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Isolation and identification of *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria from burns and wounds patients in Diyala governorate

Marwa Tawfiq Jasim
University of Diyala / college of Education pure science

Asst.prof. Dr. Adawia Fadhel Abbas Alzubaidi
University of Diyala / college of Education pure science
Corresponding author email: adwa_a2000@yahoo.com

Sami Manthoor Dawood Al-Rubaye
Diyala Health Department /Specialist General Surgeon

***Abstract*---**Patients with burns and wounds are more likely to contract an infection in the hospital than other patients due to the loss of the protective barrier (skin) and immune system disorders that appear in these patients, making the affected tissues a suitable environment for the infection. Hospital infection is one of the most severe complications that affect patients and significantly increases the rates of disease and death. Burn wound infections frequently harbor the bacteria that cause nosocomial infection, pneumonia, and other systemic infections that may eventually lead to multiple organ failures and death. Burn wound infection or sepsis may be indicated by an abrupt change in the burn wound's appearance or the burn patient's clinical status. This retrospective study was conducted at the Ba'aqubah teaching hospital.in duration march -May 2022 -, Data were collected for a number of variables including the severity of burns, demographic and clinical characteristics, laboratory data, and therapeutic devices. A total of 100 specimens,50 samples from wounds, and 50 samples from burns were collected from hospitalized burn and wound patients. The most commonly isolated species were *pseudomonas aeruginosa* which appeared in 31 isolates (11 (35.48%) isolates from burns and 20(64.52%) isolates from wounds), *Staphylococcus aureus* appeared in 25 isolates (8 (32.0%) isolates from burns and 17 (68.0%) isolates from wounds) .The highest rate of antibiotic resistance was observed in *Staphylococcus aureus* to antibiotics (Erythromycin and9(%90) to Benzylpenicillin, Oxacillin

7(70%)while the highest rate of antibiotic resistance was observed in *Pseudomonas aeruginosa* to Cefotaxime, Amikacin 8(80%). and to Meropenem, imipenem7 (70%).

***Keywords*---**bacterial burn infection, bacterial wound infection, *pseudomonas aeruginosa*, *staphylococcus aureus*, antibiotics.

Introduction

Burn and wound patients are more susceptible to hospital infection than other hospitalized patients due to the nature of their injury, as the loss of the protective barrier (skin) and defects that appear in burn and wound patients make the infected tissues a suitable environment for the growth of microbes, as about 30-80% of burn and wound patients are at risk of contracting nosocomial infection, and despite the development of burn care over the previous decades, burn and wound infection is still the leading cause of death among this group of patients (Emami *etal.*2020) .It is the wounds resulting from burns, surgeries, and injection sites that cause the entry of these organisms into the human body and thus the occurrence of skin infections. Most skin infections are caused by gram-positive bacteria such as *staphylococcus* and *streptococcus* species, while gram-negative bacteria are few present compared to gram-positive bacteria(Craft *etal.*2019) .that is caused by localized skin infections. *Staphylococcus aureus* can either be primary or secondary, as primary skin infections include scabies, folliculitis, boils, primary abscesses, and secondary skin infections that occur as a result of a pre-existing skin lesion and include abscesses, lymphatic infections, and cellulitis infections. and secondary acute and chronic wound infection(Pascal del Giudice,2020). Among the skin diseases caused by *Staphylococcus aureus* is necrotizing fasciitis (NF), which is an acute and rapidly progressive specific pathological condition, which progresses from necrosis of the superficial muscular fascicle to necrosis of the muscular fascicle of the subcutaneous tissue and the imams It can progress to sepsis, shock and death in 40% of cases under the name hospital gangrene, where necrotizing fasciitis occurs as a result of cutaneous injuries Inserts that break the body's first defense barrier (the skin(Rampal *etal.*2021), some individuals are at high risk of colonization by this type of bacteria over 80% including healthcare workers, diabetics, patients who They are treated intravenously and at the same time are Immunocompromised, long-term hospitalized surgical patients, catheter users, hemodialysis patients, and patients with metabolic diseases (Rasheed and Hussien,2021),while Ecthyma gangrenous is caused by *Pseudomonas aeruginosa.*(BUCUR *etal.*2017).It is a skin lesion that requires prompt diagnosis and treatment(Huang *etal.*2020). Which causes wound infection also is *Pseudomonas aeruginosa, Acinentobacter banumanii, Stphylococcus aureus ,Klebsiella Pneiumoniae,Enterococcus Faecalis*, then Pseudomonas aeruginosa was included in the list of dangerous pathogens(ESKAPE)(Aldawsari *etal.*2021). *Pseudomonas* algaria remains one of the therapeutic challenges due to its high mortality and associated morbidity rates and its potential to develop drug resistance throughout treatment (Al-Dahmoshi *etal.*2020).

2. Materials and Methods

2.1. Study Population: in order to obtain the most accurate assessment of the types and amounts of microorganisms present Multiple samples from several areas of the burn wound were collected frequently in the first few days to weeks following injury in the proper way to avoid any possible contamination . The study was conducted in t he Department of Microbiology at Diyala University, education college , biology department .

2.2. Collection of specimens: A total number of 100 wounds and burn swabs samples were collected from burn patients admitted to the burn and surgery Ba'aqubah teaching hospital in Diyala Governorate in Iraq Hospital from (January 2022 to May 2022). Wound samples were aseptically collected 7 days after admission to the hospital. The sample was collected from the different sites of burn, and injury, The specimen was processed according to the guidelines for the laboratory diagnosis of pathogens.

2.3. Microbiological Study of Burn and Wound Samples

Plate culture method: all specimens were inoculated less than 7 hours from their collection on nutrient agar, 5% Blood agar, MacConkey, Mannitol salt agar plates by spread plate method, under an aseptic condition in a laminar airflow cabinet. Then culture plates were incubated overnight at 37 degrees C aerobically. Isolation of microorganisms by total viable count by using colony counter. Blood agar was used for isolation and identification of all kinds of bacteria. Growth appeared in 56 samples, in order to detect the *staphylococcus* bacteria and *Pseudomonas aeruginosa* that inhabit wounds and burns, Then gram staining and microscopic examination for gram-positive, gram-negative, and morphology detection.

Confirmative Biochemical Study: Identify the bacteria from isolated samples this includes macroscopic, microscopy, Gram's staining, culture, biochemical, and antimicrobial sensitivity testing by using Vitek 2. Then identified bacterial spp was put into nutrient agar slant and subcultures at 37 degree C for 24 hours to perform antibiotic sensitivity test.

2.4. Antimicrobial sensitivity testing of Burn Wound Samples

The standard agar disc diffusion method known as the Kirby-Bauer method was applied to study of antimicrobial sensitivity testing. At first Muller Hinton agar plates were prepared. Before inoculation, the sterile swab stick was passed against the wall of the normal saline solution tube to drain out the excess fluid and moistened it. By using a sterile technique bacterial cultures were taken by sterile cotton swab stick and a uniform lawn of bacterial growth was prepared on Muller Hinton agar plates. Using sterile forceps, antibiotic discs were placed equally spread apart on the surface of the medium. 5 discs were used on each plate. The plate was incubated overnight at 37 degree C and the results were obtained no more than 24 h from incubation. The antimicrobial pattern was interpreted by the presence or absence of a clear zone around the antibiotic disc and the zone of inhibition was measured in mm by applying an ordinary ruler.

Results and Discussion

Sample collection

A total of 100 clinically significant samples from patients with wounds and burns were collected, Among them, 50 swabs or burn patients 50 swabs were for Burn patients. male were 33 (68.75%) female were 17(32.69%), *S. aureus* were 8 (32.0%), *p. aeruginosa* were 11 (35.48%) isolate, while Wound patients male were 15 (31.25%) female were 35(67.31%), *S. aureus* were 17(68.0%), *p. aeruginosa* were 20 (64.52%) included in this study as showing in table(1).

Table (1): The clinically patients with wounds and burns sample

Sample Type	Total No. of sample	No. of male	No. of female	No. of grown sample	No of *S. aureus*	Number of *p.aeruginosa*
Burn Infection	50 (50%)	33 (68.75%)	17(32.69%)	19(33.93%)	8 (32.0%)	11 (35.48%)
Wound Infection	50 (50%)	15 (31.25%)	35(67.31%)	37 (66.07%)	17(68.0%)	20 (64.52%)
Total	100	48 (48%)	52 (52%)	56 (56%)	25 (25%)	31(31%)

The results of the current study showed that the percentage of *Pseudomonas aeruginosa* bacteria in burn samples was (35.48%), which is higher than that of *Staphylococcus aureus*, which was (32%), and this may be due to the ability of *Pseudomonas aeruginosa* to live in different environments in addition to its multiple resistance to antibiotics and its possession A wide range of virulence factors that enable it to invade and bacterial colonization, as the results of the current study agree with many studies, including what was stated by(Uddin *etal.*2018).where the percentage of *Pseudomonas aeruginosa* had (30.23%) and the percentage of *staphylococcus* (20.93%).and also agreed with what was stated In(Hateet,2021) which had Pseudomonas aeruginosa bacteria more than the rest of the bacterial species by 20%, while *Staphylococcus aureus* appeared in second place compared to other bacterial species with a rate of 17.14% and this is consistent with our study in the fact that *Pseudomonas aeruginosa* bacteria were higher in burns of *Staphylococcus aureus* bacteria, as for the percentage of *Pseudomonas aeruginosa* bacteria isolated from Wound samples are (64.52%), which is higher than the percentage of *Staphylococcus aureus*, which was (68%), and this differs with many studies, including what came (Puca *etal.*2021) to have bacteria *Staphylococcus aureus* (79.4%) and *Pseudomonas aeruginosa* (40.2%) bacteria. This difference in results can be attributed to several reasons, including the nature and location of the wound, the antibiotics used to prevent infection, the level of medical care, and the measures taken to prevent hospital infections(Arshan *etal.*2020). But the results of the current study agree with what was stated by(Al-Anbaki,2020) who showed that the percentage of Gram-negative bacteria was higher than the percentage of Gram-positive bacteria in surgical wounds, where the percentage of Gram-negative bacteria was (75%), while the percentage of Gram-negative bacteria was in him, which was interpreted Studies contrary to our present results have *Staphylococcus aureus* present in Wound samples with a higher percentage of Pseudomonas aeruginosa due to the fact that

Staphylococcus aureus is part of the natural flora of the skin, which can easily cause wound infection (Arshan *etal.*2020).The results of the current study showed that the proportion of males infected with Burns (68.759), which is higher than the percentage of females, which amounted to (32.69%), and this agrees with what was stated by (Al-Anbaki,2020) where the percentage of males was 34%, and the percentage of females was 26%, and it also agrees with what (Al-Azzawi ,2005) stated, as the percentage of males was It has (71.88%) and the percentage of females was (47.37%) and this may be due to the fact that males are more mobile than females. It did not agree With the results of (Otta *etal.*2015),where the rate of injury in females was (57.7%) while in males (42%), the percentage of burn injuries in males is more than in females. The reason for this may be due to their daily chores, which may expose them to burn injuries. The results of the current study showed that the percentage of females in cases of wounds 67.31%, which is higher than the percentage of males, which amounted to (31.25%). 65.6%) and the percentage of females is 34.4%. According to previous studies, the reason for the difference in the percentages may be due to the difference in the number of samples collected, the place and time of sampling, in addition to the resistance of the isolates to antibiotics and the health conditions of the patients.

Bacterial isolates were initially diagnosed based on the phenotypic characteristics, which included colony shape, color, odor, size and texture. *Staphylococcus aureus* bacteria were grown on solid saline mannitol medium, as *Staphylococcus aureus* formed shiny golden colonies when grown on solid saline mannitol medium after an incubation period of 24 hours. The yellow color is due to the staphyloxanthin (acarotenoid produced by the *staphylococci* in order to protect them from ingestion(Rasheed and Hussien,2021), while *Pseudomonas aeruginosa* bacteria were grown on the medium of the MacConkey aquarium and the medium of the blood agar, where it appeared when grown on the medium of the MacConkey in the form of Pauso colonies. Pale green, smooth, flat, regular, not lactose-fermenting, while it appeared in the form of irregular, opaque colonies, with a glossy buttery texture and giving a fruity smell (the smell of grapes) (Al-Daraghi and Al-Badrwi ,2020).Isolates of *Staphylococcus aureus* were gram-positive, catalase-positive and oxidase-negative, while isolates of *Pseudomonas aeruginosa* were gram-negative, oxidase-positive, and catalase. The following table shows the results of the growth of *Staphylococcus* bacteria on solid manthol medium and the results of *Pseudomonas aeruginosa* culture on *Pseudomonas* mesenchymal medium. In this study: (figure 1.).

Figure (1): Examination of drug sensitivity on solid mantol and MacConkey gar for *Staphylococcus aureus* Pseudomonas aeruginosa

Drug sensitivity assay by Vitek device for *Staphylococcus aureus*

The Vitek 2 compact system was used to determine the values of the minimum inhibitory concentration of eighteen antibiotics for *Staphylococcus aureus:* Gentamicin, Tobramycin, Oxacillin, Benzylpenicillin, Levofloxacin, resistance, Texiplangexincyclin, Texiplangexinible Erythromycin, Clindamycin, Linezolid Tetracycline, Nitrofurantoin, Fusidic Acid, Rifampicin, Trimethoprim / Sulfamethoxazole, Tigecycline, where the results showed, according to the minimum inhibitory concentration of the antibiotics used, that the resistance of bacteria was high to the following antibiotics by 90% to Erythromycin and 70% for each of Benzylthromycins. And Trimethoprim / Sulfamethoxazole, as shown in the following table: Percentages of resistance and sensitivity of the minimum inhibitory concentration of antibiotics for isolates of *Staphylococcus aureus* depending on the tests of the Vitek device (Table 2)

Table (2): Percentages of antibiotic resistance and sensitivity of the MIC of isolates of *Staphylococcus aureus* to the sensitive antibiotic

Antibiotics	Resistance			Medium Resistance			Sensitive		
	NO.	Percentage	MIC Mg/ml	.NO	Percentage	MIC Mg/ml	.NO	Percentage	MIC Mg/ml
Benzylpenicillin	7	70	>=0.5			-	3	30	-
Oxacillin	7	70	>=1			-	3	30	<=0.5
Gentamicin	5	50	>=16	1	10	8	4	40	<=4
Tobramycin	3	30	>=16	4	40	8	3	30	<=4
Levofloxacin	5	50	>=4			2	5	50	<=1
Moxifloxacin	4	40	>=2			1	6	60	<=0.5
Inducible Clindamycin Resistance	3	30	+	7	70	-			
Erythromycin	9	90	>=8			1-4	1	10	<=0.5
Clindamycin	6	60	>=4	3	30	1-2	1	10	<=0.5
Linezolid	2	70	>=8	1	10	4	8	80	<=4
Teicoplanin	2	20	>=32	1	10	16	7	70	<=8
Vancomycin	2	20	>=16			8-4	8	80	<=2
Tetracycline	6	60	>=16	2	20	8	2	20	<=4
Tigecycline	4	40	>=1	/			6	60	<=0.12
Nitrofurantoin	3	30	>=78			/	7	70	<=32
Fusidic Acid	5	50	>=32	1	10	/	4	40	>=0.5
Rifampicin	6	60	>=4	2	20	2	2	20	<=8
Trimethoprim/Sulfamethoxazole	7	70	>=76	/		/	3	30	<=38

while the results of (Ramadan,2019).showed that the rate of resistance to erythromycin was 76%, and the results of the current study showed that the rate of resistance of *Staphylococcus aureus* to Oxacillin was 70 While the results of (Shaker,2018) showed that the percentage of bacterial resistance to the same antibiotic was 100%, and the current results showed that the percentage of bacteria resistance to the antibiotic Trimethoprim / Sulfamethoxazole was 70%, while the results of (Jameel,2018).indicated that the percentage of bacteria resistance to the antibiotic Trimethoprim / Sulfonamide was 100%, and the current results showed that the percentage of bacteria resistance to Benzylpenicillin was 70%. Previous studies showed that the rate of resistance of

Staphylococcus aureus to penicillins was high, as the results of (Zaidan *etal.*2009) .indicated that the percentage of bacteria resistant to penicillin Lin was 97.5%,This resistance is due to *Staphylococcus aureus* acquiring penicillin-resistant genes of chromosomal or plasmid origin, or resulting from mutations in the genes. The resistance to penicillins after something is common among Gram-positive bacteria(Levinson,2014). The difference in proportions between studies may Depends on the clinical source of the sample, sample size and antibiotics used.

Drug sensitivity assay by Vitek device for *Pseudomonas aeruginosa*

The Vitek 2 compact system was used to determine the minimum inhibitory concentration values for ten antibiotics of *Pseudomonas aeruginosa,* namely, Piperacillin / Tazobactam, Ceftazidime Cefotaxime, Meropenem Imipenem.efepime, Amikacin, Norfloxacin and Ciprofloxacin. Minimum Inhibitory Concentration of Antibiotics Used The resistance of bacteria was financial to the following antibiotics by 80% for each of Armikacing Cefotaxime and by 70% for each of Meropeneim Imipenem, as shown in table two:

Table (3) : Percentages of antibiotic resistance and sensitivity of the MIC of isolates of *Pseudomonas aeruginosa* to the sensitive antibiotic:

Antibiotics	Resistance			Medium Resistance			Sensitive		
	NO.	Percentage	MIC Mg/ ml	NO.	Percentage	MIC Mg/ ml	NO.	Percentage	MIC Mg/ ml
Piperacillin/Tazobactam	6	60	>=128			32	4	40	16>=
Cefotaxime	8	80	>=64	1	10	32	1	10	>=16
Ceftazidime	6	60	>=32			16	4	40	<=8
Cefepime	5	50	>=32	2	20	16	3	30	8
Imipenem	7	70	>=8			4	3	30	<=15
Meropenem	7	70	>=8			4	3	30	<=2
Amikacin	8	80	>=64			32	2	20	<=16
Gentamicin	6	60	>=16	2	20	8	2	20	>=4
Ciprofloxacin	4	40	>=2			1	6	60	<=0.5
Norfloxacin	4	40	>=16	1	10	8	5	50	<=4

The results of (Al-Azzawi,2018) showed in her study of 69 isolates of *Pseudomonas aeruginosa*, where the percentage of isolates resistant to Cefotaxime was 78%, the percentage of isolates resistant to Meropenem was 70%, and the percentage of isolates resistant to Imipenem was 66%, The results of a study conducted by (Ameen *etal.*2015)on 230 isolates of *Pseudomonas aeruginosa* showed that the percentage of isolates resistant to Imipenem was 49.9% and isolates resistant to Meropenem was 15.52%, according to a study conducted by (Musafer *etal.*2013) on 58 isolates of *Pseudomonas aeruginosa*. One of the most important causes of Pseudomonas aeruginosa resistance to the beta-lactam group is its production of beta-lactamase enzymes (Penicillinase), which attack the beta-

lactam ring found in the nucleus of penicillins and cephalosporins and render them ineffective in addition to their possession of other virulence factors. the isolates showed resistance to the group of anti-aminoglycosides due to bacterial production of modified enzymes such as phosphotransferase and n-acetyl transfers in addition to Other virulence factors.

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