Original Article

Assessment Of Biofilm Production Of Obligatory Aerobic And Anaerobic Bacteria For Acne Patients In Kirkuk – Iraq

Noor raad soady*[1], Asma Sumiea Karomi[2]

[1]Kirkuk Education Directorate, Ministry of Education, Kirkuk, Iraq,E-mail: scbhm020@ uokirkuk.edu.iq
[2]Department of Biology, College of Science, Kirkuk University, Kirkuk, Iraq,E-mail: dr.asmakaromi@uokirkuk.edu.iq

***Corresponding Author**:- Noor raad soady
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Abstract

The study which involved 200 patients with Acne vulgaris, was carried out in Kirkuk City from October 2021 to March 2022. To establish the bacterial species that create a biofilm and to isolate and identify the bacterial species that causes Acne vulgaris patients. Acne swab were obtained for aerobic and anaerobic culture, microtiter plate method (MPA), and congo red agar (CRA), methods were used to detect the biofilm formation.

Propionibacterium acnes plays a role in the pathogenesis of acne vulgaris, a common disorder of the pilosebaceous follicles and it has been suggested that P. acnes cells residing within the follicles grow as a biofilm.

The most common isolated bacteria *P.acne* 40.5%, followed by *S.epidermidis* 22.5%, *S.aureus* 19.5%, and 7% *S.pyogenes* . The results of the current study indicate that type of bactera isolates were strong biofilm producer by microtiter plate method (MPA) and congo red agar method , 88.9% of *P.acne*,73.3% of *S. epidermidis* , 64.1% of *S. aureus* , and 70% of *S.pyogenes* , isolates were strong biofilm producer by MPA, while the results also showed the74.0% of *P.acne* ,73.3% of *S. epidermidis* , 71.7% of *S. aureus* ,70% of *S.pyogenes* isolates were strong biofilm producer by CRA.

Keywords: Acne vulgaris, *P.acne*, biofilm.

INTRODUCTION

Propionibacterium acnes is an anaerobic gram positive non-sporing bacillus that forms a major component of the human skin flora (Zeller *et al*.,2007). Biofilms are consortia of micro-organisms that form on various surfaces, including biotic surfaces (e.g. mucosal membranes, teeth), medical devices and household surfaces. Biofilm formation is a multi-stage process (Stoodley *et al*.,2002).

On the skin surface, the microbial community is mostly constituted by bacteria belonging to the three main genera of *Corynebacteria*, *Propionibacteria* and *Staphylococci* (Grice *et al*.,2009). Interplay between members of this cutaneous microbiota is essential for the maintenance of a healthy skin. While the commensal bacterium Propionibacterium acnes (*P. acnes*), predominant in sebaceous sites, is critical in the regulation of skin homeostasis (Rosenthal *et al*.,2011) and prevents colonisation from other harmful pathogens (Szabo *et al*.,2017). it can also act as an opportunistic pathogen in acne vulgaris. New findings on *P. acnes* reveal that, contrary to what was previously thought, its proliferation is not the trigger of acne but instead, a tight equilibrium between members of the skin flora and among *P. acnes* phylotypes might play a more critical role in acne onset (Barnard *et al*.,2016) Loss of microbial diversity can indeed lead to chronic inflammatory skin diseases (Sanchez *et al*.,2015).

Colonization of the pilosebaceous follicle by P. acnes is viewed as one of the focal variables driving skin inflammation by participating in the provocative reaction of the skin, notwithstanding the cutaneous microbiota and natural resistance. Two different variables engaged with this ongoing provocative skin sickness are the expanded sebum creation, with a change of its organization, and hypercornification of the pilosebaceous follicle coming about because of hyperproliferation and unusual separation of keratinocytes of the upper piece of the follicle (Dreno,2017 ; Suh,2015).

There are numerous other contributing elements that impact the seriousness as well as the occurrence and determination of skin break out, like ecological variables, chemicals, family ancestry and stress (Lynn *et al*.,2016).

This work underlines the importance of biofilm formation in P. acnes pathogenesis, and shows that biofilm formation should be considered in the diagnosis and treatment of invasive P. acnes infections.

MATERIALS AND METHODS

The study conducted during the period October 2020 to March 2021. Included 200 with Acne vulgaris disease was carried out in Kirkuk City , Patients ages ranged from 12 to 25 years, To establish the bacterial species that create a biofilm and to isolate and identify the bacterial species that causes Acne vulgaris patients. Acne swab were obtained for aerobic and anaerobic culture.

Microtiter Plate Assay (MPA) method

Isolate was put into TSB media that had been added with 1% glucose (TSBglu) and incubated for 24 hours in 37oC and then diluted 1:100 with new medium. Every sterile polystyerene well (out of 96 wells) was filled with 0.2 ml of diluted culture and only broth was used as control for sterility test and non-specific binding of media. Culture was incubated for 24 hours in 37oC. After incubation, plate was gently tapped and then washed four times with 0.2 ml PBS in pH 7.2 to remove planktonic bacteria. Biofilms made of microorganisms that was attached on plate (sessile) binded with sodium acetate (2%) which stained by crystal violet (0.1% w/v). Stain was washed with ionized water and plate was dried. Cells that were attached usually form biofilms and wells were stained with crystal violet. They were then washed with PBS once and fixated with ethanol for 15 minutes. OD of bacteria was attached on wells and stained by crystal violet which was determined by micro ELISA autoreader (mode PR 601, quklinger S) on wave of 630 nm (OD 630 nm). This process was repeated three times to obtain optimal result. Biofilm formation is interpreted as negative if 0 - < 0.2, moderate if 0.2 - 0.4, and positive if > 0.4. In this study, the moderate results were categorized as positive (Furtuna *et al*.,2018).

Congo Red Agar (CRA) method

Congo Red Agar media consists of 37 g/L BHI broth, 10 g/L agar base, 50 g/L sucrose, 1 L water and 0.8 g/L Congo Red indicator. Congo Red indicator was prepared as concentrated liquid apart of other media constituents, and autoclaved in 121oC for 15 minutes and then added into cooled agar in 55oC. Isolate was then inoculated and incubated for 24 hours in 37oC. Positive result was shown by black colored colonies. Negative result was shown by pink color on colonies (Furtuna *et al*.,2018).

RESULTS

The results of the isolation showed that out of the 200 samples, 81 (40.5)%) were positive for *P.acnes*, 45 (22.5)%) *S.epidernidis* , 39 (19.5%) *S.aureus* , and 14 (7%) *S.pyogenes* as shown in table (1).

Table (1) Rates Of Isolated Bacteria In Acne Vulgaris Disease

bacterial isolates	NO.	%
P.acnes	81	40.5%
S. epidermidis	45	22.5%
S. aureus	39	19.5%
S.pyogenes	14	7%
E.coli	6	3%
klebsiella oxytoca	4	2%
pantoea agglomerans	1	0.5%
No growth	10	5%
Total	200	100%

The study reported that 88.9% of *P.acnes* isolates were strong biofilm producer by MPA method as in figure (1) and table (2)., while 74% of *P.acnes* isolates were strong biofilm producer by congo red agar method as in figure (2) and table (3).

Table (2) Distribution Of Different Bacterial Isolates According To Biofilm Production By MPA Method

bacterial isolates	MTP					
	Negative	%	Moderate	%	Positive	%
P.acnes	9	11.1	32	39.5	40	49.4
S. epidermidis	12	26.7	7	15.5	26	57.8
S. aureus	14	35.9	11	28.2	14	35.9
S.pyogenes	3	30	2	20	5	50
statistical analysis	Chi-square =16.719 P-value = 0.010					

Table (3) Distribution Of Different Bacterial Isolates According To Biofilm Production By CRA Method

bacterial isolates	CRA					
	Negative	%	Moderate	%	Positive	%
P.acnes	21	26	32	39.5	28	34.5
S. epidermidis	12	26.7	12	26.7	21	46.6
S. aureus	11	28.2	20	51.3	8	20.5
S.pyogenes	3	30	2	20	5	50
statistical analysis	Chi-square = 9.020 P-value = 0.172					

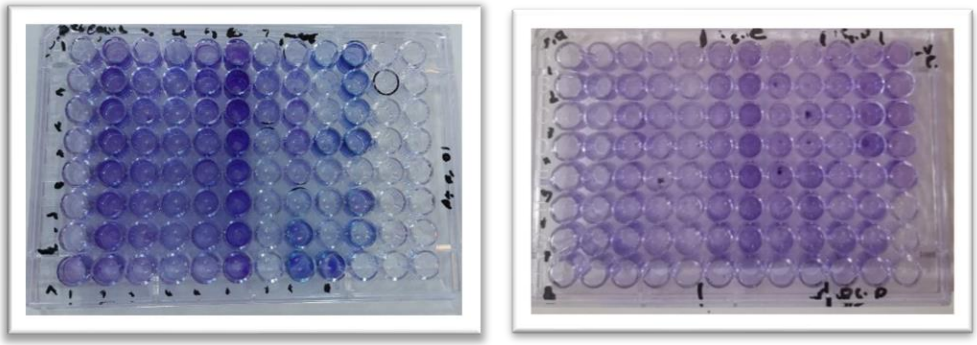
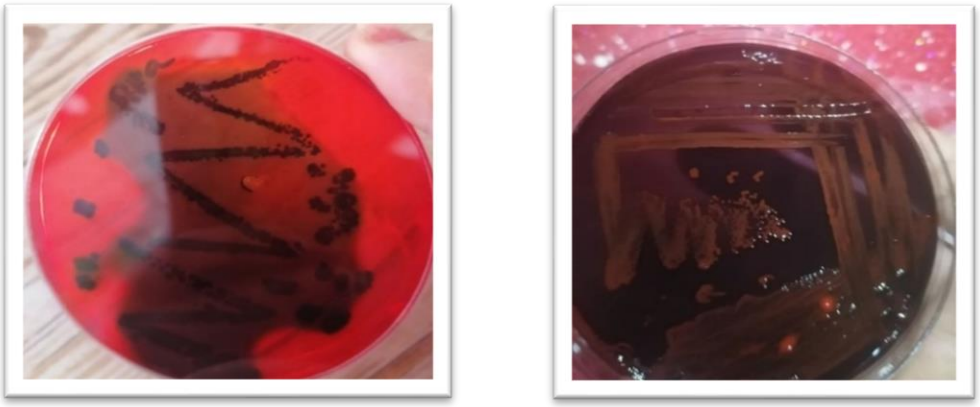


Figure (1) Distribution Of Different Bacterial Isolates According To Biofilm Production By MPA Method



A

B

C

Figure (2) Distribution Of Different Bacterial Isolates According To Biofilm Production By CRA Method

A. Strong positive
B. moderately positive
C. Biofilm non formation

DISCUSSION

The results of the current study on the ability of bacteria to form a biofilm agree with the results reached by (Okuda *et al*., 2018) among seven isolates, five were biofilm-forming isolates, where the ability of bacteria to form a biofilm is one of the most important factors of virulence granted by resistant bacteria. For most antibiotics, when the appropriate environmental conditions are present, the bacteria that do not form a biofilm and that live in the liquid environment turn into bacteria that produce the biofilm and live in the solid environment, where the bacteria make several changes and modifications to suit the new environment (Soto, 2014).

The results of the current study agreed with (Jahns *et al*., 2012) that showed the ability of P.acnes bacteria to form biofilms in sebaceous follicles and also the observation of similar biofilms also in skin diseases other than acne, such as folliculitis and hidradenitis suppurativa. These symptoms. In the terminal hair follicles (Jahns *et al*., 2014).

A study (Holmberg *et al*., 2009) showed that only invasive *P.acnes* strains can produce biofilm regardless of their relative pattern, while strains isolated from healthy skin produce less biofilm as the ability of *P.acnes* to form biofilm is also affected. The presence of plasma and surface roughness of the biomaterial.

The ability of bacteria to form biofilm is one of the most prevalent virulence factors, and it can be found in bacteria that live in the external environment or within pathogens. *S. aureus*, positive and negative coagulase are characterized by their ability to colonize the host and adhere to living surfaces through the production of biofilms. On the formation of membranes allows it to get rid of the immune defenses of the host and treatment with antibiotics (Evgueny *et al*., 2006), and the results of the study showed that among 45 isolates of S. epidermidis bacteria, 33 were biofilm-producing and 12 non-biofilm-producing isolates. S. aureus, out of 39 isolates, 25 isolates were biofilm-producing to different degrees, and 14 isolates were not biofilm-producing, and S.pyogenes bacteria were also biofilm-producing, out of 10 isolates were 7 isolates were biofilm-producing and 3 were non-biofilm-producing, as in figure (1) and table (2).

Using the Congo Red Agar (CRA) method, the results showed that a high percentage of *P.acnes* bacteria was biofilm-producing, 28 strong positive isolates were biofilm-producing out of 81 isolates, 32 moderately positive isolates and 21 non-biologically productive isolates. as in figure (2) and table (3).

As for *staphylococci*, *S. epidermidis* bacteria showed that out of 45 isolates, 21 isolates were strong positive, 12 isolates moderately positive, and 12 were not biofilm-producing. As for *S. aureus* bacteria, out of 39 isolates, 8 isolates were strong positive, and 20 isolates moderately positive isolates are medium, and 11 are not biofilm-producing, and *S.pyogenes* was also biofilm-producing. Out of the 10 isolates, 5 strong positive isolates were biofilm-producing, 2 moderately positive isolates were moderately productive, and 3 isolates were not biofilm-producing, as in figure (2) and table (3).

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