

**ORIGINAL RESEARCH**

# A study of biofilm formation in CSOM patients

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**ABSTRACT**

**Background:** Chronic suppurative otitis media (CSOM) is characterized by prolonged inflammation of middle ear mucosa and mastoid leading to TM perforation and ear discharge. CSOM is now supposed to be biofilm-related. In biofilms, bacteria are embedded in a slim-like extracellular matrix composed of proteins, polysaccharides and nucleic acids called extracellular polymeric substances. **Objectives:** This study aimed to determine the incidence of biofilms in bacterial isolates of CSOM cases, bacteriological pattern and their antibiotic susceptibility in biofilm-producing organisms in CSOM, Resistant pattern to antimicrobials among biofilm-producing strains leading to treatment failure and the outcome of early surgical intervention in such patients. **Methodology:** This prospective study was conducted in the Department of Otorhinolaryngology in association with the Department of Microbiology. Ear discharge sample is collected using aseptic precautions and culture sensitivity and tests to check for presence of biofilm is done and Early surgical intervention of the patients with biofilms positive were performed. They were evaluated for the discharge-free period, graft uptake and infection-free period during follow-up. **Results:** Out of 170 patients, 155 patients were biofilm positive, the most common organism isolated being Methicillin resistant staphylococcus aureus and early surgical intervention was performed. Postoperative graft uptake is seen in 142 (83.5%) patients. **Conclusion:** Early surgical intervention in biofilm positive CSOM patients would be helpful in high chances of graft uptake, and in regaining infection free ear

**Keywords:** CSOM, Biofilm, Early Surgical Intervention, Antibiotic resistance

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**INTRODUCTION**

In otolaryngology, chronic suppurative otitis media (CSOM) is one of the most common diseases and affects the population worldwide. The CSOM incidence is found to be affecting approximately 20 million people globally<sup>1-3</sup> CSOM is defined as mucosal inflammation in the middle ear and mastoid and persists for more than 2-3 months, leading to tympanic membrane perforation and ear discharge. This leads to profound health implications and complications intracranially or extracranially, in turn causing morbidity among the affected population. Thus, immediate attention is required to control the disease prevalence worldwide, and such outcomes make it a significant public health issue<sup>(4-7)</sup>. The occurrence of CSOM varies across different nations, but countries with low- and middle-income levels have more disease prevalence.<sup>8</sup> CSOM can develop as a complication of inadequately treated Acute Suppurative Otitis Media (ASOM) or de novo chronic

in onset. CSOM is now supposed to be biofilm-related.<sup>(9)</sup> Biofilm is a slime-like extracellular matrix made of proteins, polysaccharides, and nucleic acids, which form an extracellular polymeric substance where bacteria form aggregates and are organized into complex three-dimensional structures.<sup>(11)</sup> Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis, Escherichia coli, Aspergillus spp., Candida spp are the most common microorganisms found in CSOM patients with ear discharge, and they vary in different geographical conditions<sup>(10)</sup> Certain evidence supports the theory that Chronic otitis media with effusion, cholesteatoma, chronic suppurative otitis media and otitis media with effusion and other chronic ear diseases are biofilm related<sup>12</sup> Recently bacterial biofilms have been proven to be important in infectious diseases in the ear<sup>13</sup>. Bacterial resistance is emerging and posing problems in controlling the intractable infections. Most of the

times culture based antibiotics may not be helpful. Hence getting rid of biofilm from the infection site would be better option in such cases. The study

outcomes of early intervention in biofilm positive CSOM patients is sparse in literature, hence the study.

## BIOFILM

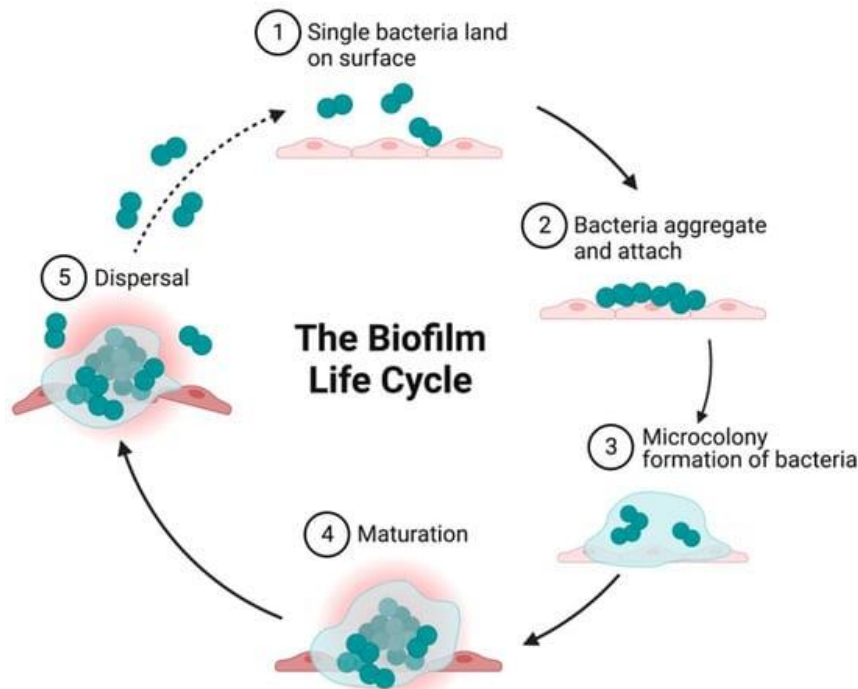


Fig.10 Life cycle of Biofilm (source <sup>14</sup>)

## MATERIALS AND METHODS

**Study design and source of data:** This prospective study is conducted in the Department of Otorhinolaryngology in association with the Department of Microbiology in a tertiary care, SHRI B M PATIL MEDICAL COLLEGE AND RESEARCH CENTRE AND HOSPITAL [BLDE (DU) UNIVERSITY] in Vijayapura between September 2022 TO March 2024

**Method of collection of data:** Patients with the clinical diagnosis of CSOM attending OPD of ENT Department of our institute who satisfied the inclusion criteria were enrolled in the study. The data was collected in the form of questionnaire in terms of complete clinical history, clinical examination and necessary investigations of the patient. Detailed examination of the patients with an emphasis on ear findings was done with the help of otoscopy to see the tympanic membrane status. Using proper aseptic precautions, a middle ear swab was collected using a sterile flocked swab. Interpretation of bacterial culture, Antibiotic susceptibility, Biofilm production testing by Tube method (fig.11) Here the isolates were inoculated in polystyrene test tube containing tryptic soy broth with 1% glucose and incubated at 37 degree for 24 hours, results were interpreted by appearance of purple ring depicting biofilm positivity and Congo red agar (fig.12) It uses a solid medium congo red agar, where directly bacterial colonies can be analysed and

slime forming strains can be identified 'black colonies on red agar' depicting biofilm positivity was done and non slime forming strains did not change colour (red coloured colonies)

The isolates were subjected to antibiotic susceptibility testing by employing Kirby-Bauer standard disc diffusion method on Muller- Hinton agar, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (M100-S24).

A preoperative pure tone audiometry and HRCT temporal bone (coronal) and (axial) view has been done in required cases. Early intervention of the patients with biofilm positive by tympanoplasty, cortical mastoidectomy, Modified radical mastoidectomy were performed. Post operatively these patients were followed up at one week, 15 days, one month, three months. They were evaluated for the discharge-free period, pain-free period, graft uptake and infection-free period during follow-up.

**Sample Size:** With the anticipated Proportion of common organisms, *Pseudomonas aeruginosa* 44% the study would require a sample size of 170 to achieve a power of 80% for detecting biofilm in CSOM cases two sided p-value of 0.05 with effect size 0.11 using G\* power software 3.1.9.7

**Statistical analysis:** All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean  $\pm$  standard deviation

(SD) were used. For categorical data, the number and percentage were used in the data summaries and diagrammatic presentation. Chi-square ( $\chi^2$ ) test was used for association between two categorical variables. The difference of the means of analysis variables between two independent groups was tested by unpaired t test. The t statistic to test whether the means are different can be calculated and If the p-value was  $< 0.05$ , then the results were considered to be significant otherwise it was considered as not statistically significant. Data were analysed using

SPSS software v.23 (IBM Statistics, Chicago, USA) and Microsoft office 2007.

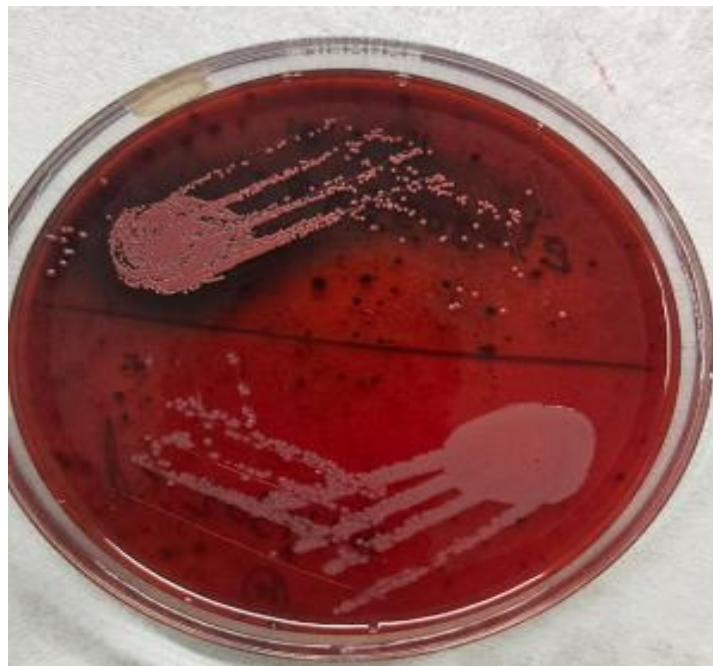
**Inclusion criteria:** 'Patients diagnosed with Chronic suppurative otitis media'.

**Exclusion criteria:** 'Patients suffering from CSOM who are on systemic antibiotics in the past seven days of presentation, who are on topical medications to the ear, Patients having ear discharge due to some other condition like otitis externa, otomycosis etc'.

### BIOFILM POSITIVE



**Fig.11 tube method positive for biofilms**



**Fig.12 Congo red agar positive for biofilms**

## RESULTS

A hospital-based prospective study was conducted with 170 patients to study the biofilm formation in CSOM

### 1. SEX AND AGE DISTRIBUTION

Out of the 170 patients enrolled in this study 91 (53.5%) were males and 79 (46.5%) were females. Males were more compared to females in the study (table.1) and the most commonly affected age group was 10 to 19 years, which included 36 (21.2%) patients and least common was <10 years (table.2)

**Table 1: Distribution of patients according to sex**

Gender	No. of patients	Percentage
Female	79	46.5
Male	91	53.5
Total	170	100.0

**Table 2: Distribution of patients according to age (years)**

Age(Years)	No. of patients	Percentage
< 10	5	2.9
10 – 19	36	21.2
20 – 29	25	14.7
30 – 39	35	20.6
40 – 49	25	14.7
50 – 59	23	13.5
60 – 69	15	8.8
70+	6	3.5
Total	170	100.0

### 2. DISTRIBUTION ON PRESENCE OF BIOFILM

In the total sample of 170 patients 155 (91.2%) were biofilm positive and 15 (8.8%) were biofilm negative (table.3)

**TABLE 3: distribution of patients based on the presence of biofilm**

BIOFILM	No of patients	Percentage
Negative	15	8.8
Positive	155	91.2
Total	170	100.0

### 3. DISTRIBUTION OF ORGANISMS IN SAMPLES

Out of 155 biofilm positive patients MRSA was found in 57 (33.55%) patients and coagulase negative staphylococcus isolated being lowest in 4 patients (2.4%) (table.4)

**Table 4: Distribution of organisms based on biofilm positivity**

Organisms	No of isolated organisms	Percentage
Citrobacterfreundi	11	6.5
Coagulase negative staphylococcus	4	2.4
Escherichia coli	10	5.9
klebsiella pneumonia	8	4.7
MRSA	57	33.5
pseudomonas aeruginosa	56	32.9
staphylococcus aureus	13	7.6
streptococcus pyogenes	4	2.4
Streptococcus pyogenes	7	4.1
Total	170	100.0

### 4. DISTRIBUTION OF PATIENTS WHO UNDERWENT EARLY SURGICAL INTERVENTION

Out of total 170 patients 155 were biofilm positive and underwent early surgical intervention and 15 were biofilm negative so were not included in early surgical intervention (table.5)

**Table.5 distribution of patients based on early surgical intervention(NA=NOT**

	No. of patients	Percentage
NA	15	8.8
No	0	0
Yes	155	91.2
Total	170	100.0

**APPLICABLE- BIOFILM NEGATIVE)****COMPARISON OF POST OP EAR DISCHARGE IN PATIENTS AT DIFFERENT INTERVALS**

Out of total 155 patients with early surgical intervention , 9(5.80%) patients had discharge at post operative 1 week , 14(9.03%) patients had discharge at post op day 15, 13(8.38%) patients had discharge on post op 1 month and 3 months(table.6)

**Table 6: Comparison of post op ear discharge in patients at different intervals**

Discharge	No	Yes	Percentage
Post op discharge 1 week	146	9	5.80%
Post op discharge 15 days	141	14	9.03%
Post op discharge 1 month	142	13	8.38%
Post op discharge 3 months	142	13	8.38%

**INFECTION FREE PERIOD AND GRAFT UPTAKE POSTOPERATIVELY**

In 155 patients who underwent early surgical intervention 142 (83.5%) patients had infection free period and successful graft uptake and 13 (7.6%) had graft uptake failure(Table.7)

**Infection free period \* graft uptake Crosstabulation**

Out of 155 patients 142(83.5%) patients had infection free period and 13(7.6%) patients persisted to have infection post operatively(table.7)

**Table.7 infection free period and graft uptake crosstabulation**

		graft uptake			
		NA	No	Yes	
infection free period	NA	15	0	0	15
		100.0%	0.0%	0.0%	8.8%
	no	0	13	0	13
		0.0%	100.0%	0.0%	7.6%
	yes	0	0	142	142
		0.0%	0.0%	100.0%	83.5%
Total		15	13	142	170
		100.0%	100.0%	100.0%	100.0%

**DISCUSSION**

In our study, 170 patients were included. Out of these patients 91 (53.5%) were males while 79(46.5%) of patients were females. In our study it was found that males were more commonly affected than females. In a study by Priya et al, 500 patients were included. Male patients were 63% (n=315) while female patients were 37% (n = 185) males were more commonly affected than females<sup>15</sup>

In our study, 10-19(21.2%) was observed to be the age group having highest CSOM incidence of all the age groups while the lowest incidence was found in patients in the age group of <10 years(2.9%). Metri Basavaraj in India reported in a study that the CSOM patients who were most commonly affected were between the age group of 1–20 years old (52.8%), followed by 21–60 years old (45.9%)<sup>16</sup>

In our study out of total 170 patients 155(91.2%) patients were biofilm positive and 15(8.8%) patients

were biofilm negative. In a study conducted by Jensen, RG, Johansen et al out of 21 patients biofilm was identified in 17 patients (81%) and 4 patients(19%) were biofilm negative<sup>17</sup>

In our study the most common organism isolated was Methicillin resistant Staphylococcus aureus(MRSA) 57(33.5%) cases and least common was Coagulase negative staphylococcus in 4 (2.4%) patients. Studies conducted by Deb et al.<sup>18</sup> (79%) Kumar et al.<sup>55</sup> (46%), Poorey et al.<sup>18</sup> (35.2%) , and Vishwanath et al.<sup>18</sup> (32.2%), reported the predominant organism to be Pseudomonas aeruginosa and Staphylococcus aureus (32.75%), was the second most common organism that was comparable to the studies conducted by Deb et al. (20%)<sup>26</sup> and Kumar et al. (33%)<sup>18</sup>

In our study early surgical intervention was done in 155 biofilm positive patients 70 patients underwent cortical mastoidectomy, 55 patients underwent

tympanoplasty and 30 patients underwent modified radical mastoidectomy. Postoperative infection free period and graft uptake was seen in 142 (83.5%) patients while 13(7.6%) patients had graft failure. Haswani et al conducted a study which showed Surgical modalities mechanically disrupt the biofilms resulting in increased host defense mechanism<sup>19</sup>

In a study conducted by mahdiani et al 82.5% of patients underwent surgical management, with majority patients undergoing intact canal wall procedure. Patient's were followed up after six months of surgical intervention that 426 patients (71.00%) were symptomatically better and presented with no complaints<sup>20</sup>

Our study shows almost same percentage of patients who had better outcomes Formation of biofilm leads to resistance and recurrence of infection in csom and explain the etiopathogenesis of CSOM. Isolation of biofilm forming patients in CSOM helps to provide proper management strategies in such CSOM cases.

Biofilm production is one of the mechanisms which leads to the bacterial resistance to antibiotics and related antimicrobial compounds (Stewart and Costerton, 2001). Biofilms eradication is difficult and also leads to recurrent infections (Donelli and Vuotto, 2014). Hence in such cases ,early intervention is more beneficial as it helps in disrupting the bacterial residue and enhances success rate in the form of reducing pain due to infection and better healing.

### STRENGTHS OF THE STUDY

1. Detection of biofilm , helps in initiating proper antibiotic and planning of further management at the earliest without delay.
2. Early intervention helps in reducing infection and gain infection free period and lesser incidence of graft failure.

### LIMITATIONS OF THE STUDY

1. The sample of middle ear swab collected should be processed within stipulated period of time, otherwise it would lead to improper culture reports.
2. Small sample size of 170 patients
3. Lack of proper Follow up visits by patients

### CONCLUSION

Occurance of biofilms is common in CSOM patients. Isolation of biofilm forming bacteria helps in better management of infection and overcome bacterial resistance. Early surgical intervention in biofilm positive csom patients would be helpful in good control of infection ,hence better graft uptake.

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