Profile of microorganisms isolated from blood and wound in the first week of post burn infections and antibacterial resistance pattern

Khalida Jhalil Ibraheem
Dept. of Biology, College of Science, Al-Mustansiriyah University

Abstract:

Infection of burn wounds is a serious problem leading to death and need critical care.

The purpose of this study is to identify common microorganisms isolated from blood and wound in the first week of post burn infections and assess their susceptibility to the commonly used antibacterial agents.

Blood and wound swabs from 62 patients with burn wound infections were collected on Third and Sixth day of post burn infections.

The microorganisms were isolated and identified by standard microbiological methods. Susceptibility test were performed by Kirby–Bauer disk diffusion method. Out of 62 patients, female (62.9%) were found to be affected more than males (37.1%) while (53.2%) of the patients were paediatric and the remaining (46.8%)were adults. The flame burn was the predominant cause of burn among patients; (51.6%) had flame burns, (33.9%) a scald, and (14.5%) had an electrical burn.

It was found that predominant aerobic and anaerobic bacteria were *Pseudomonas aeruginosa* (15.3%)and *Bacteroides spp.*(4.8%), respectively. While *Candida tropicalis* (0.8%) was the most common fungal organi followed by *Candida albicans* (0.4%). In most of the cases, the same microorganisms were found in blood and pus samples. All the bacterial isolates showed high resistance to used antibiotics.

Imipenem was the most effective antibiotic followed by Meropenem and Ceftriaxone.

الخلاصة:

تعد اصابات الحروق من المشاكل الخطيرة التي تؤدي في بعض الاحيان الى الموت والتي تحتاج الى رعاية خاصة .تهدف هذه الدراسة لتحديد الكائنات المجهرية الشائعة المعزولة من الدم والجروح خلال الاسبوع الاول من الاصابة بالحروق وتقييم حساسيتها للعوامل المضادة للميكروبات ذات الاستعمال الشائع .

تم جمع عينات دم ومسحات جروح من ٦٢ مريض مصاب بالحروق.شخصت الكائنات المجهرية المعزولة باستخدام الطرق الميكروبيولوجية القياسية.استخدمت طريقة الانتشاربالأقراص لـKirby – Bauer لإجراء فحوصات الحساسية.من بين ٦٢ مريض كانت نسبة الاصابة في الاناث (٩.٦٢ %) اكثرمن الذكور (١.٣٧%) بينما شكلت نسبة الاصابة في الاطفال (٢.٥٣%) اما النسبة المتبقية (٨.٤٦%) فكانت للبالغين.كان الحرق بواسطة اللهب هوالمسبب الرئيسي للحرق بين المرضى، حيث شكلت نسبته (٥١,٦%) فيما شكلت نسبة الحرق بواسطة السوائل الحارة او البخار (٣٣,٩%) اما الحرق بالكهرباء فكانت نسبته (١٤,٥%).

وجد ان البكتريا الهوائية واللاهوائية السائدة هي Pseudomonas aeruginosa (15.3%) و Bacteroides spp. (4.8%) على التوالي.بينما كانت اكثر الكائنات الفطرية الشائعة هي Candida tropicaliscccc (0.8%) يتبعها Candida albicans (0.4%).الكائنات المجهرية المعزولة من نماذج الدم والقيح كانت في اغلب الحالات متشابه .جميع العزلات البكتيرية اظهرت مقاومة عالية للمضادات الحيوية المستخدمة.كان المضاد Imipenem الاكثر تأثيرا يليهMeropenemوCeftriaxone .

Introduction:

Burn injury is a major problem in many areas of the world.

Infections are the major cause of morbidity and mortality in burn patients [1].

Sever dysfunction of the immune system, a large cutaneous colonization, the possibility of gastrointestinal translocation, a prolonged hospitalization and invasive diagnostic and therapeutic procedure; all contribute to infections [2].Burn wound are

highly susceptible to colonization and infection and this remains a major problem in the management of burn victims today[3].

The pathogenesis of colonization, infection and invasion of microorganisms is related to the fact that there is a disruption of the normal skin barrier at the site, as well as a large amount of necrotic tissue and protein–rich wound exudate at the burn surface, providing a rich growth medium for colonization and growth of the microorganisms, which is poorly controlled due to depressed immune responses [4] .

A variety of organisms have been isolated from burn wound infections The surface of every burn wound is contaminated to some degree by bacteria[5].

Because of this surface bacterial growth is routinely monitored in most centers to facilitate management and treatment. It has been found by many investigators that the distribution of various species of bacteria from burn wound surfaces is similar to that from blood specimens [6].

The infection of burn wound with multiple organisms the need for a drug policy by the hospitals for burn patients.

The isolated bacteria exhibited multiple resistance to antibiotics should be administered rationally in burn wards guided by the bacterial resistance pattern[8].

The purpose of the study is to identify common microorganisms isolated from blood and wound in the first week of post burn infections and assess their susceptibility to the commonly used antibacterial agents, which would enable the determination of empirical antibiotic strategies for the early treatment of imminent septic events.

Material and Method:

A total of 62 patients with burn wound infections from AL-Kindi Teaching Hospital and Baghdad Teaching Hospital were screened between September 2008 and December 2009.

Age, sex and etiology of burn wounds were recorded swabs taken from clinically deep area of burn wounds and blood were collected on 3rd and 6th day of post burn infections.

Blood was collected aseptically in brain heart infusion broth and was incubated at 37^0c for 48 hours.

It was then sub cultured on to dipped in transport medium then inoculated onto culture media.

Direct smear were prepared stained by Gram's staining method. Various culture media were used for inoculated specimens like MacConkey agar, blood agar, chocolate agar, and bile–esculin agar for aerobic and anaerobic culture, sabouraud's dextrose agar for fungal culture.

The bacterial isolates grown on media were identified by using different microscopical examination, cultural characteristics and biochemical tests [9,10].

The characterization of fungi was done by the germ tube test, morphological examination and other identification tests [11].Antimicrobial susceptibility testing was performed by Kirby – Bauar disk diffusion method according to National Committee for Clinical Laboratory Standard (NCCLS) guide lines [12].

Antibiotic discs whichused manufactured by Oxoid(England).

Types and concen trations of these antibiotic discs were as following :Imipenem (10 µg/disk), Ceftriaxone (30µg/disk), Ciprofloxacin (5µg/disk), Amikacin (30 µg/disk), Cefotaxime (30µg/disk), Augmentin (30µg/disk), eropenem (10µg/disk), Cotrimixazol (25µg/disk), hloramphenicol (30 µg/disk), Gentamycin (10µg/disk), Cloxacillin (5µg/disk), Clindamycin (2 µg/disk).

Results and Discussion:

The burn wound is considered one of the major health problem in the world, and infection is one of the frequent and sever complications in patients who sustained burns [13].

The burn wound represents a susceptible site of opportunistic colonization by organisms of endogenous and exogenous origin[15].

The risk of burn wound infection is directly correlated to the extent of the burn and is related to impaired resistance resulting from disruption of the skin's mechanical integrity and generalized immune suppression [15].

Among 62 patients with blood and burn wound infections, females 39 (62.9%)were affected more than males 23(37.1%)(Table–1).

This may be because of the reason that accidental burns are more common in females as they tend to spend more time near fire. 33(53.2%)of the patientswerepaediatric (upto18years) and the remaining 29(46.8%)were adults(Table–1).Burn wounds are more likely to occur in elderly and very young children(<5 years) with higher risk of death from burn injury [16]. In our study, the age group0 -9 years showed the highest number of cases29%compared of other age groups, whereas 6.4% of the patients were from the >46 year's age group.

Table-1: Age and sex distribution of the patients (n=62)

Age (years)	Total number (%)	Male no. (%)	Female no. (%)
0 – 9	18 (29)	7 (38.9)	11 (61.1)
10 – 18	15 (24.2)	6 (40)	9 (60)
19 – 27	11 (17.7)	4 (36.4)	7 (63.6)
28 – 36	8 (12.9)	3 (37.5)	5 (62.5)
37 – 45	6 (9.7)	2 (33.3)	4 (66.7)
46 – 54	3 (4.8)	1 (33.3)	2 (66.7)
55 – 63	1 (1.6)	0 (0)	1 (100)
Total	62	23 (37.1)	39 (62.9)

The flame burn was the predominant cause of burn among patients; 32(51.6%) had flame burns, 21(33.9%) a scald, and 9(14.5%) had an electrical burn.In our study culture positivity of pus was 96% while in 4% of cases, pus samples were sterile.

In case of blood the culture positivity was51.6% (Table–2). Santucci et al.,(2003)[17] in their study on burn wound infections found the culture positivity of blood to be 49%while the culture positivity of pus in their study was 21% .

Table-2: Percentage of culture method.

Specimen	Culture positive n (%)	Culture negative n (%)	Total
Blood	64(51.6)	60 (48.4)	124
pus	119 (96)	5 (4)	124

In the present study we did not find much difference in the pattern of organisms isolated on 3rd and 6th day of admission (Table– 3).

It was found that predominant aerobic and anaerobic bacteria were *Pseudomonas aeruginosa* (15.3%) and *Bacteroides spp.* (4.8%), respectively. The majority of studies also noted a high frequency of these microorganisms in burn wound infections [18, 19].

Candidia tropicalis (0.8%) was the most common fungal organisms isolated in burn wounds followed by *Candida albicans* (0.4%), this goes with the most research Gupta et al., 2004 [20], and Bruck et al., 1972 [21].

In most of the cases the organisms isolated from blood were the same as isolated from pus (Table–3).

This indicates the organism has entered the blood stream through the wound and is a potential threat for disseminated infection which can be life threatening.

Table–3: Profile of microorganisms isolated on 3rd and 6th day of post burn infections (n=248)

Microorganisms	Blood		Pus		Total	%
	3rd day	6th day	3rd Day	6th day		
Aerobic isolates						
Pseudomonas aeruginosa	6	9	11	12	38	15.3
Acinetobacter baumanii	1	2	2	3	8	3.2
Escherichia coli	3	3	4	6	16	6.5
Klebsiella spp.	2	2	3	4	11	4.4
Enterobacter spp.	1	2	3	3	9	3.6
Proteus mirabilis	1	1	2	3	7	2.8
Serratia spp.	1	1	1	2	5	2
Staphylococcus aureus	6	7	8	10	31	12.5
Non – coagulase *Staphylococci*	1	2	3	3	9	3.6
Group A –*Streptococci*	1	1	2	2	6	2.4
Group D –*Enterococci*	1	3	4	5	13	5.2
Anaerobic isolates						
Bacteriodes spp.	1	2	3	6	12	4.8
Peptostreptococcus spp.	1	1	2	4	8	3.2
Propionebacterium acnes	-	1	2	4	7	2.8
Fungi						
Candida albicans	-	-	-	1	1	0.4
Candida tropicalis	-	-	-	2	2	0.8
No growth	41	19	5	-	65	26.2

Increasing antimicrobial resistance among burn wound isolates is a matter of concern, with limited treatment options available for multidrug resistant strains [22].

In our study most of organisms showed multidrug resistance. Antibiogram test was done on aerobic and anaerobic bacteria (Fig–1& Fig–2).

Imipenem was the most effective antibiotic followed by Meropenem and Ceftriaxone.

The high rates of antibiotic resistance observed in the present study may be due to the wide spread usage of broad spectrum antibiotics leading to selective survival advantage of pathogens[23].

Conclusions:

Burns provide a suitable site for bacterial multiplication and infection, mainly because of the larger area involved and longer duration of patient stay in the hospital.

To ensure early and appropriate therapy in burn patients, a frequent evaluation of the wound is necessary.

Therefore, a continuous surveillance of microorganisms and a regular update of their antibiotic resistance pattern are essential to maintain good infection control programs in the burn unit, thus improving the overall infection related morbidity and mortality.

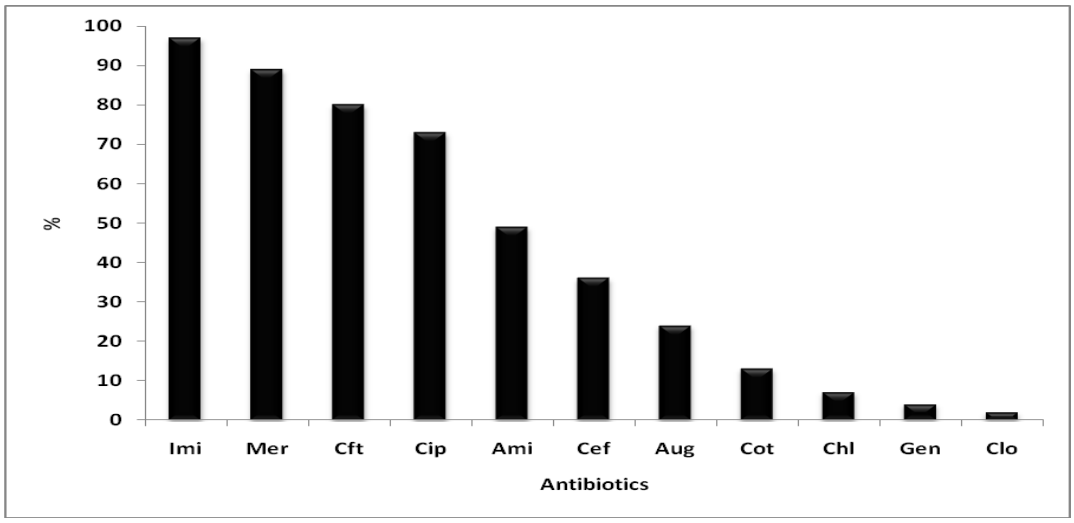

Figure-1: Percentage of antibiotic susceptibility patterns of aero-bicbacterial isolates from blood and burn wound infections

Imipenem: Imi (10 µg/disc), Meropenem: Mer (10 µg/disc), Ceftriaxone: Cft (30 µg/disc), Ciprofloxacin: Cip (5 µg/disc), Amikacin: Ami (30 µg/disc), Cefotaxime: Cef (30 µg/disc), Augmentin: Aug (30 µg/disc), Cotrimixazole: Cot (25 µg/disc), Chloramphenicol: Chl (30 µg/disc), Gentamycin: Gen (10 µg/disc), Cloxacillin: Clo (2 µg/disc),

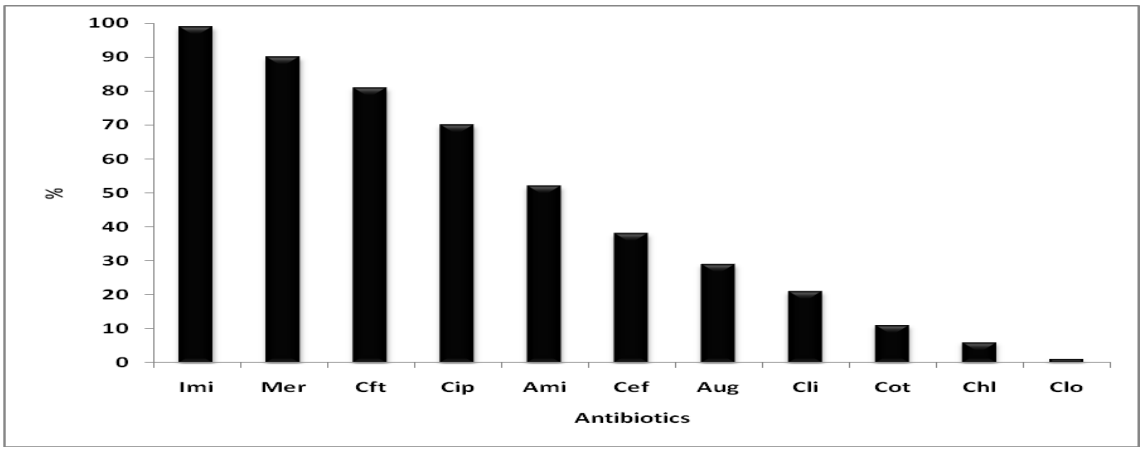

Figure-2: Percentage of antibiotic susceptibility patterns of anaerobic bacterial isolates from blood and burn wound infections

Imipenem: Imi (10 µg/disc), Meropenem: Mer (10 µg/disc), Ceftriaxone: Cft (30 µg/disc), Ciprofloxacin: Cip (5 µg/disc), Amikacin: Ami (30 µg/disc), Cefotaxime: Cef (30 µg/disc), Augmentin: Aug (30 µg/disc), Cotrimixazole: Cot (25 µg/disc), Chloramphenicol: Chl (30 µg/disc), Clindamycin: Cli (2 µg/disc), Cloxacillin: Clo (2 µg/disc).

References:

1- Reig, A.; Tejerina, C. and Cadina, J. Infection in burn patients.J. Arm. Medit. BurnsClub.1992.Vol.5. Pp: 91-95.

2- De Macedo, J. L.and Santos, J. B. Bacterial and fungal colonization of burn wound. Mem. Inst. Oswaldo. Cruz.2005.Vol.100 Pp: 535-539.

3- Pruitt, J. R.; Mcmanus, A. T.; Kim, S. H. and Goodwin, C. W.. Burn wound infection: Current status world. J. Surg. 1998.Vol.22. Pp:135-145.

4- Erol, S. Altopariak.; U.; Akcay, M. N.;Celebi, F. and Pariak, M. Changes of microbial flora and wound colonization in burned patient. J. Burns.2004.Vol.30 Pp: 357-361.

5- Lawrence, J. C. and Lilly, H. A.A quantitative method for investigating the bacteriology skin: Its application to

burn. J. Exp. Pathol. 1972. Vol. 50 Pp: 550-559.

6- Li, G.H.; Analysis of micro biological flora in the blood and wound of burn patients. Burns. 1989. Vol. 5. Pp: 199-200.

7- Roberts, J. A.; Kruger, P.; Paterson, D. L. and Lipman, J. Antibiotic resistance. J. Crit. Care Med. 2008. Vol. 36. Pp: 2433-2440.

8- Sjoberg, T.; Mzezewa, S.; Jonsson, K.; Robertson, V. and Salemark, L. Comparison of surface swab cultures and quantitative tissue biopsy cultures to predict sepsis in burn patients: Prospective study. J. Burn Care Rehabil. 2003. Vol. 24 Pp: 365-370.

9- Jousimies-somer, H. R.; Summanen, P.; Citron, D. M.; Baron, E. J.; Wexler, H. M. and Finegold, S. M. Wadsworth. KTL anaerobic bacteriology manual. Staw. Publishing, Belmont, C A. 2002.

10- Murray, P. R.; Baron, E. J.; Jorgensen, J. H.; Pfaller, M. A. and Yolken, R.H.Manual of clinical microbiology,8thed .ASM Press, Washonton, DC. 2003.

11- Freydiere, A. M.; Guinet, R. and Boiron, P. Yeast identification in the clinical microbiology laboratory: Phenotypical method. J.Med. Mycol.2001.Vol.39. Pp:9-33.

12- Clinical and Laboratory Standard Institute. Performance Standard for Antimicrobial Susceptibility Testing: Nineteenth Informational Supplement. 2009. Pp: 100-119, Waune, PA.

13- Zorgani, A.; Zaidi, M.; Ranka, R. and Shaher, A.The pattern and outcome of septicemia in burn infective care unit. J. Ann. Burn disasters. 2002. Vol. 15. Pp: 179-181.

14- Steinstrasser, L.; Thies, A. H.; Rabstein, S. and Steinau, H. U. Typical bacteria in an intensive care burn unit in severely burned patients and their importance with regard to mortality: Retrospective study 1995-2004, Handchir Mikrochir Plast. Chir. 2007. Vol.39. Pp: 338-344.

15- Applegren, P.; Bjornhagen, V.; Bragderyd, K.; Jonsson, C. E. and Ransjo, U.A prospective study of infection in burn patients. Burns. 2002. Vol. 28. Pp: 38-46.

16- Hunt, J. L.; and Purdue, G. F. The eldery burn patient. Am. J. Surg.1992. Vol. 164. Pp: 472-478.

17- Santuccci, S. G.; Gobara, S.; Santos, C. R.; Fontana, C. and Levin, A. S.Infections in a burn intensive care unit: Experience of seven years. J. Hospt. Infec. 2003. Vol.53 Pp: 6-13.

18- Kaushlk, R. Kumar, S. Sharma, R. andLal,P.Bacteriology of burn wounds-the first three years in a new burn unit at the Medical College, Chandigarh. J.Burns. 2003.Vol. 27 Pp: 595-597.

19- Revathi, G.; Puri, J. and Jain, B. K. Bacteriology of burns. Burns. 1998.Vol. 24 Pp: 347-349.

20- Gupta, N.; Haque, A.; Lattif, A. A.; Narayan, R.P. ; Mukhopadhya, G. and Prasad, R. Epidemiology and molecular typing of *Candida* isolates from burn patients. J. Mycopathologia, 2004. Vol. 158. Pp: 397-405.

21- Bruck, H. M.; Nash, G. Stein, J. M. and Lindberg, R. B.; Studies on the occurrence and significance of yeast and fungi in the burn wound. J. Ann. Surg. 1972. Vol. 176. Pp: 108-110.

22- Agnihotri, N. Gupta, V. and Joshi, R. M.; Aerobic bacterial isolate from burn wound infections and their antibiograms - a five-year study. J. Burns, 2004. Vol. 30. Pp: 241-243.

23- Gould, I. M. A review of the role of antibiotic policies in the control of antibiotic resistance. J. Antimicrob. Chemother. 1999. Vol. 43. Pp: 459-465.