

Original Research Article

Comparative study of biofilm formation in tubotympanic and atticoantral ear disease

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ABSTRACT

Background: Biofilm is an organized bacterial community that may be homogeneous or heterogeneous. It plays a significant role in the pathogenesis of chronic rhinosinusitis, chronic tonsillitis and chronic suppurative otitis media. Biofilms may explain the recurrence and recalcitrant episodes of otorrhea in chronic otitis media (COM). The objective of this study was to detect biofilm formation in chronic suppurative otitis media and compare the pattern of biofilm formation in tubotympanic and atticoantral ear diseases.

Methods: This was a prospective cross sectional study at a tertiary care hospital in Varanasi, Uttar Pradesh, India. Patients included in the study ranged from 10 to 65 years of age from both the sexes and varying socioeconomic status. Samples were collected using sterile precautions in cases of tubotympanic as well as atticoantral COM. The collected samples were directly inoculated onto MacConkey and blood agar media. LisaScan® EM microtiter plate reader was used to identify the presence of biofilm.

Results: The most common organism was *P. aeruginosa* and *S. aureus* followed by *Moraxella catarrhalis* whereas in cases with tubotympanic COM, most common organism was *S. aureus*. We found that, in atticoantral type of COM biofilm formation was significantly more common as compared to that in tubotympanic type of COM.

Conclusions: Biofilm formation in COM is even higher when atticoantral disease is present.

Keywords: Biofilm, Tubotympanic, Atticoantral, *P. aeruginosa*, *S. aureus*, *M. catarrhalis*

INTRODUCTION

Biofilms are organized bacterial communities that may be homogeneous or heterogeneous. Biofilm is a vicious layer that adheres to the inflamed tissue surfaces like mucosal lining or osteitic bone and they play a significant role in the pathogenesis of chronic rhinosinusitis, chronic tonsillitis, chronic suppurative otitis media and develop

device related infections. Despite this clinical importance, only a limited number of studies have been done to examine the presence of biofilms in tissues from patients with different types of middle ear infections, COM of tubotympanic type or cholesteatomas.¹

Etiology of COM is multifactorial. Bacterial infection is a major factor in inflammation that lasts longer in COM. Bacteria that are involved in the etiology of COM like

P. aeruginosa, *S. aureus*, gram positive and negative strains have the ability to secrete polysaccharide exopolymer which form a glycocalyx matrix. This matrix along with the bacterial colony together forms the biofilm.² Bacterial biofilms have some defense abilities towards an unfavorable environment like resistance to antibiotics. Bacterial biofilm in COM can provide signals that result in increased exudation of fluid from epithelial surface, turnover rate of epithelial cells and keratin production. This accelerates the formation of cholesteatoma and/or local inflammation in COM, resulting in more complications and difficulty in treatment.²⁻⁴ Management aimed at surgical debridement and cleaning up the pathologic tissue is an effective standard treatment.⁵

The aim of our study was to determine the formation of biofilm in COM and to compare the pattern of biofilm formation between tubotympanic and atticointral COM.

METHODS

This was a prospective cross sectional study done in the patients of COM presenting for treatment to the department of otorhinolaryngology, institute of medical sciences, Banaras Hindu university, Uttar Pradesh between December 2018 to March 2020. Sample size taken was a total of 60 patients of COM (divided into 2 groups: group 1 had 30 patients of atticointral disease and group 2 had 30 patients of tubotympanic disease.

Inclusion criteria

Patients of active COM aged between 10-65 years irrespective of gender, socio-economic status who consented to participate in the study were included.

Exclusion criteria

Patients with pregnancy, malignancy, tuberculosis were excluded.

Pus sample was collected with sterile swab from the external auditory canal in tubotympanic COM cases. In atticointral COM cases tissue from periphery of cholesteatoma site was collected intra-operatively in sterile vials containing phosphate buffered solution (pH 7.2).

All samples were sent to the microbiology department of the institute immediately after collection.

Isolation and characterization of aerobic bacteria

The collected samples were directly inoculated onto MacConkey and blood agar media. The plates were kept for overnight incubation at 37°C. Post incubation their colony morphology and cultural characteristics were observed post-incubation. Further phenotypic identification was done through gram staining and

biochemical reaction. The pure isolated colonies were stocked in peptone medium and stored at room temperature for further processing.

Detection of biofilm formation

To assess biofilm formation, stocked bacterial isolates were subcultured onto MacConkey and blood agar media and the plates were kept for overnight incubation at 37°C. The isolated pure colonies were suspended into normal saline and the suspension was adjusted according to 0.5 McFarland $\sim 1.5 \times 10^8$ CFU/ml. In 96 well microtiter plate, 190 μ l Luria Bertani broth media and 10 μ l of a bacterial suspension was added. The plate was overnight incubated at 37°C. Following day, the wells were rinsed with sterile phosphate buffer saline and air dried. Each well was stained with 100 μ l of 0.1% crystal violet. Excess stain in the well was rinsed with 95% ethanol followed by 20 min incubation at room temperature. The optical density at 578 nm (OD578) of each well was then measured using LisaScan® EM micro-titer plate reader. For the determination of biofilm formation OD value of standardized low, moderate and high biofilm producing *S. aureus* strains were taken a reference, while well containing only media were used as negative control. Reference strain OD578=low biofilms 0.040, moderate biofilms 0.060, high biofilms 0.091.

Statistical analysis was performed using statistical package for the social sciences (SPSS), version 23.0. IBM Corp., NY. The data was analyzed using Chi square test and Fisher's exact test.

RESULTS

The following information were collected after going through clinical examination, surgical intervention, bacterial culture and biofilm formation. The following observations were made.

In our study, we observed that, in cases of atticointral type of COM, most predominant bacteria found were *P. aeruginosa* and *S. aureus* equally as both amounted to 26.7%, followed by *M. catarrhalis* amounting to 16.7%, *P. mirabilis* amounting to 10% and *Enterococcus* to 3.3% and 10% cases had no bacterial growth (Figure 1).

In cases of tubotympanic type of COM, most predominant bacteria found was *S. aureus* amounting to 33.3%, followed by *M. catarrhalis* (13.3%), *P. aeruginosa* and *P. mirabilis* both amounting to 10% and 30% cases had no bacterial growth (Table 1).

Since $p=0.308$ there is no significant correlation between bacterial growth in our study groups.

In our study, we observed that, in cases with atticointral type, 83.3% patients presented with biofilm formation whereas in cases with tubotympanic type, 60% patients presented with biofilm formation (Figure 2).

Table 1: Correlation between COM types and bacterial growth.

Bacteria	Groups			
	Atticoantral COM (group 1)		Tubotympanic COM (group 2)	
	No.	%	No.	%
<i>M. catarrhalis</i>	5	16.7	4	13.3
<i>P. aeruginosa</i>	8	26.7	3	10.0
<i>S. aureus</i>	8	26.7	10	33.3
<i>K. pneumoniae</i>	2	6.7	1	3.3
<i>P. mirabilis</i>	3	10.0	3	10.0
<i>Enterococcus</i>	1	3.3	0	0.0
Sterile	3	10.0	9	30.0
Total	30	100	30	100

$\chi^2=6.939$; $p=0.308$.

Table 2: Correlation between COM types and biofilm formation.

Biofilm	Group cross tabulation			
	Atticoantral COM		Tubotympanic COM	
	No.	%	No.	%
Present	25	83.3	18	60.0
Absent	5	16.7	12	40.0
Total	30	100	30	100

$\chi^2=4.02$; $p=0.044$.

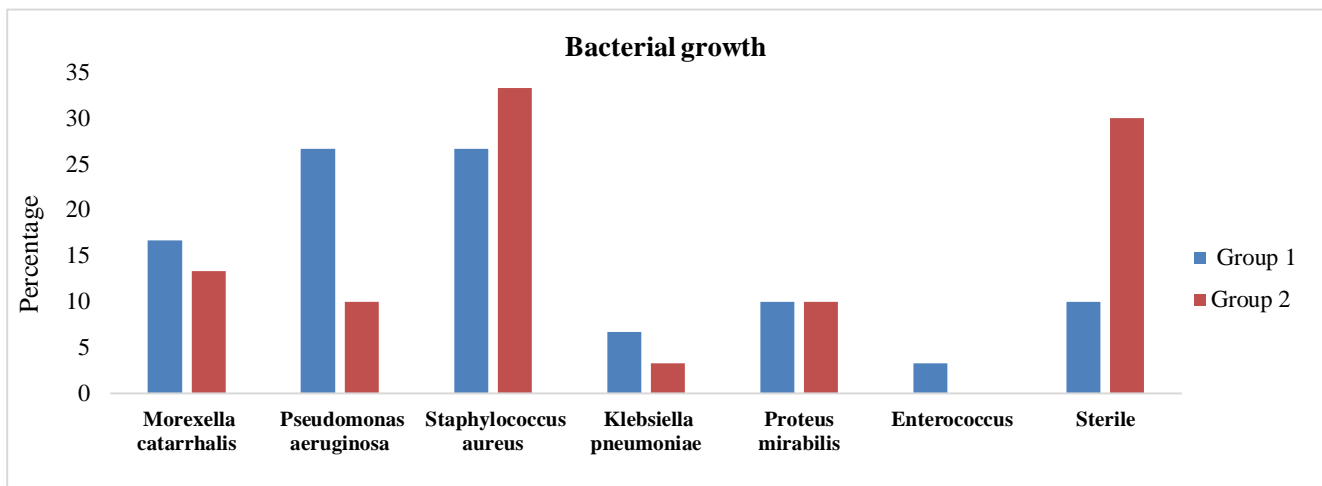


Figure 1: Bacterial growth.

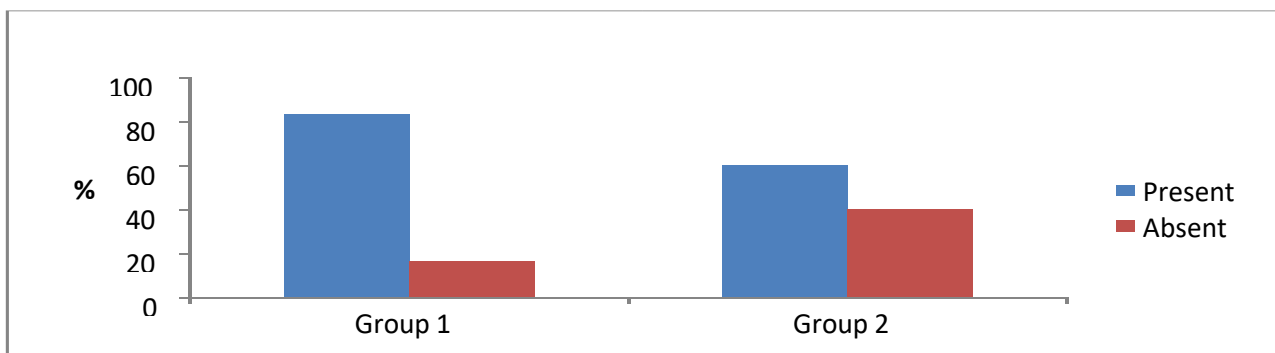


Figure 2: Biofilm formation.

Since $p=0.044$, hence there was a significant correlation between the two groups of COM.

DISCUSSION

In our study, we observed that, in cases of atticointral type of COM, the most predominant bacteria found were *P. aeruginosa* and *S. aureus* both amounting to 26.7%, followed by *M. catarrhalis* amounting to 16.7%, *P. mirabilis* amounting to 10% and *Enterococcus* to 3.3%, and 10% cases had no bacterial growth.

In our study, we observed that, in cases of tubotympanic type of COM, the most predominant bacteria found were *S. aureus* amounting to 33.3% , followed by *M. catarrhalis* amounting to 13.3%, and then *P. aeruginosa* and *P. mirabilis* both amounting to 10% and 30% cases had no bacterial growth. Since $p=0.308$ there is no significant difference between bacterial growth in our study group.

In majority of the studies, *P. aeruginosa* was the most commonly recovered organism from the chronically draining ear. Various researchers over the past few decades have recovered pseudomonas from 48-98% of patients with CSOM. Also some studies have shown that *S. aureus* is the second most common organism isolated from chronically diseased middle ears. Reported data estimate infection rates from 15-30% of culture-positive draining ears.

The remainder of infections is caused by a large variety of gram negative organisms. *Klebsiella* (10-21%) and *Proteus* (10-15%) species are slightly more common than other gram negative organisms. Sivarajan et al (1991), conducted a study on the bacteriological profile in 146 cases of CSOM and found that *Pseudomonas* was the highest followed by *Staphylococcus* and the *Proteus*.⁶

In our study, we found out that in cases with atticointral type of COM, on bacterial culture, the most common organism were *P. aeruginosa*, *S. aureus* followed by *M. catarrhalis* whereas in cases with tubotympanic COM, most common organism was *S. aureus*. In cases with atticointral type of COM 10% specimen culture were sterile whereas in cases with tubotympanic type of COM 30% cases were sterile.

In our study, we observed that, in cases with atticointral type, 83.3% patients presented with biofilm formation whereas in cases with tubotympanic type, 60% patients presented with biofilm formation. Since $p=0.044$, hence there was a significant difference between the two groups of COM, with biofilm formation being more common in atticointral type of COM.

In the study by Romling et al almost all bacteria can be found as part of a biofilm. Some bacteria like *S. aureus*, *P. aeruginosa* and *E. coli* are more likely to be found in a biofilm community than others.⁷

In the study by Gu et al (2014) bacterial biofilm were found in 85%, 92%, 16% of patient with middle ear cholesteatoma, COM and tympanic membrane perforation, respectively.¹

Artono et al conducted a study using scanning electron microscopy (SEM) with 33 patients and divided into 16 unsafe type and 17 patient safe type, the test result showed that in unsafe type CSOM 12 (75%) was positive with biofilm bacteria and 4 (25%) negative bacteria biofilm. Safe type CSOM showed 6 (35.35%) with positive biofilm bacteria and 11 (67.9%) negative biofilm bacteria.⁵

Lampikoski et al conducted a study using confocal scanning laser microscopy (CSLM) of the 17 chlesteatoma patient, mastoid mucosal biofilm formation could be seen in 14 cases (82%). In CSOM group, 5 (42%) of 12 patient showed mastoid mucosal biofilm. In control group (patient undergoing cochlear implantation, with no history of COM), 1 (9%) patient of 11 showed mastoid mucosal biofilm.⁸

Kaya et al (2013) showed 7 out of 10 CSOM patient had biofilm (70%), 6 of the 11 groups of non-suppurative COM showed biofilm (54.5%). Cholesteatoma group showed biofilm in 8 of 13 patient (61.5%).⁹

In our study, in cases with atticointral type of COM biofilm formation was found to be significantly more common as compared to tubotympanic type of COM. A long-term follow up after surgical intervention could yield more information about relationship of biofilm and treatment outcome, which was the major limitation of this study.

CONCLUSION

In our study, we found out that in cases with atticointral type of COM, on bacterial culture, the most common organism was *P. aeruginosa* and *S. aureus* followed by *M. catarrhalis* whereas in cases with tubotympanic COM, most common organism was *S. aureus*. We found that, in atticointral type of COM biofilm formation was significantly more common as compared to that in tubotympanic type of COM. Therefore, we can conclude that there is a significant correlation in biofilm formation between atticointral and tubotympanic type of COM. Biofilm formation in COM is even higher when atticointral disease is present.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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