Detection of the Some Dominant Aerobic Microorganisms in Burn Injury and Testing their Susceptibility for Different Antibiotics in Najaf

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Abstract

Burn injury and wound is a pig problem that assists the pathogens to grow and cause infection. 149 burn injury swabs were taken from different site of the patients' body ages (11-53 years old). All samples were tested to isolate the sigificant microorganisms that found in burn injury. Antibiotics test was performed by using disc diffusion way that stated by Kirby-Baucer. Results appeared that the most frequent pathogen in burn injury was *Pseudomonas aeruginosa* 33(22.14%) and followed by *E.coli* 31(20.8%), *Staphylococcus aureus* 28(18.79%), *Proteus vulgaris* 26(17.48%), *Coagulase-negative Staphylococci* 12(8.05%), *Proteus mirabilis* 10(6.71%), *Klebsiella pneumonia* 5(3.35%) and *Candida spp.* 4(2.68%) respectively. Antibiotic test showed that *Staphylococcus aureus* was a high resistance against Gentamycin and ciprofloxacin, but it was sensitive to Vancomycin and Imipenem. Coagulase negative *Staphylococci* showed a resistance to Gentamicin, Ciprofloxacin and Ceftazidime, but it was sensitive to Amikacin, Oxacillin, Cefotaxim, Vancomycin, Imipenem and Cefepime. *P. aeruginosa* showed resistance against Cefotaxime, Aztreonam, Gentamicin and Ceftazidime. Whlist it showed a high sensitivity against Imipenem, Cefepime and Tobramycin. *E.coli* showed a high resistance against Ceftriaxone, Ciprofloxacin and Cephalothin. whilst it showed a high sensitivity against Ticarcillin/clavulanic acid, Imipenem, Cefepime and Tobramycin. *Proteus vulgaris* showed a high resistance against Ceftriaxone, Ceftazidime, Amikacin, Cephalothin and Gentamicin. Whilst it showed a high sensitivity against Cefotaxime, Aztreonam, Ticarcillin/clavulanic acid and Imipenem. *Proteus mirabilis* showed resistance against Amikacin, Ciprofloxacin, Ceftriaxone, Gentamicin and Cephalothin. Whilst it showed a high sensitivity against Ceftazidime, Cefotaxime, Aztreonam, Ticarcillin/clavulanic acid, Imipenem, Cefepime and Tobramycin. *Klebsiella pneumonia* showed resistance against Ciprofloxacin, Ceftazidime, Cefotaxime and Aztreonam. whilst it showed a high sensitivity against Ceftriaxone, Amikacin, Gentamicin, Cephalothin, Ticarcillin/clavulanic acid, Imipenem, Cefepime and Tobramycin. The current study was aimed to determine the most frequent pathogens in burn injury and test their antibiotics susceptibility in hospitalize patients in Najaf city.

***Keywords**: Burn injury, Burn infection, Antibiotics.*

Introduction

Burn infection considered one of the most medical issue in the world[1]. there are many reasons could lead for it, such as heat, chemical agent, electricity… etc. [2]. The big problem in burn is the infection when the skin is destroyed, many pathogens and opportunistic microbes are initiated the infection[3] and this lead to sepsis especially in the modern countries[4], and this is the cause of high morbidity and mortality in hospitalized burn patients[5,6]. Burn injury considered a harmful form of trauma so, the patients with burn injury must take essential care to prevent mortality and morbidity[7]. The term of multi-drug resistant Gram-positive bacteria and Gram-negative bacteria is used to the most microorganisms linked to injuries infection,

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according to many studies, these microorganisms include *Staphylococcus aureus* which is found in 20-40% and *Pseudomonas aeruginosa* which is found in 5-15% and other microorganisms like Escherichia coli, *Klebsiella pneumonia* and *Acinetobacter* spp. consider as a nosocomial pathogens[8-10]. Topical antibacterial reduce bacteria growth but not prevent growth other potentially invasive bacteria and fungi. These are contaminated the wound patients and their source is initiated from gastrointestinal or upper respiratory tract or the hospital environment itself[11]. After wounds contamination and adherence, these microbes begin to penetrate the viable tissue and invasive it, invasiveness of microbes is depend on the size of local wound and who the patients' health are, some patients have immunosuppression state [12]. If sub-eschar tissue is colonized, disseminated infection is likely to happen, and Some microorganisms are changed with time, some of them become so aggressive and other microorganism lose it ability to cause infection and this depend on the host immunity [13]. New microorganisms are arrived to the burn ward with new patients. These organisms live with resident flora of the burn for period of time so, they acquire new feature like antibiotics resistance. Presentation of new topical agents and systemic antibiotics influence the flora of the wound could be interested in future to prevent the mortality and morbidity by opportunistic flora [14]. The current study was aimed to determine the most frequent pathogens in burn injury and test their antibiotics susceptibility in hospitalize patients in Najaf city.

Materials and Methods

Population in study

The current study included 149 samples (112 males and 39 females) which collected from patients with burn injury in different sites by assistance of physician or the team care in the patient's room during the period 5 March 2020 to 12 August 2020 from the burn center in Alsadr hospital in Najaf city, Iraq. the patients in this study didn't have any chronic diseases or any other infection, the patients ages were 11 to 53 years old. all patient were agreed to cooperate and participate in this study in order to provide service to the community.

Sample collection

When the samples were taken from patients under complete aseptic conditions by sterile cotton swabs with sterile normal saline, they put in to brain heart infusion broth (Himedia-India) and they stayed no longer than one hour in the broth (to prevent the growth of contaminated microorganism especially *Aspergillus* spp.), and directly transported to the medical microbiology lab in Altoosi University College and then cultured in to different media (Himedia-India) for microbiology diagnosis that was achieved according to Macfaddin, and Forbes et al[13,15].

Identification of aerobic microorganism

For each sample in brain heart infusion broth, three petri dishes with Nutrient agar Blood agar, Maccokey, agar mannitol salt agar and Sabouraud dextrose agar (with chloramphenicol). All agars were incubated at 37°C for 24-48h. Subculture for single colonies were achieved to obtain pure colony for each microorganism. Gram's stein and biochemical tests such as oxidase, catalase, Indole, methyl red, vogas proskaur, simmons citrate, gelatin and sugar fermentation in addition to colony form were accomplished to complete identify of microorganisms[13,15].

Antibiotics Test

The method of antibiotics test was performed by using disc diffusion way that stated by Kirby-Baucer. The pure colony for each bacteria was streaked like mat onto Muellur Hinton agar (Himedia-India). For gram positive bacteria, thirteen different antibiotics disc were used which provided from Oxoid-USA, these antibiotics were as follow: Amoxicillin/ Clavulanic acid (20/10 µg), Gentamicin (10 µg), Amikacin (30 µg), Oxacillin (1 µg), Ciprofloxacin (1 µg), Ceftriaxone (30 µg), Ceftazidime (30µg), Cefotaxim (30µg), Cephalothin (30µg), Ticarcillin/clavulanic acid (75/10µg), Vancomycin (30µg), Imipenem (50µg) and Cefepime (10µg). While for gram negative bacteria, twelve different antibiotics disc were used which provided from Oxoid-USA, these antibiotics were as follow: Ciprofloxacin (5µg), Ceftriaxone (30µg), Ceftazidime (30µg), Amikacin (30µg), Gentamicin (10µg), Cefotaxime (30µg), Aztreonam (30µg), Cephalothin (30µg), Ticarcillin/ clavulanic acid (75/10µg), Imipenem (10µg), Cefepime (10µg) and Tobramycin (10µg). The sensitivity and resistance of bacteria was determined based on the inhibition zone that form on Muellur Hinton agar after

18-24h from culture. The inhibition zone that form was compared with CLSI 2017[14].

Statistical Analysis

The percentages of the number of was achieved by SPSS version 17 windows7.

Results

Identification of Microorganisms in burn injury

The present study included 149 burn injury patients. Eight different microorganisms were isolated in this study. Forty (26.84%) patients swabs were Gram positive bacteria (*Staphylococcus aureus* (18.79%) and *Coagulase-negative Staphylococci* (8.05%)), while fifty four (70.48%) patients swabs were gram negative bacteria (*Pseudomonas aeruginosa* (22.14%), *E.coli* (20.8%), *Proteus vulgaris* (17.44%), *Proteus mirabilis* (6.71%) and *Klebsiella pneumonia* (3.35%)). *Candida spp*. was isolated in four (2.68%) swabs patients. Table 1.

Table 1. the most frequent microorganisms isolated from burn injury patients

Microorganisms	Frequency N=149	% Percentage
Pseudomonas aeruginosa	33	22.14
E.coli	31	20.8
Staphylococcus aureus	28	18.79
Proteus vulgaris	26	17.48
Coagulase-negative Staphylococci	12	8.05
Proteus mirabilis	10	6.71
Klebsiella pneumoniae	5	3.35
Candida spp.	4	2.68

Antibiotics susceptibility test

Gram positive bacteria

Staphylococcus aureus Showed a high resistance against Gentamycin and ciprofloxacin, but it was a high sensitivity against Vancomycin and Imipenem. whilst it was had a mild resistance to *Cephalothin* and Amikacin. Table 2.

Coagulase negative Staphylococci showed a mild resistance against Gentamicin, Ciprofloxacin and Ceftazidime. but it was a high sensitivity against Amikacin, Oxacillin, Cefotaxim, Vancomycin, Imipenem and Cefepime. Whilst it was had a mild resistance to Gentamicin, Ciprofloxacin, Ceftazidime, table 2.

Table 2. Antibiotics test for Gram positive bacteria isolated from burn injury and their .concentrations.

Antibiotics	Resistance of Staphylococcus aureus N=28 (18.79%)	Resistance of Coagulase negative Staphylococci N=12 (8.05%)
Amoxicillin/ Clavulanic acid (20/10 µg)	5 (17.8%)	1 (8.33%)
Gentamicin (10 µg)	11 (39.28%)	2 (16.66%)
Amikacin (30 µg)	4 (14.28%)	S*
Oxacillin (1 µg)	1 (3.57%)	S
Ciprofloxacin (1 µg)	7 (25%)	2 (16.66%)
Ceftriaxone (30 µg)	2 (7.14%)	1 (8.33%)
Ceftazidime (30µg)	2 (7.14%)	2 (16.66%)
Cefotaxim (30µg)	1 (3.57%)	S
Cephalothin (30 µg)	5 (17.85%)	1 (8.33%)
Ticarcillin/clavulanic acid (75/10µg)	2 (7.14%)	S
Vancomycin (30 µg)	S	S
Imipenem (50 µg)	S	S
Cefepime (10 µg)	2 (7.14%)	S

* S= Sensitive

Gram Negative Bacteria

Pseudomonas aeruginosa showed resistance against Cefotaxime, *Aztreonam*, Gentamicin and Ceftazidime, but it showed a high sensitivity against Imipenem, Cefepime and Tobramycin. Whilst it was had a mild resistance to Amikacin, *Ceftriaxone*, *Cephalothin and Ciprofloxacin* Table 3.

E.coli showed a high resistance against Ceftriaxone, Ciprofloxacin and Cephalothin. But it showed a high sensitivity against Ticarcillin/clavulanic acid, Imipenem, Cefepime and Tobramycin. Whilst it was had a mild resistance to Amikacin, Gentamicin, Cefotaxime, Aztreonam, and Ceftazidime. Table 3.

Proteus vulgaris showed a high resistance against Ceftriaxone, Ceftazidime, Amikacin, *Cephalothin* and Gentamicin. But it showed a high sensitivity against Cefotaxime, Aztreonam, Ticarcillin/clavulanic acid and Imipenem. Whilst it was had a mild resistance to *Ciprofloxacin and* Tobramycin Table 3.

Proteus mirabilis showed resistance against Amikacin, Ciprofloxacin, Ceftriaxone, *Gentamicin* and Cephalothin. But it showed a high sensitivity against Ceftazidime, Cefotaxime, Aztreonam, Ticarcillin/ clavulanic acid, Imipenem, Cefepime and Tobramycin.

Whilst it was had a mild resistance to Amikacin Table 3.

Klebsiella pneumonia showed resistance against Ciprofloxacin, Ceftazidime, Cefotaxime and Aztreonam. whilst it showed a high sensitivity against Ceftriaxone, Amikacin, Gentamicin, Cephalothin, Ticarcillin/clavulanic acid, Imipenem, Cefepime and Tobramycin. Table 3.

Table 3. Antibiotics test for Gram Negative bacteria isolated from burn injury and their concentrations.

Antibiotics	*P. aeruginosa* N=33 (22.14%)	*E.coli* N=31 (20.8%)	*Proteus vulgaris* N=26 (17.44%)	*Proteus mirabilis* N=10 (6.71%)	*Klebsiella pneumonia* N=5 (3.35%)
Ciprofloxacin (5µg)	4 (12.12%)	10 (32.25%)	2 (7.69%)	1 (10%)	1 (20%)
Ceftriaxone (30µg)	6 (18.18%)	11 (35.48%)	10 (38.46%)	1 (10%)	S
Ceftazidime (30µg)	7 (21.21%)	4 (12.9%)	10 (38.46%)	S	1 (20%)
Amikacin (30 µg)	6 (18.18%)	6 (19.35%	9 (34.61%)	2 (20%)	S
Gentamicin (10µg)	7 (21.21%)	6 (19.35%	7 (26.92%)	1 (10%)	S
Cefotaxime (30µg)	9 (27.27%)	5 (16.12%)	S	S	1 (20%)
Aztreonam (30µg)	8 (24.24%)	5 (16.12%)	S	S	1 (20%)
Cephalothin (30µg)	5 (15.15%)	7 (22.58%)	8(30.76%)	1 (10%)	S
Ticarcillin/clavulanic acid (75/10µg)	1 (3.03%)	S	S	S	S
Imipenem (10µg)	S	S	S	S	S
Cefepime (10µg)	S	S	1 (3.84%)	S	S
Tobramycin 10µg	S	S	2 (7.69%)	S	S

Discussion

Burns infection death are the most notified event that occur between admitted hospitalize patients which represent over 50% of burn deaths. Although many burns centers gave specialize medical care for patients, but the nosocomial infections still the big problem for care teams[18]. In the current study, the most frequent pathogen in burn infection was *P. aeruginosa* (22.14%) in Najaf city, followed by *E.coli* (20.8%), *Staphylococcus aureus* (18.79%), *Proteus vulgaris* (17.44%), *Coagulase-negative Staphylococci* (8.05%),

Proteus mirabilis (6.71%) and *Klebsiella pneumonia* (3.35%). These results were in agreement with finding stated by Aljanaby et al, they found in their a three years cross-sectional study that the most frequent pathogen was *P. aeruginosa* (27.6%)[19]. This could be belong to the fact that *P. aeruginosa* is one of the most significant microbes which is distributed in the nature. *P. aeruginosa* has ability to produce infection because the high rate of virulence and antimicrobial resistance[20,21] But the current study disagree with the finding that stated by Al-Kanaany, she find the majority isolated organism in burn infection was *Staphylococcus* and followed by *Pseudomonas*. this difference is not significant because in the current study *S. aureus* was isolated as a third frequent pathogens in burn infection. although Both *P. aeruginosa* and S. aureus have high diffusion rate and consider as a nosocomial pathogens in admitted Hospitalize patients. In this study *E. coli, Proteus vulgaris*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Candida* were isolated and this result are in agreement with previous studies[22,23]. present of these bacteria may belong to environmental factor that contaminate the wounds, some patients could take these bacteria from hospital. Modern study showed that there was a relation-ship between admitted hospitalize patient for long time and the prevalence of pathogenic bacteria in burn[19].

Results showed the most isolated bacteria which has multi-antibiotics, resistance is *P. aeruginosa* and followed by *S. aureus*, *E.coli* and *Proteus vulgaris*. this either could belong to their ability to acquire the genes that responsible for drug resistance from other bacteria in the environment though plasmid or other vector by conjugation or any methods of gene transfer, or belong to ability of Gram negative bacteria especially *P. aeruginosa* to form biofilm that protect bacteria from the effect of host immunity and antibiotics[24-26]. *P. aeruginosa* is one of the most important pathogens causing different infections such as bacteremia and burn infection[27]. *P. aeruginosa* alone has more than 70% mortality of Burn infection[28].

Conclusions

From the presented study, the author conclude that the most dominant pathogens in burn infection was *Pseudomonas aeruginosa* and followed by *Staphylococcus aureus*, *Proteus vulgaris*, *Coagulase-negative Staphylococci*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Candida spp.* We also conclude that Imipenem (10µg) is an excellent choice for burn infection as a first choice, it has 100% against all the pathogens that isolated in the current study, while Ticarcillin/ clavulanic acid (75/10µg) and Cefepime (10µg) consider the second choice.

Conflict of Interest: Nil.

Source of Funding: Self**.**

Ethical Clearance: Taken from Altoosi University College committee**.**

Acknowledgements: The author acknowledges Alsadr hospital-burns center teams for their assistance in sample collection. The author is dedicated this work to the soul of his best friend Assist. Prof. Dr. Raad Alharmoosh, Co-dean of Altoosi college University.

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