Research Article

INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230-8407 [LINKING]

EVALUATION OF C. ACNES CAPACITY TO BUILD BIOFILM AND A POSSIBLE LINK BETWEEN IT AND RESISTANCE TO WIDELY USED MEDICATIONS

Dr. Pranab Kumar Basak,[1] Dr Prakash sajja[2*]

[1]Assistant Professor, Department of Dermatology, MR Bangur Hospital, Kolkata, West Bengal

[2*]Professor, Dept of Dermatology Venerology and Leprosy, Sri Lakshmi Narayana Institute of Medical sciences Osudu, Puducherry

Corresponding author

Email id: drsajja@gmail.com

How to cite: Basak PK, Sajja P. Evaluation of c. acnes capacity to build biofilm and a possible link between it and resistance to widely used medications. International Research Journal of Pharmacy, 2021,12:3:58-62.

Doi:10.7897/2230-8407.1203136

==========

ABSTRACT

Background: Concern over antibiotic resistance in acne vulgaris patients has lately surfaced worldwide, especially in India. Although Cutibacterium acnes has been shown to be capable of forming biofilm, its involvement in acne vulgaris remains unclear and contentious.

Aim: The purpose of this study was to evaluate Cutibacterium acnes capacity to build biofilm and any possible relationships with antibiotic resistance.

Methods: 176 individuals with acne vulgaris who presented to the Institute during the designated study period were evaluated in this study. The collected samples underwent biofilm testing using the microtiter plate assay and analysed using MALDI-TOF-MS (Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry).

Results: According to the study, 43.1% (n=38) of the samples included C. acnes. The most resistant antibiotics were azithromycin, clindamycin, ampicillin, doxycycline, and minocycline, which were found in 73.7%, 65.8%, 15.8%, 31.6%, and 5.3% of patients, respectively. While 63.2% of C. acnes exhibited a limited ability to build biofilms, 37% of the isolates showed resistance to at least two antibiotics. Over 60% of the isolates exhibited a limited ability to produce biofilm and resistance to at least two different antibiotic classes.

Conclusion: Based on a number of variables, the current study finds that although C. acnes may produce biofilms, its effectiveness against antibiotic resistance may be deemed low. Alternative processes that might result in antibiotic resistance, such as genetic or metabolic plasticity, must be taken into account.

Keywords: Cutibacterium, biofilms, C. acnes, antibiotic resistance

INTRODUCTION

Acne vulgaris is a prevalent ailment that impacts around 9% of the global population, placing a substantial strain on the healthcare system worldwide. Cutibacterium acnes, the primary pathogen linked to acne vulgaris, is essential for preserving the equilibrium of the skin microbiota, which ultimately contributes to the development of chronic inflammatory skin diseases.[1]

The primary treatment method for acne vulgaris in recent decades has been the availability of oral and topical antibiotics. However, the widespread and careless use of antibiotics has often led to the formation of antibiotic-resistant strains of Candida vulgaris, which has presented a serious obstacle to the existing treatment regimens.[2]

Biofilms, which are organized collections of bacterial cells, may contribute to the development of antibiotic resistance in Candida acnes, according to recent evidence from the literature. Biofilm formation can serve as a barrier that prevents antibiotics from penetrating and makes resistance problems worse.[3]

In light of these circumstances, the goal of the current investigation was to evaluate C. acnes capacity for biofilm formation and clarify any possible relationships with the antibiograms. This might greatly advance our knowledge of the mechanisms of antibiotic resistance in acne vulgaris and pave the way for creative approaches to investigate C. acnes biofilms and their potential therapeutic applications.

MATERIALS AND METHODS

The goal of the current descriptive cross-sectional study was to evaluate Cutibacterium acnes capacity to build biofilm and any possible relationships with antibiotic resistance. The research participants came from the Institute's Department of Dermatology. Before participating in the study, all participants and school officials gave their verbal and written informed consent.

Participants who met the inclusion criteria, had a clinically verified diagnosis of acne vulgaris, and attended the Institute's Department of Dermatology during the research period were evaluated. Participants with acne vulgaris who were older than 12 years and who were not receiving treatment with topical or oral isotretinoin, complementary and alternative medicine (CAM), or hormone prescription met the study's inclusion requirements. Participants with hormonal acne were not allowed to participate in the trial.

Following final inclusion, each subject received a thorough general, cutaneous, and systemic examination, and the information acquired was documented on a standardized proforma that had already been prepared. Following the clinical diagnosis of acne vulgaris, the severity of the condition was evaluated using the Investigator's Global Assessment Scale System (IGA), which was scored from 0 to 4. Grade 0: Clear; Grade 1: A few papules and comedones; Grade 2: Less than half of the face is affected, with numerous visible comedones and pustules; Grade 3: More than half of the face is affected, with visible comedones, papules, pustules, and one nodule; Grade 4: Comedones, papules, pustules, nodules, and cysts are present throughout the entire face. Ethanol was used to clean the skin's surface before the sample was taken, which was determined by the type of lesion.

Using a sterile needle, pustules and comedones were taken from the lesion and placed in thioglycolate transport medium. Cutibacterium was then isolated using the appropriate media. Using Matrix Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry, colony morphology, and Gram’s staining, distinctive big white to yellow dry colonies were recognized as Cutibacterium species after incubation.

Following identification, the disc diffusion method was used to test the colonies of C. acnes for antibiotic susceptibility. Antimicrobial drugs such as doxycycline (30 mcg/disc), minocycline (30 mcg/disc), azithromycin (15 mcg/disc), ampicillin (10 mcg/disc), and clindamycin (10 mcg/disc) were used to test for susceptibility.

Comedones and pustules were removed from the lesion using a sterile needle and put in thioglycolate transport medium. After that, Cutibacterium was isolated using the proper medium. Time of Flight with Matrix-Assisted Laser Desorption Ionization Mass Following incubation, Cutibacterium species were identified by spectrometry, colony morphology, and Gram's staining. These characteristics included large, dry colonies that ranged from white to yellow.

After identification, the colonies of C. acnes were tested for antibiotic susceptibility using the disc diffusion technique. To test for susceptibility, antimicrobial medications including ampicillin (10 mcg/disc), clindamycin (10 mcg/disc), azithromycin (15 mcg/disc), minocycline (30 mcg/disc), and doxycycline (30 mcg/disc) were utilized.

ANOVA, the chi-square test, the student's t-test, Fisher's exact test, the Mann Whitney U test, and SPSS (Statistical Package for the Social Sciences) software version 24.0 (IBM Corp., Armonk, NY, USA) were used to statistically evaluate the obtained data. A p-value of less than 0.05 was regarded as the significance level.

RESULTS

The goal of the current study was to evaluate Cutibacterium acnes capacity to build biofilm and any possible relationships with antibiotic resistance. 176 individuals with acne vulgaris who presented to the Institute throughout the designated research period were evaluated in this study.

The collected samples underwent biofilm testing using the microtiter plate assay and analysed using MALDI-TOF-MS (Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry). Among the 76 strains evaluated, 65.8% (n=50) and 34.2% (n=26) of the study participants were resistant to clindamycin, 31.6% (n=24) and 68.4% (n=52) were sensitive to ampicillin, 5.3% (n=4) were resistant and 94.7% (n=72) were sensitive to minocycline, 15.8% (n=12) were resistant and 84.2% (n=64) were sensitive to doxycycline, and 73.7% (n=56) were resistant and 26.3% (n=20) were sensitive to azithromycin (Table 1).

Regarding the number of isolates with multi-antibiotic resistance for antibiotics on antibiotic-naïve subjects, among 76 study subjects, 18.4% (n14) isolates had no antibiotic resistance, 13.2% (n10) isolates had one antibiotic resistance, 36.8%

(n28) subjects had two antibiotic resistance, 21.1% (n16) isolates had three antibiotic resistance, and 10.5% (n8) study subjects had four antibiotic resistance (Table 2).

According to the study's findings, 3% (n=2) and 3% (n=2) of the isolates were resistant to a combination of antibiotics; 3% (n=2) were resistant to ampicillin; 3% (n=2) were resistant to minocycline, clindamycin, azithromycin, and ampicillin; 3% (n=2) were resistant to minocycline, clindamycin, azithromycin, and ampicillin; 5% (n=4) were resistant to 5% (n=4), 5% (n=4), clindamycin, 8% (n=6), ampicillin; 13% (n=10) were resistant to no antibiotics; 29% (n=22) were resistant to azithromycin and clindamycin, azithromycin, and ampicillin (Table 3).

When evaluating the biofilm capability of the C. acnes research isolates, it was found that, out of the 100% (n=76) isolates from the study, 63.2% (n=48) had weak biofilm capacity and 36.8% (n=28) had moderate biofilm capacity (Table 4).

DISCUSSION

176 individuals with acne vulgaris who presented to the Institute throughout the designated research period were evaluated in this study. The collected samples underwent biofilm testing using the microtiter plate assay and analysed using MALDI-TOF-MS (Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry).

Among the 76 strains evaluated, 65.8% (n=50) and 34.2% (n=26) of the study participants were resistant to clindamycin, 31.6% (n=24) and 68.4% (n=52) were sensitive to ampicillin, 5.3% (n=4) were resistant and 94.7% (n=72) were sensitive to minocycline, 15.8% (n=12) were resistant and 84.2% (n=64) were sensitive to doxycycline, and 73.7% (n=56) were resistant and 26.3% (n=20) were sensitive to azithromycin. These findings were similar to those of earlier research on C. acnes strains and their antibiotic sensitivity and resistance conducted by Zhu T et al. in 2019 and Nakase K et al. in 2017, where authors evaluated participants using data similar to the current study.

According to the study's findings, among the 76 study participants, 18.4% (n14) of the isolates had multi-antibiotic resistance to antibiotics, 13.2% (n=10) to multiple antibiotics, 36.8% (n=28) to multiple antibiotics, 21.1% (n=16) to three antibiotics, and 10.5% (n=8) to four antibiotics. These findings aligned with those of Luk NM et al. (2013) and Ishida N et al. (2008), who found similar numbers of isolates with multi-antibiotic resistance for antibiotics on antibiotic-naïve participants in their respective investigations.

For a variety of antibiotic combinations that demonstrated resistance to the isolates, it was observed that 3% (n=2) strains were resistant to minocycline, doxycycline, clindamycin, and azithromycin, 3% (n=2) strains were resistant to ampicillin, 3% (n=2) strains were resistant to minocycline, clindamycin, azithromycin, and ampicillin, 5% (n=4) strains were resistant to doxycycline, clindamycin, azithromycin, and ampicillin, 8% (n=6) strains were resistant to Doxycycline, clindamycin, ampicillin, and 13% (n=10) strains were resistant to no antibiotics, 18% (n=14) strains were resistant to antibiotics, and 29% (n=22) strains were resistant.

These results were comparable to those of the current study and were in line with the findings of Bettoli V et al. (2006) and Mandoza N et al. (2013), who found that different antibiotic combinations demonstrated resistance to the isolates described by the authors in their investigations. Regarding the evaluation of the biofilm capacity in the isolates from the C. acnes study, out of the 100% (n=76) isolates from the study, 63.2% (n=48) had poor biofilm capacity and 36.8% (n=28) had moderate biofilm capacity.

These results were in line with the findings of Mongaret C et al[13] in 2020 and Burkhart CG et al[14] in 2007 where biofilm capacity in C. acnes study isolates comparable to the present study was also reported by the authors in their respective studies.

CONCLUSION

The present study, within its limitations, concludes that despite of C. acnes having capability to form biofilms, its efficacy towards antibiotics resistance can be considered as modest depending on the various factors. It is vital to consider alternate mechanisms as biochemical or genetic plasticity which can lead to antibiotic resistance.

REFERENCES

1. Scholz CFP, Kilian M. The natural history of cutaneous propionibacteria, and reclassification of selected species within the genus Propionibacterium to the proposed novel genera Acidipropionibacterium gen. nov., Cutibacterium gen. nov. and Pseudopropionibacterium gen. nov. Int J Syst Evol Microbiol 2016;66:4422-32.
2. Tan JK, Bhate K. A global perspective on the epidemiology of acne. Br J Dermatol 2015;172:3-12.
3. Sadhasivam S, Sinha M, Saini S, Kaur SP, Gupta T, Sengupta S, et al. Heterogeneity and antibiotic resistance in Propionibacterium acnes isolates and its therapeutic implications: Blurring the lines between commensal and pathogenic phylotypes. Dermatol Ther 2016;29:451-4.

4. Dréno B, Pécastaings S, Corvec S, Veraldi S, Khammari A, Roques C. Cutibacterium acnes (Propionibacterium acnes) and acne vulgaris: A brief look at the latest updates. J Eur Acad Dermatol Venereol 2018;32:5-14.
5. Clinical and laboratory standards institute. Analysis and presentation of cumulative antimicrobial susceptibilty test data; Approved guideline. 4th ed. Pennsylvania: Clinical and laboratory standards institute; 2014.
6. Holmberg A, Lood R, Mörgelin M, Söderquist B, Holst E, Collin M, et al. Biofilm formation by Propionibacterium acnes is a characteristic of invasive isolates. Clin Microbiol Infect 2009;15:787-95.
7. Zhu T, Zhu W, Wang Q, He L, Wu W, Liu J, et al. Antibiotic susceptibility of Propionibacterium acnes isolated from patients with acne in a public hospital in Southwest China: Prospective cross-sectional study. BMJ Open 2019;9:e022938.
8. Nakase K, Hayashi N, Akiyama Y, Aoki S, Noguchi N. Antimicrobial susceptibility and phylogenetic analysis of Propionibacterium acnes isolated from acne patients in Japan between 2013 and 2015. J Dermatol 2017;44:1248-54.
9. Luk NM, Hui M, Lee HC, Fu LH, Liu ZH, Lam LY, et al. Antibioticresistant Propionibacterium acnes among acne patients in a regional skin centre in Hong Kong. J Eur Acad Dermatol Venereol 2013;27:31-6.
10. Ishida N, Nakaminami H, Noguchi N, Kurokawa I, Nishijima S, Sasatsu M. Antimicrobial susceptibilities of Propionibacterium acnes isolated from patients with acne vulgaris. Microbiol Immunol 2008;52:621-4.
11. Bettoli V, Borghi A, Rossi R, Ferroni M, Rigolin F, Virgili A. Antibiotic resistance of propionibacteria. Four years' experience of a large number of cases in Italy. Dermatology 2006;212:206-7.
12. Mendoza N, Hernandez PO, Tyring SK, Haitz KA, Motta A. Antimicrobial susceptibility of Propionibacterium acnes isolates from acne patients in Colombia. Int J Dermatol 2013;52:688-92.
13. Mongaret C, Varin-Simon J, Lamret F, El-Mahdy TS, Brasme L, VernetGarnier V, et al. Cutibacterium acnes biofilm study during bone cells interaction. Microorganisms 2020;8:1409.
14. Burkhart CG, Burkhart CN. Expanding the microcomedone theory and acne therapeutics: Propionibacterium acnes biofilm produces biological glue that holds corneocytes together to form plug. J Am Acad Dermatol 2007;57:722-4.

S. No	Antibiotic	Resistant		Sensitive	
		N	%	N	%
1.	Clindamycin (n=76)	50	65.8	26	34.2
2.	Ampicillin (n=76)	24	31.6	52	68.4
3.	Minocycline (n=76)	4	5.3	72	94.7
4.	Doxycycline (n=76)	12	15.8	64	84.2
5.	Azithromycin (n=76)	56	73.7	20	26.3

Table 1: Antibiotic susceptibility pattern for all antibiotics in study participants

S. No	Number of antibiotics having resistance	Number of isolates with antibiotic resistance	Percentage
1.	0	14	18.4
2.	1	10	13.2
3.	2	28	36.8
4.	3	16	21.1
5.	4	8	10.5
6.	Total isolates=76		

Table 2: Number of isolates with multi-antibiotic resistance for antibiotics on antibiotic naïve subjects

Antibiotics	Number of strains	Percentage
Minocycline, doxycycline, clindamycin, azithromycin	2	3
Ampicillin	2	3
Minocycline, Clindamycin, azithromycin, Ampicillin	2	3
Doxycycline, clindamycin, azithromycin, Ampicillin	4	5
Azithromycin	4	5
Clindamycin	4	5
Doxycycline, clindamycin, azithromycin	6	8
Azithromycin, ampicillin	6	8
Clindamycin, azithromycin, Ampicillin	10	13
No antibiotics	14	18
Clindamycin, azithromycin	22	29
Total	76	100

Table 3: Various antibiotics combination showing resistance to the isolates

S. No	Biofilm capacity	Number of isolates	Percentage
1.	**Weak**	48	63.2
2.	**Moderate**	28	36.8
3.	**Total**	76	100

Table 4: Biofilm capacity in C. acnes study isolates