari MS, Alshaya HK, Alkhalaf AA, et al. The Common Microbial Causes of Otitis Media with Especial Focus in Saudi Arabia. *Res Otolaryngol*. 2017; 6(5): 67-72. doi: 10.5923/j.otolaryn.20170605.03

14. Al-Mazrou KA, Shibl AM, Kandeil W, Pirçon J-Y, Marano C. A Prospective, Observational, Epidemiological Evaluation of the Aetiology and Antimicrobial Susceptibility of Acute Otitis Media in Saudi Children Younger Than 5 Years of Age. *J Epidemiol Glob Health*. 2014; 4(3): 231-238. doi: 10.1016/j.jegh.2014.03.002

15. Ngo CC, Massa HM, Thornton RB, Cripps AW. Predominant Bacteria Detected from the Middle Ear Fluid of Children Experiencing Otitis Media: A Systematic Review. *PLOS ONE*. 2016;11(3): e0150949. doi: 10.1371/journal.pone.0150949

16. Post JC, Aul JJ, White GJ, et al. PCR-Based Detection of Bacterial DNA After Antimicrobial Treatment is Indicative of Persistent, Viable Bacteria in the Chinchilla Model of Otitis Media. *Am J Otolaryngol*. 1996; 17(2): 106-111. doi: 10.1016/s0196-0709(96)90005-8

17. Pumarola F, Marès J, Losada I, et al. Microbiology of Bacteria Causing Recurrent Acute Otitis Media (AOM) and AOM Treatment Failure in Young Children in Spain: Shifting Pathogens in the Post-Pneumococcal Conjugate Vaccination Era. *Int J Pediatr Otorhinolaryngol*. 2013; 77(8): 1231-1236. doi: 10.1016/j.ijporl.2013.04.002