Scholars LITERATURE

La Prensa Medica Argentina

Research Article

DOI: https://doi.org/10.47275/0032-745X-196
Volume 106 Issue 2

Role of Bacterial Biofilm in Resistant Cases of Chronic Rhinosinusitis and Otitis externa

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Abstract

Bacterial biofilms consist of a complex, organized community of bacteria that anchor to both biotic and abiotic surfaces. They are composed of layers of embedded, live bacteria within extruded ex-polymeric matrix. This configuration allows for evasion of host defenses and decreased susceptibility to antibiotic therapy while maintaining the ability to deliberately release planktonic bacteria, resulting in recurrent acute infections. Thus, bacterial biofilms were hypothesized to contribute to the progression and persistence of chronic rhinosinusitis and otitis externa.

Keywords: Biofilm; Chronic Rhinosinusitis; Otitis Externa

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Citation: El Sayed M, Ghazal A, ELdsoky I, et al. (2020) Role of Bacterial Biofilm in Resistant Cases of Chronic Rhinosinusitis and Otitis externa. Prensa Med Argent, Volume 106:2. 196. DOI: https://doi.org/10.47275/0032-745X-196.

Received: November 07, 2019; **Accepted:** December 25, 2019; **Published:** January 03, 2020

Introduction

Biofilm is a 3D specialized population of adherent microorganisms, surrounded by an extracellular polymeric substance (EPS). In most environments, including human illnesses biofilm communities tend to be polymicrobial. Biofilms have numerous advantages, such as passive resistance, metabolic cooperation, by-product influence, quorum sensing systems, an increased gene pool with more efficient DNA sharing, and many other synergies that provide them with a competitive advantage, by providing multiple bacterial and/or fungal species in a single community. The greater the variety, the better the biofilm is in terms of its longevity [1].

The high antibiotic resistance and to host immune mechanisms, such as less vulnerability to phagocytosis and opsonization , is one of the most important features of biofilms [2,3].

In vitro studies have shown that, owing to the physical barrier imposed by the polysaccharide matrix that prevents the spread of compounds or inactivates the biocide action of certain agents, certain strains of bacteria in the biofilm state may be more than 1000 times more resistant to antibiotics in relation to the minimum resistance mechanisms [4,5].

There have been several different approaches to examining and appreciation the complex existence of biofilms.

The conventional techniques involved both scanning electron microscope and transmission electron microscopy (SEM and TEM, respectively).

These approaches provide comprehensive imaging of biofilms' complex architecture, growth phases, and polymicrobial nature.

Nevertheless, owing to difficulties in fixing, the presence of artifacts during fixation processes and the difficulties in recognizing individual bacterial organisms, both methods may be limited to clinical usefulness. The distinction among mucus, clot, and biofilm is also hard for SEM, though TEM only makes a two-dimensional portion of the biofilm [6].

Fluorescent in situ hybridization (FISH) with confocal laser scan microscopy (CLSM) is used to surmount these problems and to simplify the method in biofilm recognition. In addition to being able to make three-dimensional biofilm structures, CLSM with FISH also has the benefit of speciating the visualized bacteria. Crystal violet stain is a low-cost assay to evaluate the growth of in-vitro biofilm but does not represent in-vivo biofilm growth.

One of the most popular chronic disorders is chronic rhinosinusitis. This disease greatly affects the life quality of its patients and is a social and economic burden on the society. In contrast to the general public, sufferers with persistent or chronic rhinosinusitis have a worsening sense of overall health and vitality. CRS reflects a variety of inflammatory and infectious processes that affect the nose and paranasal sinuses at the same time and is distinguished by at least two symptoms.

Nasal congestion or nasal discharge, face pain and reduced sense of smell (anterior/posterior nasal drip) are all involved. Furthermore, one of the major presentations being examined is the existence of polyps and mucosal edema. It seems that the period of the illness is more than 12 weeks [7].

Otitis externa, also known the ear of the swimmer, includes diffuse inflammation of the external ear canal which could extend to the pinna distally and to the tympanic membrane proximally [8]. Otitis externa, referred to as chronic otitis externa, persists for 3 months or longer and is mostly a product of allergies, chronic dermatological disorders, or acute otitis externa that is inadequately treated.

Material and Methods

40 patients were included in this study. Culture of forty cases, twenty cases of otitis externa and twenty cases of chronic rhinosinusitis. All the blood samples, MacConkey Agar, are inoculated. Plates incubated overnight at 37℃. After bacterial identification and storage. Quantitative determination of biofilm formation [9].

Exclusion criteria

- Patients that receive antibiotics 48 hrs. before the collection of samples.
- Patients that have clinical proof of fungal infection.
- Patients with mixed infection i.e., otitis media and otitis externa.

This technique has been used for all isolated bacterial pathogens. Overnight culture with fresh TSB was diluted at 1:100 in the tryptic Soya Broth (TSB). Three wells with a lid filled with 0.2 ml per bacterial suspension of a sterile 96-well flat-bottomed plastic tissue culture plate (Cellstar, greiner bio-one). Sterile broth with no bacteria was used for a negative control [10].

At 37°C, for 24 hours the plates have been covered and incubated aerobically. Following incubation, the contents of the wells have been aspirated and each well with 25 ml of sterile physiological saline was washed three times. For the removal of all non-adherent bacteria, the plate was vigorously shaken. The residual bacterial attached were fixed for fifteen min utilizing 0.2 ml of absolute methanol each well followed by elimination of methanol and air drying. With 0.2 ml of 0.2% Hucker crystal violet solution, the plates were stained for 5 min. With flowing tap water, the residual stain was rinsed.

Table 1: Distribution of the studied cases according to diagnosis (n=40).

OD <ODc	Non-adherent and no biofilm formation
ODc< OD < 2*ODc	Weakly adherent
2*ODc<OD < 4*ODc	Moderately adherent
4*ODc< OD	Strong adherent

Table 2: Distribution of the studied organisms for CRS cases (n=20).

CRS	No.	%
Organism		
Staph Aureus	3	15
MRSA	10	50
Citrobacter	1	5
Klebsiella	2	10
Strept	1	5
E-coli	1	5
Staph. Epidermidis	2	10

The plates have been permitted to dry in the air. With 160 pl of 30% (v/v) glacial acetic acid, the dye attached to the adherent cells was resolubilized. Every well's optical density (OD) was calculated at 590 nm that used an automated plate reader.

The cut-off OD (ODc-) was measured as 3 standard deviations above the negative control wells' average OD.

Results

Results were interpreted as follow [11]:

Table 3: Distribution of the studied organisms according to level of biofilm for CRS cases (n=20).

CRS	No.	%
Biofilm		
Non	9	45
Weak	5	25
Moderate	4	20
Strong	2	10

Table 4: Distribution of the studied organisms for OE ^ cases (n=22).

OE	No.	%
Organism		
Pseudomonas	10	45.5
Proteus	6	27.3
Diphteroid	1	4.5
Staph .Aureus	4	18.2
MRSA	1	4.5

Table 5: Distribution of the studied organisms according to level of biofilm for OE cases (n=22).

OE	No.	%
Biofilm		
Non	3	13.6
Weak	12	54.5
Moderate	7	31.8
Strong	2	10

Discussion

Most common isolates from CRS patients were MRSA. Forty samples have been collected in Karmouz health and staphylococcus aureus representing 65% of isolates. Insurance hospital collected between 11/2016 to 4/2017. Twenty cases of CRS and twenty cases of OE. In our study biofilm forming bacteria in CRS were found in (11 of 20) about 55% using microtiter plate assay. Results show different organisms in CRS and different organisms in OE with two cases with mixed infection. A further study has not specifically demonstrated the existence of biofilms in the greatest series of patient specimens to date [12], but rather evaluated the ability of bacteria recovered from CRS sufferers to develop in vitro biofilms using the Calgary biofilm assay. The biofilm formation rate was 28.6% of the 157 specimens collected in a tertiary rhinology clinic.

The speciation of cultures has shown that S. Aureus has been the most isolated organism (33%), but 20% of sufferers had either purely pseudomonal infections or polymicrobial infections including *P. aeruginosa*.

40 sufferers receiving FESS for CRS had mucosal specimens collected intraoperatively in a prospective research by Psaltis AJ et al. (2007) and were analyzed for mucosal biofilms using CLSM, revealing

Table 6: Relation between level of biofilm and organism for CRS cases (n=20).

CRS	Biofilm								X^2	^{MC}p
	No (n=9)		Weak (n=5)		Moderate (n=4)		Strong (n=2)			
	No.	%	No.	%	No.	%	No.	%		
Organism										
Staph Aurous	2	22.2	0	0	0	0	1	50	16.445	0.845
MRSA	4	44.4	3	60	2	50	1	50		
Citrobacter	0	0	1	20	0	0	0	0		
Klebsiella	0	0	0	0	2	50	0	0		
Strept	1	11.1	0	0	0	0	0	0		
E-coli	0	0	1	20	0	0	0	0		
Staph. Epidermidis	2	22.2	0	0	0	0	0	0		

Table 7: Relation between level of biofilm and organism for OE cases (n=22).

OE	Biofilm						x^2	^{MC}p
	No (n=3)		Weak (n=12)		Moderate (n=7)			
	No.	%	No.	%	No.	%		
Organism								
Pseudomonas	0	0	6	50	4	57.1	9.23	0.286
Proteus	0	0	4	33.3	2	28.5		
Diphteroid	1	33.3	0	0	0	0		
Staph .Aureus	1	33.3	2	16.6	1	14.2		
MRSA	1	33.3	0	0	0	0		

50 % of their research group with proof of biofilms (20/40) [13].

CLSM and FISH analysis were used in another study to analyze intraoperative specimens obtained from 18 CRS patients and five controls receiving septoplasty [14]. 78% (14/18) of sufferers with detectable bacteria in a biofilm matrix were identified by the study.

In combination with bacterial cultures, Ferguson BJ, et al. (2005) utilized TEM to display biofilms on 50% (2/4) of patient specimens obtained intraoperatively in assumed CRS, both of that resulted in *P. aeruginosa* [6].

The other two patients were found to have a CRS nonbacterial etiology to the CRS. Intraoperative specimens of ethmoid bullae were collected from five patients who undergo functional endoscopic sinus surgery (FESS) by Ramadan HH, et al. (2005), which all displayed morphological requirements for biofilms on SEM [15]. There were also different degrees of anomalies on the mucosal surface of all samples, ranging from disarrayed cilia to the total absence of cilia and goblet cells.

A follow-up analysis by Sanclement JA, et al. (2005) found that 80% (24/30) of patients had biofilms [16].

The inconsistencies in the above findings can or may arise from the different detection methods used and/or differences in the populations of patients studied. Moreover, the fact that the collection of small specimens was not indicative of the whole sino-nasal cavity may be attributable to the data inconsistency. The clear presentation of biofilms on the sino-mucosal specimens of CRS patients, irrespective of these variations, indicates that these complex structures could perform a role in the pathogenesis or persistence of chronic rhinosinusitis.

On the other hand, we will discuss biofilm in Otitis Externa. Using microtiter plate assay, most common isolates from OE patients were *Pseudomonas* 45% and Proteus 27% and staphylococcus Aureus 22.7%.

In another study, microbiological results for OE swabs shows that the incidences of *Pseudomonas* have varied from a low 12% to a high of 80% and the incidence of staphylococcus has been reported as low as 8.5% and as high as 29% [17,18].

In our study biofilm were identified in about 19 of 22 isolates representing 86.3% of chronic otitis externa patients. Biofilms have been found in 23 out of 25 patients (92%) with chronic otitis externa. In contrast, biofilms in the acute otitis externa group were isolated only in 3 instances (20%). The frequency variations were statistically significant. This evidence indicates that the development and maintenance of chronic external otitis is determined by biofilm [19,20].

Conclusion

Bacterial biofilms are highly ordered structures inside a protective extracellular matrix consisting of bacterial communities. One of the potential etiologies for the incidence and persistence of inflammation in CRS and OE is bacterial biofilms.

References

1. Wolcott R, Costerton JW, Raoult D, Cutler SJ (2013) The polymicrobial nature of biofilm infection. Clin Microbiol Infect 19: 107-112.
2. Vuong C1, Voyich JM, Fischer ER, Braughton KR, Whitney AR, et al. (2004) Polysaccharide intercellular adhesin (PIA) protects Staphylococcus epidermidis against major components of the human innate immune system. Cell Microbiol 6: 269-275.
3. Leid JG, Shirtliff ME, Costerton JW, Stoodley P (2002) Human leukocytes adhere, penetrate, and respond to Staphylococcus aureus biofilms. Infect Immun 70: 6339-6345
4. Stewart PS, Costerton JW (2001) Antibiotical resistance of bacteria in biofilms. Lancet 358: 135-138.
5. Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C,et al. (2003) The application of biofilm science to the study of and control of chronic bacterial infections. infections. J Clin Invest 112: 1446-1477.
6. Ferguson BJ, Stolz DB (2005) Demonstration of biofilm in human bacterial chronic rhinosinusitis. Am J Rhinol 19: 452-457.

7. Al-Mutairi D, Kilty SJ (2011) Bacterial biofilms and pathophysiology of chronic rhinosinusitis. Curr Opin Allergy Clin Immunol 11: 18-23.

8. Rowlands S, Devalia H, Smith C, Hubbard R, Dean A (2001) Otitis externa in UK general practice: a survey using the UK General Practice Research Database. Br J Gen Pract 51: 533-538.

9. O'Toole GA, Kolter R (1998) Flagellar and twitching motility are necessary for pseudomonas aeruginosa biofilm development. Mol Microbiol 30: 295-304.

10. Perez LR, Barth AL (2011) Biofilm production using distinct media and antimicrobial susceptibility profile of Pseudomonas aeruginosa. Braz J Infect Dis 15: 301-304.

11. Stepanović S1, Vuković D, Hola V, Di Bonaventura G, Djukić S, et al. (2007) Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS 115: 891-899.

12. Prince AA, Steiger JD, Khalid AN, Dogrhamji L, Reger C, et al. (2008) Prevalence of biofilm-forming bacteria in chronic rhinosinusitis. Am J Rhinol 22: 239-245.

13. Psaltis AJ, Ha KR, Beule AG, Tan LW (2007) Confocal scanning laser microscopy evidence of biofilms in patients with chronic rhinosinusitis. Laryngoscope 117: 1302-1306.

14. Sanderson AR, Leid JG, Hunsaker D (2009) Bacterial biofilms on the sinus mucosa of human subjects with chronic rhinosinusitis. Laryngoscope 116: 1121-1126.

15. Ramadan HH, Sanclement JA, Thomas JG (2005) Chronic rhinosinusitis and biofilms. Otolaryngol Head Neck Surg 132: 414-417.

16. Sanclement JA, Webster P, Thomas J, Ramadan HH (2005) Bacterial biofilms in surgical specimens of patients with chronic rhinosinusitis. Laryngoscope 115: 578-582.

17. Stroman DW, Roland PS, Dohar J, Burt W (2001) Microbiology of normal external auditory canal. Laryngoscope 111: 2054-2059.

18. Agius A, Pickles J, Burch K (1992) A prospective study of otitis externa. Clin Otolaryngol 17: 150-154.

19. Fusconi M, Chiarini F, Taddei AR, De Virgilio A, Gallinelli C, et al. (2010) Chemical ear peeling: a simple technique for the treatment of chronic external otitis: how we do it. Clin Otolaryngol 35: 424-429.

20. Ceri H, Olson ME, Stremick C, Read RR, Morck D, et al. (1999) The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin Microbiol 37: 1771-1776.