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ORIGINAL ARTICLE

Polymerase Chain Reaction (PCR) Versus Bacterial Culture in Detection of Organisms in Otitis Media with Effusion (OME) in Children

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Abstract The aim of this study was to compare between polymerase chain reaction (PCR) and bacterial culture in detection of *Streptococcus Pneumonia* and *M. Catarrhalis* in otitis media with effusion (OME) in children. Fifty patients having OME were included in this study between 2003 and 2008. Myringotomy and tympanostomy tube insertion were done in every patient and the middle ear effusion samples were aspirated. The samples were subjected to bacteriological study in the form of culture and molecular study in the form of PCR using JM201/202–204 primer probe set for both *S. pneumonia* and *M. catarrhalis*. The results of Bacterial cultures are as follows: five cases (10%) were culture positive for *S. pneumonia*. Six cases (12%) were culture positive for *M. catarrhalis*. Only one case (2%) showed positively for both *S. pneumonia* and *M. catarrhalis*. Polymerase chain reaction test shows that 18 cases (36%) were positive for *S. pneumonia*, 22 cases (44%) were positive for *M. catarrhalis*, 6 cases (12%) were positive for both organism and 4 cases (8%) were negative. The difference between the proportion of culture positive and PCR positive specimens for both organisms individually and collectively was significant ($P < 0.001$). From our study we can conclude that PCR is more accurate than bacterial culture in detection of organisms in middle ear fluid in OME and that *M. catarrhalis* plays a significant rule in OME as it is the sole organism identified more than the other one by PCR.

Keywords (OME) · Bacterial culture · Polymerase chain reaction (PCR)

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Introduction

Otitis media is an inflammatory process of the middle ear secondary to any aetiology. It generally refers to an infective process caused by either bacteria or virus or both [1].

Otitis media is the most common reason for ambulatory antibiotic use; about half of all oral antimicrobial prescriptions written for children under 10 years of age are for this indication. The peak incidence appears to be between 6 and 36 months with another peak at 4–7 years of age. The occurrence then declines to a minimum after the age of 12 years. [2].

Otitis media with effusion (OME) is the major form of chronic relapsing inflammatory disease of the middle ear. Chronic OME is associated with hearing diminution, delayed speech development and may cause permanent middle ear damage with mucosal changes [3].

The fluid in the middle ear can be serous (a generally a thin transudate), secretory (mucoid secretions consisting of mucopolysaccharides from goblet cells and glands in a metaplastic or hyperplastic middle ear mucosa), or purulent (from active infection).The differentiation is not possible if the membrane is opacified. Otitis media with effusion can be further classified as acute (less than 3 weeks), sub acute (3 weeks to 3 months), or chronic (greater than 3 months) [4].

The polymerase chain reaction (PCR) is an in vitro method for nucleic acid synthesis that enables the specific replication of a target segment of DNA, providing a rapid, highly sensitive, and specific means of nucleic acid detection and isolation.

In the past, chronic MEE was thought to be sterile but studies have shown a 30 to 50% incidence of positive middle ear cultures in children with chronic MEE. While PCR testing has revealed that over 75% of the specimens are PCR positive for bacterial DNA. The most likely organisms are, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *M. catarrhalis*, and group A strep [5].

The PCR can detect the presence of bacterial DNA in a significant percentage of culturally sterile middle ear effusion. The large number of bacterial genomic equivalents present in the ear is suggestive of an active process [6].

Craig et al. [7] used the PCR in specimens negative viral and bacterial culture for influenza A virus and *S. pneumonia*. They showed that PCR was sensitive and specific method for detecting influenza and *S. pneumonia*. They stated that the negative cultures may possibly be explained by (1) Inherent insensitivity of culture techniques in detecting small quantities of microorganisms (2) The presence of inhibitory substances such as interferons and antibodies, which exist in middle ear effusion and prevent both bacterial and viral cultivation; (3) The possibility that live bacteria or virus did not exist in the specimen at the time of collection [7].

Post et al. [6] concluded that the PCR based assay system can detect the presence of bacterial DNA in a significant percentage of culturally sterile middle ear effusion. While this finding is not a proof of an active bacterial infection process, the large number of bacterial genomic equivalents present in the ears is suggestive of an active process.

Kalcioglu et al. [8], used PCR and detected positive results in 18, while culture positive in 3, from 54 samples with chronic middle ear effusion.

Patients and Methods

The study was done in the ENT department El Minia University, Clinical pathology department, Al Azhar University, ENT department, Fayioum University.

Clinical Features

All cases were suffering from deafness. Full history were taken from the patients and then they were classified according to (1) Age (2) Sex, (3) History of previous myringotomy (4) Site of the lesion (unilateral or bilateral) (5) History of adenotonsillectomy (6) Type of the fluid (mucoid or serous).

MEE samples were aspirated from patients underlying myringotomy with the insertion of tympanostomy tube for OME. For all patients considered, the effusion (MEE) has persisted for at least 2 months prior to the date of the operation.

A total of 50 patients were studied between 2003 and 2008 at the ENT department, El Minia University, ENT department Fayium University and Clinical pathology department Al Azhar University. The age range of the patients was 3–10 years.

Microbial Culture

An aliquot of solution was lifted from the tip of storage tube with a sterile cotton wool swab and inoculated on two blood agar plates. One plate was incubated under anaerobic condition, the other in aerobic conditions in presence of 5% CO2. Both were incubated at 37°. If no growth was observed within 48 h, plates were left for longer incubation under the same conditions for up to 5 days. Identification of bacterial growth was done according to the standard method.

Isolation of Nucleic Acid

Part of the sample was centrifuged overnight at 12,000 rpm, then the supernatant were poured off and 1 ml of DNA extraction buffer was added to the pellet and mixed well till the pellet was completely dissolved then incubated in water bath at 65°C for 30 min.

Centrifugation was done for 15 min at 14,000 rpm and the upper 500 µl was transferred to a new tube containing 500 µl isopropanol.

Mixing was done and the samples were left for 5–25 min at room temp.then spinned for 20 min at 13,000 rpm. Gently the supernatent was poured off.

The pellet was washed two times with 70% Ethanol and air dried. Lastly the pellet was resuspended in 50 µl sterile water or 1× TE buffer (10 mM Tris, 1 M Na2 EDTA,pH 8.0). Five to ten micro litre were loaded on agarose gel.

PCR Amplification of the DNA

PCR amplification was carried in 100 µl reaction mixture each containing 56.5 µl of sterile distilled water, 16 µl of deoxyribonucleoside triphosphate(0.2 mM), 10 µl of 10× PCR buffer(100 mM tris Hcl {pH 8.3} 0.500 mM KCl, 15 mM MgCl2), one µl of each one of the primer pairs to

be used (0.3 µg/µl), 0.5 µl of taq DNA polymerase (5 U/µl), 15 µl of the DNA extract.

Amplification was carried in a (TECHNE PCR, Model: FTC 3102D, Cambridge, UK) and the PCR programme for both was as follows:

Denaturation at 95°C for 1 min.

Annealing at 55°C for 1 min.

Extension at 72°C for 1 min.

This was repeated for 34 times.

Then final extension was at 72°C for 10 min.

The amplicons were separated on 1.5% agarose gel stained with Ethidium bromide at a final concentration of 0.5 µg/ml. The gels were then visualized on a UV-light transilluminator and photographed with polaroid type 667 film.

Results

Age of the Patients

Twenty cases (46%) were 3 years old, ten cases (23%) were 4 years old, eight cases (16%) were 5 years old, seven cases (14%) were 6 years old, three cases (6%) were 7 years old and one case each (2%) for 8 and 9 years (Table 1).

Sex

Thirty-three cases were males (66%) while 17 cases were female (34%) (Table 1).

Previous Myringotomy

13 cases had done previous myringotomy (26%) while 37 cases had not (74%) (Table 2).

Unilateral or Bilateral

38 cases (76%) were bilateral while 12 (24%) cases were unilateral (Table 2).

Type of Fluid

In bilateral cases, 37 from 38 cases (97%) were mucoid while one case (3%) was serous. In unilateral cases ten (83%) from 12 cases were mucoid while 2 (17%) cases were serous (Table 2).

Previous Adenotonsillectomy

Nine cases (18%) had previous adenotonsillectomy while 41(82%) cases had not (Table 2).

PCR and Culture Result

Comparative analysis of OME samples were done by culture and PCR using JM201/202-204 primer probe set for *S. pneumonia* and *M. catarrhalis*.

Bacterial Cultures

Five cases (10%) was culture positive for *S. pneumonia*. While six cases (12%) was culture positive for *M.*

Table 3 comparison between PCR and culture

	Bacterial culture		PCR		*Z*	*P*
	No	%	No	%		
S. pneumonia	5	10	18	36	3.09	<0.001
M. catarrhalis	6	12	22	44	3.56	<0.0001
Both organism	1	2	6	12	1.96	<0.025
Negative	38	76	4	8	6.89	<0.0001

Table 1 Distribution of cases according to age and sex

	Age							Sex	
	3 years	4 years	5 years	6 years	7 years	8 years	9 years	Male	Female
Number	20	10	8	7	3	1	1	33	17

Table 2 Distribution of cases according to previous myringotomy, side, type of fluid, and adenotonsillectomy

	Previous Myringotomy		Side		Type of fluid				Adenoton.	
	Done	Not done	Unilat.	Bil.	Unilat.		Bil		Done	Not done
					Mucoid	Serous	Mucoid	Serous		
Number	13	37	12	38	10	2	37	1	9	41

Table 4 PCR versus culture positive and negative cases

Result of samples	Bacterial culture		PCR		*Z*	*P*
	No	%	No	%		
Positive	12	24	46	92	44.7	<0.00001
Negative	38	76	4	8		

catarrhalis. One case (2%) was positive for both *S. pneumonia* and *M. catarrhalis* (Table 3).

PCR

In middle ear effusion tested by PCR, 18 cases (36%) were PCR positive for *S. pneumonia* while 22 cases (44%) were positive for *M. catarrhalis*, 6 cases (12%) were positive for both organism while 4 cases (8%) were negative for PCR (Table 3).

There was no specimen showing culture positive and PCR negative for *S. pneumonia*, whereas 13 cases of the PCR positive specimens were culture negative for *S. pneumonia*.

There was no specimen showing culture positive and PCR negative for *M. catarrhalis* whereas 16 cases of PCR positive specimens were culture negative for *M. catarrhalis* (Table 4).

In the six cases which were positive PCR for both organisms, five of them was culture negative for both organisms.

The difference between the proportion of culture positive and PCR positive specimens for both organism individually and collectively was significant ($P < 0.001$).

The incidence of positive PCR over cultures was shown more for *M. catarrhalis* (44%) than for *S. pneumonia* (36%). This may indicate that *M. catarrhalis* plays more rule in OME as it is the sole organism identified more than the other one by PCR.

Discussion

Otitis media with effusion is one of the main causes of hearing loss in children. It is associated with delay language development in children younger than 10 years. The loss is usually conductive, with an average air conduction threshold of 27.5 dB. Otitis media with effusion has also been associated with sensory neural hearing loss. Both prostaglandins and leukotrienes have been found in high concentration in middle ear effusion, and their ability to cross the round window membrane has been demonstrated. Chronic exposure to these metabolites of arachidonic acid may cause a temporary and sometimes permanent sensory neural hearing loss [9].

Middle ear effusion was thought sterile until Senturia et al. 1958 first reported the presence of bacteria. De Maria et al. 1989 detected gram negative endotoxin in 80% of middle ear effusion including many culture negative. Although the existence of endotoxin of gram negative bacteria was present in MEE, the bacteria can not be identified because endotoxin is not specific for one gram negative organism. The bacteriological studies of MEE using highly sensitive technique of molecular biology such as PCR have demonstrated that traditional culture method are inadequate to detect many valuable bacteria present in MEE. Culture of MEE yield positive result for only 20-30% of patients while by PCR up to 75% of MEE were positive for pathogenic bacteria [10].

Otitis media with effusion is considered a direct extension of the inflammatory process that occur during long lasting or recurrent episodes of acute otitis media, which was confirmed by the facts that almost all cases of OME followed episodes of acute otitis media. These observation suggest that OME has an infectious etiology [11].

Arguedas et al. [12] found that *S. pneumonia* and *M. catarrhalis* were the most common isolated organisms from MEE followed by *H. influenzae*.

In our study bacterial culture for *S. pneumonia* were positive in five cases (10%) and for *M. catarrhalis* in six cases (12%) and only one cases (2%) showed positive for both organism. All positive culture cases were only 24%. On the other hand positive results by PCR for both organisms were 18 cases (36%) for *S. pneumonia* while 22 cases (44%) for *M. catarrhalis* and 6 cases (12%) for both organism and 4 cases (8%) were negative. The PCR test results show high significance over culture for both *S. pneumonia* and *M. catarrhalis* ($P < 0.0001$).

For *S. pneumonia*, culture positive cases were 5, while PCR positive cases were 18 which is statistically significant difference ($P < 0.001$). For *M. catarrhalis*, culture positive specimen were 6, while PCR were 22 and this shows a highly statistically significant difference ($P < 0.0001$). In comparison between both organisms it was found that only two positive cases in culture and six positive cases by PCR and this was statistically significant ($P < 0.025$)

These results were in consistence with Gok et al. 2001. They found that PCR assay of samples showed that 35 samples (94.5%) contained bacterial DNA while by bacterial culture 9 samples (24%) showed bacterial growth and they suggested PCR technique is more specific and sensitive in detection of MEE compared with conventional methods.

Bacteriological studies of OME using highly sensitive technique of molecular biology such as PCR have demonstrated that traditional culturing method are inadequate to detect may valuables bacteria present in OME. Culture

of middle ear effusion yield positive results for only 20–30% of patients, while by PCR up to 75% of middle ear fluid effusions were positive for pathogenic bacteria [11].

The *S. pneumonia* was detected more frequently in middle ear that required ventilation tube insertion at least twice compared with those requiring ventilation tube insertions only once.

Conclusion

Traditional bacteriological culture of middle ear fluid for detection of organisms is inadequate method while bacteriological studies of OME using highly sensitive technique of molecular biology as PCR is highly sensitive and accurate method. *M. catarrhalis* plays a significant rule in OME as it is the sole organism identified more than the other one by PCR.

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