

ORIGINAL ARTICLE

Comparison of PCR Assay and Culture for Detecting Bacteria in Middle Ear Fluid of Children with Otitis Media with Effusion

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Objective; Otitis media is one of the most common causes for visiting children. The aim of the present study was to compare the efficacy of conventional culture and polymerase Chain Reaction (PCR) methods in determination of the bacterial pathogen in the middle ear effusion of children with otitis media undergoing myringotomy.

Materials & Methods; In a prospective analytical study 49 middle ear fluid samples from 32 children having the age range 2 to 10 years old obtained by myringotomy were included in the study. Statistical analyses were made by using the chi-square method (SPSS version13.0) and p-value of ≤ 0.05 was accepted as being statistically significant.

Results; From 49 middle ear effusions, 12 (24.4%) were serous, 22 (44.9%) mucous and 15 (30.6%) were sero-mucoid. Bacteria were cultured in 12 (24.5%) out of 49 samples and pathogens under investigation were found in 10 (20.4%). PCR was positive for bacteria in 18 (36.7%). Among them non-typeable *Haemophilus influenzae* was identified in 13 (26.5%) and *Streptococcus pneumoniae* in 5 (10.2%).

Conclusion; The results indicate that PCR assay is more sensitive, less cumbersome and more specific in detection of bacteria in middle ear effusion compared with conventional bacterial culture methods.

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Otitis media is one of the most common reason for the children visiting the physicians, and receiving antibiotics^[1]. Otitis media with effusion (OME) is the most common cause of hearing loss in childhood^[2]. If not treated it may lead to impairment on speech and language development^[3].

Its pathogenesis still remains unclear, though there are powerful clues supporting its relation with acute otitis media^[4]. However still, conventional culture methods fails identifying the active agents of otitis media in many cases.

The aim of the present study was to compare the accuracy of conventional culture and PCR methods in detection of the bacterial pathogens in the middle ear effusion of children with otitis media undergoing myringotomy.

Patients and Methods

Forty nine middle ear fluid samples obtained from 32 children between the ages of 2 and 10 years who had undergone myringotomy were included in the study. The study was carried in Imam Hospital of Ahwaz Jondi-Shapour University, Department of Otorhinolaryngology between May 2004 and March 2005. The children having allergy history were excluded from the study.

Before the myringotomy incision external auditory canal was scrubbed with 70% alcohol by applying it for one minute. The middle ear aspirates were collected through the myringotomy incisions.

Bacteriological studies were initiated as soon as the specimens were obtained. Part of the sample was

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stored at -20°C for later PCR analysis. PCR method was utilized for identification of DNA genome of Haemophilus influenza and Streptococcus pneumoniae in the middle ear effusion media. The patients had no signs of acute otitis media or respiratory tract infection and were not on antibiotic therapy.

For ordinary bacterial culture, chocolate agar media was used for the H. influenzae culture, sheep's blood agar media and chocolate agar media for the Streptococcus pneumoniae culture. The genomic DNA was extracted by mixing soul of stored middle ear effusion with 900 µL lysis solution, followed by a 10 minutes centrifugation at 15000 rpm at room temperature. DNA was extracted by using the wizard Genomic DNA purification Kit. During PCR essays, P6 protein was used as a primer for H.influenzae and PBP 2B for S pneumoniae. Medical records of the patients were evaluated for the history of ventilation tube insertion due to OME, characteristics of middle ear effusion. Statistical analyses were made by using the chi-square method (SPSS version 13.0) and p value of ≤0.05 was accepted as being statistically significant.

Results

A total number of 32 patients (twenty boys and twelve girls) aged between 2-10 years (3.2±2.3) and their 49 ears were enrolled in this study. In seventeen patients both ears demonstrated effusions, whereas in fifteen patients the effusion was unilateral. The most common complaint as expressed by the parents was hearing loss. Of thirty two children, twenty two

patients (75%) had hearing loss, three patients (9.3%) had otalgia and two patients (6.2%) had fullness of the ear upon admission. The remainder three patients who had no history of any complaints were identified during routine ORL look-up. Of 49 middle ear effusions, 12 (24.4%) were serous, 22 (44.9%) were mucous and 15 (30.6%) were sero-mucoid in character. Bacteria were cultured in 12 (24.4%) out of 49 samples and the bacteria were identified among 10 (20.4%) (positive culture) children.

PCR was positive for bacteria in 18 (36.7%) specimens included 13 (26.5%) for non-typeable Haemophilus influenzae, 5 (10.2%) for Streptococcus pneumonia (Table 1). There was significant difference in positive results between conventional culture and PCR (p: 0.05) but this difference was not significant for two studied types of pathogens (H. influenza and Streptococcus pneumoniae) and conventional culture in detection of these two pathogens was as accurate as PCR.

Discussion

Bacterial infection has been known as an important factor Senturia et al^[1]. Until now almost all of these studies are relied upon culturing of middle ear effusions samples^[5-7]. Haemophilus influenza and Streptococcus pneumonia have been reported as the most common bacterial pathogens^[1,2]. However, the impact of these two bacteria varied among the studies.^[2,4]. Two possible causes for these low positive rates can be the prolonged use of antibiotics before ventilation tube insertion which slows down the

Table 1. Comparison of the results of positive conventional culture and positive PCR to detect major bacterial pathogens in otitis media with effusion

Bacteria	Number (%) of positive culture specimens	Number (%) of positive PCR specimens	p value
H. Influenza	8(16.3)	13(26.5)	0.55
S.Pneumonia	2(4.1)	5(10.2)	0.09
Others	2(4.1)	-	-
Total	12(24.5)	18(36.7)	0.05*

*p<0.05 significant

proliferation of bacteria, and that secretory immunoglobulins and lysozymes in middle ear effusion inhibits the proliferation of bacteria.

By using PCR method it was possible to detect the bacteria in the middle ear effusion in higher rates (77.3% to 94.5%) also being consistent in many studies^[3,4,7,8]. In this study, bacteria could be detected in 20.4% of the cases by using conventional cultures while bacterial DNA was detected in 36.7% by using PCR method.

The middle ear fluid samples which remained negative by PCR, in spite of having positive cultures for *Streptococcus pneumoniae*, were tested for their ability to inhibit amplification reaction and no inhibition was found. The negative PCR results for these samples may be due to the small number of bacteria initially present in the swab or loss of DNA during the extraction procedures.

The other cause of negative culture in OME may be the presence of atypical forms of bacteria such as L-forms in OME^[9]. L-forms remain in a latent state within the host but cause immunological responses. These forms could not be detected by conventional culture as they do not form colonies in standard culture environment. PCR may be an alternative for detecting pathogen DNA. However, there are some limitations for PCR method as it amplifies DNA of both living and dead bacteria^[10].

Furthermore, recent studies have revealed the role of biofilms in chronic and recurrent otitis media with negative culture^[11,12]. Presence of biofilms in almost all cases of OME has been shown in Hall-Stoodley study that may explain the lack of efficacy of antibiotic therapy in OME^[12]. Even the L-forms can be detected both by PCR and conventional cultures, electron microscope is needed to evaluate it. It is possible to isolate the biofilms by PCR method.

The most important obstacle in detecting microbial DNA in body fluids is the presence of heme or urea which both act like polymerase inhibitors^[13]. This might be the cause of false negative results. Isolation of DNA from non-purified samples might

eliminate these polymerase inhibitors. However, a small part of target DNA can be lost during this procedure^[14]. In addition higher rate of positive results may be due to DNA of non-viable bacteria in PCR method. In our study, we did all PCR assays after the isolation of DNA in order to minimize the number of false negativities.

The present study demonstrated that effusion contains bacteria which displays an important role in the pathogenesis of OME. It is possible to mention that PCR assay is more sensitive in detection of bacteria in middle ear effusion as compared with conventional culture method. The presence of biofilm related infection should be considered in cases with negative conventional culture and PCR. We wish to emphasize the presence of latent form of bacteria that limits cultures for some pathogens responsible for otitis media.

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