Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 2
Open Access

The Prevalence and Molecular Detection of the Main Bacterial Pathogens among Patients with Chronic Otitis Media with Effusion

Ghaidaa Abdul Kareem Goodi[1] and Kais Kassim Ghaima[2*]

[1]Ministry of Health, Baghdad, Iraq, ghaidaaa1992@gmail.com

[2] Institute of Genetic Engineering & Biotechnology for Postgraduate Studies- University of Baghdad- Baghdad- Iraq, kaisskasim22@ige.uobaghdad.edu.iq

Article Info

Article type:

Research

Article History:

Received: 2024-03-15

Revised: 2024-05-20

Accepted: 2024-06-18

Keywords:

Chronic otitis media, Effusion, Bacteria, PCR.

A B S T R A C T

Aim: This study investigates the diagnosis of chronic otitis media bacterial infection using molecular methods that are difficult to culture and conventional culture methods.

Methodology: 110 clinical specimens (ear discharge) and healthy control were gathered from individuals suffering from persistent otitis media (COMCOM) with effusion, with 46 (41.8%) females and 64 (58.2%) males. All samples had their DNADNA extracted to identify the bacterium. Out of the 110 ear exudate samples that were analyzed, 23(20.9%) specimens included bacterial pathogens, including *Pseudomonas aeruginosa* (no=17, 15.5%) and *Staphylococcus aureus* (no=6, 5.5%) diagnosed by VITEKVITEK 2 Compact.

Results: The presence of the primers at the precise nucleotide positions in the 16S rRNA genes (*H. influenzae*, *M. catarrhalis*, *S. pneumoniae*) was detected by PCR. Every sample of extracted DNADNA was included in the PCR reaction. PCR was used to identify isolates of 16 *M. catarrhalis* (14.5%), 6 *S. pneumoniae* (5.5%), and 4 *H. influenzae* (3.6%) from 110 OME samples—outcomes of PCR and traditional culture for 110 OME samples from COMCOM patients. According to the current data, *P. aeruginosa* (17/49; 34.69%) and *M. catarrhalis* (16/49; 32.65%) were the most common species, whereas *Haemophilus influenzae* (4/49; 8.16%) had the lowest prevalence rate.

Conclusion: *P. aeruginosa* and *M. catarrhalis* were found in COMCOM patients at high frequencies, and molecular Identification of bacterial infections that are difficult to diagnose by conventional culture methods was empathetic.

INTRODUCTION

The ear, like vision, is one of the most important sources of biological information. It is a vulnerable organ of the human body that is primarily responsible for detecting, transmitting, and transforming sounds. The ear also plays a vital role in maintaining our body's equilibrium (Cao *et al.,* 2023). Inflammation of the middle ear, or otitis media, is the most common and severe infection worldwide; it can manifest as non-suppurative otitis media with effusion (OMEOME), suppurative acute otitis media (AOMAOM), or chronic otitis media (COMCOM) (WHO, 2021). The varied condition known as Chronic Otitis medium (COMCOM) is characterized by ongoing inflammation of the middle ear and/or mastoid cavity; a significant portion of people with Chronic Otitis medium (COMCOM) may experience gradual hearing loss, which can impact their quality of life (Al-Obaydi, 2011; Rahim & Ali, 2019; Elzinga *et al*., 2021Leichtle *et al*., 2024). Otitis medium with effusion (OMEOME) can develop as an inflammatory reaction to AOMAOM or impaired Eustachian tube function. Several papers have linked bacteria to OMEOME (Korona-

Glowniak *et al.,* 2020). Globally, more than 360 million people experience severe hearing loss. It is possible to prevent over 60% of this hearing loss, with infections responsible for up to 40% of this avoidable hearing loss (Brown *et al.,* 2018). Because of its many resistance mechanisms, Pseudomonas aeruginosa is the most common unwanted illness that causes morbidity and death in hospitalized patients (AL-Ataar, 2015; AL-Fridawy *et al.,* 2020). Pseudomonas aeruginosa is the most common bacteria in Chronic Suppurative Otitis Media (CSOM). It is pathogenic and may form biofilms (Alwan *et al.,* 2021; Al-Sheikhly *et al.,* 2020; Abd et al., 2020; Janitra *et al.,* 2023). In both clinical and community settings, Staphylococcus aureus is a primary source of severe human infections (AlKhazraji *et al.,* 2020). The efflux pump system and most clinical isolates of Staphylococcus aureus are essential for multidrug resistance (Shamkhi *et al.,* 2019; Ahmed & Al-Daraghi, 2022). acute otitis media can also be caused by two more common bacteria, *Haemophilus influenzae* and *Moraxella catarrhalis*, which can cause CSOMCSOM. Otitis media is also frequently caused by *streptococcus pneumoniae* (Khairkar *et al.,* 2023).

Streptococcus pneumoniae, Haemophilus influenzae, and *Moraxella catarrhalis* are the three bacterial pathogens that are most frequently reported; recent research has demonstrated the role of pathogen-formed biofilms in the chronicity and recurrence of otitis media (Silva & Sillankorva, 2019). The significance of molecular techniques lies in their ability to discover novel bacterial species in middle ear infection samples and raise the frequency of positive culture results; molecular Identification improves the sensitivity of pathogen detection; for example, qPCR was found to be more sensitive than culture to detect these pathogens (Silva *et al.,* 2021).

MATERIALS AND METHODS

Subjects' selection and sample collection

Between August 2022 and April 2023, otolaryngologists collected 110 clinical specimens (ear discharges) and a healthy control under sterile settings from patients who were inpatients or outpatients seeking ENTENT consultations at Ghazi Alhariri Hospital for specialist surgery in the Baghdad Medical City. The individuals whose specimens were obtained were between 15 and 55. All patients were asked questions regarding their age, the onset of the disease, whether they had a unilateral or bilateral ear infection, their symptoms, any previous illnesses they may have had, and if they were taking any medication throughout the interviews

Ear swabs sample analysis.

Following a 70% ethanol cleaning of the auditory canal, sterile cotton swabs were used to massage the pus removed from the ears to collect samples of ear discharge. Each patient was given a sterile, dry cotton swab and a transport medium. Dry swabs were added to Eppendorf tubes with 1 mL of phosphate buffer saline (PHPH 7) mixed well. The tubes were then frozen to extract bacterial DNADNA and prepare it for PCR analysis. On the other hand, mannitol salt agar, cetrimide agar, blood agar, and MacConkey agar media were grown directly on a transport medium.

Genes Selection

In this study, the following genes are detected using conventional PCR after DNADNA is extracted using the Monarch Genomic DNADNA Purification Kit (New England Biolabs, USAUSA). The 16S gene is used as a primer for *Hemophilus influenzae* (526 bp fragments) and 16S (235 bp fragments) for *Moraxella catarrhalis*. Additionally, as indicated in Table (1), 16S (500 bp) segments for *Streptococcus pneumoniae* (Hendolin *et al*., 2000) are also employed.

Table 1. Conditions of PCR reaction for detecting bacterial infections in a sample of Iraqi chronic otitis media.

The bacterial species	Target Gene	Primer name	Oligonucleotide primer Sequence (5-3)	Product size(bp)	The Reference
Haemophilus. influenza	16S rRNA	F	CGT ATT ATC GGA AGA TGA AAG TGC	526	(Hendolin *et al*., 2000)
		R	CTA CGC ATT TCA CCG CTA CAC		

Morexella catarrhalis	16S rRNA	F	CCC ATA AGC CCT GAC GTT AC	235	
		R	CTA CGC ATT TCA CCG CTA CAC		
Streptococcus pneumoniae	16S rRNA	F	AAG GTG CAC TTG CAT CAC TAC	500	

Amplification reaction

Five microliters of DNADNA template are amplified using a PCR reaction tube filled with 25 microliters, 12.5 microliters of Go Taq green master mix (NEB, USAUSA), and 1.5 microliters of whole primer (10 pmol/µL) and whole gene. The final volume is 25 microliters with 4.5 nucleases of free water. After thawing at four °C, the extracted DNADNA, primers, and PCR premix were vortexed and quickly centrifuged, moving the contents to the bottom of the tubes; then, after several attempts, the polymerase chain reaction was enhanced. Everything was absent from the negative control except DNADNA, to which NFWNFW was introduced instead of template DNADNA. On a thermal cycler (SimpliAmp (ThermoFisher Scientific), USAUSA), PCR protocols were created (Table 2).

Table 2. Conventional PCR materials reaction.

Material	Volume (µL)
MMMM	12.5
Forward	1.5
Reverse	1.5
DNA	5
NFWNFW	4.5
Total	25

Conditions of the PCR reaction for 16S rRNA detection according to Hendolin *et al*. (2000). After many attempts to maximize the PCR settings, the reaction performs (modified somewhat) in a DNADNA thermal cycler. Table (3) adopts the following program.

Table 3. Conditions of PCR reaction for 16S rRNA detection.

Cycle Step	Temperature (°C)	N. of cycles	Time
Initial Denaturation	94°C	1 cycle	3 minutes
Denaturation	94°C	35 cycles	30 seconds
Annealing	51[1] 53[2]		45 seconds
Extension	70°C		30 seconds
Final Extension	70°C	1 cycle	5 minutes

[1] for *Haemophilus. Influenza*, [2] for *Morexella catarrhalis* and *Streptococcus pneumoniae* 16S Rrna

Culture and Identification

Pseudomonas aeruginosa and Staphylococcus aureus were isolated from the ear discharge samples using selected agar plates, Blood, MacConkey, and Chocolate culture medium. All bacterial isolates were identified using recognized microbiological methods after a 24-hour aerobic incubation at 37°C (MacFaddin, 2000). The species of bacteria were identified using the microbiological automated system VITEK® 2 Compact (BioMérieux, France). Gram-negative bacteria were identified using IDGNIDGN (REF 21341) cards, whereas Gram-positive bacteria were identified using ID-GP (REF 21342) cards (Pincus, 2006).

RESULTS AND DISCUSSION

Isolation and Identification of pathogenic bacteria of Chronic Otitis Media infection

Following the morphology in Gram's staining, cultural traits, and biochemical properties, the isolates were isolated

and identified. The following selective media were used to cultivate 110 clinical specimens of ear discharge swabs: Blood agar, MacConkey agar, mannitol salt agar, and Cetrmide agar. The results demonstrated that the bacteria in the blood agar colony have sticky textures, a white-to-gray color, the capacity to hemolyze blood, and a specific form of hemolysis. P. aeruginosa on blood agar frequently displays beta hemolysis, a metallic sheen, and blue or green pigment, according to Korgaonkar *et al*. (2013). The colonies showed lactose non-fermenting on MacConkey agar, with small, pale colonies (Figure 1).

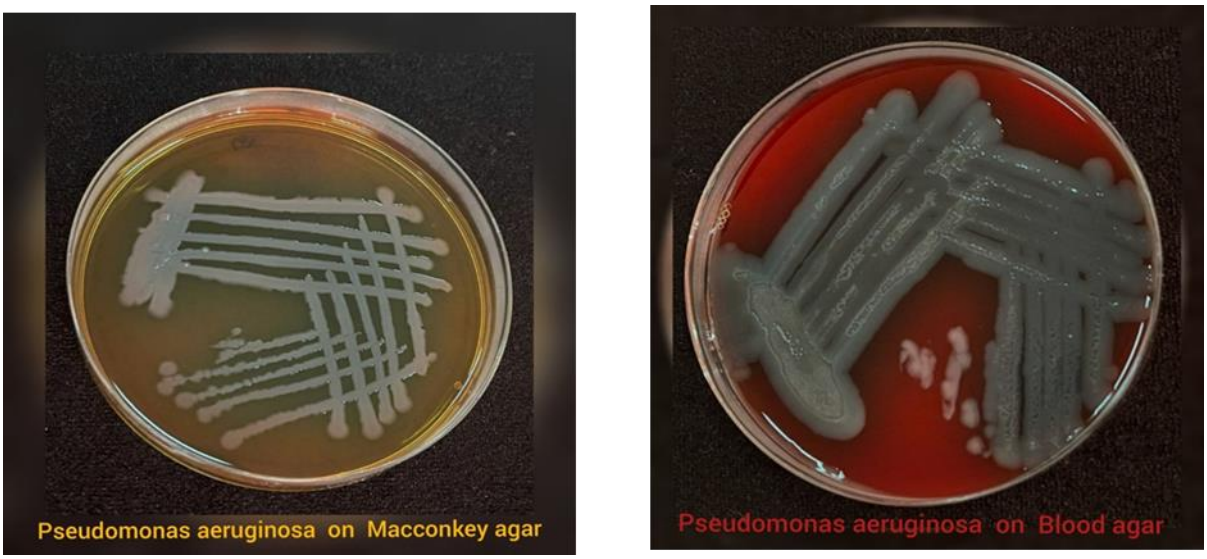

Figure 1. Colonies of *P. aeruginosa* on MacConkey Agar and Blood agar (37˚C/24 hrs).

Pyocyanin (blue) and pyoverdine (green), two *P. aeruginosa* metabolites combined, give colonies on Cetrimide Agar a characteristic color. Cetrimide Agar is a selective and differential medium used for *Pseudomonas aeruginosa* isolation and Identification from clinical and non-clinical specimens. *Pseudomonas aeruginosa* colonies that range in hue from yellow-green to blue are good outcomes (Figure 2).

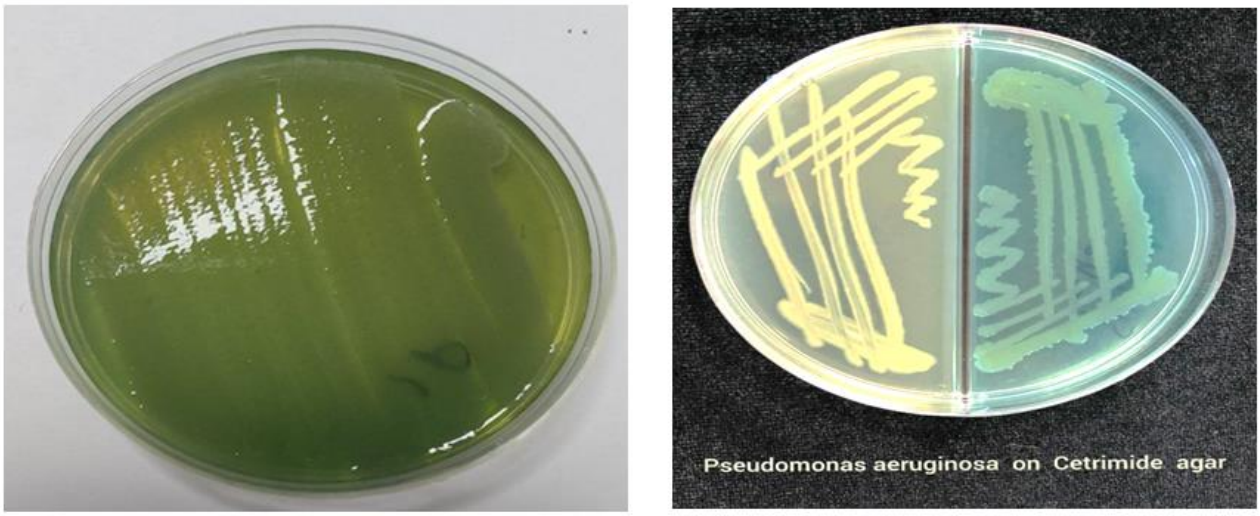

Figure 2. Colonies of *P. aeruginosa* on Cetrimide Agar(37˚C/ 24 hrs).

Staphylococcus aureus grows between 15° and 45°C, reaching a maximum concentration of 15% in NaCl. However, prolonged exposures are not advised at temperatures more than forty-two and less than ten degrees. The plates ought to be refrigerated at 4°C for no more than one week; S. aureus is toxic to alcohol, detergents, and high

osmolarity. Mannitol salt agar with 7.5% NaCl has been utilized for selectivity because S. aureus can ferment mannitol and produce the yellow pigment staphyloxanthin and characteristic gold-colored colonies (Figure 3).

Figure 3. Colonies of *S. aureus* on Mannitol Salt Agar (37°C / 24 hrs).

Out of the 110 ear exudate samples that were analyzed, 23(20.9%) specimens included bacterial pathogens, including *Pseudomonas aeruginosa* (no=17, 15.5%) and *Staphylococcus aureus* (no=6, 5.5%). These findings were based on the results of the biochemical assays. *Staphylococcus aureus* was identified in six of the isolates by the use of coagulase, catalase, and Gram stain assays. Additionally, 17 isolates gave off a fishy odor and produced greenish pigmentation on McConkey agar media. Additionally, they were verified to be *Pseudomonas aeruginosa* by being shown to be motile and highly oxidase-positive at 37 °C. Additionally, all isolates were identified using the API 20E method for *P. aeruginosa* identification of Enterobacteriaceae isolates.

Identification of bacterial pathogens by molecular method Genomic DNADNA extraction

Genomic DNADNA was isolated to molecularly detect isolates of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* using a genomic DNADNA purification kit (NEB, USAUSA). extraction of genomic DNADNA from 110 Otitis media infection specimens (effusion) that were verified as bands using gel electrophoresis. Figure (4) displays the DNA-DNA extraction findings. The amount of DNA-DNA was measured using the Qubit fluorometer, which counts the amount of proteins or nucleic acids in a sample using fluorescent dyes. In the Qubit system, fluorescent hues bind specifically to target analytes such as RNA-RNA, protein, miRNA, double-stranded DNA-DNA (dsDNA), and single-stranded DNA-DNA (ssDNA), allowing for more accurate measurement. The DNA-DNA concentration of each isolate ranged from 50 to 100 ng/µl. Figure (4) shows the process of genomic DNA-DNA electrophoresis; after EtBr staining on a 1% agarose gel at 70 volts for 45 minutes, the bands were visible under a UVUV light.

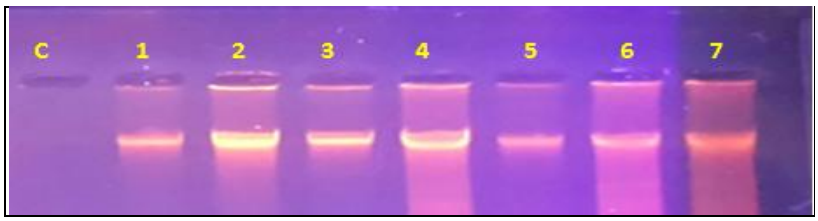

Figure 4. Agarose gel electrophoresis of extracted DNADNA to check purity and integrity. Lane 1-7: DNADNA of different Otitis media specimens, Lane C: Negative control. (70 V/ 30 min.).

Identification of *S. pneumoniae*, *M. catarrhalis*, and H*. influenzae* by Polymerase Chain Reaction (PCR)

PCR was used to detect the presence of the specific nucleotide locations of the primers in the 16S rRNA genes (*H. influenzae*, 177–200; *M. catarrhalis*, 416–435; *S. pneumoniae*, 106–127) in order to identify *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae* isolates in the specimens of Otitis media infections. Every sample of extracted DNADNA was included in the PCR reaction. The PCR results have been verified by analyzing the bands on gel electrophoresis and comparing their molecular weight with a 100 bp DNADNA ladder. Figures (5), (6), and (7) display the outcomes of the PCR reaction for the 16S rRNA genes (*H. influenzae*, 526 bp); *M. catarrhalis*, 235 bp; and *S. pneumoniae*, 500 bp).

Figure 5. Agarose gel electrophoresis of PCR products for *16S rRNA* genes (*H. influenzae*, (526bp); *M. catarrhalis*, (235bp); *S. pneumoniae*, (500bp). Lane M: 100bp DNADNA ladder. (70V for 2hr).

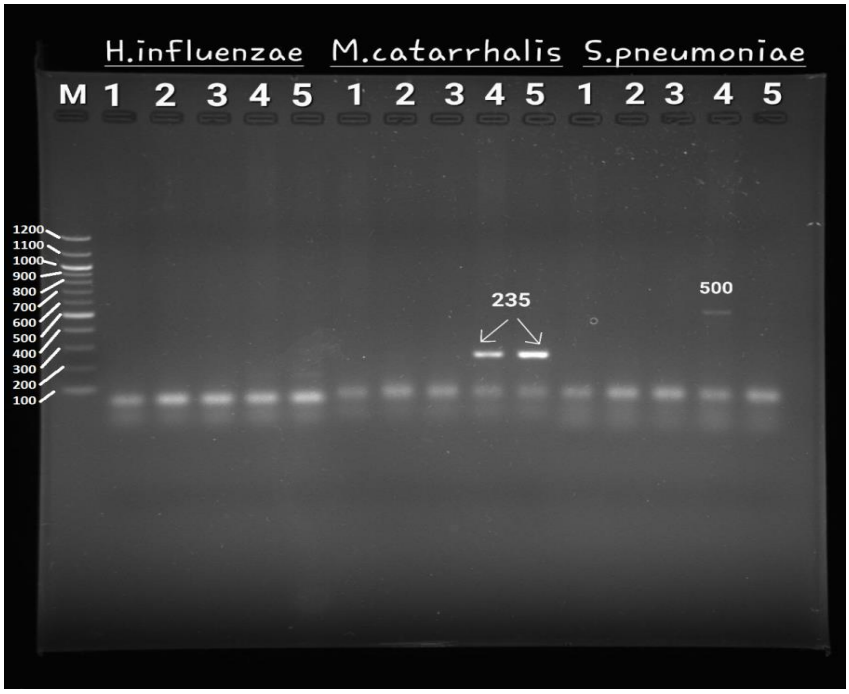

Figure 6. Agarose gel electrophoresis of PCR products for *16S rRNA* genes (*H. influenzae*, (526bp); *M. catarrhalis*, (235bp); *S. pneumoniae*, (500bp). Lane M: 100bp DNADNA ladder. (70V for 2hr).

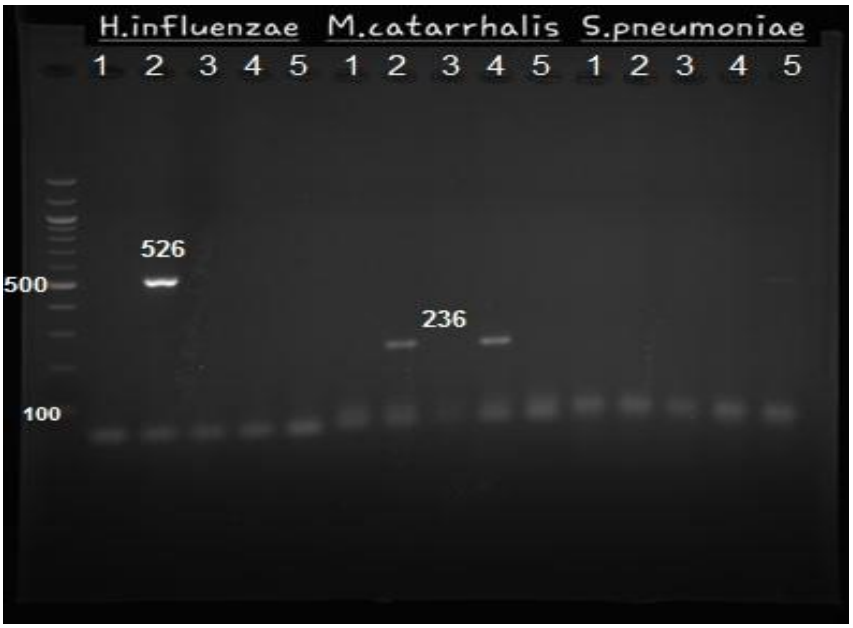

Figure 7. Agarose gel electrophoresis of PCR products for *16S rRNA* genes (*H. influenzae*, (526bp); *M. catarrhalis*, (235bp); *S. pneumoniae*, (500bp). Lane M: 100bp DNADNA ladder. (70V for 2hr).

Matar *et al.* (1998) discovered that the PCR assay for OMOM pathogen detection was quicker, cost-effective, and more sensitive than culture-based methods. It is also PCR-Southern hybridization-based. At 16600 daltons, the outer membrane protein (OMPOMP) P6 of *H. influenzae* is a lipoprotein linked to peptidoglycans. Because of its high level of sequence conservation among strains, its encoding gene is regarded as both a desirable vaccine candidate and an excellent target for diagnosis in *H. influenzae* (Michel *et al.,* 2011).

Distribution of pathogenic bacteria among chronic otitis media patients

PCR was used to identify isolates of 16 M. catarrhalis (14.5%), 6 *S. pneumoniae* (5.5%), and 4 *H. influenzae* (3.6%) from 110 OME samples. Table (4) presents the statistical analysis of 110 OME samples from patients with OMOM and the results of standard culture, biochemical testing, and PCR. According to the findings, of the five distinct species that were the subject of the investigation, 49 isolates (44.5%) were found to harbor bacterial pathogens.

Table 4. Distribution of the pathogenic bacteria among patients with COMCOM.

Bacteria	**Total**
Haemophilus influenza	4 (8.16%)
Moraxella catarrhalis	16 (32.65%)
Streptococcus pneumoniae	6 (12.2%)
Pseudomonas aeruginosa	17 (34.69%)
Staphylococcus aureus	6 (6 (12.2%)
Total	49

According to the current data, *P. aeruginosa* (17/49; 34.69%) and *M. catarrhalis* (16/49; 32.65%) were the most common species, *whereas Haemophilus influenzae* (4/49; 8.16%) had the lowest prevalence rate.
The Baysallar *et al*. (2013) study used multiplex PCR to identify 7 *S. pneumoniae*, 9 *H. influenzae*, and 11 *M. catarrhalis* isolates from 67 MEEMEE samples. This suggests that the PCR test enables quick screening and is a viable method for identifying the most prevalent fastidious bacteria that cause OMEOME. The high rate of PCR detection of Otitis Media with effusion in contrast to the culture method was found in the study done in Shiraz, Iran, and the discrepancies with previous studies may be attributed to the paucity of *H. influenzae* vaccine in the Middle

East (Shishegar *et al.,* 2011). The presence or absence of live organisms cannot be determined by PCR analysis of bacterial DNADNA recovered from culture-negative materials in research, including kids who had recurrent AOMAOM and OMEOME. It was demonstrated that genetic material deteriorated two days after bacterial death. Therefore, it is likely that the DNADNA found in this study also came from living bacterial species. When identifying the bacterial infection that caused OMEOME, the PCR assay's specificity and sensitivity were greater than the culture approach's. *M. catarrhalis*, *S. pneumoniae*, and *H. influenzae* were found in 12.8%, 20%, and 20% of samples, respectively, using the PCR test (Farajzadah Sheikh *et al*., 2015), and these findings disagreed somewhat with our findings.

S. pneumoniae and *H. influenzae* were the most frequently found, and molecular methods were utilized to detect bacteria much more frequently than traditional culture methods; this means that molecular methods may be an additional helpful diagnosis method of lower respiratory tract infections (LRTIsLRTIs) (Tchatchouang *et al.,* 2019). Assays for real-time PCR demonstrated exceptional sensitivity and no cross-reactivity with other species, real-time PCR was much more common than culture in identifying the bacteria responsible for chronic obstructive pulmonary disease in cases of *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus* species, and *Moraxella catarrhalis*, Real-time PCR revealed some infections that were not discovered by culture (Curran *et al*., 2007).

The results of the current investigation were consistent with the findings of Mittal *et al*. (2015), which stated that the two most common bacteria that cause OMOM are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. According to local research conducted in Basrah by Alsaimary et al. (2010), the most prevalent microorganisms found in the sixty patients with chronic suppurative otitis media (CSOMCSOM) were Gram-negative bacteria, which comprised 65 (54.2%) men and 55 (45.8%) females. The male-to-female ratio was 1.2:1. The most frequent causal agent was *Pseudomonas aeruginosa* (19.04%), followed by *Klebsiella spp*. (14.3%) and *Staphylococcus aureus* (16.7%). *P. aeruginosa* and other bacteria were more prevalent in the high percentage (74%), where mixed infections were discovered.

According to Mofatteh *et al.* (2018), the most common bacteria recovered from 185 patients with a clinical diagnosis of chronic suppurative otitis media were *Staphylococci spp*: the successful antibiotic, Ciprofloxacin in treating bacterial chronic suppurative otitis media. A retrospective analysis was carried out in the otolaryngology clinic of Liuzhou Maternity and Child Healthcare Hospital. During the study, 228 children between 0 and 15 were found to have AOMAOM. Male to female ratio was 1:0.6. Out of 228 specimens, 181 (79.4%) tested positive for pathogenic bacteria. The most prevalent bacteria found in these specimens were *S. pneumoniae* (36.4%), then *S. aureus* (16.2%), *P. aeruginosa* (4.4%), and *H. influenzae* (3.9%) (Ding *et al.,* 2018).

A middle ear bacterial infection is the most frequent cause of OMOM. According to Qureishi *et al.* (2014), *Moraxella catarrhalis*, *Haemophilus influenzae*, and *Streptococcus pneumonia* are the leading causes of AOMAOM. However, the most frequent aerobic microbial isolates in patients with CSOMCSOM are *Pseudomonas aeruginosa* and *Staphylococcus aureus*, then Klebsiella *pneumoniae* and *Proteus vulgaris* (Sattar *et al.,* 2012). *P. aeruginosa* is the most prevalent pathogen that causes CSOMCSOM, followed by *S. aureus*, according to a various nations research, including India, Nepal, Singapore, and Nigeria (Madana *et al.,* 2011; Ahn *et al.,* 2012). However, according to research from Saudi Arabia, Iran, and Pakistan (Gilgit*), P. aeruginosa* was the traditional pathogen, then S. *aureus* (Ahmad *et al.,* 2013; Khalil *et al.,* 2013). The actions in the patient group investigated varied, and regional variance may be the cause of the discrepancies throughout the research. Pseudomonas spp. was the high prevalent pathogen in the middle ear, while *Streptococcus spp*. they dominated the tonsil microbiota, with relative abundance rates of 82.7 and 69.2 %, respectively, in a cross-sectional study of the bacterial microbiota in middle ear, adenoid, and tonsil specimens from a pediatric patient with chronic serous OMOM using 16S rRNA gene-based pyrosequencing analysis (Liu *et al*., 2011). Table (5) summarizes the age distribution of pathogenic microorganisms identified from COMCOM infections.

Table 5. Distribution of the pathogenic bacteria according to age groups.

Bacteria	**15-35 Years**	**36-55 years**	**56-80 years**	**Total**	**Chi-square (χ2)**
Haemophilus influenza	1 (25.00%)	1 (25.00%)	2 (50.00%)	4 (8.16%)	0.722 NS

Moraxella catarrhalis	10 (62.50%)	5 (31.25%)	1 (6.25%)	16 (32.65%)	5.482 *
Streptococcus pneumonia	3 (50.00%)	3 (50.00%)	0 (0.00%)	6 (12.2%)	0.107 NS
Pseudomonas aeruginosa	11 (64.71%)	5 (29.41%)	1 (5.88%)	17 (34.69%)	7.944 **
Staphylococcus aureus	4 (66.67%)	2 (33.33.00%)	0 (0.00%)	6 (6 (12.2%)	4.023 *
Total	29	16	4	49	---
Chi square (χ2)	5.627 *	2.388 NS	0.569 NS	9.806 **	---
*** (P≤0.05), ** (P≤0.01),**					

Table 6. The distribution of pathogenic bacteria isolated from patients with COMCOM infections is according to gender and age ranges.

Factor		**Number (%)**	**Chi-square (χ2)**
Gender	Female	23 (46.9%)	0.080 NS
	Male	26 (53.1%)	
Age (year)	15-35	24 (49%)	9.257 **
	35-55	18 (36.7%)	
	56-80	7 (14.3%)	
Total	---	49	---
**** (P≤0.01), NS: Non-Significant.**			

There was a statistically significant difference (p>0.05) in the total number of positive samples recorded in females (23; 46.9%) and males (26; 53.1%), although the difference was not statistically significant. Adults between the ages of 15 and 35 had 24 (49%) positive samples overall out of 110 positive samples, which was statistically considerably higher (P<0.01) than the 18 (36.7%) and 7 (14.3%) positive samples for the age group 35–55 and 56–80, respectively.

It has been shown that middle ear infections affect about 75% of children under three. According to Bakar et al. (2018), OMOM complications are the primary cause of avoidable hearing loss, especially in environments with limited resources. Up to 75% of children will experience acute otitis media (AOMAOM), the most frequent bacterial illness in children under the age of five. Up to 80% of bacterial AOMAOM is caused by *Streptococcus pneumoniae* and *Haemophilus influenzae*, the non-typable strains (NTHi) (Duff et al., 2024; Casey & Pichichero, 2004).

According to research done on children under a year old, 27% of patients with a symptomatic upper respiratory tract infection progressed to develop AOMAOM (Chonmaitree *et al*., 2015). Although it often appears in the early years of life, CSOMCSOM can continue throughout maturity. The illness affects 65–330 million individuals worldwide, mostly in underdeveloped nations. According to estimates, there are 31 million new cases of CSOMCSOM annually, with 22.6% occurring in children under the age of five (Monasta *et al.,* 2012). AOMAOM is very common; 75 percent of children have had at least one ear infection before starting school. The absence of middle ear drainage causes fluid stasis, resulting in AOMAOM if the fluid becomes colonized with bacterial or viral pathogens. Children are more likely than adults to get viral infections, and their ETsETs are shorter and more horizontal than adults (Coticchia et al., 2013). According to the study, the most incredible occurrence happened between six and twelve months of life. Male sex, daycare attendance, family history of recurrent AOMAOM, and early occurrence of AOMAOM were all linked to a significantly increased risk of AOMAOM, according to multivariable analysis of demographic and environmental data. The proportion of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* that cause AOMAOM also varied dynamically (Kaur *et al.,* 2017). The variation in AOMAOM incidence in this research compared to the incidence previously reported in other countries may be related to the differences in healthcare systems, societal structure, and diagnostic techniques between physicians.

CONCLUSION

S. pneumoniae and *H. influenzae* were shown to be the most prevalent pathogenic bacteria among adult patients with Chronic Otitis medium with effusion, followed by *P. aeruginosa* and *M. catarhalis*, which are challenging to detect using a conventional culture approach. However, one-third of the MEFMEF samples had more than one bacterial infection. PCR ultimately became the most precise method for identifying the OMEOME etiology in the effusion samples.

REFERENCES

1. Cao, C., Song, J., Su, R., Wu, X., Wang, Z. and Hou, M. (2023). Structure-constrained deep feature fusion for chronic otitis media and cholesteatoma identification. *Multimedia Tools and Applications*. pp.1-21.
2. Korgaonkar, A., Trivedi, U., Rumbaugh, K. P., and Whiteley, M. (2013). Community surveillance enhances Pseudomonas aeruginosa virulence during polymicrobial infection. *Proceedings of the National Academy of Sciences*, *110*(3), pp.1059–1064.
3. Elzinga, H.B.E., van Oorschot, H. D., Stegeman, I. and Smit, A. L. (2021). Relation between otitis media and sensorineural hearing loss: a systematic review. *BMJBMJ open*. 11(8).
4. Baysallar, M. Güçlü, A. Ü., Yetİşer, S., Kılıç, A., Gözen, A. G. and Açıkel, C.(2023). Comparison of culture and PCR methods in detection of Haemophilus influenzae, Streptococcus pneumoniae and Moraxella catarrhalis in children with otitis media with effusion. Türkiye Klinikleri tıp Bilimleri Dergisi.33(1),pp. 54-58.
5. Janitra, S.N., Purnami, N., Handoko, E. and Widodo, A.D.W. (2023). Effect of Acetic Acid on Clinical Isolated Pseudomonas aeruginosa Biofilm in Chronic Suppurative Otitis Media: In vitro Study. *Research Journal of Pharmacy and Technology*, *16*(9), pp. 4138-4142.
6. Shishegar, M., Faramarzi, A., Kazemi, T., Bayat, A. and Motamedifar M. (2011). Polymerase chain reaction, bacteriologic detection, and antibiogram of bacteria isolated from otitis media with effusion in children, Shiraz, Iran. *Iranian Journal of Medical Sciences,* 36(4), pp.273–280.
7. Pincus, D. H. (2006). Microbial Identification using the bioMérieux Vitek® 2 system. *Encyclopedia of Rapid Microbiological Methods. Bethesda, MD: Parenteral Drug Association*, pp.1-32.
8. Alsaimary, I.E., Alabbasi, A.M., and Jassim M. Najim, J.M. (2010). Antibiotics susceptibility of bacterial pathogens associated with otitis media, Journal of Bacteriology Research, 2(4), pp.41-50.
9. Kaur, R., Morris, M. and Pichichero, M.E. (2017). Epidemiology of Acute Otitis Media in the Postpneumococcal Conjugate Vaccine Era. *Pediatrics*. 140(3),pp.e20170181.
10. Coticchia, J.M., Chen, M., Sachdeva, L., Mutchnick, S. (2013). New paradigms in the pathogenesis of otitis media in children. Front Pediatr.,1,pp. 52.
11. Brown, C. S., Emmett, S. D., Robler, S. K. and Tucci, D. L. (2018). Global hearing loss prevention. *Otolaryngologic Clinics of North America*, *51*(3), pp.575-592.
12. Silva, M. D. and Sillankorva, S. (2019). Otitis media pathogens–A life entrapped in biofilm communities. *Critical reviews in microbiology*, *45*(5-6), pp.595-612.
13. Silva, M.D., Lima, A., Marçal, N., Dias. L., Gama, M. and Sillankorva S. (2021). Identification of the Bacterial Pathogens in Children with Otitis Media: A Study in the Northwestern Portuguese District of Braga. Microorganisms, 10(1), pp.54.
14. Monasta L., Ronfani L., Marchetti F., Montico M., Vecchi Brumatti L., Bavcar A., Grasso D., Barbiero C., Tamburlini G. (2012). Burden of disease caused by otitis media: systematic review and global estimates PLoS One, 7, pp.e36226
15. Mariam, Khalil A., Ahsanullah M., Mehtab J., Raja I., Gulab S., Farmanullah, Abdul L. (2013). Prevalence of bacteria in chronic suppurative otitis media patients and their sensitivity patterns against various antibiotics in human population of Gilgit. *Pak J Zool,* 45, pp.1647–1653.
16. Khairkar, M., Deshmukh, P., Maity, H. and Deotale, V. (2023). Chronic suppurative otitis media: a comprehensive review of epidemiology, pathogenesis, microbiology, and complications. *Cureus*, 15(8).

17. Hendolin, P. H., Paulin, L. and Ylikoski, J. (2000). Clinically applicable multiplex PCR for four middle ear pathogens. *Journal of Clinical Microbiology*, *38*(1), pp. 125–132.
18. MacFaddin, J, F. (2000). Biochemical tests for Identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Philadelphia, PA.
19. World Health Organization(WHO) (2021). World Report on Hearing (World Health Organization). License: CC BY-NC-SA3.0IGO. Available from: https://apps.who.int/iris/handle/10665/339913. Accessed October 12, 2021.
20. Matar, G.M., Sidani, N., Fayad, M. and Hadi, U. (1998). Two-step PCR-based assay for Identification of bacterial etiology of otitis media with effusion in infected Lebanese children. *J Clin Microbiol.* 36(5), pp.1185–8.
21. Michel, L.V., Kalmeta, B., McCreary, M., Snyder, J. and Craig P. (2011). Vaccine candidate P6 of nontypable Haemophilus influenzae is not a transmembrane protein based on protein structural analysis. *Vaccine* 29, pp.1624–1627.
22. Shishegar, M., Faramarzi, A., Kazemi, T., Bayat, A. and Motamedifar, M. (2011). Polymerase chain reaction, bacteriologic detection, and antibiogram of bacteria isolated from otitis media with effusion in children, Shiraz, Iran. *Iran J Med Sci*. 36(4), pp.273–280.
23. Farajzadah-Sheikh, A., Saki, N. and Roointan, M. (2015). Identification of Alloiococcus otitidis, Streptococcus pneumoniae, Moraxella catarrhalis and Haemophilus influenzae in Children with Otitis Media With Effusion. *Jundishapur J Microbiol*. 8(3), pp.e17985.
24. Tchatchouang, S., Nzouankeu, A. and Kenmoe, S. (2019). Bacterial Aetiologies of Lower Respiratory Tract Infections among Adults in Yaoundé, Cameroon. *Biomed Res Int*. 2019, pp.4834396.
25. Curran, T., Coyle, P.V., McManus, T.E., Kidney, J. and Coulter, W.A. (2007). Evaluation of real-time PCR for the detection and quantification of bacteria in chronic obstructive pulmonary disease. *FEMS Immunol Med Microbiol.* 50(1), pp.112–118.
26. Mittal, R., Lisi, C.V. and Gerring, R. (2015). Current concepts in the pathogenesis and treatment of chronic suppurative otitis media. *J Med Microbiol*. 64(10), pp.1103–1116.
27. Ding, Y.L., Fu, J. and Chen, J. (2018). Molecular characterization and antimicrobial susceptibility of Staphylococcus aureus isolated from children with acute otitis media in Liuzhou, China. *BMCBMC Pediatr*. 18(1), pp.388.
28. Qureishi, A., Lee, Y., Belfield, K., Birchall, J. P., and Daniel, M. (2014). Update on otitis media–prevention and treatment. *Infection and drug resistance*, pp.15–24.
29. Madana, J., Yolmo, D., Kalaiarasi, R., Gopalakrishnan, S. and Sujatha S. (2011). Microbiological profile with antibiotic sensitivity pattern of cholesteatomatous chronic suppurative otitis media among children. *Int J Pediatr Otorhinolaryngol,* 75, pp.1104–1108.
30. Ahn, J.H., Kim, M.N., Suk, Y.A. and Moon, B.J. (2012). Preoperative, intraoperative, and postoperative results of bacterial culture from patients with chronic suppurative otitis media. *Otol Neurotol.,* 33, pp.54–59.
31. Ahmad, S. (2013). Antibiotics in chronic suppurative otitis media: A bacteriologic study Egyptian Journal of Ear, Nose, *Throat and Allied Sciences,* 14, pp.191–194.
32. Liu, C.M., Cosetti, M.K., Aziz, M., Buchhagen, J.L., Contente-Cuomo, T.L., Price, L.B., Keim, P.S. and Lalwani, A.K. (2011). The otologic microbiome: a study of the bacterial microbiota in a pediatric patient with chronic serous otitis media using *16SrRNA* gene-based pyrosequencing. *Arch Otolaryngol Head Neck Surg.,* 137(7), pp.664-8.
33. Casey, J.R. and Pichichero, M.E. (2004). Changes in frequency and pathogens causing acute otitis media in 1995–2003. *Pediatric Infectious Disease Journal,* 23, pp.824–828
34. Chonmaitree, T., Alvarez-Fernandez, P. and Jennings, K. (2015). Symptomatic and asymptomatic respiratory viral infections in the first year of life: Association with acute otitis media development. *Clin Infect Dis.,* 60(1), pp.1–9.

35. Mofatteh, M.R., Shahabian-Moghaddam, F., Yousefi, M. and Namaei, M.H. (2018). A study of bacterial pathogens and antibiotic susceptibility patterns in chronic suppurative otitis media. *J Laryngol Otol.*;132(1),pp.41-45.
36. Abu-Bakar, M.B., McKimm, J. and Haque, M. (2018). Otitis media and biofilm: An overview. *Int. J. Nutr. Pharmacol. Neural. Dis*., *pp.70-78*.
37. Sattar A., Alamgir A., Hussain Z., Sarfraz S., Nasir J., Badar-e-Alam (2012). Bacterial spectrum and their sensitivity pattern in patients of chronic suppurative otitis media J Coll Physicians Surg Pak., 22, 128–129.
38. Leichtle, A., Lupatsii, M., Graspeuntner, S., Jeschke, S., Penxová, Z., Kurabi, A., and Bruchhage, K.L. (2024). Anti-inflammatory response to 1, 8-Cineol and associated microbial communities in Otitis media patients. *Scientific Reports*, *14*(1), pp.16362.
39. Duff, A. F., Jurcisek, J. A., Kurbatfinski, N., Chiang, T., Goodman, S. D., Bakaletz, L. O., and Bailey, M. T. (2024). Oral and middle ear delivery of otitis media standard of care antibiotics, but not biofilm-targeted antibodies, alter chinchilla nasopharyngeal and fecal microbiomes. *npj Biofilms and Microbiomes*, *10*(1), pp.10.
40. Korona-Glowniak, I., Wisniewska, A., Juda, M., Kielbik, K., Niedzielska, G., & Malm, A. (2020). Bacterial aetiology of chronic otitis media with effusion in children-risk factors. *Journal of Otolaryngology-Head & Neck Surgery*, *49*(1), pp.24.
41. Alwan, M.K., Ghaima, K.K. and Khalaf, Z.S. (2021). Effect of Combined Antibiotics and Biofilm Formation in Some Bacterial Pathogens from Otitis Media among Children in Baghdad, Iraq. *Medico Legal Update*, *21*(1), pp.592-598.
42. Rahim, G.H. and Ali, M.R. (2019). Tympanostomy Tube Complication in Otitis Media with Effusion. *AL-Kindy College Medical Journal*, *15*(2), pp.58-61.
43. Al-Obaydi, R. A. (2011). Tympanogenic Labyrinthitis (Cochlear Deafness) due to chronic suppurative otitis media (CSOMCSOM). *AL-Kindy College Medical Journal*, *7*(1), pp.75–78.
44. AL-Ataar, Z.I. (2015). The prevalence and antimicrobial resistance of Pseudomonas species in patients with chronic suppurative otitis media. *Al-Kindy College Medical Journal*, *11*(1), pp.49-52.
45. Ahmed, Z. F., and Al-Daraghi, W. A. H. (2022). Molecular detection of medA virulence gene in Staphylococcus aureus isolated from Iraqi patients. *Iraqi journal of biotechnology*, *21*(1).
46. AL-Fridawy, R.A.K., Al-Daraghi, W.A.H., and Alkhafaji, M.H. (2020). Isolation and Identification of multidrug resistance among clinical and environmental Pseudomonas aeruginosa isolates. *Iraqi journal of biotechnology*, *19*(2).
47. Abd Al-Mayali, M., & Salman, E. D. (2020). Bacteriological and Molecular Study of Fluoroquinolones Resistance in Pseudomonas aeruginosa Isolated from Different Clinical Sources. *Iraqi Journal of Science*, 61(9), pp.2204-2214.
48. Al-Sheikhly, MARHMARH, Musleh, L.N., and Al-Mathkhury, H.J. (2020). Gene expression of pelA and pslA in Pseudomonas aeruginosa under gentamicin stress. Iraqi Journal of Science*, 61(2),* pp.295–305.
49. AlKhazraji, N.Z., Al Jubouri, A.S., and Al Marjani, M.F. (2020). Detection of antiseptic resistant genes and biofilm formation in multidrug-resistant Staphylococcus aureus in Baghdad hospitals. Iraqi Journal of Biotechnology, 19(2), pp.21-27.
50. Shamkhi, G. J., Saadedin, S. M., and Jassim, K. A. (2019). Detection the prevalence of some chromosomal efflux pump genes in Methicillin resistant Staphylococcus aureus isolated from Iraqi patients. *Iraqi Journal of Biotechnology*, 18(3), pp.33-42.