JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Prevalence and antibiogram of *Pseudomonas aeruginosa* and *Staphylococcus aureus* clinical isolates from burns and wounds in Duhok City, Iraq

Samir Ali[1,2], Mahde Assafi[1]

[1] Department of Biology, College of Science, University of Duhok, Duhok, Kurdistan Region-Iraq
[2] PhD Candidate - ERASMUS+ Mobility Programme - Department for Innovation in Biological, Agro-food and Forest Systems, Università degli Studi della Tuscia, Viterbo, Italy

Abstract

Introduction: this study aimed to isolate *P. aeruginosa* and *S. aureus*, investigate the antimicrobial resistance of collected isolates, and investigate the distribution of *exoU* and *mecA* genes in *P. aeruginosa* and *S. aureus* isolates.

Methodology: Out of 150 samples, 32 isolates were identified as *P. aeruginosa*, 48 isolates were identified as *S. aureus.* All isolates were checked for AST. Then, a PCR was applied to detect *exoU* and *mecA* genes in *P. aeruginosa* and *S. aureus*.

Results: 12.0% and 29.3% of the samples showed co-isolates and single isolates of studied pathogens, respectively. Regarding burn samples, *S. aureus* was the most prevalent pathogen (38.0%, 38/100) among males (41.8%, 23/55), followed by *P. aeruginosa* (27.0%, 27/100) among females (28.9%, 13/45). The highest burn infection rates of *S. aureus* (50.0%) and *P. aeruginosa* (32.7%) were recorded among age groups (≥ 50) and (18-49), respectively. Comparatively, wound samples were less infected with these pathogens. *P. aeruginosa* isolates usually exhibited high resistance to gentamicin, tobramycin, and netilmicin, whereas, imipenem showed low resistance at 46.87%. *S. aureus* isolates were susceptible to trimethoprim-sulphamethoxazole and rifampin. 56.25% of *P. aeruginosa* isolates were *exoU* positive and 37.5% of *S. aureus* isolates were *mecA* positive. Results of the cefoxitin inhibition zone with *mecA* gene amplification, 33.3% isolates were MRSA, 4.2% isolates were nmrMRSA, and 62.5% isolates were MSSA. Most of the resistant isolates of *P. aeruginosa* carried the *exoU* gene, 80% resistant isolates to imipenem were *exoU* positive.

Conclusions: *S. aureus* was more predominant than *P. aeruginosa* in burns and wounds infections.

Key words: *P. aeruginosa*; *S. aureus*; burns/wounds infections; antimicrobial resistance; *exoU* gene; *mecA* gene.

J Infect Dev Ctries 2024; 18(1):82-92. doi:10.3855/jidc.18193

(Received 09 March 2023 – Accepted 09 July 2023)

Introduction

The main epithelial barrier between the human body and the hostile environment is the skin which is one of the largest immune organs in the human body [1]. A painful traumatic injury of the skin by burns or wounds can cause lowering host immunity, increased hospital prolonging, and ultimately risks of hospital-acquired infections [2].

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic pathogen responsible for hospital-acquired infections [3], especially bacteremia, and urinary, respiratory, and gastrointestinal tract infections [4], additionally, *P. aeruginosa* infections can be severe in burnt people, especially with a compromised immune system [5]. Abbasi *et al.,* (2017) revealed that this pathogen is the third nosocomial pathogen after *S. aureus* and *Escherichia coli* (*E. coli*) [6]. Although *P. aeruginosa* produces numerous virulence factors, for instance, pili, flagella, proteases, elastase, lipases, iron chelators, and a variety of various toxins, including pyocyanin and exotoxin A [7], The type III secretion system (T3SS) toxins (*exoS*, *exoT*, *exoU*, *exoY*) were identified as the major virulence determents that transport via T3SS system from pathogen cytosol directly into the cytoplasm of the eukaryotic host cell [8]. *S. aureus* is considered one of the most clinically important zoonotic pathogens, it can cause skin and soft tissue infections, and bloodstream infections [9].

Antimicrobial resistance is one of the most important global threats to general public health [10,11]. The spreading of multidrug-resistant (MDR) pathogens reduces the efficacy of antimicrobial drugs, thereby prolonging hospital stays and increasing treatment costs and fatalities. Also, one of the most important pathogens related to antimicrobial resistance worldwide is *Staphylococcus aureus* (*S. aureus*) [12]. It

can resist almost all lactams (e.g., methicillin, oxacillin, and flucloxacillin) and other major antimicrobial classes [13]. One of the most common resistance mechanisms in *S. aureus* is methicillin resistance, which is usually conferred by alteration of penicillin-binding protein-2a (PBP-2a), this protein is located in the bacterial cell wall and has a low affinity for β-lactams [14]. Historically, methicillin-resistant *S. aureus* (MRSA) was first reported in 1961 [15]. The gold standard for identifying MRSA strains is by detection of the *mecA* gene which is located on the staphylococcal chromosome cassette *mec* (SCC*mec*) in *S. aureus* [16].

Herein, the objectives of the present study were: (i) to isolate and identify *P. aeruginosa* and *S. aureus* from hospitalized patients with burns and wound infections using conventional and molecular methods, (ii) to investigate the antibiogram of collected isolates using Kirby-Bauer disc-diffusion technique, and (iii) to investigate the distribution of virulence and resistance genes of these isolates using species-specific primers.

Methodology

Study setting

This prospective study was conducted on patients at Burns and Plastic Surgery Hospital, Duhok City, Iraq between October, 2021 and February, 2022. A total of 150 swab samples from admitted patients of different ages and genders having wounds (accident and surgery) or burns were aseptically collected with the help of nurses. The admitted patients did not receive antibiotics previously. For the immediate and post-treatment, all patients received a treatment based on a dressing protocol including disinfection, cleansing, application of topical antibiotic silver sulfadiazine 1% (Awamedica, Iraq), and application of nonadherent gauze, followed by a tibio-breech bandage. Usually, this tropical antibiotic is applied daily. Demographic data collected in this study included sample source, patient age, and gender.

Ethical approval and consent to participate

The PhD proposal with informed consent was approved by the ethics committee of the College of Science, University of Duhok, and Duhok Public Health Directorate, Duhok City, Kurdistan Region-Iraq. Before collecting the clinical swabs, written formal consents for participation were obtained from all eligible patients or parents/guardians of eligible children (Reference number of research ethics was 18082021-8-12 on 7 Jun 2022).

Sample collection and processing

A total of 150 clinical swabs, 100 (55 males: 45 females) burn swabs and 50 (32 males: 18 females) wounds (pus or discharge) swabs were aseptically collected. All swab samples were labelled and immediately transported in ice- packed boxes to the laboratory for bacteriological analysis. The samples were identified as *P. aeruginosa* and *S. aureus* by application of selective culture media, differential staining, and different confirmatory tests. The collected swabs were cultured on cetrimide agar (Neogen, USA) and mannitol salt agar (Neogen, USA) under aerobic and sterile conditions and the plates were incubated at 37 °C for 24 hours. After the incubation period, purified colonies on both culture media were selected according to macroscopical characteristics [17,18]. Afterwards, the Gram staining technique (Atom, UK) and motility test (slide method) were performed for all purified isolates. Lastly, the obtained results were confirmed using biochemical tests including catalase (slide method) (Scharlau, Spin) and oxidase (using strip) (Bioanalysis, Turkey) tests for confirmatory identification of *P. aeruginosa*, while catalase (slide method) and coagulase (tube method) tests for confirmatory identification of *S. aureus*. For further processing, confirmed isolates of both bacterial species were preserved frozen in 2mL eppendorf tubes at -20 °C in nutrient broth supplemented with 25% (v/v) glycerol (Scharlau, Spin) [19].

Antibiogram

Antimicrobial susceptibility testing (AST) against study isolates of *P. aeruginosa* and *S. aureus* was carried out using the Kirby-Bauer disc-diffusion technique [20] and according to Clinical and Laboratory Standard Institute (CLSI, 32nd edition). A set of 10 antibiotic discs belonging to different antibiotic classes were tested against *P. aeruginosa* isolates, which included: piperacillin (100µg), ceftazidime (30µg), aztreonam (30µg), imipenem (10µg), meropenem (10µg), gentamicin (10µg), tobramycin (10µg), netilmicin (30µg), levofloxacin (5µg), and ofloxacin (5µg). Similarly, for testing the antimicrobial susceptibility of *S. aureus* isolates, 10 antibiotics discs belonging to different antibiotic classes were applied: penicillin G (10 units), cefoxitin (30µg) as surrogate antibiotic of oxacillin, gentamicin (10µg), azithromycin (15µg), tetracycline (30µg), levofloxacin (5µg), ofloxacin (5µg), clindamycin (2µg), trimethoprim/sulphamethoxazole (1.25/23.75µg), and rifampin (5µg). Practically, a cotton swab of an overnight bacterial broth culture (equivalent to 0.5

Table 1. Specific genes, primers sequences, and expected products for PCR assays for amplification of studied genes in *P. aeruginosa* and *S. aureus* isolates.

Microorganism	Gene	Gene type	Primer sequence 5´-3´	Amplicon size	References
P. aeruginosa	PA-SS	Housekeeping gene	F- GGGGGATCTTCGGACCTCA R- TCCTTAGAGTGCCCACCCG	956 bp	[24]
S. aureus	NUC	Housekeeping gene	F- GCGATTGATGGTGATACGGTT R- AGCCAAGCCTTGACGAACTAAAGC	280 bp	[25]
P. aeruginosa	*exoU*	Virulence gene	F- AGCGTTAGTGACGTGCG R- GCGCATGGCATCGAGTAACTG	1572 bp	[26]
S. aureus	*mecA*	Resistance gene	F- TAGAAATGACTGAACGTCCG R- TTGCGATCAAATGTTACCGTAG	154 bp	[27]

McFarland standard "density of 1.5×10^8 cell/mL") was inoculated on the entire surface of Muller-Hinton agar (Biomark laboratories, India) plate. After the plates were dried at room temperature, antibiotic discs (Bioanalysis, Turkey) were placed on each 100-mm plate, then the plates were incubated at 35 ± 2 ºC for 18 hours. After incubation, the plates were read for detection of antibiograms, the diameter of inhibition zones was measured in millimeters and the antibiogram was determined according to the CLSI criteria [21]. Isolates are described as MDR in case they show resistance to one antimicrobial agent in three different antimicrobial categories [22].

Extraction of bacterial DNA

Bacterial genomic DNA from *P. aeruginosa* and *S. aureus* isolates was extracted using AddPrep Bacterial Genomic DNA Extraction Kit (Addbio, Korea) according to manufacturer's instructions. Then, DNA concentration was measured by NanoDrop spectrophotometer instrument (Thermos Scientific, USA), and DNA purity was measured by reading the 260/280 absorbance ratio [23]. Then, extracted DNA from pure cultures was stored frozen at -20 °C.

Molecular detection

In this current study, uniplex polymerase chain reaction (PCR) technique was used for the identification and investigation of virulence and resistance genes of *P. aeruginosa* and *S. aureus* using published specific primers for each gene as shown in Table 1.

Each PCR reaction was done in a total volume of 20 µL as follows: 10µL of Add Taq Mater (20mM Tris-HCl (pH 8.8), 100Mm KCl, 0.2%Triton® X-100, 4Mm $MgCl_2$, Protein stabilizer, sediment, loading dye and 0.5 Mm each of dATP, dCTP, dGTP, and dTTP) (Addbio, Korea), 6µL of deionized distilled water, 2µL of DNA template (50 ng/µL) and 1µL of each forward and reverse primer (10 pmol/µL) (Macrogen, Korea). The PCR condition for each studied gene was carried out as shown in Table 2. Following amplification, aliquots of 5µL from each PCR product were analyzed by gel electrophoresis (composed of 1% agarose in TBE buffer (Addbio, Korea)) stained with safe gel stain dye (Addbio, Korea) (5µL/50 mL agarose gel) for 40-45 minutes at 85 voltages. Finally, the PCR products were visualized under a UV transilluminator instrument. The 100bp plus DNA ladder (GeneDireX, Taiwan) was used as the DNA molecular weight standard in this study.

Statistical analysis

Data obtained from this study were primarily input using Microsoft Excel Worksheet 2016 (Microsoft, USA), and descriptive statistics (mean, median, percentage) were calculated. Data analysis was performed using GraphPad Prism 8 (GraphPad Software, USA). Fisher's exact was applied to assess significant associations between two categorical variables. A p value < 0.05 was taken to indicate statistical significance.

Results

Socio-demographic characteristics of patients

A total of 150 participants were enrolled in the current study (63, 42% females and 87, 58% males) with a sex ratio of 0.72: 1. The ages of the participants ranged from 1 year to 85 years with a mean of 28.03

Table 2. Conventional PCR programs of PA-SS, NUC, *exoU*, and *mecA* genes amplification in collected isolates.

Steps	Temperature (°C)				Times (Minute)				Cycles
	PA-SS	NUC	*exoU*	*mecA*	PA-SS	NUC	*exoU*	*mecA*	
Initial denaturation	95	94	94	92	2	2	4	5	1X
Denaturation	94	94	94	92	0.5	1	0.5	1	
Annealing	58	55	57	56	0.5	0.5	0.5	1	30-35X
Extension	72	72	72	72	1	1.5	2.5	1	
Final extension	72	72	72	72	5	3.5	5	5	1X

years and a median age of 24 years. Most of the study participants were in the age group of (18-49) years (76, 50.7%).

Bacterial identification

According to the results of morphological, biochemical, and molecular characterization among the 100 burns and 50 wounds swabs, co-isolates of studied pathogens were found in 18 (12.0%) clinical samples, and single isolates were found in 44 (29.3%) samples. Eighty-eight samples (58.7%) showed the absence of studied bacterial pathogens as shown in Figure 1A. *S. aureus* was the commonest isolated pathogen (48, 60.0%) followed by *P. aeruginosa* (32, 40.0%) with a significant statistical difference (*p* value 0.049) as shown in Figure 1B. There were also significant statistical differences between the frequency rate of each pathogen and total clinical samples of burns and wounds as in Table 3 and 4.

Figure 1. A: Frequency rates of single isolates and co-isolates of studied bacterial pathogens; **B:** Frequency rates (%) of S. aureus and P. aeruginosa strains in burns and wounds.

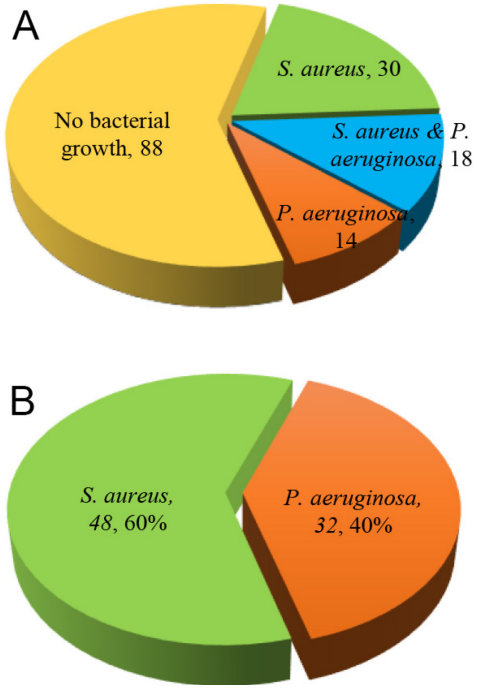


Table 3. Proportions (%) of S. *aureus* isolates among variable genders and ages.

Variables		Specimens			*p* value
		Burn	Wound	Total	
Samples n	TS	100	50	150	0.027*
	PS	38	10	48	
Gender n (%)					
Male	TS	55	32	87	0.252
	PS	23 (41.8)	9 (28.1)	32	
Female	TS	45	18	63	0.025*
	PS	15 (33.3)	1 (5.6)	16	
Age group (Years) n (%)					
≤ 17	TS	40	10	50	0.704
	PS	12 (30.0)	2 (20.0)	14	
18-49	TS	52	24	76	0.318
	PS	22 (42.3)	7 (33.3)	29	
≥ 50	TS	8	16	24	0.027*
	PS	4 (50.0)	1 (6.3)	5	

TS: total samples; PS: positive samples; *: statistically significant.

Table 4. Proportions (%) of *P. aeruginosa* isolates among variable genders and ages.

Variables		Specimens			*p* value
		Burn	Wound	Total	
Samples, n	TS	100	50	150	0.019*
	PS	27	5	32	
Gender, n (%)					
Male	TS	55	32	87	0.179
	PS	14 (25.5)	4 (12.5)	18	
Female	TS	45	18	63	0.050
	PS	13 (28.9)	1 (5.6)	14	
Age group (Years), n (%)					
≤ 17	TS	40	10	50	0.665
	PS	8 (20.0)	1 (10.0)	9	
18-49	TS	52	24	76	0.176
	PS	17 (32.7)	4 (16.7)	21	
≥ 50	TS	8	16	24	N/A
	PS	2 (25.0)	0 (0.0)	2	

TS: total samples; PS: positive samples; *: statistically significant; N/A: not applicable.

Table 5. Percentages of antimicrobial susceptibility rates of isolated *P. aeruginosa* from burns and wounds.

Class of antibiotics	Antibiotics	Susceptible n (%)	Intermediate n (%)	Resistance n (%)
Penicillins	Piperacillin (PRL)	8 (25.0)	10 (31.25)	14 (43.75)
Cephems	Ceftazidime (CAZ)	11 (34.37)	2 (6.25)	19 (59.37)
Monobactams	Aztreonam (ATM)	6 (18.75)	3 (9.37)	23 (71.87)
Carbapenems	Imipenem (IPM)	17 (53.12)	0 (0.00)	15 (46.87)
	Meropenem (MEM)	8 (25.0)	1 (3.12)	23 (71.87)
Aminoglycosides	Gentamicin (CN)	5 (15.62)	0 (0.00)	27 (84.37)
	Tobramycin (TOB)	6 (18.75)	0 (0.00)	26 (81.25)
	Netilmicin (NET)	6 (18.75)	0 (0.00)	26 (81.25)
Fluoroquinolones	Levofloxacin (LEV)	10 (31.25)	0 (0.00)	22 (68.75)
	Ofloxacin (OFX)	10 (31.25)	0 (0.00)	22 (68.75)

Regarding *S. aureus* infections, burn samples were more infected with this pathogen, especially among males (41.8%), but no significant difference between the pathogen frequency rate and total clinical samples of burns and wounds was noticed (*p* value 0.252). In females and compared to wounds, burn samples (33.3%) were more infected with this pathogen with a significant statistical difference between the pathogen frequency rate and total clinical samples of burns and wounds (*p* value 0.025). The highest infection rate (50.0%) was recorded among the age group (≥ 50) of burn patients with a significant statistical difference between the frequency rate of this pathogen in total samples of burns and wounds (*p* value 0.027).

In *P. aeruginosa* infections (Table 4), burn samples were more infected with this pathogen especially among females (28.9%) with on significant difference between the pathogen frequency rate and total clinical samples of burns and wounds (*p* value 0.050). In the male gender and compared to wounds, burn samples (25.5%) were more infected with this pathogen with no significant difference between the pathogen frequency rate and total samples of burns and wounds (*p* value 0.179). The highest infection rate (32.7%) was recorded among the age group (18-49) of burn patients with significant statistical differences between the frequency rate of this pathogen and total samples of burns and wounds (*p* value 0.176). However, compared to burns, the occurrence of *S. aureus* and *P. aeruginosa* pathogens was less in wounds.

Antimicrobial susceptibility testing

In the current study, most of the *P. aeruginosa* isolates exhibited higher resistance rates to 10 antimicrobials tested as shown in Table 5. High resistance rates were seen among *P. aeruginosa* isolates toward aminoglycosides (gentamicin 84.37%, tobramycin 81.25%, and netilmicin 81.25%). Whereas, 53.12% of *P. aeruginosa* isolates were susceptible to imipenem. *S. aureus* isolates were usually susceptible to 10 antimicrobials tested, high susceptible rates were seen among *S. aureus* isolates to trimethoprim-sulphamethoxazole (62.50%), and rifampin (60.42%). While, 91.67% and 70.83% of *S. aureus* isolates were resistant to penicillin G and azithromycin, respectively (Table 6). Before admitting, the patients did not receive any antibiotics. The tested antibiotic, trimethoprim-sulphamethoxazole used in the current study and the tropical antibiotic silver sulfadiazine which was used in the immediate and post treatments are both belonging to the sulfonamide or sulfa drug group.

PCR amplification of screened genes

Table 7 summarized the finding of the molecular detection (using conventional PCR) of studied genes in 32 and 48 isolates which were phenotypically identified as *P. aeruginosa* and *S. aureus,* respectively.

Table 6. Percentages of antimicrobial susceptibility rates of isolated *S. aureus* from burns and wounds.

Class of antibiotics	Antibiotics	Susceptible n (%)	Intermediate n (%)	Resistance n (%)
Penicillinase-labile penicillins	Penicillin G (P)	4 (8.33)	0 (0.00)	44 (91.67)
Penicillinase-stable penicillins	Cefoxitin (FOX) †	28 (58.33)	0 (0.00)	20 (41.67)
Aminoglycosides	Gentamicin (CN)	26 (54.17)	2 (4.17)	20 (41.67)
Macrolides	Azithromycin (AZM)	14 (29.17)	0 (0.00)	34 (70.83)
Tetracyclines	Tetracycline (T)	28 (58.33)	0 (0.00)	20 (41.67)
Fluoroquinolones	Levofloxacin (LEV)	27 (56.25)	3 (6.25)	18 (37.5)
	Ofloxacin (OFX)	26 (54.17)	1 (2.08)	21 (43.75)
Lincosamides	Clindamycin (CD)	24 (50.00)	5 (10.42)	19 (39.58)
Folate pathway antagonists	Trimethoprim-sulphamethoxazole (SXT)	30 (62.50)	1 (2.08)	17 (35.42)
Ansamycins	Rifampin (RA)	29 (60.42)	2 (4.17)	17 (35.42)

† Cefoxitin resistance as a surrogate maker for the detection of methicillin (oxacillin)-resistant *Staphylococcus aureus* (MRSA) strains.

Table 7. Molecular detection of studied genes in (32) *P. aeruginosa* and (48) *S. aureus* isolates in this study.

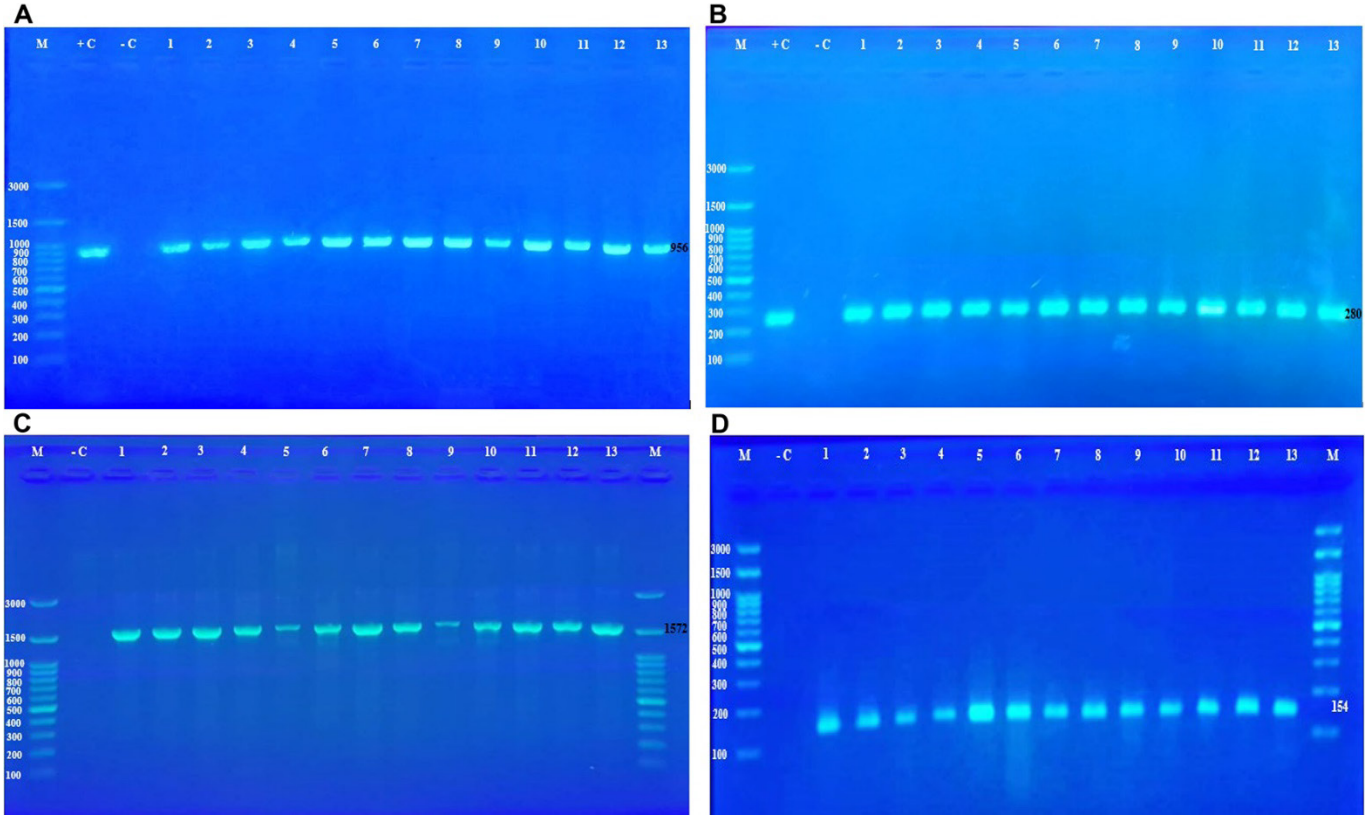
Microorganism	Gene	Gene type	n PCR positive / n tested isolates (%)
P. aeruginosa	PA-SS	Housekeeping gene	32/32 (100)
	exoU	Virulence gene	18/32 (56.25)
S. aureus	NUC	Housekeeping gene	48/48 (100)
	mecA	Resistance gene	18/48 (37.5)

Table 8. Comparison of the results by PCR amplification of *mecA* gene and standard disc-diffusion method of cefoxitin for demonstration of *S. aureus* isolates strains.

Microbe	Total isolates	PCR results of *mecA* gene	No. of isolates at cefoxitin inhibition zone † (mm) of			n (%) of *S. aureus* strains
			0 mm	10-21 mm	≥ 22 mm	
S. aureus	48	Positive	16	-	-	16 (33.3) MRSA
		Positive	-	2	-	2 (4.2) nmrMRSA
		Negative	-	-	28	30 (62.5) MSSA
		Negative	-	2	-	

† According to CLSI (32nd edition, 2022) criteria, the cefoxitin inhibition zone (≥ 22mm: susceptible, ≤ 21mm: resistant).

Figure 2. A: Uniplex PCR amplification products of P. aeruginosa PA-SS gene; lane M: 100 + DNA ladder; lane + C: positive control; lane -C: negative control; lanes 1-13: positive samples, which indicated by the 956 bp product; **B:** Uniplex PCR amplification products of S. aureus NUC gene; lane M: 100 + DNA ladder; lane + C: positive control; lane -C: negative control; lanes 1-13: positive samples, which indicated by the 280 bp product; **C:** Uniplex PCR amplification products of P. aeruginosa exoU gene; lanes M: 100 + DNA ladder; lane -C: negative control; lanes 1-13: positive samples, which indicated by the 1572 bp product; **D:** Uniplex PCR amplification products of S. aureus mecA gene; lanes M: 100 + DNA ladder; lane -C: negative control; lanes 1-13: positive samples, which indicated by the 154 bp product.



In this current study, 32/32 (100%) and 18/32 (56.25%) of *P. aeruginosa* isolates were PA-SS positive and *exoU* positive respectively, while the remaining 14/32 (43.75%) of *P. aeruginosa* isolates failed to produce the band of 1572 bp specific for *exoU* gene as shown in Figures 2A and 2C.

Amongst *S. aureus* isolates, 48/48 (100%) and 18/48 (37.5%) were NUC positive and *mecA* positive respectively, while 30/48 (62.5%) of *S. aureus* isolates failed to produce the band of 154 bp specific for *mecA* gene as shown in Figures 2B and 2D

Moreover, *S. aureus* strains were emphasized by the results of PCR of *mecA* gene amplification and cefoxitin disc-diffusion methods. However, 16 (33.3%) isolates, 2 (4.2%) isolates and 30 (62.5%) isolates of *S. aureus* were identified as MRSA, non-multidrug resistant methicillin-resistant *Staphylococcus aureus* (nmrMRSA), and methicillin-susceptible *Staphylococcus aureus* (MSSA) strains, respectively as shown in Table 8. Actually, out of 20 isolates of cefoxitin-resistant *S. aureus,* only 2 isolates failed to produce the band of 154 bp specific for the *mecA* gene. Whereas all 30 cefoxitin-susceptible *S. aureus* isolates were *mecA*-gene negative.

Table 9 illuminated the correlation between antimicrobial susceptibility patterns and possession of *exoU* gene in *P. aeruginosa* isolates*,* both resistant and intermediate-resistant isolates were considered as resistant in this study. The results concluded that most of the resistant isolates to antimicrobial agents tested in this study carried the *exoU* gene, in particular, 12 (80%) of 15 resistant isolates to imipenem carried the *exoU* gene with the significant statistical difference between *exoU*-positive strains and a total number of imipenem-resistant and imipenem-susceptible isolates (*p* value 0.015).

Discussion

Burn infections are the most serious health problem worldwide. Skin damage by burns or wounds leads to exposing a huge portion of its tissue to infectious agents, and this damaged tissue will be a suitable place for residing opportunistic microbes responsible for inflammation and infection immediately after colonization and bacterial secretions [28]. The current study investigated the prevalence and susceptibility of *P. aeruginosa* and *S. aureus* isolates. The identification of burn/wound-infected patients was based on morphological characteristics, microscopic examination, biochemical tests specific for each isolate, and molecular detection of housekeeping gene specific for each isolate. The results revealed that the prevalence of housekeeping genes PA-SS in *P. aeruginosa* and NUC in *S. aureus* isolates was (100%).

S. aureus was frequently co-isolated with opportunistic pathogen *P. aeruginosa* in polymicrobial infections such as burns and wounds infections [29]. Co-infections caused by multiple bacterial species are more virulent and/or more difficult to treat than infections caused by either bacterium alone [30]. Although many bacterial species were found in polymicrobial infections, the most common association was between *S. aureus* and *P. aeruginosa* [31].

In the current study, *S. aureus* was found to be the most common pathogen causing burns and wound infections. Globally, *S. aureus* is an important cause of hospital-acquired and community-acquired infections [32-34], due to its ability to produce various virulence factors, including capsule, protein A, a variety of exotoxins and enterotoxins. The prevalence of *S. aureus* and *P. aeruginosa* in burn/wound-infected patients was 60.0% and 40.0%, respectively. However, there are arguments about which bacteria is the leading infective pathogen in burns and wounds infections. Our findings were in agreement with previous studies, which have shown that *S. aureus* was the most prevalent pathogen

Table 9. Antimicrobial susceptibility patterns and possession of *exoU* gene in *P. aeruginosa* isolates.

Class of antibiotics	Antibiotics	No. of isolates based on susceptibility and possession of *exoU*				*p* value
		No. of resistant isolates	*exoU* + n (%)	No. of susceptible isolates	*exoU* + n (%)	
Penicillins	Piperacillin	24	16 (66.67)	8	2 (25.0)	0.096
Cephems	Ceftazidime	21	14 (66.67)	11	4 (36.36)	0.142
Monobactams	Aztreonam	26	16 (61.45)	6	2 (33.33)	0.364
Carbapenems	Imipenem	15	12 (80.0)	17	6 (35.29)	0.015*
	Meropenem	24	14 (58.33)	8	4 (50.0)	0.703
Aminoglycosides	Gentamicin	27	15 (55.56)	5	3 (60.0)	> 0.999
	Tobramycin	26	15 (57.69)	6	3 (50.0)	> 0.999
	Netilmicin	26	15 (57.69)	6	3 (50.0)	> 0.999
Fluoroquinolones	Levofloxacin	22	15 (68.18)	10	3 (30.0)	0.062
	Ofloxacin	22	15 (68.18)	10	3 (30.0)	0.062

*: statistically significant.

than *P. aeruginosa* in burns and wounds [31,35]. However, the results were disagreed with the other previous studies by Singh *et al.* [36] from India, Bayram *et al.* [37] from Turkey, Anuradha *et al.* [38] from India, Chaudhary *et al.* [39] from Pakistan, and Ghafil & Fleih [40] from Iraq, where *P. aeruginosa* prevalence was more than that of *S. aureus*. This difference in prevalence rates might be attributed to geographical variations, climatic features, antimicrobials abuse, and tropical antibiotics tested in the immediate and post burn treatments. In this study, more males (41.8%) with burn infections were affected by *S. aureus* compared to females (33.3%), and higher occurrence of *S. aureus* among burn patients found in age group (≥ 50) (50.0%) followed by age group (18-49) (42.3%). Regarding *P. aeruginosa* infections, burns samples were more infected with this pathogen especially among females (28.9%) with the highest burn infection rate (32.7%) recorded among the age group (18-49) followed by age group (≥ 50) (25.0%). These results indicated that burn infections in the elderly were quite more than infections in a younger population due to age-related alternations in immunity.

Most *S. aureus* isolates were susceptible to antimicrobial drugs tested in this study; high susceptibility rates were observed among *S. aureus* isolates to trimethoprim-sulphamethoxazole 62.50% followed by rifampin (60.42%). While, 91.67% and 70.83% of *S. aureus* isolates were extremely resistant to penicillin G and azithromycin, respectively. The reason of high susceptibility of *S. aureus* to trimethoprim-sulphamethoxazole might be using of the antibiotic silver sulfadiazine which was used in the immediate and post treatments. So, it might result in providing selection condition for trimethoprim-sulphamethoxazole. These results were consistent with the finding of studies by Bhat & Vasaikar [35], Ansari *et al.* [41], and Chen *et al.* [42], which have shown that *S. aureus* isolates were extremely resistant to penicillin G. However, according to another study conducted by Ahmed *et al.* [43] from Pakistan, *S. aureus* isolated from burn wounds were highly resistant to trimethoprim-sulphamethoxazole.

P. aeruginosa has multidrug resistance mechanisms, for instance, it can develop antimicrobial resistance through chromosomal mutations, and the acquisition of resistance genes encoding β-lactamases [44], subsequently, the action of antimicrobial drugs becomes limited. In this current study, *P. aeruginosa* isolates usually exhibited higher resistance rates to antimicrobial drugs tested. However, *P. aeruginosa* isolates were highly resistant to gentamicin, followed by tobramycin and netilmicin. Whereas, *P. aeruginosa* isolates were more susceptible to imipenem antibiotics which relatively revealed that this antibiotic was the last choice of therapy for burns and wounds infections. This was in accordance with the study conducted in Northwest Iran by Azimi *et al.* [45], where *P. aeruginosa* isolates have the highest rate of resistance to gentamicin and tobramycin, and with the studies conducted by Bhat & Vasaikar [35] from South Africa, Bayram *et al.* [37] from Turkey, and Bobai *et al.* [46] from Nigeria, where *P. aeruginosa* isolates were more sensitive to imipenem. But according to another study conducted by Chaudhary *et al* [39] from Pakistan, where *P. aeruginosa* isolates were less sensitive to imipenem (23.3%). The treatment becomes very difficult in burn infections caused by this pathogen and the mortality rate is likely to reach up to 40-50% [47]. However, imipenem and meropenem are used for a long period as the last choice antibiotics for the treatment of multidrug-resistant *P. aeruginosa* infections when other antimicrobial drugs have failed [48]. Nevertheless, this study highlighted *P. aeruginosa* as a multi-drug resistant pathogen in burns and wounds, this could have the following explanations: the present antibiotics policy in the Burns and Plastic Surgery Hospital – Duhok city has caused an increased rate of *P. aeruginosa* to commonly used antibiotics; the overuse of available antibiotics which are used as prophylactic or therapeutic measures; and introducing broad spectrum antibiotics such as imipenem that can be helpful in treatment of burn/wound infected patients.

In this current study, conventional PCR amplification was employed for the detection of the methicillin resistance gene (*mecA*) in all *S. aureus* isolates by using species-specific primers. In addition to the detection of the *mecA* gene in this study, the cefoxitin disc-diffusion method was performed to identify MRSA strains, according to the results of cefoxitin inhibition zone with *mecA* gene amplification, 33.3%, 4.2%, and 62.5% isolates of *S. aureus* were identified as MRSA, nmrMRSA, and MSSA strains, respectively. The prevalence of MRSA in this study found to be 37.5% which was comparable to that previously found in Pakistan by Junaid *et al.* [49] and in Northeast Ethiopia by Tsige *et al.* [50], but incomparable to that previously found in Iran by Emaneini *et al.* [51] (63.6%) and in Iraq by Aalaa and Abd Al-Abbas, (2019) [52] (93.0%), where *S. aureus* strains isolated from burns carried the *mecA* gene.

Cefoxitin incorrectly does not identify only 2 out of 20 isolates as resistant that were *mecA* negative. This was in accordance with the study conducted by Zhu *et*

al. [53], where only 3 out of 115 cefoxitin-resistant isolates were *mecA* negative. The explanation for two *mecA*-negative isolates with cefoxitin-inhibition zone (10-21mm) was maybe regarded with the concentration of antibiotic power in cefoxitin-30µg disc. On the other hand, Ba *et al.* [54] mentioned specific alterations in different amino acids present in penicillin-binding proteins cascade (PBPs 1, 2, and 3) which may be the basis of resistance. However, a study conducted by Swenson *et al.* [55], which had shown that the cefoxitin disc-diffusion test is preferred over the oxacillin disc-diffusion test for detecting *mecA*-mediated methicillin (oxacillin) resistance in *S. aureus*. In addition, *mecC* designed as the recently reported *mecA* homologue in the detection of the MRSA strains. Based on our results, we recommend other researches to establish the prevalence of the *mecA* and *mecC* genes among phenotypically identified MRSA strains and their effectiveness against different antibiotics in clinical specimens.

Also, conventional PCR amplification was used for the detection of the virulence gene (*exoU*) in *P. aeruginosa* isolates and 56.25% of *P. aeruginosa* isolates were *exoU* positive. *exoU* toxin as a cytotoxic protein has phospholipase activity towards the cell membranes of mammalian host cells resulting in rapid and complete cell lysis (cellular necroptosis) [56]. However, all *P. aeruginosa* strains do not have the T3SS toxins, for instance, the *exoU* gene was found in 28-42% of isolates from acute infections [57]. According to the results about the correlation between antimicrobial susceptibility patterns and possession of *exoU* gene in *P. aeruginosa* isolates, most of the resistant isolates to antimicrobial agents tested in this study carried *exoU* gene, in particular, (80%) of resistant isolates to imipenem were carried *exoU* gene. Our findings are in agreement with previous studies by Subedi *et al.* [58] and Takata *et al.* [59], which have shown that most of the carbapenem- and fluoroquinolone-resistant strains of *P. aeruginosa* were *exoU* positive. *exoU*-positive *P. aeruginosa* strains tend to harbour mutations in quinolone resistance-determining regions that lead to fluoroquinolone resistance [60]. Strains possessing the *exoU* gene were more resistant to the antibiotics tested in this study. However, other researchers should investigate the presence of other *exo* (*exoS*, *exoT*, and *exoY*) genes among phenotypically identified *P. aeruginosa* and their correlations with antibiotic resistance.

Conclusions

It was noticed that *S. aureus* was more predominant than *P. aeruginosa* in burns and wounds infections. Burns samples were more infected with *S. aureus* especially among males. *S. aureus* isolates were susceptible to trimethoprim-sulphamethoxazole and rifampin, while more resistant to penicillin G and azithromycin. *P. aeruginosa* isolates exhibited higher resistance rates to antimicrobial tests especially gentamicin, tobramycin, and netilmicin while having a low resistance rate to imipenem. The cefoxitin disc-diffusion test was used successfully for detecting *mecA*-mediated methicillin (oxacillin) resistance in *S. aureus* isolates. Most of the *P. aeruginosa* isolates exhibit resistance to antimicrobial agents especially imipenem was *exoU* positive.

Acknowledgements

We cordially thank the staff of the Burns and Plastic Surgery Hospital in Duhok city for their support during samples collection.

References

1. Karpelowsky JS, Rode H (2008) Basic principles in the management of thermal injuries. S Afr Fam Pract 50: 24-31. doi: 10.1080/20786204.2008.10873712.
2. Bhatt P, Rathi KR, Hazra S, Sharma A, Shete V (2015) Prevalence of multidrug resistant *Pseudomonas aeruginosa* infection in burn patients at a tertiary care centre. IJB 23: 56-59. doi: 10.4103/0971-653X.171656.
3. Mehrad B, Clark NM, Zhanel GG, Lynch III JP (2015) Antimicrobial resistance in hospital-acquired gram-negative bacterial infections. Chest 147: 1413-1421. doi: 10.1378/chest.14-2171.
4. Spagnolo AM, Sartini M, Cristina ML (2021) *Pseudomonas aeruginosa* in the healthcare facility setting. RRMM 32: 169-175. doi: 10.1097/MRM.000000000000027.
5. Wen MM, Abdelwahab, IA, Aly RG, El-Zahaby SA (2021) Nanophyto-gel against multi-drug resistant *Pseudomonas aeruginosa* burn wound infection. Drug Deliv 28: 463-477. doi: 10.1080/10717544.2021.1889720.
6. Abbasi A, Maddah SM, Mahboubi A, Khaledi A, Vazini H, Esmaeili D (2017) Investigate the inhibitory effects of Satureja khuzestanica essential oil against housekeeping fabD and *exoA* genes of *Pseudomonas aeruginosa* from hospital isolates using RT-PCR technique. Ann Med Health Sci Res 7: 246-250.
7. Harper DR, Parracho HMRT, Walker J, Sharp R, Hughes G, Werthén M, Lehman S, Morales S (2014) Bacteriophages and biofilms. Antibiotics 3: 270-284. doi: 10.3390/antibiotics3030270.
8. Galle M, Carpentier I, Beyaert R (2012) Structure and function of the type III secretion system of *Pseudomonas aeruginosa*. Curr Protein Pept Sci 13: 831-842. doi: 10.2174/138920312804871210.
9. Assafi MS, Mohammed RQ, Hussein NR (2015) Nasal carriage rates of *Staphylococcus aureus* and CA-methicillin resistant *Staphylococcus aureus* among university students. J

Microbiol Res 5: 123-127. doi: 10.5923/j.microbiology.20150504.01.
10. Assafi MS, Polse RF, Hussein NR, Haji AH, Issa AR (2017) The prevalence of *S. aureus* nasal colonisation and its antibiotic sensitivity pattern amongst primary school pupils. SJUOZ 5: 7-10. doi: 10.25271/2017.5.1.291.
11. Hussein NR, Assafi MS, Ijaz T (2017) Methicillin-resistant *Staphylococcus aureus* nasal colonisation amongst healthcare workers in Kurdistan Region, Iraq. J Glob Antimicrob Resist 9: 78-81. doi: 10.1016/j.jgar.2017.01.010.
12. Hado HA, Assafi MS (2021) Molecular fingerprinting of methicillin resistant *Staphylococcus aureus* strains isolated from human and poultry in Duhok, Iraq. IJVS 35: 99-103. doi: 10.33899/IJVS.2020.126375.1310.
13. Gajdács M (2019) The continuing threat of methicillin-resistant *Staphylococcus aureus*. Antibiotics 8: 52. doi: 10.3390/antibiotics8020052.
14. Fishovitz J, Hermoso JA, Chang M, Mobashery S (2014) Penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus*. IUBMB Life 66: 572-577. doi: 10.1002/iub.1289.
15. Jevons MP (1961) "Celbenin"-resistant staphylococci. Br Med J 1: 124-125
16. Brown DF (2001) Detection of methicillin/oxacillin resistance in staphylococci. J Antimicrob Chemother 48: 65-70. doi: 10.1093/jac/48.suppl_1.65.
17. Kateete DP, Kimani CN, Katabazi FA, Okeng A, Okee MS, Nanteza A, Joloba MA, Najjuka FC (2010) Identification of *Staphylococcus aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Ann Clin Microbiol Antimicrob 9: 1-7. doi: 10.1186/1476-0711-9-23.
18. Al-Bayati SS, Al-Ahmer SD, Shami AM, Al-Azawi AH (2021) Isolation and identification of *Pseudomonas aeruginosa* from clinical samples. Biochem Cell Arch 21: 3931-3935.
19. Nanvazadeh F, Khosravi AD, Zolfaghari MR, Parhizgari N (2013) Genotyping of *Pseudomonas aeruginosa* strains isolated from burn patients by RAPD-PCR. Burns 39: 1409-1413. doi: 10/1016/j.burns.2013.03.008.
20. Bauer AW, Kirby WMM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 45: 493-496.
21. CLSI (2022) Performance standards for antimicrobial susceptibility testing, 32nd edition. CLSI supplement M100. USA: Clinical and Laboratory Standards Institute 50-75 p.
22. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbath S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18: 268-281. doi: 10.1111/j.1469-0691.2011.03570.x.
23. Desjardins PR, Conklin DS (2011) Microvolume quantitation of nucleic acids. Curr Protoc Mol Biol A3: 3J. doi: 10.1002/0471142727.mba03js93.
24. Spilker T, Coenye T, Vandamme P, LiPuma JJ (2004) PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. J Clin Microbiol 42: 2074-2079. doi: 10.1128/JCM.42.5.2074-2079.2004.
25. Othman HE, Merza NS, Jubrael JM (2014) Nucleotide sequence analysis of methicillin resistance *Staphylococcus aureus* in Kurdistan Region-Iraq. SJUOZ 2: 65-73.
26. Feltman H, Schulert G, Khan S, Jain M, Peterson L, Hauser AR (2001) Prevalence of type III secretion genes in clinical and environmental isolates of *Pseudomonas aeruginosa*. Microbiology 147: 2659-2669. doi: 10.1099/00221287-147-10-2659.
27. Siripornmongcolchai T, Chomvarin C, Chaicumpar K, Limpaiboon T, Wongkhum C (2002) Evaluation of different primers for detecting mecA gene by PCR in comparison with phenotypic methods for discrimination of methicillin-resistant *Staphylococcus aureus*. Southeast Asian J Trop Med Public Health 33: 758-763.
28. Butzelaar L, Ulrich MMW, Van Der Molen AM, Niessen FB, Beelen RHJ (2016) Currently known risk factors for hypertrophic skin scarring: A review. J Plast Reconstr Aesthet Surg 69: 163-169. doi: 10.1016/j.bjps.2015.11.015.
29. Gjødsbøl K, Christensen JJ, Karlsmark T, Jørgensen B, Klein BM, Krogfelt KA (2006) Multiple bacterial species reside in chronic wounds: a longitudinal study. Int Wound J 3: 225-231. doi: 10.1111/j.1742-481X.2006.00159.x.
30. Pastar I, Nusbaum AG, Gil J, Patel SB, Chen J, Valdes J, Stojadinovic O, Plano L, Tomic-Canic M, Davis SC (2013) Interactions of methicillin resistant *Staphylococcus aureus* USA300 and *Pseudomonas aeruginosa* in polymicrobial wound infection. PLoS One 8: e56846. doi: 10.1371/journal.pone.0056846.
31. Bessa LJ, Fazii P, Di Giulio M, Cellini L (2015) Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. Int Wound J 12: 47-52. doi: 10.1111/iwj.12049.
32. Hussein NR, Alyas A, Majeed M, Assafi MS (2015) Prevalence rate and prevalent genotypes of CA-MRSA in Kurdistan Region: First Report from Iraq. Int J Pure Appl Sci Technol 27: 44-49.
33. Hussein NR, Basharat Z, Muhammed AH, Al-Dabbagh SA (2015) Comparative evaluation of MRSA nasal colonization Epidemiology in the urban and rural secondary school community of Kurdistan, Iraq. PLoS One 10: e0124920. doi: 10.1371/journal.pone.0124920.
34. Goudarzi M, Kobayashi N, Dadashi M, Pantůček R, Nasiri MJ, Fazeli M, Pouriran R, Goudarzi H, Miri M, Amirpour A, Seyedjavadi SS (2020) Prevalence, genetic diversity, and temporary shifts of inducible clindamycin resistance *Staphylococcus aureus* clones in Tehran, Iran: a molecular–epidemiological analysis from 2013 to 2018. Front Microbiol 11: 663. doi: 10.3389/fmicb.2020.00663.
35. Bhat V, Vasaikar S (2010) Bacteriological profile and antibiogram of aerobic burn wound isolates in Mthatha, Eastern Cape, South Africa. S Afr J Infect Dis 25: 16-19. doi: 10.1080/10158782.2010.11441404.
36. Singh NP, Goyal R, Manchanda V, Das S, Kaur I, Talwar V (2003) Changing trends in bacteriology of burns in the burns unit, Delhi, India. Burns 29: 129-132. doi: 10.1016/s0305-4179(02)00249-8.
37. Bayram Y, Parlak M, Aypak C, Bayram I (2013) Three-year review of bacteriological profile and antibiogram of burn wound isolates in Van, Turkey. Int J Med Sci 10: 19-23. doi: 10.7150/ijms.4723.
38. Anuradha M, Vasudevanaidu KH, Suneetha N (2015) Phenotypic and molecular characterisation of *Pseudomonas aeruginosa* among hospital acquired infections in a tertiary care hospital, Tirupathi. World J Pharm Res 4: 931-941.
39. Chaudhary NA, Munawar MD, Khan MT, Rehan K, Sadiq A, Bhatti HW, Rizvi ZA (2019) Epidemiology, bacteriological

profile, and antibiotic sensitivity pattern of burn wounds in the burn unit of a tertiary care hospital. Cureus 11: e4794. doi: 10.7759/cureus.4794.
40. Ghafil JA, Flieh MT (2021) Isolation and identification of bacterial burn wound infection in Iraqi patient. Indian J Forensic Med Toxicol 15: 1351-1357. doi: 10.37506/ijfmt.v15i4.16897.
41. Ansari S, Nepal HP, Gautam R, Rayamajhi N, Shrestha S, Upadhyay G, Acharya A, Chapagain ML (2014) Threat of drug resistant *Staphylococcus aureus* to health in Nepal. BMC Infect Dis 14: 157. doi: 10.1186/1471-2334-14-157.
42. Chen K, Lin S, Li P, Song Q, Luo D, Liu T, Zeng L, Zhang W (2018) Characterization of *Staphylococcus aureus* isolated from patients with burns in a regional burn center, Southeastern China. BMC Infect Dis 18: 51. doi: 10.1186/s12879-018-2955-6.
43. Ahmed J, Ali A, Hussain M, Ahmad H (2013) Antimicrobial susceptibility pattern of bacteria isolated from burn wounds in a tertiary care hospital in Pakistan. Afr J Microbiol Res 7: 3627-3631. doi: 10.5897/AJMR.1319.
44. Lopez-Garcia A, del Carmen Rocha-Gracia R, Bello-López E, Juárez-Zelocualtecalt C, Sáenz Y, Castañeda-Lucio M, López-Pliego L, González-Vázquez MC, Torres C, Ayala-Nuñez T, Jiménez-Flores G, de la Paz Arenas-Hernández MM, Lozano-Zarain P (2018) Characterization of antimicrobial resistance mechanisms in carbapenem-resistant *Pseudomonas aeruginosa* carrying IMP variants recovered from a Mexican hospital. Infect Drug Resist 11: 1523-1536. doi: 10.2147/IDR.S173455.
45. Azimi S, Kafil HS, Baghi HB, Shokrian S, Najaf K, Asgharzadeh M, Yousefi M, Shahrivar F, Aghazadeh M (2016) Presence of exoY, exoS, exoU and exoT genes, antibiotic resistance and biofilm production among *Pseudomonas aeruginosa* isolates in Northwest Iran. GMS Hyg Infect Control 11: Doc04. doi: 10.3205/dgkh000264.
46. Bobai M, Lawal D, Nura SM, Joshua IA (2022) Phenotypic and molecular characterisation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from patients' wounds in Barau Dikko Teaching Hospital, Kaduna, Nigeria. AJOPRED 14: 096-118.
47. Estahbanati HK, Kashani PP, Ghanaatpisheh F (2002) Frequency of *Pseudomonas aeruginosa* serotypes in burn wound infections and their resistance to antibiotics. Burns 28: 340-348. doi: 10.1016/s0305-4179(02)00024-4.
48. Tsao LH, Hsin CY, Liu HY, Chuang HC, Chen LY, Lee YJ (2018) Risk factors for healthcare-associated infection caused by carbapenem-resistant *Pseudomonas aeruginosa*. J Microbiol Immunol Infect 51: 359-366. doi: 10.1016/j.jmii.2017.08.015.
49. Junaid KASHAF, Mustafa AUL, Arshad SANA, Farraj DAAL, Younas S, Ejaz H (2019) Burn wound infections: A serious threat of multidrug-resistant *Staphylococcus aureus*. PJMHS 13: 804-807.
50. Tsige Y, Tadesse S, Tefera MM, Amsalu A, Menberu MA, Gelaw B (2020) Prevalence of methicillin-resistant *Staphylococcus aureus* and associated risk factors among patients with wound infection at referral hospital, northeast Ethiopia. J Pathog 2020: 3168325. doi: 10.1155/2020/3168325.
51. Emaneini M, Bigverdi R, Kalantar D, Soroush S, Jabalameli F, Noorazer Khoshgnab B, Asadollahi P, Taherikalani M (2013) Distribution of genes encoding tetracycline resistance and aminoglycoside modifying enzymes in *Staphylococcus aureus* strains isolated from a burn center. Ann Burns Fire Disasters 26: 76-80.
52. Aalaa AC, Abd Al-Abbas MJ (2019) PCR-RFLP by AluI for coa gene of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from burn wounds, pneumonia and otitis media. Gene Rep 15: 100390. doi: 10.1016/j.genrep.2019.100390.
53. Zhu LX, Zhang ZW, Wang C, Yang HW, Zhang Q, Cheng J (2006) Evaluation of the CLSI cefoxitin 30-µg disk-diffusion method for detecting methicillin resistance in staphylococci. Clin Microbiol Infect 12: 1039-1042. doi: 10.1111/j.1469-0691.2006.01522.x.
54. Ba X, Harrison EM, Edwards GF, Holden MTG, Larsen AR, Petersen A, Skov RL, Peacock SJ, Parkhill J, Paterson GK, Holmes MA (2014) Novel mutations in penicillin-binding protein genes in clinical *Staphylococcus aureus* isolates that are methicillin resistant on susceptibility testing, but lack the mec gene. J Antimicrob Chemother 69: 594-597. doi: 10.1093/jac/dkt418.
55. Swenson JM, Tenover FC, Cefoxitin Disk Study Group (2005) Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* spp. J Clin Microbiol 43: 3818-3823. doi: 10.1128/JCM.43.8.3818-3823.2005.
56. Sato H, Frank DW (2004) ExoU is a potent intracellular phospholipase. Mol Microbiol 53: 1279-1290. doi: 10.1111/j.1365-2958.2004.04194.x.
57. Hauser AR (2009) The type III secretion system of *Pseudomonas aeruginosa*: infection by injection. Nat Rev Microbiol 7: 654-665. doi: 10.1038/nrmmicro2199.
58. Subedi D, Vijay AK, Kohli GS, Rice SA, Willcox M (2018) Association between possession of *ExoU* and antibiotic resistance in *Pseudomonas aeruginosa*. PLoS One 13: e0204936. doi: 10.1371/journal.pone.0204936.
59. Takata I, Yamagishi Y, Mikamo H (2018) Association of the exoU genotype with a multidrug non-susceptible phenotype and mRNA expressions of resistance genes in *Pseudomonas aeruginosa*. J Infect Chemother 24: 45-52. doi: 10.1016/j.jiac.2017.08.018.
60. Agnello M, Finkel SE, Wong-Beringer A (2016) Fitness cost of fluoroquinolone resistance in clinical isolates of *Pseudomonas aeruginosa* differs by type III secretion genotype. Front Microbiol 7: 1591. doi: 10.3389/fmicb.2016.01591.

Corresponding author

Samir Ali, MS
Department of Biology, College of Science
University of Duhok
Duhok, Iraq
Tel: +964(0)7507816475
E-mail: samir.alali@unitus.it; samir.ali@uod.ac

Conflict of interests: No conflict of interests is declared.