Biofilm Formation by Bacterial Isolates from Burn Infected Patients

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Abstract

In this study, wound swabs and blood samples were collected from (100) patients suffering from burn wound infections with the age range between (1.5-45) years old from both sexes. The period of collection were extended from November 2011 through March 2012.

All samples were subjected to identified the bacterial cultivation, and different types of bacteria were isolated which caused burn wounds infections. The predominant bacteria was *P.aerugenusa* for gram negative, and for gram positive was *S.aureus*.

Biofilm formation were studied for all bacterial isolates and the results shown that The most bacteria that form biofilm were *A.baumannii* and *Enterobacter spp.* (80%) where as lawest one was *K. pneumoniae* (20%).

الخلاصة

تم خلال هذا البحث جمع و دراسة ١٠٠ عينه (مسحات جلد) و ٦٥ عينة دم من مرضى الحروق (٥٩ اناث و ٤١ ذكور) و ٧٠ مسحه من ردهات الحروق وممن أحيلوا إلى مستشفى الحلة التعليمي العام (من تشرين الثاني ٢٠١١ خلال اذار٢٠١٢) . تراوحت أعمار المرضى الذين شملتهم الدراسة من ١.٥– ٤٥ سنه.

خضعت جميع العينات للزرع البكتيري للتعرف على المزارع البكتيرية واجريت الاختبارات الكيموحيوية على جميع عزلات البكتريا المسببة لالتهاب الحروق، وكانت السائدة من البكتريا السالبة لصبغة غرام هي *P.aerugenosa* والبكتريا الموجبة لصبغة غرام هي *S. aureus* .

تم دراسة تكوين الغشاء الحيوي ايضا لاغلب البكتريا وكانت *A.baumannii* و *Enterobacter spp.* هي الاكثر انتاجا لهذا الغشاء الحيوي(٨٠%)، في حين كانت *K.pneumoniae* هي الاقل تكوينا له (٢٠%).

Introduction

Burn wound infections are problematic because it delays healing, encourages scarring and may result in bacteremia, sepsis or multiple-organ dysfunction syndrome whereby organs from several systems are unable to maintain homeostasis on their own, requiring immediate medical attention[1].

Bacteria and fungi are the most common pathogens of burn wounds. These microbes form multi-species biofilms on burn wounds within 48 – 72 hours of injury [1]. Organisms originate from the patient's own skin, gut and respiratory flora, as well as from contact with contaminated health care environments and workers [2].

The epidemology of burn wound varies in different populations according to the influencing factor, which includes sex, age, gentic factors, exposure smoking, environmental factors and upper respiratory tract infections (URTI).

Biofilms are complex communities of surface attached aggregates of microorganisms embedded in a self-secreted

extracellular polysaccharide matrix, or slime [3]. Once formed, biofilms act as efficient barriers against antimicrobial agents and the host immune system, resulting in persistent colonization and/or infection at the site of biofilm formation[4]. Although biofilms are best known for their role in foreign device-related infections, recent studies have confirmed the importance of biofilms in the pathogenesis of burn wound infections [5]. Bacteria within a biofilm typically undergo a phenotypic change whereby microbial virulence factor production is altered and metabolic rate and motility are reduced [4]. Channels formed within the protective environment of the biofilm facilitate the transport of nutrients and microbial waste products [6]. Intercellular signaling molecules produced by bacteria within the biofilm are able to traverse these channels and influence the overall growth pattern and behavior of the biofilm in response to various host and environmental factors [7].

Aim of the Study

1- Isolation and Identification of different type of bacteria from burn infected patients.
2- Study of biofilm formation for bacterial isolates.

Materials and Methods

Collection of specimens:

Skin burned swabs and blood samples were collected from 100 patients suffering from burn infection from both sexes who are admitted to burn ward in AL_Hilla general hospital and environmental swabs from burn ward were also obtained. The period from November 2011 through March 2012.

The bacterial isolates were identified after staining with gram stain, specific biochemical tests were done to reach the final identification according to [8,9].

Biofilm formation:

Tissue culture plate method (TCP):

In present study, we screened some isolates for their ability to form biofilm by TCP method as described by *Christensen et al* [10] with a modification in duration of incubation from (18-24 hours) according to Mathur *et al* [11].

Results and Discussion

Isolation of Bacteria:

A total of (235) samples consisting of (100) skin swabs, (65) blood specimens and (70) burn ward swabs were subjected for culturing on bacterial culture media. The results shown in table (1) revealed that 166 (70.6%) samples gave positive bacterial culture whereas 69 (29.4%) showed no bacterial growth. Regarding skin swabs, 89(89%) were positive bacterial cultures consisting of single growth 68 (76.4%), and mixed bacterial growth 21 (23.6%). Meanwhile, no bacterial growth was found in 11 (11%) of skin swab cultures. The single and mixed bacterial growth results of skin swabs are shown in table (1). These results agree with that obtained by Bagdonas *et al* [12] who found that 86.5% of skin swabs were positive for bacterial growth. Also, Al-Akayleh [13] reported that negative bacterial growth was found in approximately 18% of the cultures of skin swab.

Table-1 Number and Percentage of Bacterial Isolates from Burn Patients and Burn Unit

Result	Source of Culture			
	Skin No. (%)	Blood No. (%)	Burn Unit No. (%)	Total of Samples No. (%)
Culture Positive	89 (89%)	32 (49.2%)	45 (64.3%)	166 (70.6%)
Single Growth	68 (76.4%)	25 (78.1%)	32 (71.1%)	125 (75.3%)
Mixed Growth	21 (23.6%)	7 (21.9%)	13 (28.9%)	41 (24.7%)
Culture Negative	11 (11%)	33 (50.8%)	25 (35.7%)	69 (29.4%)
Total	100 (100%)	65 (100%)	70 (100%)	235 (100%)

The high percentage of positive bacterial cultures of the skin swab may be attributed to the fact that the burn wound has a much higher incidence of infections compared with other forms of trauma because of extensive skin barrier disruption as well as alteration of cellular and humoral immune responses [14].

Regarding the blood culture table (1), results showed that positive bacterial cultures were found in 32 (49.2%) of burned patients. This result is higher than that obtained by De Macedo *et al* [15] in Brazilia who showed that (19.4%) of burned patients developed proven sepsis. In this study, the high rate of positive blood culture may be attributed to the high level of nosocomial infections acquired from medical devices in burn wards, the crowding of wards with burned patients and the unavailability of recent techniques in the sterilization of burn wards.

The burn unit samples showed positive cultures in 45 (64.3%). 32 (71.1%) of them were of single growth, the other 13 (28.9%) were mixed bacterial cultures table (1). These findings reflect the higher percentage of bacterial contamination of the burn unit which explains the higher percentages of positivity of both skin and blood cultures found in this study.

Types of Bacterial Isolates:

It is shown in table(2) and table (3) the frequency of bacteria in skin swab, blood and burn ward cultures, it is clear from the total number of isolates that gram negative bacteria are more frequent than gram positive type. This agrees with Kamel and El-Megeed [16] who found that Gram negative bacteria represent about 65% of micro-organisms that cause burn wound infection and that this type of bacteria has assumed a primary lethal role among the cases of burn wound infection and septicaemia.

The predominance of gram negative bacteria is clear from the high frequency of *P.aeruginosa* in each source of the cultures. This agrees with Singh *et al* [17] who found the most commonly isolated organisms from burn patients were *Pseudomonas* species followed by *S. aureus* and *Klebsiella* species and these results are also in accordance with other studies [18].

In the blood cultures table (2), *Enterobacter* is second frequent than *P.aeruginosa*. The source of *Enterobacter* bacteraemia is either from burn wound or as a result of bacterial translocation which is caused by failure of the gut barrier, the imbalance of intestinal flora and impaired host immune defenses, all these conditions are caused and enhanced by burn injury [19]. It is clear that the burn wound can be contaminated by microorganisms that migrate from the gastrointestinal, urinary and respiratory tracts [20]. This indicates the idea of autoinfection that the burn patients suffer from in addition to the infection acquired from the burn unit itself [21].

Table-2 Frequency of Gram negative Bacteria

Bacterial Isolates	Skin	Blood	Burn unit
	Frequency	Frequency	Frequency
P.aeruginosa	**29**	**13**	**17**
Enterobacter spp.	**13**	**7**	**5**
E.coli	**14**	**3**	**5**
A.baumannii	**11**	**2**	**4**
K. pneumoniae	**11**	**1**	**4**
Proteus spp.	**4**	**——**	**2**

In this study the more frequent Gram positive bacteria isolated from the blood is *S. aureus* followed by *S. epidermidis*, β-hemolytic *streptococci* and *E. faecalis*, the later three have the same frequency as demonstrated in table (3). These results were approximately near with that of De Macedo *et al* [15] who showed that the most common bacteria isolated from blood culture were *S.aureus* followed by *S. epidermidis*. Furthermore, *S. epidermidis* should be considered as an important pathogen for sepsis in burns. The organism, being ubiquitous in a hospital environment, and burn wounds being the ideal medium for its multiplication, it is hardly surprising that this bacteria would be the cause of 20.7% of septic episodes [15].

Emmerson [22], noted that *S. aureus* is still one of the most frequently encountered single bacterial species in hospitals and continues to be a frequent cause of burn wound sepsis.

Table-3 Frequency of Gram Positive Bacteria

Bacterial Isolates	Skin	Blood	Burn unit
	Frequency	Frequency	Frequency
S. aureus	16	7	17
S. epidermidis	4	2	——
E. faecalis	5	2	4
Streptococcus spp.	3	2	——
Total	28	13	21

Biofilm formation:

Bacterial biofilms cause chronic diseases that are difficult to control and in the present study, differentiation of bacteria as biofilm producers and non-biofilm producers using (ELISA) was based on OD570 measurements. A total of (59) isolates from deferent types of bacteria were tested for there ability to produce biofilm. From these isolates (9),(23) were form strong and moderate biofilm respectively(54.2%) and (27) isolates (45.8%) were non biofilm formation as shown in table (4),(5).

Table 4 Production of biofilm in gram positive bacteria

Bacterial Isolates	Biofilm			
	Strong	Moderate	Weak	%of biofilm formation
S. aureus	2	5	3 (30%)	70%
S.epidermidis	2	1	2 (40%)	60%
E. faeclis	1	2	2 (40%)	60%
Streptococcus spp.	—	2	2 (50%)	50%

Table 5 Production of biofilm in gram negative bacteria

Bacterial Isolates	Biofilm			
	Strong	Moderate	Weak	%of biofilm formation
P. aeruginosa	—	4	6 (60%)	40%
Enterobacter spp.	1	3	1 (20%)	80%
E. coli	—	2	3 (60%)	40%
A. baumannii	3	1	2 (20%)	80%
K. pneumoniae	—	1	4 (80%)	20%
Proteus spp.	—	2	2 (50%)	50%

70%(7/10) of *S.aureus* and 60%(3/5) *S.epidermides* were formed biofilm where as 30%(3/10) and 40%(2/5) from both species respectively were not have biofilm, This result approximately agree with Mathur *et al* [11], who found that 53.9% from *S.aureus* were formed biofilm after incubation to 24 hours.

S.aureus is especially capable of adhering to a large variety of matrix components to initiate colonization. This adherence is frequently mediated by protein adhesions of the family known as MSCRAMM (microbial surface components recognizing adhesive matrix molecules). The collagen-binding protein, fibronectin-binding proteins, and fibrinogen-binding proteins belong to this family [23].

The emergence of *S. epidermidis* as pathogen has been synonymous with the now widespread use of intravascular catheter in modern medicine. The enherent capacity of this organism to cause infection derives primarily from its ability to form mucoid biofilm on the inert synthetic surfaces of indwelling medical devices [24].

On the other hand (50%) of *Streptococcus spp.* and (60%) of *E. faecalis* were formed biofilm and (50%) , (40%) were non biofilm formation respectively. This result agree with Toledo-Arana *et al* [25], which found that 57% of *E. faecalis* isolates derived from various clinical isolates produced biofilms. Mohamed *et al* [26], refer to factors that may influence the process of biofilm formation in *E. faecalis* and the first report of involvement of multiple genes, such as fsr, gelE, epa, atn, in biofilm formation by *E. faecalis* and was published in 2004.

Regarding (40%) from both *P. aeruginosa* and *E. coli* and (80%) from both *Enterobacter ssp.* and *A. baumannii* were formed biofilm and (50%), (20%) of *Proteus spp.* and *K. pneumoniae* were produce biofilm respectively as showen in table (5). This result nearly compatible with Vishnu *et al* [27], who found that (33.05%) from *Pseudomonas* were formed biofilm and Jayachandran and Prashanth [28] found that 62% from *A. baumannii* were biofilm positive.

K. pneumoniae is often involved in biofilm-related infections, Madam *et al* [29] found that 62.5% of *K.*

pneumoniae produce biofilm and this results higher than the results obtained in this study, the reason may be due to small sample size (N= 5) may account for this. The reason for this higher rate in biofilm- positive strains in Madam *et al* may be due to the biofilm is almost invariably a multispecies microbial community and these species can share their genetic material at a high rate [30].

Regarding, (40%) of *E.coli* were formed biofilm and these results bring into line with the results obtained by Wizemann *et al* [31], who observed that some isolate of *E.coli* were formed biofilm because it encodes a peptide methionine sulphoxide reductase (MsrA) that responsible for biofilm formation in these bacteria.

On the other hand Macleod and Stickler [32], found that 33% from *Proteus spp*. were formed biofilm and these result approximately similar to the result that obtained in this study. Jones *et al* [33], suggest that the formation of swarmer cells could represent a method by which *P. mirabilis* can disperse from the mature biofilm to seed new surfaces and blocked catheters more
quickly than the wild-type strain in the catheterized bladder model.

Recommendations

More research is needed to clarify the biofilm formation by bacteria in experimental model, and additional studies on the biofilm formation to characterize the molecular and cellular between biofilm formation as virulence factors and infection.

Conclusions

The Gram negative bacteria are more predominant as causative agents for burn infections than the Gram positive type and the burn unit is an important aetiology for burn wound infection. The most bacterial that form biofilm were *A.baumannii* and *Enterobacter spp.* (80%) where as lawest one was *K. pneumoniae* (20%).

References

1- Church, D., Elsayed, S., Reid, O., Winston, B., Lindsay, R. (2006) Burn Wound Infections. Clinical Microbiology Reviews, 19 (2), 403 – 434.

2- Murray, C., Hospenthal, D.R. (2008). "Burn Wound Infections". *Emedicine* http://emedicine.medscape.com/article/213595-overview (Accessed 14 Apr 2009).

3- Stoodley, P., K. Sauer, D. G. Davies, and J. W. Costerton. 2002. Biofilms as complex differentiated communities. Annu. Rev. Microbiol. 56:187–209.

4- Edwards, R., and K. G. Harding. 2004. Bacteria and wound healing. Curr. Opin. Infect. Dis. 17:91–96.

5- Trafny, E. A. 1998. Susceptibility of adherent organisms from *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains isolated from burn wounds to antimicrobial agents. Int J. Antimicrob. Agents 10:223–228.

6- Harrison-Balestra, C., A. L. Cazzaniga, S. C. Davis, and P. M. Mertz. (2003). A wound-isolated *Pseudomonas aeruginosa* grows a biofilm in vitro within 10 hours and is visualized by light microscopy. Dermatol. Surg. 29:631–635.

7- Mack, D., P. Becker, I. Chatterjee, S. Dobinsky, J. K. Knobloch, G. Peters, H. Rohde, and M. Herrmann.(2004). Mechanisms of biofilm formation in *Staphylococcus epidermidis* and *Staphylococcus aureus*: functional molecules, regulatory circuits, and adaptive responses. Int. J. Med. Microbiol. 294:203–212.

8- McFadden, J. F. (2000). Biochemical tests for the identification of medical bacteria. 3rd Ed. The Williams and Wilkins- Baltimor, USA.

9- Forbes, B. A., Daniel, F. S. and Alice, S. W. (2007). Baily and Scott's Diagnostic microbiology. 12th ed. Mosby. Elsevier Company, USA.

10- Christensen GD, Simpson WA, Younger JA, Baddour LM, Barrett FF, Melton DM. (1985). Adherence of cogulase negative *Staphylococci* to plastic tissue cultures:a quantitative model for the adherence of *staphylococci* to medical devices. *J Clin Microbiol*22:996-1006.

11- Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A, (2006) [1] New Drug Discovery Research, Ranbaxy Research Laboratories, Gurgaon-122 001,Haryana,India; [2] Department of Biosciences, Jamia Millia Islamia University, Jamia Nagar, New Delhi-110 025, India. 24(1):25-29.

12- Bagdonas, R.; Tamelis, A.; Rimdeika, R. and Kiudelis, M.(2004). Analysis of burn patients and the isolated pathogens. Lithuanian Surgery. 2(3):190-3.

13- Al-Akayleh, A.T. (1999). Invasive burn wound infection. Annals of burns and fire disaster. 12(2).

14- Sanyal, S.C.; Mokaddas, E.M.; Gang, R.X. and Bang, R.L.(1998). Microbiology of septicemia in burn patients. Annals of burns and fire disaster. 11(1).

15- De Macedo, J.L.S.; Rosa, S.C. and Castro, C. (2003). Sepsis in burned patients. Revista da Sociedade Brasileria de Medicina Tropical. 36(6):647-52.

16- Kamel, A.H. and EL Megeed, E.A. (1997). The role of aztreanam in the control of Gram-negative burn wound infection. Annals of burns and fire disasters. 10(1).

17- Singh NP, Goyal R, Manchanda V, Das S, Kaur Z, Talwar V. (2003). Changing Trends in bacteriology of burns in the burns units, Delhi, India. Burns. 29:129-32.

18- Ozumba UC, Jiburum BC. (2000) Bacteriology of Burn Wounds in Enugu, Nigeria. Burns. 26:178-80.

19- Herek, O.; Ozturkk, H.; Ozyurt, M.; Albay, A. and Cetinkursun,S.(2000). Effects of treatment with immunoglobulin on bacterial translocation in burn wound infection. Annals of Burns and Fire Disasters.13 (1).

20- Bowler, P.G.; Duerden, B.I.; and Armstrong, D.G. (2001). Wound microbiology and associated approaches to wound management. Clin. Microbial. Rev. 14: 244-69.

21- Collier, M.(2003). Understanding wound inflammation. Nurs. Times. 99 (25).

22- Emmerson, M.(1994). Noocomial Staphylococcal outbreak. Scandinavian Journal of infectious diseases. Suppl.93: 47-54.

23- Gotz F. *Staphylococcus* and biofilms. (2002). Mol Microbiol. 43: 1367-1378.

24- Xu KD, McFeters GA, Stewart PS.(2000). Biofilm resistance to antimicrobial agents. Microbiology. 146: 547-549.

25- Toledo-Arana, A., Valle, J., Solano, C., Arrizubieta, M. J., Cucarella, C., Lamata, M., Amorena, B., Leiva, J., Penades, J. R. & Lasa, I. (2001). The enterococcal surface protein, Esp, is involved in *Enterococcus faecalis* biofilm formation. Appl Environ Microbiol 67, 4538–4545.

26- Mohamed, J. A., Huang, W., Nallapareddy, S. R., Teng, F. & Murray, B. E. (2004). Influence of origin of isolates, especially endocarditis isolates, and various genes on biofilm formation by *Enterococcus faecalis*. Infect Immun 72, 3658–3663.

27- Vishnu Prasad S , Mamatha Ballal, PG Shivananda (2009). Slime production a virulence marker in *Pseudomonas aeruginosa* strains isolated from clinical and environmental specimens: A

comparative study of two methods. Pathology and Microbiology.(52):191-193.

28- Jayachandran S. and Prashanth K.(2008). Correlation between biofilm production and multiple drug resistance in Imipenam resistant clinical isolates of *Acinetobacter baumannii*. Indian J Microbiol. 26, 333-337.

29- Madam.M, I. Petersenb, T. Cheastyc.(2008). Biofilm-forming *Klebsiella pneumoniae* strains have greater likelihood of producing extendedspectrum b-lactamases. Elsevier. 10, 369-371.

30- Watnick P. and Kolter R.(2000). Biofilm, city of microbes. J Bacteriol. 182:2675e2679.

31- Wizemann, T.M., Moskovitz, J., Pearce, B.J., Cundell, D., Arvidson, C.G., So, M.(1996) Peptide methionine sulfoxide reductase contributes to the maintenance of adhesins in three major pathogens. *Proc Natl Acad Sci USA* 93: 7985–7990.

32- Macleod SM. and Stickler DJ. (2007). Species interactions in mixed-community crystalline biofilms on urinary catheters. *J Med Microbiol* 56: 1549–1557.

33- Jones SM, Yerly J, Hu Y, Ceri H, Martinuzzi R.(2007). Structure of *Proteus mirabilis* biofilms grown in artificial urine and standard laboratory media. FEMS Microbiol. Lett. 268:16-21.