



Prevalence and Antibiogram Susceptibility of *Pseudomonas aeruginosa* among Burn Wound Patients in Sana'a city –Yemen

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ABSTRACT

Background and aim: Burn injuries are prevalent globally, and *Pseudomonas aeruginosa* is the primary etiological agent in these patients. This study aimed to determine the prevalence of *P. aeruginosa* isolated from burn wound specimens and evaluate its antibiogram susceptibility patterns.

Methods: Swabs were collected from 424 patients with burns, between October 2023 and May 2024. Standard microbiological methods were employed for isolation, identification, and susceptibility testing of *P. aeruginosa*.

Results: The most frequent isolate was *P. aeruginosa* (168; 39.6%), followed by *Klebsiella pneumoniae* (115; 27.1%), and *Staphylococcus aureus* (82; 19.3%). The lowest isolation frequencies were recorded for *Acinetobacter spp.* and *Proteus spp.* (0.7% each). Significant statistical associations ($P < 0.05$) were found between antibiotic resistance and clinical procedures, such as surgical skin transplantation and debridement. *P. aeruginosa* isolates showed high resistance (90%) to third- and fourth-generation cephalosporins (cefepime, ceftazidime, and ticarcillin). Resistance to carbapenems was moderate (imipenem, 58%; meropenem, 62%), whereas colistin resistance remained exceptionally low (3%).

Conclusion: The isolation rates of *P. aeruginosa* from burn wound infections (BWI) were high in Sana'a. Resistance is strongly associated with length of hospital stay, surgical intervention, and prior antibiotic exposure.

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1. INTRODUCTION

Burn injuries represent one of the most destructive types of trauma worldwide. Burns compromise the physical skin barrier and suppress immunological responses, leaving the body vulnerable to opportunistic infections [1, 2]. Microbial infection is a hazardous and potentially fatal complication that occurs when a significant portion of the skin is affected. Infection of burn wounds is the leading cause of mortality and disability in both industrialized and developing nations [3]. Necrotic tissue and protein-rich exudates provide an ideal niche for microbial colonization [4]. A complex microbial ecology typically emerges within

5–7 d post-injury [5]. *P. aeruginosa* accounts for approximately 45% of burn-related deaths globally [6, 7]. Burn wounds are susceptible to *P. aeruginosa* infection due to the presence of dead, denatured tissues and a moist environment [8]. Infection of burn wounds with opportunistic organisms, particularly multi-drug resistant (MDR) *P. aeruginosa*, is often facilitated by breakdown of the protective skin barrier and extended hospitalization [9]. Owing to their ability to form biofilms and their inherent resistance to multiple antimicrobial classes, infections caused by this organism are increasingly difficult to manage [10, 11]. A recurrent infection with the same strain of *P. aeruginosa* is linked to colonization with that organism [12, 13]. This study of active surveillance

has the following goals: a) determine the prevalence of *P. aeruginosa* colonization in burn wound patients, b) determine the risk factors for *P. aeruginosa* colonization, and c) determine the antibiotic susceptibility of the isolated *P. aeruginosa*.

Methods:

Site area

This cross-sectional study was conducted at the Burn Center in Al-Jumhori Hospital, Sana'a, Yemen.

Subject's definition

The study included burn patients admitted for more than 48 h at the Al-Jumhori Hospital Burn Center in Sana'a, Yemen.

Inclusion criteria

A. Patients who were clinically presumptive for various healthcare-associated infections (HAIs) were included in this study.

B. Patients who had been admitted for more than 48 h at any age and of both sexes.

Exclusion criteria

A. Repeated specimens from the same individual.

B. Hospitalization for less than 48 hours.

C. Patients currently undergoing active antibiotic treatment (at the time of initial sampling).

Survey procedure and laboratory analysis

Sample size

The sample size at a confidence level of 95% was 377, calculated using the Epi Info 6 version software (CDC, Atlanta, USA). Based on the population, the burn center size is 20,000 per year (according to burn center statistics at Al-Jumhori Hospital). According to a previous study in Yemen, the prevalence of *Pseudomonas aeruginosa* in burn wound infection patients was 49% [14]. The sample size was increased to 424.

Laboratory Methods

Specimen collection

Specimens (wound or pus) were collected using Levine's technique. Purulent exudates, pus, and discharges were collected aseptically from the depth of the incision using a syringe or sterile cotton swab dipped in sterile normal saline. The cotton swab was inserted into a tube containing brain heart infusion transport medium and transferred within 30 min [15].

Isolation and identification of organisms: Clinical and demographic data were collected from all the participants. Swabs were inoculated on blood agar and MacConkey agar, and incubated at 37°C for 18–24 h. *P. aeruginosa* is strictly aerobic, non-spore-forming, gram-negative, rod-shaped, motile, oxidase positive,

catalase positive, methyl red negative, Voges Proskauer negative, indole negative, gelatin positive, and citrate positive [16]. Confirmatory tests for *P. aeruginosa* included pyocyanin production on cetrimide agar, and growth at 42°C. [[17].

Antibiogram Susceptibility Test:

All the susceptibility testing procedures followed the 2022 CLSI guidelines using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. [18]. A bacterial suspension of *Pseudomonas aeruginosa* was prepared according to the 0.5 McFarland turbidity standard and then cultured on Mueller Hinton agar petri plates.

The tested antibiotics included piperacillin (100 µg), piperacillin tazobactam (100/10 µg), cefepime (30 µg), cefoperazone (75 µg), ceftazidime (30 µg), imipenem (10 µg), meropenem (10 µg), azlocillin (75 µg), ticarcillin (75 µg), carbenicillin (100 µg), aztreonam (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), netilmicin (30 µg), colistin (10 µg), gatifloxacin (5 µg), lomefloxacin (5 µg), levofloxacin (5 µg), and ofloxacin (5 µg) [18]. The zones of inhibition were measured following incubation at 37 °C for 24 h, and the results were reported as susceptible, intermediate, or resistant.

Statistical analysis

Statistical Package for Social Science (SPSS) version 26 and Epidemiological Information (Epi Info) version 6.04 computer software programs (Epi Info, CDC, Atlanta, USA) were used for statistical analysis of the results. Using Pearson's chi-square test, associations between independent and dependent variables were examined, and odds ratios (OR) and 95% confidence intervals (CI) were calculated. Fisher's exact test was performed when necessary. A p-value of 0.05 or less was regarded as significant.

Ethical consideration

Ethical approval for the study was obtained from the Faculty of Medicine and Health Sciences Research Review Committee. Written permission was also obtained from the administrative managers of Al-Jumhori Hospital. Informed consent was obtained from all patients before completing the questionnaire.

2. RESULTS

Table (1) shows the frequency of bacterial isolates in patients with burn wounds. *Pseudomonas aeruginosa* had the highest frequency of identified gram-negative bacteria 168 (39.6%), followed by *Klebsiella pneumoniae* 115 (27.1%), *Citrobacter spp.* 34 (8.0%), and *Escherichia coli* 10 (2.4%). The lowest frequency of isolated bacteria was *Acinetobacter spp.* and *Proteus spp.* at three (0.7%) and *Enterobacter spp.* at two (0.5%). The most



frequently isolated gram-positive bacteria was *S. aureus* 82 (19.3%), followed by *Streptococcus* spp. 7 (1.7%).

Table (2) shows the distribution of bacterial isolates among patients with burn wounds in Sana'a City. Of the 424 patients with burn wounds, the majority of patients were in the age group > 36 years old, 102 (24.1%) were age group 16-25 years old, 88 (20.8%) were age group 3-10 years old, 75 (17.7%) were age group 26-35 years old, and 72 (17%). Then age group 0-2 years old 44 (10.4%) were age group 11-15 years old 43 (10.1%). Two hundred and sixty-nine (63.4%) patients were males, and 155 (36.6%) were females.

Table (3) shows the risk factors and clinical interference among the bacterial isolates in patients with burn wounds. The number of patients who underwent burn wound debridement (87%) and surgical skin transplantation (16%) were statistically significant ($p=0.001$), while those who underwent central line intravenous (62%) were not statistically significant ($p=0.07$). The number of bacterial isolates among burn wound patients according to the duration of hospitalization was statistically significant ($P=0.001$).

Table (4) shows the association between the antibiogram susceptibility of isolated *Pseudomonas aeruginosa* among patients with burn wounds. Out of 168 patients with *Pseudomonas aeruginosa*, resistance to antibiotics was statistically significant for cefepime 152 (90.5%), ceftazidime 151 (90%), and ticarcillin 155 (92.3%). Imipenem 98 (58.3%), meropenem 104 (62%), and colistin 5 (3%).

3. DISCUSSION

In this study, 63% of the patients with burn infections were male, while 37% were female. This is consistent with findings in Iraq (57% male), and females were 42.62% [19]; in Turkey, males were 55% and females were 45% [20], and in Saudi Arabia, 74% and 25.8% [21]. Moreover, another study in Taif, Saudi Arabia, found that 72.2% of men and 27.7% of women had burn wound infections [22].

In contrast, this result disagrees with previous studies that reported that females were predominant: in Yemen, males constituted 42% and females 58% [23]; in India, women made up 60% and men 40% [24]; and in Accra, Ghana, 42.9% of the patients were men and 57% were women [25]. Burn injuries are a significant global health concern, and epidemiological data suggest that males are more frequently exposed to burns than females. The higher prevalence in males is likely due to occupational exposure in high-risk environments, such as construction and industrial facilities. [21].

Regarding age, burn wound patients were the highest (24.1%) in the > 36 years age group. This finding is consistent with those of research conducted in Iraq [19], Morocco [26], Accra, Ghana [25], Yemen [27], and Iran

[28]. This can be due to the fact that adults at this age are often in dangerous environments at work and at home. In this study, out of 424 wound burn patients, 374 (88%) were taking antibiotics, with a statistically significant association with bacterial isolates ($P=0.001$). This could be due to prolonged hospital stays for medical care, and there is a connection between *P. aeruginosa* colonization and extended infection duration, which requires patients to take more antibiotics [29]. Risk factors for acquiring an antibiotic-resistant bacterium include receiving antibiotics prior to the onset of a disease, prolonged hospitalization, previous hospitalization, medical treatment, and comatose state [30]. Compared to bacteria acquired from the patient's gut microbiota, bacteria transmitted from the hospital environment have a higher level of antibiotic resistance [31, 32].

In this study, patients undergoing skin transplantation and debridement of burn wounds (370 patients, 87%, and 67 patients, 16%, respectively) had a highly statistically significant association ($P=0.001$). Based on the findings of this investigation, excision within 48 h can be advised, even though an effect on mortality could not be demonstrated. Additionally, a group of burned children had previously shown comparable outcomes in terms of a noticeably shorter hospital stay and lower rate of burn wound infection [33].

According to hospitalization periods of 3–7 days, 28% of burn wound patients had bacterial isolates, which was statistically significant ($P=0.001$). This result agrees with that reported by Wanis *et al.* [34], where the incidence of *P. aeruginosa* isolated from burn infections was associated with the length of hospital stay. These findings are in line with reports from similar studies conducted in Ethiopia [35], Nigeria [36], the Netherlands [7], and Iraq [37]. The association between hospital stay duration and *P. aeruginosa* infection may indicate that patients are more susceptible to acquiring and colonizing the notoriously significant nosocomial opportunistic pathogen, *P. aeruginosa*, the longer they stay in the hospital and the more exposed they are to infection [16].

At least 168 (38.6%) of the 424 burn wound patients enrolled in this study had *P. aeruginosa* infections, which is consistent with findings from earlier studies conducted in Ethiopia [38], Pakistan (24.9%) [39], Malaysia (22.4%) [40], China (21%) [41], Iraq (30.2%) [42], Ghana, Yemen (46.5%) [43, 44], and India (55%) [45]. These findings disagree with previous studies in Turkey and India, where the rates were significantly different [46–49] and [36].

In a study of burn wound patients, isolates of *Pseudomonas aeruginosa* demonstrated widespread resistance to traditional cephalosporins and penicillins, while maintaining moderate susceptibility to carbapenems and remaining highly sensitive to colistin. The high rate of resistance observed against third- and fourth-generation cephalosporins (90%) is alarming. However, our findings align with those of Tchakal *et al.* and studies con-



Table[1]: The frequency of bacterial isolates among burn wound patients in Sana'a city.

Bacterial isolates		
	Frequency	%
<i>Pseudomonas aeruginosa</i>	168	39.6
<i>Klebsiella spp</i>	115	27.1
<i>Staph. aureus</i>	82	19.3
<i>Citrobacter spp</i>	34	8.0
<i>E. coli</i>	10	2.4
<i>Strep. spp</i>	7	1.7
<i>Acinetobacter spp</i>	3	0.7
<i>Proteus spp</i>	3	0.7
<i>Enterobacter spp</i>	2	0.5
Total	424	100.0

Table[2]: Distribution of Bacterial Isolates among Burn Wound Patients in Sana'a City.

Sociodemographic Characteristics		Bacterial isolates				Total N=424		p
		<i>P.aeruginosa</i> N=168		Other bacterial (N=256)		No.	%	
		No.	%	No.	%			
Age	0–2	15	8.9	29	11.3	44	10.3	0.3
	3–10	24	14.3	51	19.9	75	17.7	
	11–15	17	10.1	26	10.2	43	10.1	
	16–25	35	20.8	53	20.7	88	20.8	
	26–35	36	21.4	36	14.1	72	16.9	
	>36	41	24.4	61	23.8	102	24.0	
Gender	Male	106	63	163	64	269	63.4	0.9
	Female	62	37	93	36	155	36.5	

Table[3]: The association between risk factors and clinical interference among bacterial isolates in burn wound patients, Sana'a city.

Risk Factors and Clinical Interfering		Bacterial isolates				OR	x ²	p		
		<i>P.aeruginosa</i> N=168		Other bacterial (N=256)					Total N=424	
		No.	%	No.	%				No.	%
Antibiotic use	Yes	135	80	239	93	374	88	0.3	16.4	0.001
	No	33	20	17	7	50	12			
Burn wound debridement	Yes	131	78	239	93	370	87	0.3	21.6	0.001
	No	37	22	17	7	54	13			
Surgical skin transplant	Yes	14	8	53	21	67	16	0.3	11.7	0.001
	No	154	92	203	79	357	84			
Central line intravenous	Yes	96	57	168	66	264	62	0.7	3.1	0.07
	No	72	43	88	34	160	38			
Medical discharge	Alive	143	85	203	79	346	82	1.5	2.3	0.1
	Dead	25	15	53	21	78	18			
Duration hospitalization	< 3	26	15	6	2	32	8	7.6	28.6	0.001
	4–7	110	65	171	67	281	66			
	8–14	25	15	63	25	88	21			
	> 15	07	4	16	6	23	5			

**Table[4]:** The association between antibiogram susceptibility of isolated *Pseudomonas* among patients with burn wound patients.

Antibiogram pattern		<i>P. aeruginosa</i>		Antibiogram pattern		<i>P. aeruginosa</i>	
		No.	%			No.	%
Cefepime	R	152	90	Tobramycin	R	125	74
	I	05	3		I	09	5
	S	11	7		S	34	20
Ceftazidime	R	151	90	Amikacin	R	132	79
	I	06	4		I	06	4
	S	11	6		S	30	18
Imipenem	R	98	58	Meropenem	R	104	62
	I	10	6		I	14	8
	S	60	36		S	50	30
Ticarcillin	R	155	90	Colistin	R	05	3
	I	02	1		I	00	0
	S	11	7		S	163	97
Carbenicillin	R	162	96	Levofloxacin	R	131	78
	I	03	2		I	09	5
	S	03	2		S	28	17
Piperacillin/Tazobactam	R	140	84	Lomefloxacin	R	145	86
	I	04	2		I	03	2
	S	24	14		S	20	12
Aztreonam	R	150	89	Gatifloxacin	R	124	74
	I	06	4		I	09	5
	S	12	7		S	35	21
Ciprofloxacin	R	132	79	Ofloxacin	R	144	86
	I	05	3		I	02	1
	S	31	18		S	22	13
Piperacillin	R	158	94	Netilmicin	R	120	72
	I	02	1		I	04	2
	S	08	5		S	44	26

ducted in Iran [50, 51]. In contrast, imipenem resistance was observed in 54.9% of *P. aeruginosa* isolates [49]. This result disagrees with the reported resistance to meropenem in India (18.2%) [52], Ethiopia (19.4% to 65%) [53, 54], Saudi Arabia (36.4%) [55], and Poland (41%) [56]. This result also contradicts the reported resistance to imipenem in Ethiopia (18%) [16], India (18.2%) [52], Nigeria (17.5%) [8], Kenya (31.7%) [57], Iraq (32%) [42], Turkey (46%) [47], Saudi Arabia (42.4%) [55], and Iran (30%) [58].

These findings agree with those of previous studies that reported imipenem resistance in India (61%) [9], Pakistan (66.7%) [39], Malaysia (73.9%) [40], Iran (94.7%) [59], Iran (70%) [58], and India (78%) [60]. In this study, the lower levels of colistin resistance were in line with another study's finding that the drug caused the lowest rate of resistance (2%) [53]. A 2015 study conducted by the European Antimicrobial Resistance Surveillance Network (EARS-Net) revealed that hospitals across Europe had the lowest level of colistin resistance (1%). Similarly, 1.1% of hospitals in the United States have colistin resistance [61]. Overall, our results are consistent with those

of earlier studies that demonstrated *P. aeruginosa* strains' comparatively low resistance of *P. aeruginosa* strains to colistin. Accordingly, this antibiotic may be suitable for treating *P. aeruginosa*-related hospital infections, particularly in immunocompromised patients such as those in the burn unit [62].

Conclusion:

It can be concluded from this study that the findings indicate *Pseudomonas aeruginosa* isolation rates from BWI are high. Antibiotic resistance was mostly associated with the length of hospital stay, skin transplantation, debridement of burn wounds, and prior antibiotic use.

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Authors' contributions

Ahlam Maher collected clinical samples, performed all diagnostic and laboratory procedures, analyzed the data, and wrote the manuscript. Prof. Ahmed and Dr. Huda supervised the research and provided guidance throughout the study and manuscript preparation. All the au-

thors have read and approved the final version of the manuscript.

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