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Study the Antimicrobial Drug Resistance to some Microbes Isolates from Skin and Blood in Burn Patients

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Abstract

A total of (170) Skin swabs were taken after 72 and 144 hours of burn injury and (45) blood specimens for culture from (85) patients with burn size (10-90%). This study showed that cases of infection are increased with the increasing of stay period in Hospital and burn size. It has been found that Gram negative bacteria are more frequent than Gram positive type and yeast. *Pseudomonas aeruginosa* is the most frequent species among microbial isolates. Besides, it has been shown through this study that the antibiotics: Ciprofloxacin, Amikacin, Norfloxacin, and Imipenem are the most influential on Gram negative bacterial isolates and Chloramphenicol, Vangomycin and Nitrofurantion on Gram positive bacterial isolates. Furthermore, Amphotericin B and Clotrimazole on yeast.

KEYWORDS: Burn injury, Microbial, Antibiotic sensitivity.

Introduction

A burn is an injury to an organic tissue caused by direct or indirect action of heat by means of exposure to flames, heated liquids, contact with hot objects and exposure to corrosive chemicals, radiation and contact with an electric current. Burn injury destroys the physical skin barrier that is considered as most important tool against invasion by microorganisms and consequently, this injury provides novel sites for bacterial colonization, infection and clinical sepsis [1-2-3].

Bacteria are the most common pathogens of burn patients. These microorganism form multi-species biofilms on burn wounds through (48-72) hours of injury [4]. These microorganisms are originated from the patient's own skin (hair follicles and sweat glands), gut and respiratory flora (endogenous), as well as from contact with health care personnel and environment (exogenous). A burn patient is extremely susceptible to hospital infections that contribute to excess morbidity and mortality [5].

Invasion of microorganisms into the tissue layers below the dermis lead to bacteremia, sepsis and multiple organ dysfunctions [6]. Bacteremia is presence of bacteria in blood stream. Septicemia is bacteremia with replicating bacteria to cause an infection. Extensive bacteremia and infection can release toxins into the blood that leading to sepsis and causing systemic inflammatory response syndrome, bacteremia to progress to septicemia [7].

Bloodstream infection and the subsequent development of sepsis are among the most common infection complications occurring in burn patients in the intensive care unit [8].

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Methodes

1. Patients

A total of (85) burned patients, whose ages range between (1-75) years and with burn size (10-90%) were included in this study which lasted from November (2012) to May (2013). Those patients were admitted to the burn unit at Al-Sadder Teaching Hospital in Najaf.

2. Specimens Collection

Skin swabs were taken after 72 and 144 hours from the pus of the burned area in the morning before the bathing of the affected area (before hydrotherapy). Each swab was placed in a sterile tube with media till reaching the laboratory to be inoculated on culture media (blood agar, MacConkey agar and Mannitol salt agar) and incubated aerobically for 24- 48 hours at 37C˚, in addition to (sabouraud dextrose agar) for 24- 48 hours at 32C˚ [9].

Blood were taken from (45) patients, (1-4) ml from infants and children and (8-10) ml from adult after (5-10) days of burn injury [10]. The blood put in blood culture bottles that were incubated aerobically at 37C˚ in BacT/ALERT 3D for 2-7 days and then to be cultured on different types of culture media as above. Blood samples were collected carefully by sterilizing hands with alcohol, wear palm of the hand and sterilization site pull blood for reduce the probability of contamination with commensal types of skin flora [9].

3. Bacterial Diagnosis

Isolation and identification of bacteria were carried out by morphological and cultural characteristics, biochemical tests and identification by Vitec 2 Compact [9-11-12-13].

4. Antibiotic Sensitivity Test

The antimicrobial susceptibility test carry out according to Kirby-Bauer method. Zone size was compared to standard zones depending on clinical and laboratory standard institute (CLSI).

Results

Burn Percentage and Results of Cultures

The results in Table (1) show a number of microbes that colonized to the burn wound and bloodstream reaches to high rate when percentage of burn becomes more than 40% of the total body surface area (TBSA) and reach to maximum rate in patients with burn more than 60% of the (TBSA).

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Table (1): The Relation between Burn Percentage and Results of Cultures.

Burn Percentage %	No. of Positive Culture		No. of Single Growth		No. of Mixed Growth	
	Skin	Blood	Skin	Blood	Skin	Blood
1 – 10	7	0	5	0	2	0
11 –20	18	1	11	1	7	0
21 –30		2				
31 – 40	14		7	1	7	1
41 – 50		2				
51 – 60	14		8	1	6	1
61 –70		2				
71 –80	13		1	1	12	1
81 – 90		2				
	2		1	0	1	2
		1				
	3		0	0	3	1
		0				
	1		0	0	1	0
		5				
	5		0	0	5	5
Total	**77/85**	**15/45**	**33**	**4**	**44**	**11**

*only eight patients give negative skin swab culture.

Types of Bacterial Isolates

The results shown in Table (2) revealed that a total of (170) skin swabs were (69.4%) of which positive culture after three days of admission while (90.6%) of which showed positive culture after six days of admission. Besides, the presence that (33.33%) of blood samples showed positive culture.

Table (2): Number and Percentage of Negative and Positive Culture after Three and Six Days.

Result of Culture	Time of Culture		Total No. (%)
	Three Days No. (%)	Six Days No. (%)	
Negative	26 (30.6%)	8 (9.4%)	34 (20%)
Positive	59 (69.4%)	77 (90.6%)	136 (80%)
Total	**85 (100%)**	**85 (100%)**	**170 (100%)**

Further, as seen in Fig (1) which showed that the Gram negative bacteria were more frequent (76.19%) in skin specimens and (68.96%) in blood specimens than Gram positive bacteria (18.36%) and (31.04%) in both specimens. As for *Candida albicans*, it was occupied a small part; where it found in eight patients (5.44%) of skin swabs only.

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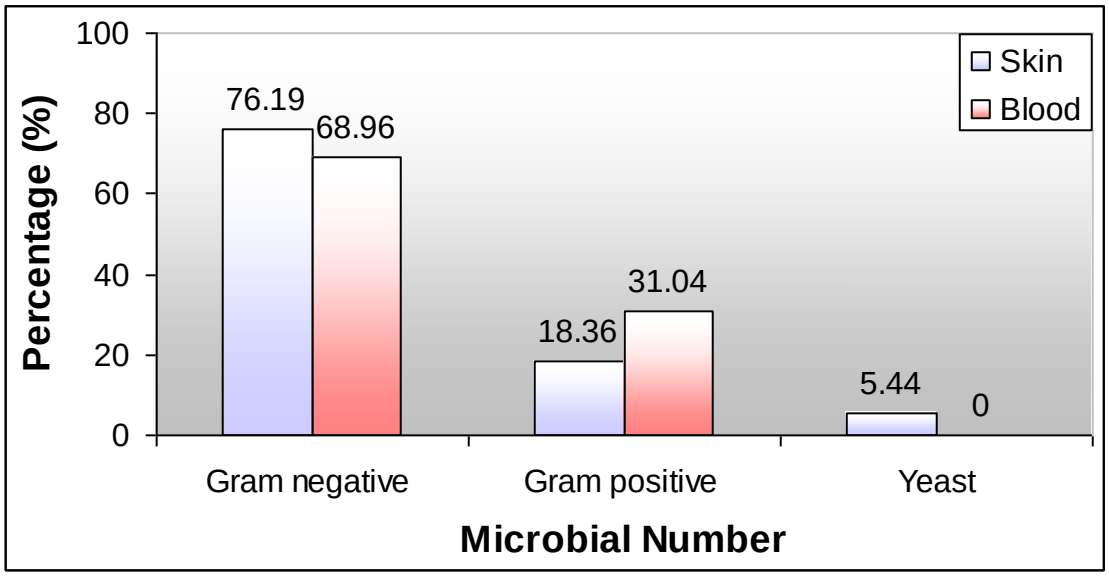

Figure (1): Bacteria and Yeast Percentage in Skin and Blood Samples.

Table (3): Types and Numbers of Bacteria and Yeast were Isolated from Skin and Blood Samples.

Bacterial and Yeast Isolates	No. (%)	
	Skin	Blood
Pseudomonas aeruginosa	53 (36.05 %)	9 (31.03 %)
Klebsiella pneumonia	31 (21.08 %)	7 (24.13 %)
Staph. Aureus	25 (17 %)	5 (17.24 %)
Enterobacter cloacae	12 (8.16 %)	3 (10.34 %)
Acinetobacter baumannii	10 (6.8 %)	1 (3.44%)
Candida albicans	8 (5.44%)	-
Pantoea spp.	4 (2.72 %)	-
Staph. Epidermidis	2 (1.36%)	4 (13.79 %)
Serratia marcescens	2 (1.36 %)	-
Total	**147 (100%)**	**29 (100%)**

In addition, as seen above in Table (3) it was found that *P. aeruginosa* occupied large part of microbial isolated from the wound swabs (36.05%), followed by *K. pneumoniae* (21.08%), and *S. aureus* (17%). Moreover, in blood samples *P. aeruginosa* was found in percentage (31.03%), followed by *K. pneumoniae* (24.13%), and *S. aureus* (17.24%).

Antibiotics Sensitivity Test

As shown in Table (4) and Table (5) the results of antibiotic sensitivity test for types of Gram negative and Gram positive bacteria. Most bacterial isolates showed high resistance towards Ceftazidin, Cefotaxim, Ampicillin, Trimethoprim-Sulfamethoxazole, Nitrofurantion, , Tobramycin, and Gentamicin; Gram negative bacteria was resistant with percentage (99.2%, 99.2%, 98.4%, 97.7%, 96.2%, 90.1%, 85.6%) respectively, while Gram positive bacteria resist with percentage (100%, 100%, 97.2%, 88.8%, 13.8%, 80.5%, 80.5%); moreover, Gram positive bacteria was very sensitive to Nitrofurantion (86.2%). The bacterial isolates showed a varying resistance to each of Norfloxacin (53.7%), Amikacin (54.5%), Ciprofloxacin (46.2%) for Gram negative bacteria and Amikacin (77.7%), Norfloxacin (69.4%), Ciprofloxacin (69.4%) for Gram positive bacteria. Besides, Gram negative bacteria was very

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resistant to Cefepime (96.2%) and Piperacillin (95.4%), in other word was less resist to Meropenem (69.6%) and Imipenem (59%). Regarding Gram positive bacteria, it was very resistant to Cefoxitin (100%) and Oxacillin (86.1%); in other word was less resist to Vangomycin (5.5%) and Chloramphenicol (2.7%). *Candida albicans* was (100%) sensitive to Amphotericin B and Clotrimazole and (20%) to Fluconazole Fig (2).

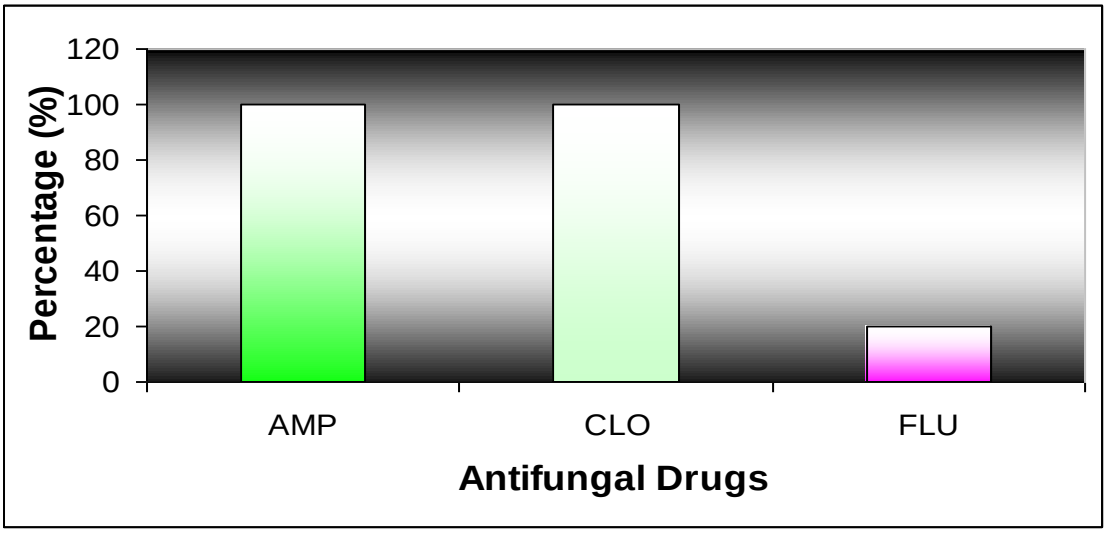

Figure (2): The Percentage of Susceptibility of *Candida albicans* to Antibiotics.

Amphotericin B (AMP) , Clotrimazole (CLO) , Fluconazole (FLU).

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Table (4): Antibiotic Resistance of Gram Negative Bacteria (Number (%)).

Type of Bacteria	NO.	Types of Antibiotics													
		AK	AMP	CAZ	CN	CIP	CTX	F	FEP	IPM	MEM	NOR	PRL	SXT	TOB
Pseudomonas aeruginosa	62	37 (59.6)	62 (100)	62 (100)	62 (100)	32 (51.6)	62 (100)	62 (100)	62 (100)	41 (66.1)	51 (82.2)	37 (59.6)	62 (100)	62 (100)	58 (93.5)
Klebsiella pneumoniae	38	11 (28.9)	38 (100)	38 (100)	25 (65.7)	18 (47.3)	38 (100)	33 (86.8)	36 (94.7)	12 (31.5)	16 (42.1)	20 (52.6)	38 (100)	36 (94.7)	34 (89.4)
Enterobacter cloacae.	15	13 (86.6)	15 (100)	15 (100)	15 (100)	0 (0)	15 (100)	15 (100)	15 (100)	14 (93.3)	14 (93.3)	3 (20)	15 (100)	15 (100)	15 (100)
Acinetobacter baumannii	11	11 (100)	11 (100)	11 (100)	11 (100)	11 (100)	11 (100)	11 (100)	11 (100)	11 (100)	11 (100)	11 (100)	11 (100)	11 (100)	11 (100)
Pantoea spp.	4	0 (0)	2 (50)	3 (75)	0 (0)	0 (0)	3 (75)	4 (100)	3 (75)	0 (0)	0 (0)	0 (0)	0 (0)	3 (75)	0 (0)
Serratia marcescens	2	0 (0)	2 (100)	2 (100)	0 (0)	0 (0)	2 (100)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)	1 (50)
Total	132	72 (54.5)	130 (98.4)	131 (99.2)	113 (85.6)	61 (46.2)	131 (99.2)	127 (96.2)	127 (96.2)	78 (59)	92 (69.6)	71 (53.7)	126 (95.4)	129 (97.7)	119 (90.1)

(AK) Amikacin, (AMP) Ampicillin, (CAZ) Ceftazidime, (CN) Gentamicin, (CIP) Ciprofloxacin, (CTX) Cefotaxime, (F) Nitrofurantion, (FEP) Cefepime, (IPM) Imipenem, (MEM) Meropenem, (NOR) Norfloxacin, (PRL) Piperacillin, (SXT) Trimethoprim-Sulphamethoxazole, (TOB) Tobramycin.

Table (5): Antibiotic Resistance of Gram Positive Bacteria (Number (%)).

Type of Bacteria	NO.	Types of Antibiotics												
		AK	AMP	CAZ	C	CN	CIP	CTX	CX	F	FOX	NOR	SXT	TOB	VA
Staph. aureus	30	28 (93.3)	30 (100)	30 (100)	1 (3.3)	29 (96.6)	25 (83.3)	30 (100)	30 (100)	5 (16.6)	30 (100)	25 (83.3)	29 (96.6)	29 (96.6)	2 (6.6)
Staph. epidermidis	6	0 (0)	5 (83.3)	6 (100)	0 (0)	0 (0)	0 (0)	6 (100)	6 (100)	0 (0)	1 (16.6)	0 (0)	3 (50)	0 (0)	0 (0)
Total	36	28 (77.7)	35 (97.2)	36 (100)	1 (2.7)	29 (80.5)	25 (69.4)	36 (100)	36 (100)	5 (13.8)	31 (86.1)	25 (69.4)	32 (88.8)	29 (80.5)	2 (5.5)

(AK) Amikacin, (AMP) Ampicillin, (CAZ) Ceftazidime, (C) Chloramphenicol, (CN) Gentamicin, (CIP) Ciprofloxacin, (CTX) Cefotaxime, (F) Nitrofurantion, (CX) Cefoxitin, Cefotaxime, (FOX) Oxacillin, (NOR) Norfloxacin, (SXT) Trimethoprim-Sulphamethoxazole, (TOB) Tobramycin, (VA) Vangomycin.

Discussion

The percentage of the burn is considered as important risk factors for infection This agrees with (**Bang** *et al*., 2002) in **Spain** who revealed that invasive cultures increased as burn size increased > 60% (TBSA). Burn patients are at a high risk of infection as a result of the protective skin barrier disruption, the immunocompromizing effects of burns and prolonged hospital stays. The risk of infection with microbes becomes at a high rate with the

increase of skin damage because the skin forms a protective barrier against invasions by microbes that otherwise an easy access for microbial invasion [14].

The presence study show that burn wound infection much effected with stay period in Hospital. This agrees with result [14]; who found that (66%) of skin swabs gave positive culture after three days of admission while the result became (88%) after seven days of admission and closer to those of [15]; who found that the positive culture which was on 3 and 5 days after admission was (75% and 90%) respectively. Furthermore, it has found (33.33%) of blood samples give positive culture; this result is closer to the study that obtianed by [15]; who found (24%) of patients developed severe sepsis. The burn wounds initially sterile; however, Gram-positive bacteria from hair follicles and sweat glands, which may survive thermal injury, colonize the wound within 48 hours of injury, or after stay in hospital, the patients comes into contact with new infective agents, becomes contaminated, and subsequently develops an infection [16].

In this study, Gram negative bacteria were more frequent than Gram positive bacteria. This result was closer to that obtained by [17-18], in India and Iran who found; the incident of Gram negative bacteria in (80%) of skin specimens. Besides, this result is similar to result that obtained by [19]; who found Gram-negative bacilli accounted for (67%) of blood samples. The reasons for this high prevalence of Gram negative bacteria may be due to resistant of these bacteria to many types of antibiotics or due to the virulence factors both increase its ability to cause infection [20]. Further, in present study, the most commonly microorganisms isolated from burn patients were *P. aeruginosa*. These results compititive with those found in a study in Iraq [21-22]. The reasons for high prevalence of *P. aeruginosa* may be due to factors such as the acquisition of nosocomial pathogens in patients with recurrent long term hospitalization complicating illnesses, in addition to multidrug resist of *P. aeruginosa* [23]. The minimal nutritional requirements of *P. aeruginosa*, as evidenced by its ability to grow in distilled water and its tolerance to a wide variety of physical conditions [24]. As for the *P.aeruginosa, S. aureus* found in the most patients and prevailing on others types of Gram positive bacteria, these fact due to production of coagulase it protect the bacteria from phagocytosis by coating the cell with fibrin and resistance of *Staphylococcus aureus* to β-lactam antibiotics [25].

In present study, Gram negative bacteria showed intermediate resistant toward Imipenem, Norfloxacin, Amikacin, and Ciprofloxacin, and resist to another antibiotics. This agreement with result [21-27-28]. Besides, Gram positive bacteria were very sensitive to chloramphenicol, Nitrofurantion, and Vangomycin, and was resistant to other antibiotics. This agreement with [26-30]. *C. albicans* was higher sensitive to Amphotericin B and Clotrimazole and lower to Fluconazole. This results were near to those of [31-32]. In this study, some bacterial isolates was resistant to antibiotics, this may be due to development new resistant genes or by mutation through previously exposure to these antibiotics. There are many mechanisms of resistance such as reduced uptake or decreased cell permeability, alterations at the ribosomal binding sites, production of hydrolyzing enzymes and alterations in the target enzymes[33]

Conclusions

This study revealed that there is competition between burn size and number of microbes that cause wound and blood infection. Burn wound infection much effected with a stay period in Hospital. G- bacteria are more predominant as causative agent than the G+ bacteria. In vitro, Ciprofloxacin, Amikacin, Norfloxacin, and Imipenem are the most influential on Gram negative bacterial isolates and Chloramphenicol, Vangomycin and Nitrofurantion on Gram positive bacterial isolates. Furthermore, Amphotericin B and Clotrimazole on yeast.

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