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Polymerase Chain Reaction (PCR) versus bacterial culture in detection of organisms in otitis media with effusion (OME) in children

Balegh Aly[1], Mostafa Hamad[1], Mervat Mohey[2], Sameh Amen[3]
ENT department, Faculty of Medicine, El Minia University[1], Clinical pathology department, Al Azhar Universityt[2], ENT department, Faculty of Medicine Fayiom University[3].

Abstract

Objective: The aim of this prospective 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.
Methods: Fifty patients having OME were included in this study between 2003-2008. Myringotomy and tympanostomy tube insertion was 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.
Results: Bacterial cultures: Five cases (10%) were culture positive for S.pneumonia. Six cases (12%) were culture positive for M.catarrhalis .Only one case (2%) was positive for both S.pneumonia and M.catarrhalis. PCR testing: 18 cases (36%) were PCR 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 by PCR. The difference between the proportion of culture positive and PCR positive specimens for both organisms individually and collectively was significant (P<0.001).
Conclusion:PCR is more accurate than bacterial culture in detection of organisms in middle ear fluid in OME and that M.catarrhalis has more rule in otitis media with effusion as it is more commonly identified by PCR.

Keywords: otitis media with effusion, bacterial culture, polymerase chain reaction.

Introduction

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, and may cause permanent middle ear damage with mucosal changes (1).

The fluid in the middle ear can be serous (a generally 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. OME can be further classified as acute (less than three weeks), subacute (three weeks to three months), or chronic (greater than three months) (2).

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 middle ear effusion (MEE) was thought to be sterile but studies have shown a 30 to 50% incidence of positive middle ear cultures. Polymerase chain reaction (PCR) testing has revealed that over 75% of the specimens are positive for bacterial DNA (3).

The most likely organisms are S. pneumoniae, H. influenzae, M. catarrhalis, and group A strept. (4).

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 (5).

Craig et al, 1995 used the PCR in negative viral and bacterial culture for influenza A virus and S.pneumonia. They showed that PCR was a sensitive and a specific method for detecting influenza and S. pneumonia (6). 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 MEE 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 (6).

Post et al, 1995 concluded that the PCR based assay system can detect the presence of bacterial DNA in a significant percentage of culturally sterile MEE (5). 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.

Patients and Methods

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

Clinical features:

All cases were suffering from deafness. Patients were diagnosed to have OME with full history taking, otoscopic examination, tympanometry, pure tone and play audiometry.

MEE samples were aspirated from patients undergoing myringotomy with the insertion of tympanostomy tube for OME. For all patients considered, MEE has persisted for at least 3 months prior to the date of the operation.

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°C. If no growth was observed within 48h, 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 12,000rpm. The supernatant was poured off and 1ml of DNA extraction buffer was added to the pellet and mixed well till complete dissolution. This was incubated in water bath at 65°C for 30min. Centrifugation was done for 15min at 14,000 rpm and the upper 500µl was transferred to a new tube containing 500µl 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 supernatant was poured off. The pellet was washed two times with 70% Ethanol and air dried. Lastly the pellet was resuspended in 50µl sterile water or 1X TE buffer (10mM Tris, 1M Na2 EDTA, pH 8.0). Five to ten µl were loaded on agarose gel.

PCR amplification of the DNA

PCR amplification was carried in 100µl reaction mixture each containing 56.5µl of sterile distilled water, 16µl of deoxyribonucleoside triphosphate(0.2mM), 10µl of 10X PCR buffer (100mM tris Hcl {pH8.3}.500mM KCl, 15mM MgCl2), one µl of each one of the primer pairs to be used (0.3µg/µl), 0.5?l of taq DNA polymerase(5U/µl),15µl 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 1min.
Annealing at 55°C for 1min.
Extension at 72°C for 1min.
This is was repeated for 34 times
Then final extension at 72°C for 10min.

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

Results:

The age of the patients ranged from 3-9 years (mean = 4.4 years). Thirty three cases were males (66%) while 17 cases were female (34%). Thirteen cases had previous myringotomy (26%) while 37 cases had not (74%). Nine cases (18%) had previous adenotonsillectomy while 41(82%) cases had not. Thirty eight cases (76%) were bilateral, while 12 cases (24 %) were unilateral. 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.

PCR and culture result:

Bacterial cultures:

Five cases (10%) was culture positive for S.pneumonia. While six cases (12%) was culture positive for M.catarrhalis .One case (2%) was positive for both S.pneumonia and M.catarrhalis.

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 .

There was no specimen showing culture positive and PCR negative for S.pneumonia. In the other hand, 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. On the other hand, 16 cases of PCR positive specimens were culture negative for M.catarrhalis.

	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 organisms	1	2%	6	12%	1.96	<0.025
Negative	38	76%	4	8%	6.89	<0.0001

Table (1) comparisonbetween PCR and culture

In the 6 cases which were positive PCR for both organisms, 5 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 otitis media with effusion as it w the most common organism identified by PCR in our study.

Result of samples	Bacterial culture		PCR		Z	P
	NO	%	NO	%		
positive	12	24	46	92%	44.7	<0.00001
negative	38	76	4	8%		

Table (2) PCR versus culture positive and negative cases

Discussion:

Otitis media with effusion is one of the main causes of hearing loss in children. The loss is usually conductive, with an average air conduction threshold of 27.5dB (7).

Otitis media with effusion is considered a direct extension of the inflammatory process that occurs during episodes of acute otitis media. This observation suggest that otitis media with effusion has an infectious etiology (8).

Tong et al, 2000, detected gram negative endotoxin in 80% of middle ear effusion including many culture negative (9). 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 a single organism. The bacteriological studies of MEE using highly sensitive technique of molecular biology such as PCR have demonstrated higher sensitivity. Culture of MEE yield positive result in 20-30% of patients while PCR results are as high as 75% (3).

Arguedas et al, 2003, found that S.pneumonia and M.catarrhalis were the most common isolated organisms from MEE followed by Haemophilus influenzae (10). In our study bacterial culture for S.pneumonia were positive in 5 cases (10%) and for M.catarrhalis in 6 cases (12%) and only one cases (2%) showed positive for both organisms. 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, 22 cases (44%) for M.catarrhalis and 6 cases (12%) for both organisms. Only 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 2 positive cases in culture and 6 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 (11). In conclusion, traditional bacteriological culture of MEE for detection of organisms is usually inadequate, while polymerase chain reaction (PCR) is highly sensitive and accurate method. M.catarrhalis may play a more important role in OME as it is more commonly identified by PCR.

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