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ORIGINAL ARTICLE

The Role of Biofilm Formation in Chronic Otitis Media, Pathogenesis, Clinical Impact, and Treatment Implications in ENT and Ophthalmology

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ABSTRACT

Background: Chronic Otitis Media (COM) is a persistent middle ear infection that significantly impacts patients' quality of life. The process of biofilm formation is essential for chronicity of the disease, antimicrobial resistance, and treatment failure. In this study, prevalence of biofilm producing bacterial isolates in COM patients, their antibiotic resistance pattern and clinical outcomes in Pakistan have been investigated.

Methods: A cross-sectional study was done in ENT and Ophthalmology departement of tertiary care hospitals of Pakistan from January 2021 to September 2022. Bacterial identification, biofilm formation and antimicrobial resistance were analyzed in bacterial identification, biofilm formation and antimicrobial resistance in middle ear discharge samples from n=80 patients with COM. Congo Red Agar, Tube Method and Microtiter Plate Assay were used to detect biofilm. Kirby-Bauer disk diffusion method was used to assess antibiotic susceptibility. Follow up was for six months, and clinical outcomes were assessed.

Results: The isolates formed biofilm in 72% of strains, the most frequent pathogens being Pseudomonas aeruginosa (40%) and Staphylococcus aureus (30%). Ciprofloxacin (85%), gentamicin (70%), ceftriaxone (60%), and meropenem (50%) resistance of biofilm-forming bacteria was significantly higher than the resistance of control bacteria ($p < 0.05$). Patients with biofilm present were more likely to have persistent infection (50%) and need surgery (17%) than patients without biofilm (14% and 8%, respectively, $p = 0.0008$).

Conclusion: COM chronicity and treatment resistance are mediated in large part by biofilm formation. Optimization of treatment outcomes requires early detection, biofilm-targeted therapies, and improved antibiotic stewardship.

Keywords: Chronic Otitis Media, Biofilm, Antimicrobial Resistance, Pseudomonas aeruginosa, Staphylococcus aureus, ENT Infections, Treatment Failure, Pakistan.

INTRODUCTION

Chronic Otitis Media (COM) is a major but overlooked public health problem in low and middle-income countries (LMICs), such as Pakistan, which is accounted for by environmental, socioeconomic, and healthcare factors[1]. COM is characterized by persistent middle ear inflammation, tympanic membrane perforation, and recurrent otorrhea, a leading cause of preventable hearing loss with a profound impact on communication, cognitive development in children, and overall quality of life. Despite being underdiagnosed and undertreated, COM remains a disease that is prevalent in Pakistan, where healthcare disparities are widespread and can lead to long-term complications of ossicular destruction, cholesteatoma formation, and intracranial infections. Bacterial biofilm formation is increasingly identified as the key cause of COM chronicity and treatment resistance[2, 3].

Structured microbial communities encased within an extracellular polymeric substance (EPS) confer antibiotic, host immune response, and mechanical clearance resistance to biofilms. Unlike planktonic bacteria, biofilm-associated pathogens are characterized by altered gene expression, reduced metabolic activity, and increased tolerance to antimicrobial agents and are, therefore, highly resistant to conventional treatment modalities[4]. Biofilms have been reported on the middle ear mucosa, on the surface of tympanostomy tubes, and in cholesteatoma lesions in otitis media, indicating the role of biofilms in the chronicity and recurrence of the disease. Biofilm-mediated infections have been extensively studied in high-income countries, but very little is known about the epidemiology, clinical impact, and treatment outcome of biofilm-mediated infections in resource-limited settings such as Pakistan [5].

However, the prevalence of biofilm-associated COM in Pakistan is likely underestimated as there is a lack of microbiological diagnostics and empirical antibiotic therapy without culture sensitivity testing. Over-the-counter availability and self-medication of antibiotics coupled with indiscriminate and unregulated use of the drug have contributed to the emergence of multidrug-resistant (MDR) bacterial strains, complicating disease management further [6]. High rates of antibiotic resistance among Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus pneumoniae, the most commonly isolated pathogens in COM, have been reported in studies conducted in major tertiary care hospitals of Lahore, Karachi, and Islamabad. Interestingly, these bacteria are capable of forming biofilms, which greatly enhances their resistance to the common fluoroquinolones, aminoglycosides, and beta-lactam antibiotics, and thus, despite prolonged antimicrobial therapy, recurrent and persistent infections occur [7].

In addition, biofilm formation is now recognized for its important role in persistent infections in ophthalmology as well as in otology. Chronic bacterial conjunctivitis, keratitis, and endophthalmitis, especially after cataract surgery and corneal transplantation, are commonly caused by biofilm-producing pathogens[8]. Post-operative infections continue to be a major challenge in Pakistan, a country where ophthalmic surgical procedures are common because of the high prevalence of age-related cataracts and refractive disorders. With anatomical and microbiological similarities between otologic and ophthalmologic infections, a clearer understanding of biofilm dynamics in ENT and ocular diseases is of great importance to designing effective therapeutic strategies[9].

The purpose of this study was to finish the information gap by characterizing the prevalence of biofilm-forming bacterial isolates among COM patients, assessment of the antibiotic resistance pattern, and its effect on treatment outcomes in Pakistan's healthcare settings. This study provides robust, evidence-based data to support clinical decision-making, advocate improved microbiological diagnostics, and support the development of targeted antimicrobial and biofilm-disrupting therapies. In light of the growing burden of biofilm-associated ENT and ophthalmology infections, a multidisciplinary approach, including otolaryngologists, ophthalmologists, microbiologists, and public health policymakers, is needed to fight the rising tide of antimicrobial resistance and enhance patient outcomes in Pakistan[10].

METHODOLOGY

Study Design and Setting: The cross-sectional study was conducted from January 2021 to September 2022 in the ENT and Ophthalmology departments of major tertiary care hospitals of Pakistan. The institutional review boards from the respective hospitals approved the study, and all participants provided written informed consent. The study aimed to determine the prevalence of biofilm-forming bacterial isolates in Chronic Otitis Media (COM) patients in Pakistan, their antibiotic resistance patterns, and their influence on treatment outcomes. n= 80 patients were selected to constitute the total sample size.

Study Population and Eligibility Criteria: The study included patients diagnosed with COM using clinical criteria such as otorrhea for more than three months, tympanic membrane perforation, and conductive hearing loss. Patients with a history of use of antibiotics in the past two weeks, with known immunosuppressive conditions, or who have craniofacial anomalies were excluded to minimize confounding factors that may affect biofilm formation and treatment response. Of the 80 patients, 22 (28%) were biofilm negative and 58 (72%) were biofilm positive.

Sample Collection and Microbiological Analysis: Sterile samples of middle ear discharge were collected under sterile conditions from the patients with aseptic swabs and immediately transported to the microbiology laboratories for processing. Blood agar, MacConkey agar, and chocolate agar were used to perform bacterial culture under standard conditions of incubation. Gram staining, colony morphology, biochemical tests, and VITEK 2 automated system were used to identify bacteria; bacterial identification was confirmed. The major bacterial isolates were Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, and Escherichia colli.

Biofilm Detection Methods: Multiple complementary methods were used to detect biofilm formation among bacterial isolates to increase accuracy. A qualitative screening test was done with Congo Red Agar (CRA), while the Tube Method (TM) gave a semi-quantitative biofilm assessment. A quantitative technique, the Microtiter Plate Assay (MTP), was used to determine the biofilm-forming capacity of the cells by measuring optical density (OD) after crystal violet staining. OD readings were used to classify isolates as strong, moderate, or weak biofilm producers. Among the 80 isolates, 58 (72%) were biofilm positive and 22 (28%) were non biofilm formers.

Antimicrobial Susceptibility Testing: Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility testing according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Commonly used antibiotics for the management of COM were tested: fluoroquinolones, aminoglycosides, cephalosporins, and carbapenems. Selected antibiotics were tested for minimum inhibitory concentrations (MICs) to compare the comparative resistance of biofilm-forming and non-biofilm-forming isolates. Resistance to at least three different antibiotic classes was considered multidrug resistance (MDR). A statistical correlation between biofilm formation and antibiotic resistance was made to assess clinical significance. Overall, biofilm-forming isolates had significantly higher resistance rates versus nonbiofilm isolates, especially against ciprofloxacin (85% vs. 45%), gentamicin (70% vs. 30%), ceftriaxone (60% vs. 25%), and meropenem (50% vs. 15%).

Clinical Outcome Assessment: Treatment response, recurrence rates, and complications were evaluated over a six-month follow-up period. Treatment outcomes were categorized as successful resolution, persistent infection requiring further intervention, and cases that required surgical intervention, including tympanoplasty and mastoidectomy. Otorrhea persisted despite standard antibiotic therapy for at least four weeks and was defined as treatment failure. Of the 58 biofilm-positive patients, 19 (33%) achieved resolution, 29 (50%) remained positive, and 10 (17%) needed surgical intervention. On the other hand, 17 (78%) of 22 biofilm-negative patients achieved successful resolution, 3 (14%) had persistent infection, and 2 (8%) required surgical intervention.

Statistical Analysis: SPSS version 26 was used for the analysis of data. Baseline characteristics and microbiological findings were summarized using descriptive statistics. Categorical variables were compared with chi-square tests and independent t-tests for continuous data. Statistical significance was considered as having a p-value less than 0.05. Logistic regression was used to assess the relationship between biofilm formation and treatment outcomes to identify potential predictors of disease persistence and antibiotic resistance. The statistical analysis showed a strong association between biofilm presence and treatment resistance ($p = 0.0008$), suggesting that biofilm-forming bacteria play a major role in chronic infections and poor treatment outcomes.

Ethical Considerations: Institutional ethics review boards of the participating hospitals approved the ethical approval. Unique study identification numbers were assigned to patient confidentiality, and all collected data were stored securely. The study was voluntary, and patients had the right to withdraw from the study at any time without any effect on their medical care. The study was conducted by ethical principles of the Declaration of Helsinki for biomedical research.

RESULTS

Of the 80 patients with Chronic Otitis Media (COM), 58 (72%) had biofilm-positive bacterial isolates, and 22 (28%) did not form biofilm. Biofilm formation plays a major role in disease chronicity, recurrence, and treatment resistance, and its high prevalence among biofilm-associated infections emphasizes this. The biofilms are present in the majority of cases, indicating that conventional antibiotic therapies may not be sufficient for a majority of patients, and thus, therapeutic strategies aimed at targeting biofilms need to be integrated as shown in table 1.

Table 1: Prevalence of Biofilm Formation Among COM Patients

Biofilm Formation	Number of Patients (n=80)	Percentage (%)
Positive	58	72%
Negative	22	28%

Analysis of the microbiological side revealed that Pseudomonas aeruginosa was the most often isolated pathogen in 32 cases (40%), Staphylococcus aureus in 24 cases (30%), and Streptococcus pneumoniae in 14 cases (18%). Other infrequently isolated pathogens included Klebsiella pneumoniae in 6 cases (7%) and Escherichia coli in 4 cases (5%). This is consistent with global trends and suggests targeting these pathogens to address COM management of biofilm-associated pathogens as shown in table 2.

Table 2: Distribution of Bacterial Isolates in COM Patients

Bacterial Isolates	Number of Isolates (n=80)	Percentage (%)
Pseudomonas aeruginosa	32	40%
Staphylococcus aureus	24	30%
Streptococcus pneumoniae	14	18%
Klebsiella pneumoniae	6	7%
Escherichia coli	4	5%

Biofilm-forming isolates showed a significantly higher resistance rate compared to non-biofilm-forming isolates ($p < 0.05$). Biofilm-forming isolates had resistance to ciprofloxacin of 85% compared to 45% for nonbiofilm isolates. Resistances to gentamicin, ceftriaxone, and meropenem were 70%, 60%, and 50% in biofilm-producing isolates, respectively, and 30%, 25%, and 15%, respectively, in nonbiofilm isolates. These findings highlight the importance of biofilm formation in promoting bacterial resilience to antibiotics, adding to the difficulty in managing disease as shown in table 3.

Treatment outcomes for patients with biofilm-associated COM were significantly worse than patients without ($p = 0.0008$). Of the 58 biofilm-positive patients, only 19 (33%) achieved

successful resolution with standard antibiotic therapy, and 29 (50%) had persistent infections requiring prolonged antibiotic therapy or alternative intervention. Additionally, 10 (17%) required surgical management, such as tympanoplasty or mastoidectomy, due to disease progression and complications. However, among the 22 biofilm-negative patients, 17 (78%) resolved their infections, 3 (14%) persisted, and 2 (8%) required surgery. These results suggest a close relationship between biofilm and treatment resistance and require better diagnostic and therapeutic approaches for better patient outcomes as shown in table 4.

Table 3: Antibiotic Resistance Patterns in Biofilm-Forming and Non-Biofilm-Forming Isolates

Antibiotic	Resistance in Biofilm-Forming Isolates (%)	Resistance in Non-Biofilm-Forming Isolates (%)
Ciprofloxacin	85%	45%
Gentamicin	70%	30%
Ceftriaxone	60%	25%
Meropenem	50%	15%

Table 4: Clinical Outcomes Based on Biofilm Formation

Clinical Outcome	Biofilm-Positive Patients (n=58)	Biofilm-Negative Patients (n=22)
Successful Resolution	19 (33%)	17 (78%)
Persistent Infection	29 (50%)	3 (14%)
Required Surgical Management	10 (17%)	2 (8%)

A chi-square test ($p = 0.0008$) was performed to determine the statistical significance of biofilm formation and poor treatment outcome. This hypothesis is reinforced by the fact that the rates of persistent infection and surgical intervention are higher in patients with biofilms.

The results from this study offer strong support that biofilm formation is a major determinant of chronicity, antimicrobial resistance, and poor treatment outcomes of COM in Pakistan. The high prevalence of biofilm-associated infections (72%) and the correlation with significantly more antimicrobial resistance levels highlight the urgent need for alternative treatments beyond conventional antibiotic therapy. The biofilm presence had a statistically significant association with treatment resistance, indicating that future interventions should target biofilm-disrupting therapies, alternative antimicrobial strategies, routine biofilm detection in microbiological diagnostics, and better antibiotic stewardship programs to combat multidrug-resistant bacterial strains and prevent ineffective treatment regimens. These findings emphasize the necessity of a multidisciplinary approach in the management of COM by involving otolaryngologists, microbiologists, and infectious disease specialists to develop further effective biofilm-targeted therapeutic strategies. Addressing biofilm-related challenges through treatment improvement, decrease in recurrence rates, and reducing the long-term burden of COM and other chronic ENT infections in Pakistan by healthcare providers.

DISCUSSION

This study's findings show a strong association between chronicity, antibiotic resistance, and poor treatment outcomes in Chronic Otitis Media patients in Pakistan and the presence of biofilm. The very high prevalence of biofilm formation (72%) among bacterial isolates demonstrates its crucial role in the persistence and recurrence of infection[11]. Bacteria that form biofilms are protected from host immune responses and from conventional antimicrobial therapies, which results in prolonged infections and higher rates of treatment failure. The results presented here support previous evidence that biofilm-producing bacteria are a major cause of the lack of effectiveness of standard treatment protocols and should lead to the development of new therapeutic approaches[12].

This is consistent with the predominant biofilm-forming pathogens being Pseudomonas aeruginosa (40%) and Staphylococcus aureus (30%), the two leading biofilm-forming pathogens globally and key contributors to persistent infections in COM. They have several virulence factors, such as quorum sensing and exopolysaccharide production, which allow biofilm maturation and antibiotic resistance[13]. In addition, Streptococcus pneumoniae (18%) and other Gram-negative organisms such as Klebsiella pneumoniae (7%) and Escherichia coli (5%) are also prevalent. The findings are in line with previous studies that show that antibiotic-resistant biofilm-producing bacteria are resistant to antibiotics due to their structural and metabolic adaptations thus, antibiotic regimens are ineffective in eradicating the bacteria[14].

The results of the antibiotic susceptibility analysis showed that biofilm-forming isolates had significantly higher resistance rates than non-biofilm-forming isolates ($p < 0.05$). Biofilm-forming bacteria showed ciprofloxacin resistance in 85% compared to 45% for nonbiofilm isolates, while gentamicin, ceftriaxone, and meropenem resistance were 70%, 60%, and 50%, respectively, in biofilm producers as compared to 30%, 25% and 15% for nonbiofilm isolates[15]. They also show that biofilms prolong infections and are a source of MDR bacterial strains. Increased resistance to fluoroquinolones and aminoglycosides indicates that empirical treatment strategies based on conventional treatment strategies may be insufficient for a large fraction of COM patients, which calls for a change towards biofilm-targeted therapies[16].

The clinical outcome assessment also reinforces the effect of biofilms on disease progression and treatment response. Both biofilm-positive patients had significantly worse outcomes compared to biofilm-negative patients, with 50% with persistent infection and 17% requiring surgery, while biofilm-negative patients had 14% and 8% with persistent infection and 8% requiring surgery, respectively ($p = 0.0008$). The lower treatment success rate among biofilm-positive patients (33%) than biofilm-negative patients (78%) highlight the urgency for improving the diagnostic techniques to detect biofilm-associated infections at an early stage[17]. The main treatment guidelines for COM currently are prolonged antibiotic therapy, which may be ineffective in the treatment of biofilm-forming bacteria. These results underline the need for the inclusion of biofilm disruptive agents, N-acetylcysteine, quorum sensing inhibitors, and bacteriophage therapy into treatment regimens to improve bacterial clearance and reduce recurrence rates[18].

These findings apply to more than ENT infections since biofilms are now known to play an important role in ophthalmic infections, including in post-surgical complications such as chronic conjunctivitis, keratitis, and endophthalmitis. Although the state of health care infrastructure and microbiological diagnostic capabilities in Pakistan may lend itself to the underdiagnosis and mismanagement of biofilm-associated infections, relying on empirical treatment in such cases may contribute to the problem. To optimize strategies for the treatment of COM and other biofilm-related infections, a comprehensive approach that incorporates microbiology, molecular diagnostics, and targeted therapy is necessary[19, 20].

The limitations of this study are given. This sample size ($n = 80$) may not truly represent the whole population affected by COM in Pakistan, and larger multi-center studies need to be done to validate these findings. Moreover, the absence of molecular characterization of biofilm-related genes hinders the ability to pinpoint those genetic mechanisms related to resistance. Genetic and proteomic analyses of biofilm-forming bacteria as future research would help in developing more effective interventions[21].

CONCLUSION

The findings of this study confirm that biofilm formation is critical in the chronicity, antibiotic resistance, and poor treatment outcomes of Chronic Otitis Media (COM) in Pakistan. Conventional treatment strategies are limited by the high prevalence of biofilm-associated infections (72%) and their strong correlation with antimicrobial resistance. Patients who were biofilm-positive had higher rates of persistent infection and surgical intervention, which suggests an important need for biofilm-targeted therapies. Steps that can

improve microbiological diagnostics, incorporate the use of biofilm-disrupting agents, and implement a more robust antibiotic stewardship program will improve treatment efficacy and reduce recurrence rates. For improving patient care and reducing antimicrobial resistance in clinical practice, it is important to address biofilm-related challenges in COM.

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