Microbes and Infectious Diseases 2024; 5(4): 1353-1366

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Distribution of toxin encoding genes among *Staphylococcus aureus* clinical isolates and their correlation with antibiotic resistance

Rania Alam Eldin Mohamed *[1], Nouran Magdy Moustafa [1,2], Shaimaa Abou Bakr Gaber [3], Yara said Elsaadawy [1]

1- Medical Microbiology and Immunology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt,

2- Basic Medical Science Department, Faculty of medicine, Dar Al Uloom University, Riyadh, Saudi Arabia.

3- Clinical Pathology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

ARTICLE INFO

Article history:
Received 14 May 2024
Received in revised form 2 June 2024
Accepted 7 June 2024

Keywords:
MRSA
Antimicrobial susceptibility
sea
hla
pvl

ABSTRACT

Background: *Staphylococcus aureus (S. aureus)* represents a substantial public health concern due to its progressive development of antimicrobial resistance. Its virulence is significantly contributed to the released toxins. This study aimed to assess toxin encoding genes (*sea*, *hla* and *pvl*) and the prevalence of Methicillin-resistant *S. aureus* (MRSA) among *S. aureus* isolates gathered from Ain Shams University (ASU) Hospital and determine possible correlation of these genes with antibiotic resistance. **Methods:** A total of 94 isolates were obtained from various clinical departments and samples. The process of identifying the isolates was carried out utilizing standard procedures. Disc diffusion method was utilized for testing antimicrobial susceptibility. Conventional PCR was utilized for detection of *sea*, *hla* and *pvl* genes. **Results:** MRSA prevalence among collected isolates was 78.7%, ICU was significantly more frequent in MRSA. Vancomycin, linezolid and doxycycline were the most effective tested antibiotics. Isolates showed high prevalence of three genes: *sea*, *hla* genes 93.6%, *pvl* gene 86.2%. The three genes collectively detected in 79.8% of isolates. Both *hla* and *pvl* genes were significantly more frequent in MRSA. Positive *hla* and *pvl* genes isolates had significant more frequent resistance to beta lactams, erythromycin, ciprofloxacin and levofloxacin. In addition, positive *pvl* gene isolates had significant more frequent resistance to trimethoprim-sulfamethoxazole and gentamycin. **Conclusion**: This research reports high prevalence of MRSA isolates in ASU hospitals. A high resistance rate to antibiotics was observed in positive *pvl*, then in *hla* positive isolates. Presence of either gene may indicate increased rate of resistance to antimicrobials.

Introduction

Staphylococcus aureus (S. aureus), is a highly prevalent bacterial pathogen that is responsible for an enormous variety of infections, spanning from simple cutaneous infections to severe invasive infections. In addition to nosocomial bacteremia, *S. aureus* causes infections of the respiratory tract, including pneumonia, prosthetic joints, cardiovascular systems and surgical sites [1]. *S. aureus* bacteremia has been identified as a leading cause of mortality, surpassing the combine fatality

DOI: 10.21608/MID.2024.287792.1943

* *Corresponding author:* Rania alam eldin Mohamed

E-mail address: *raniaalaam44@yahoo.com*

rate of tuberculosis, viral hepatitis and acquired immune deficiency syndrome [2].

Staphylococcus aureus-induced moderate to severe cutaneous infections as wound infections, abscesses, & furuncles, are typically not fatal but may result in significant pain and morbidity. They impose a significant burden on public health as a result of their frequency [3].

As opposed to numerous other bacterial pathogens that frequently rely on a limited number of virulence factors to induce disease, *S. aureus* possesses a vast array of virulence factors, involving protease production, toxins, adhesins and immune evasion factors, toxins. All of these agents induce pathogenesis via facilitating the bacterial attachment to tissues [4]. Several *S. aureus* secrete one or numerous staphylococcal exotoxins as staphylococcal enterotoxins, hemolysins and leukocidins. Staphylococcal enterotoxins (SEs) are associated with food poisoning and considered as one of the most virulent components of the super antigen family that causes nonspecific T-cell stimulation and proliferation [1].

Cytolytic toxins, namely hemolysins and leukocidins induce cell lysis by formation of β-barrel apertures in the cell membrane, causing cellular contents leakage. Panton-Valentine leucocidin (PVL) and α-hemolysin (α-HL) are two examples of these toxins [5]. Alpha hemolysin toxin is fatal to human epithelial cells and a major contributor to the pathogenesis of *S. aureus* cutaneous infections [6]. Necrotizing pneumonia, osteomyelitis, diffuse cellulitis, necrotizing pneumonia, and soft tissue and epidermal infection are caused by PVL [7]. As PVL attacks leucocytes including neutrophils, the main component of the innate immunity system, eradication of the organism becomes difficult. PVL toxin together with other toxins augment the lethal and destructive tendency of the organism [8]. Rising reports suggest that the prevalence of PVL-positive strains poses a significant threat to public health, despite the fact that the currency of PVL-producing *S. aureus* isolates differs globally [9].

Concerns regarding the treatment of *S. aureus* infections are evident given the organism's propensity to swiftly develop resistance. Both nucleic acid affection/cell membrane distortions and enzymatic inactivation can accelerate the antibiotic resistance development. The vast majority *S. aureus* strains are penicillin resistant which is presently recognized as a global issue [10]. Methicillin-resistant *S. aureus* (MRSA) is accountable for a vast array of distressing conditions, from minor cutaneous infections to critical illnesses [11]. The development of methicillin resistance relies on the acquisition of a mobile genetic element known as staphylococcal cassette chromosome mec (SCCmec). This element harbors the *mecA* resistance gene, which encodes a variant of the penicillin-binding protein (PBP2) that has a reduced affinity for β-lactam antibiotics [12]. MRSA is accountable for a multitude of infectious diseases as a result of its capacity to produce extracellular and surface virulence factors that possess adhesive properties; these include α-HL, PVL, and SEs [13].

It has been established that the prevalence, epidemiology, and molecular properties of MRSA strains can vary substantially between hospitals and even wards within the same institution. Hence, it is imperative to monitor the fluctuating MRSA epidemiology in regional healthcare establishments that serve a particular patient demographic in order to gather data that could inform empirical treatment during patient care. The objective of this research is to shed light on the MRSA prevalence discovered in clinical isolates of *S. aureus* from patients admitted to ASU Hospital. Furthermore, detect presence of certain toxin-encoding genes; staphylococcal enterotoxin A (*sea*), hemolysin A (*hla*) and *pvl* among methicillin-sensitive *S. aureus* (MSSA) and MRSA isolates and determine possible correlation of these genes with antibiotic resistance.

Methods

Ethical approval

The Research Ethical Committee of Ain Shams University, Faculty of Medicine had approved this research code No: FMASU R 20/2024.

Study settings and design

This observational cross-sectional analytic research was performed on 94 *S. aureus* strains, which were obtained from diverse clinical samples involving wound swab, sputum, blood and pus samples from clinical laboratory at ASU Hospital, Egypt between October 2023 and March 2024.

Samples transport and processing

The gained samples from patients were directly transported to the laboratory in iceboxes within 2 hours of collection and were instantly processed according to standard procedures of

microbiology. The samples were cultured on blood agar and mannitol salt agar (MSA) (Oxoid, England) and incubated for 24 hours at 37°C. Bacterial colonies showing typical characteristics of *S. aureus* (beta-hemolytic on blood agar and yellow colonies on MSA) were subjected to further phenotypic identification.

Phenotypic identification of *S. aureus* isolates

The conventional techniques were utilized for identification of the isolates including Gram staining and biochemical reactions (Coagulase, and DNase).

The DNase test was conducted by inoculation of the isolates into deoxyribonuclease (DNase) agar (HiMedia, Mumbai, India) and allowing them to incubate at 37^{O} C for 24 hr. About 15 mL of HCl was subsequently added. DNase-positive colonies were observed in the transparent regions surrounding the bacterial colonies after removing excess acid with a vacuum pipette. Inoculates were maintained in trypticase-treated soy agar pending for additional analysis [14].

For the tube coagulase test, colonies of each isolate were added to 2 mL of citrated human plasma in a sterile test tube. At 35 ^{O} C, the containers were incubated for 4 hrs. and clot formation was observed [14].

Antibiotic sensitivity testing

Using the Kirby-Bauer agar disc diffusion method as specified by the Clinical Laboratory Standards Institute (CLSI), antimicrobial sensitivity was ascertained. Antibiotic discs (supplied from Oxoid, England) containing the subsequent drug concentrations were utilized: Sulphamethoxazole + Trimethoprim (SXT, 1.25/23.75µg), oxacillin (1 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), gentamycin (10 µg), imipenem (10 ug), ampicillin/sulbactam (20 µg), piperacillin (100 µg), amoxicillin/ clavulanic acid (30 µg), ceftriaxone (30 ug), linezolid (30 µg), tetracycline (30 µg), Piperacillin -Tazobactam (30/6 ug) & vancomycin (30 µg). Antibiotic discs were applied onto Muller-Hinton agar (Himedia). After 24 hrs. of aerobic incubation at 37 °C, the inhibition zone diameter (in millimeters) on the plates were examined. The interpretation of the results followed CLSI breakpoints [15].

Detection of MRSA isolates

Kirby-Bauer agar disc diffusion method using cefoxitin disc (30 ug) was used to detect MRSA isolates. Measuring the inhibition zone diameter around cefoxitin disc was based on the CLSI guidelines [15].

Detection of toxin encoding genes *sea, hla* and *pvl* genes by conventional polymerase chain reaction (PCR)

DNA extraction: the QIAamp DNA Mini reagent (Qiagen, Germany, GmbH) was utilized. A volume of 200µl of the sample suspension was incubated with 200µl of lysate buffer and 200µl of proteinase K at 56^{o}C for a duration of 10 minutes. 200 µl of 100% ethanol were added to the lysate after it had been incubated. Following that, the sample underwent centrifugation and rinsing as directed by the manufacturer. To elute nucleic acid, 100 µl of the elution buffer included in the kit was utilized.

PCR amplification:

As specified in **Table 1**, Metabion (Germany) supplied the primers. A 25-µl reaction was carried out utilizing the subsequent components: 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 5 µl of DNA template, 5.5 µl of water, and 1 µl of each primer with a concentration of 20 pmol. The experimental procedure was carried out using an Applied Biosystem 2720 thermal cycler. The first stage, denaturation, was conducted at 94°C for a duration of 5 minutes.

Subsequent PCR cycles (35 cycles) commence with a discrete denaturation phase lasting 30 seconds at 94°C, following the initial denaturation. Following this, the primers were annealed for 40 seconds at 60°C. The extension of the primers was then completed for 45 sec at 72°C. the final extension step was obtained for 10 min after at 72°C.

Analysis of the PCR products:

At ambient temperature, PCR products were separated by means of electrophoresis on a 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer, employing gradients of 5V/cm. To perform gel analysis, 30 µl of the multiplex products and 20 µl of the uniplex products were loaded into each gel position. The determination of fragment size was performed using a gene ruler 100 bp ladder (Fermentas, Thermofisher, Germany). The gel was photographed using a gel documentation system (Alpha Innotech, Biometra), and the data were analyzed using computer software (automated image capture protein simple, formerly cell bioscience, USA) **(Figure 1)**.

Statistical methods

Utilizing Microsoft Office Excel 2007 & IBM SPSS version 22.0, the gathered data was analyzed. Bonferroni test was used for each pairwise comparison adjustment. We used the Chi-square test and Fisher's exact test to compare qualitative data presented as frequency (%). A P-value ≤0 0.05 was considered statistically significant.

Results

Ninety-four *S. aureus* isolates were collected from diverse wards and different clinical samples. The majority of isolates were collected from general medicine and surgery wards 48.9% (46 isolates) followed by ICU 44.7% (42 isolates). Fewer numbers were collected from outpatient clinics 6.4% (6 isolates). Isolates were collected from blood 39.4% (37 isolates), pus 24.5% (23 isolates), sputum 19.1% (18 isolates) and wound swabs 17.0% (16 isolates). The prevalence of MRSA among isolates was 78.7% (74 isolates) whereas MSSA detected only in 21.3% (20 isolates). It was revealed that ICU was significantly more frequent in MRSA (40/42) while outpatient-clinics were significantly more frequent in MSSA (6/6). General medicine and surgery wards were non-significantly more frequent in MSSA. Only sputum samples were significantly more frequent in MRSA (18/18) **(Table 2)**.

Antimicrobial susceptibility testing revealed that *S. aureus* isolates (94 isolates) had the highest resistance towards penicillin, ampicillin, piperacillin and oxacillin with resistance rates of (100.0%, 93.6%, 84.0% and 81.9% respectively). All other beta lactams showed resistance rate (78.7%). Moderate resistance was detected towards erythromycin, levofloxacin, tetracycline, gentamycin, trimethoprim-sulfamethoxazole (58.5%, 44.7%, 39.4%, 36.2%, 34% respectively) A lower resistance rate was also detected against moxifloxacin and clindamycin (6.4%, 18.3% respectively). No resistance was detected towards vancomycin, linezolid and doxycycline **(Figure 2)**.

By comparing resistance pattern among MRSA and MSSA, all MRSA isolates had higher resistance to all tested antibiotics than MSSA isolates with high statistical significance except for moxifloxacin and clindamycin **(Table 3)**.

PCR analysis of the 94 clinical *S. aureus* isolates revealed high prevalence of both *sea* and *hla* genes 93.6% (88 isolates) while *pvl* gene presents in 86.2% (81 isolates). Moreover, 79.8% (75 isolates) have the three genes together. The prevalence of *hla* gene and *pvl* gene were significantly more frequent in MRSA. *Sea* gene was non significantly more frequent in MSSA isolates **(Table 4)**.

Isolates with positive *pvl* and *hla* genes had significant more frequent distribution among ICU isolates (51.9%, 47.7%) and isolates obtained from general medicine and surgery wards (48.1%, 52.3%) respectively. However, isolates with positive *sea* gene had non-significant distribution among different departments **(Table 5)**.

Isolates with positive *pvl*, *sea*, *hla* genes had significant more frequent distribution among blood samples (45.7%, 42.0%, 42.0%) and significant low frequent distribution among wound samples (9.9%, 13.6%, 11.4%) respectively. However, there is no statistical significance regarding gene presence in isolates obtained from pus or sputum samples **(Table 5)**.

Regarding antibiotic resistance in relation to different genes, isolates with positive *hla*, *pvl* genes had significant more frequent resistance to most antibiotics; oxacillin (87.5%, 86.4%), ceftriaxone, cefoxitin, imipenem, ampicillin-sulbactam, amoxicillin-clavulanate, piperacillin-tazobactam, and piperacillin (84.1%, 82.7%), erythromycin (62.5%, 63.0%), ciprofloxacin and levofloxacin (47.7%, 51.9%) respectively. In addition, isolates with positive *pvl* gene had significant more frequent resistance to gentamycin (42.0%) and trimethoprim-sulfamethoxazole (39.5%). Isolates with positive *sea* gene showed non-significant less frequent resistance to all antibiotics except tetracycline, gentamycin and trimethoprim-sulfamethoxazole **(Table 6)**.

Table 1. Target genes, primers sequences, amplicon sizes and cycling conditions

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
Sea	F:5′-TTGGAAACGGTTAAAACGAA-3′	120	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	Mohammed et al. [16]
	R:5′-GAACCTTCCCATCAAAAACA-3′							
hla	F:5′-GAAGTCTGGTGAAAACCCTGA-3′	704 bp	94˚C 5 min.	94˚C 30 sec.	53˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Fei et al. [17]
	R: 5′-TGAATCCTGTCGCTAATGCC-3′							
pvl	F:5′-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A-3′	433	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Park et al. [18]
	R:5′-GCA TCA AST GTA TTG GAT AGC AAA AGC-3′							

Table 2. Distribution of MRSA and MSSA isolates among different departments and clinical samples included in the study

Sample source		All isolates (N=94)	Organism		*p*-value
			MRSA (N=74)	MSSA (N=20)	
Depart-ment	ICU	42 (44.7%)	40 (54.1%)a	2 (10.0%)b	§<0.001*
	General medicine and surgery wards	46 (48.9%)	34 (45.9%)a	12 (60.0%)a	
	Outpatient clinic	6 (6.4%)	0 (0.0%)a	6 (30.0%)b	
Type of the sample	Blood	37 (39.4%)	26 (35.1%)a	11 (55.0%)a	§0.011*
	Pus	23 (24.5%)	20 (27.0%)a	3 (15.0%)a	
	Sputum	18 (19.1%)	18 (24.3%)a	0 (0.0%)b	
	Wound swabs	16 (17.0%)	10 (13.5%)a	6 (30.0%)a	

Table 3. Comparison between MRSA and MSSA isolates regarding antibiotic resistance

Antibiotic resistance	Isolates		p-value
	MRSA (N=74)	MSSA (N=20)	
Penicillin	74 (100.0%)	20 (100.0%)	NA
Amoxicillin-clavulanate	74 (100.0%)	0 (0.0%)	**§<0.001***
Piperacillin-Tazobactam	74 (100.0%)	0 (0.0%)	**§<0.001***
Piperacillin	74 (100.0%)	5 (25.0%)	**§<0.001***
Ceftriaxone	74 (100.0%)	0 (0.0%)	**§<0.001***
Cefoxitin	74 (100.0%)	0 (0.0%)	**§<0.001***
Imipenem	74 (100.0%)	0 (0.0%)	**§<0.001***
Oxacillin	74 (100.0%)	3 (15.0%)	**§<0.001***
Ampicillin-sulbactam	74 (100.0%)	0 (0.0%)	**§<0.001***
Erythromycin	48 (64.9%)	7 (35.0%)	**#0.016***
Tetracycline	36 (48.6%)	1 (5.0%)	**#<0.001***
Gentamycin	34 (45.9%)	0 (0.0%)	**#<0.001***
Ciprofloxacin	42 (56.8%)	0 (0.0%)	**#<0.001***
Levofloxacin	42 (56.8%)	0 (0.0%)	**#<0.001***
Moxifloxacin	6 (8.1%)	0 (0.0%)	§0.336
Trimethoprim-sulfamethoxazole	32 (43.2%)	0 (0.0%)	**#<0.001***
Clindamycin	13 (17.6%)	0 (0.0%)	§0.063
Vancomycin	0 (0.0%)	0 (0.0%)	NA
Linezolid	0 (0.0%)	0 (0.0%)	NA
Doxycycline	0 (0.0%)	0 (0.0%)	NA
Ampicillin	74 (100.0%)	14 (70.0%)	**§<0.001***

Table 4. Prevalence of toxin encoding genes among different isolates

Variables	All isolates (N=94)	Organism		p-value
		MRSA (N=74)	MSSA (N=20)	
***sea* gene**	88 (93.6%)	68 (91.9%)	20 (100.0%)	§0.336
***hla* gene**	88 (93.6%)	74 (100.0%)	14 (70.0%)	**§<0.001***
***pvl* gene**	81 (86.2%)	67 (90.5%)	14 (70.0%)	**§0.029***
***sea* and *hla* genes**	7 (7.4%)	7 (9.5%)	0 (0.0%)	§0.339
***sea* and *pvl* genes**	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
***hla* and *pvl* genes**	6 (6.4%)	6 (8.1%)	0 (0.0%)	§0.336
***sea, hla* and *pvl* genes**	75 (79.8%)	61 (82.4%)	14 (70.0%)	§0.225

Data presented as number (%). §Fishers Exact test.

Table 5. Distribution of *sea, hla* and *pvl* genes among different departments and samples

Source		*sea* gene			*hla* gene			*pvl* gene		
		Positive (N=88)	**Negative (N=6)**	**p-value**	**Positive (N=88)**	**Negative (N=6)**	**p-value**	**Positive (N=81)**	**Negative (N=13)**	**p-value**
Department	**ICU**	42 (47.7%)	0 (0.0%)	§0.052	42 (47.7%)a	0 (0.0%)b	**§<0.001***	42 (51.9%)a	0 (0.0%)b	**#<0.001***
	General medicine and surgery wards	40 (45.5%)	6 (100.0%)		46 (52.3%)a	0 (0.0%)b		39 (48.1%)a	7 (53.8%)a	
	Outpatient clinics	6 (6.8%)	0 (0.0%)		0 (0.0%)a	6 (100.0%)b		0 (0.0%)a	6 (46.2%)b	
Type of the sample	**Blood**	37 (42.0%)a	0 (0.0%)b	**§0.003***	37 (42.0%)a	0 (0.0%)b	**§<0.001***	37 (45.7%)a	0 (0.0%)b	**§<0.001***
	Pus	21 (23.9%)a	2 (33.3%)a		23 (26.1%)a	0 (0.0%)a		18 (22.2%)a	5 (38.5%)a	
	Sputum	18 (20.5%)a	0 (0.0%)a		18 (20.5%)a	0 (0.0%)a		18 (22.2%)a	0 (0.0%)a	
	Wound smear	12 (13.6%)a	4 (66.7%)b		10 (11.4%)a	6 (100.0%)b		8 (9.9%)a	8 (61.5%)b	

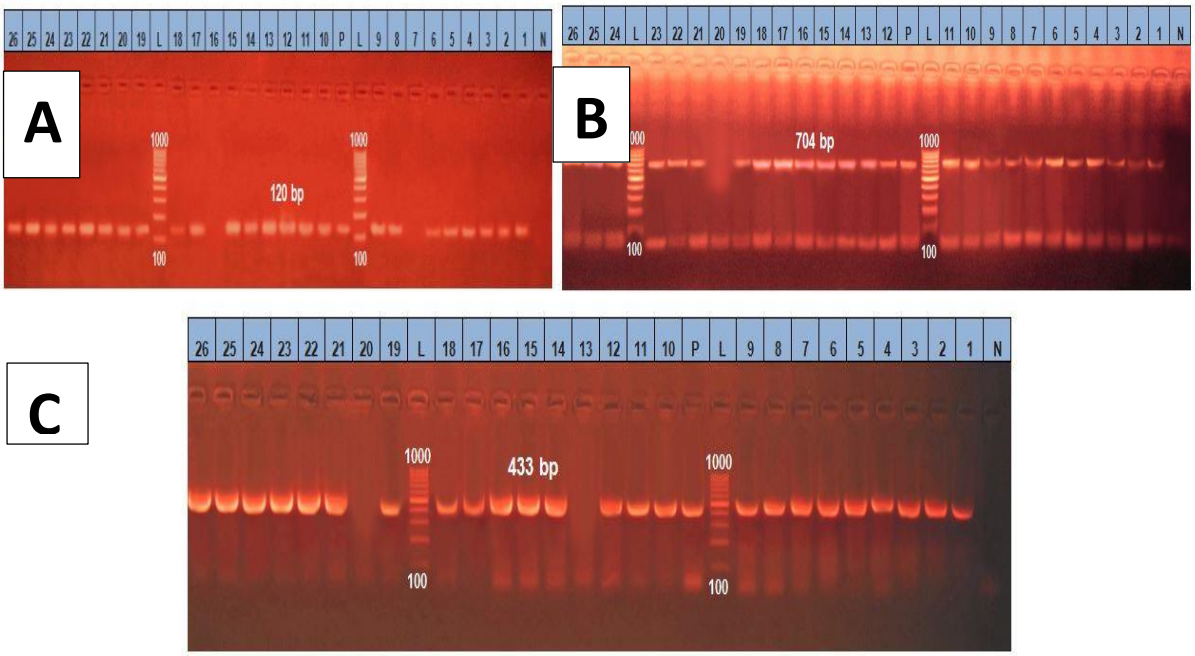
Data presented as number. §Fishers Exact test. *Significant. Homogenous groups had the same symbol "a,b".

Table 6. Prevalence of antibiotic resistance *among S. aureus* isolates in relation to presence or absence of *sea, hla* and *pvl* genes

Antibiotic resistance	*sea* gene			*hla* gene			*pvl* gene		
	Positive (N=88)	**Negative (N=6)**	**p-value**	**Positive (N=88)**	**Negative (N=6)**	**p-value**	**Positive (N=81)**	**Negative (N=13)**	**p-value**
Ceftriaxone	68 (77.3%)	6 (100.0%)	§0.336	74 (84.1%)	0 (0.0%)	**§<0.001***	67 (82.7%)	7 (53.8%)	**§0.029***
Cefoxitin	68 (77.3%)	6 (100.0%)	§0.336	74 (84.1%)	0 (0.0%)	**§<0.001***	67 (82.7%)	7 (53.8%)	**§0.029***
Imipenem	68 (77.3%)	6 (100.0%)	§0.336	74 (84.1%)	0 (0.0%)	**§<0.001***	67 (82.7%)	7 (53.8%)	**§0.029***
Erythromycin	51 (58.0%)	4 (66.7%)	§0.999	55 (62.5%)	0 (0.0%)	**§0.004***	51 (63.0%)	4 (30.8%)	**#0.029***
Tetracycline	37 (42.0%)	0 (0.0%)	§0.078	37 (42.0%)	0 (0.0%)	§0.078	33 (40.7%)	4 (30.8%)	#0.459
Oxacillin	71 (80.7%)	6 (100.0%)	§0.587	77 (87.5%)	0 (0.0%)	**§<0.001***	70 (86.4%)	7 (53.8%)	**§0.012***
Ampicillin-sulbactam	68 (77.3%)	6 (100.0%)	§0.336	74 (84.1%)	0 (0.0%)	**§<0.001***	67 (82.7%)	7 (53.8%)	**§0.029***
Gentamycin	32 (36.4%)	2 (33.3%)	§0.999	34 (38.6%)	0 (0.0%)	§0.084	34 (42.0%)	0 (0.0%)	**§0.003***
Ciprofloxacin	38 (43.2%)	4 (66.7%)	§0.402	42 (47.7%)	0 (0.0%)	**§0.031***	42 (51.9%)	0 (0.0%)	**#<0.001***
Levofloxacin	38 (43.2%)	4 (66.7%)	§0.402	42 (47.7%)	0 (0.0%)	**§0.031***	42 (51.9%)	0 (0.0%)	**#<0.001***
Moxifloxacin	6 (6.8%)	0 (0.0%)	§0.999	6 (6.8%)	0 (0.0%)	§0.999	6 (7.4%)	0 (0.0%)	§0.591
Trimethoprim-sulfamethoxazole	30 (34.1%)	2 (33.3%)	§0.999	32 (36.4%)	0 (0.0%)	§0.092	32 (39.5%)	0 (0.0%)	**§0.004***
Clindamycin	13 (14.8%)	0 (0.0%)	§0.591	13 (14.8%)	0 (0.0%)	§0.591	13 (16.0%)	0 (0.0%)	§0.202
Amoxicillin-clavulanate	68 (77.3%)	6 (100.0%)	§0.336	74 (84.1%)	0 (0.0%)	**§<0.001***	67 (82.7%)	7 (53.8%)	**§0.029***
Piperacillin-Tazobactam	68 (77.3%)	6 (100.0%)	§0.336	74 (84.1%)	0 (0.0%)	**§<0.001***	67 (82.7%)	7 (53.8%)	**§0.029***
Piperacillin	68 (77.3%)	6 (100.0%)	§0.336	74 (84.1%)	0 (0.0%)	**§<0.001***	67 (82.7%)	7 (53.8%)	**§0.029***
Ampicillin	82 (93.2%)	6 (100.0%)	§0.999	82 (93.2%)	6 (100.0%)	§0.999	75 (92.6%)	13 (100.0%)	§0.591

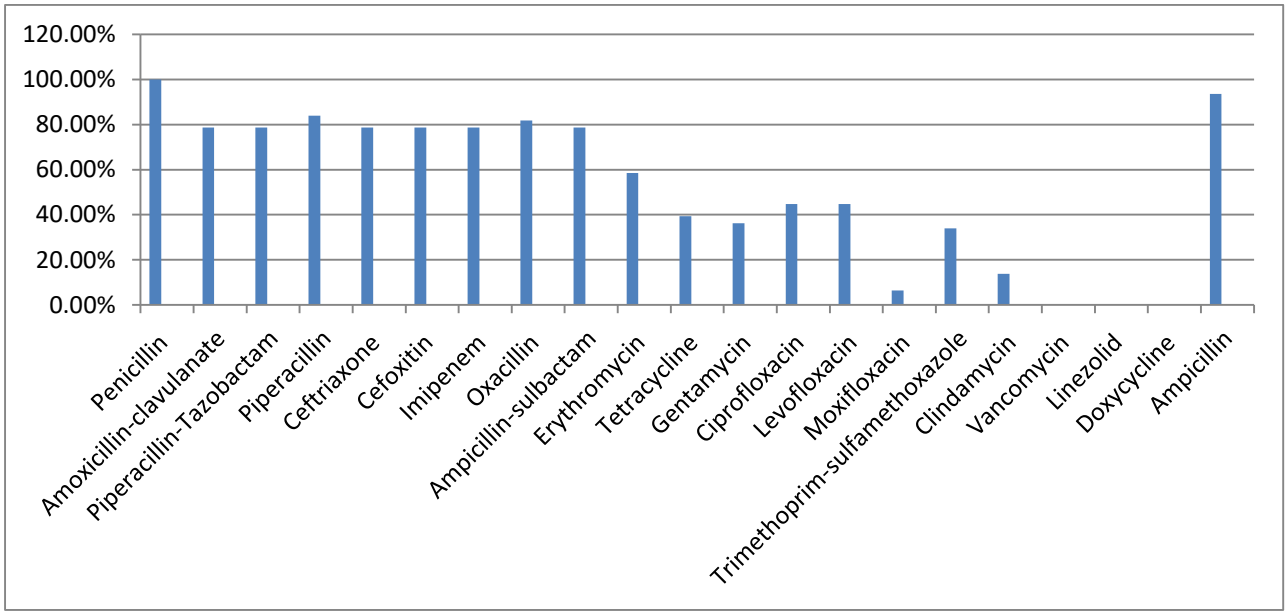
Data presented as number (%). #Chi square test. §Fishers Exact test. *Significant.

Figure 1. Detection of amplification product of different toxin encoding genes



A. *sea* gene by PCR; lane N: negative control, lane P: positive control and all lanes are positive PCR products (120 bp) except lane 7&16.
B. *hla* gene by PCR; lane P: positive control, lane N: negative control and all lanes are positive PCR products (704 bp) except lane 20.
C. *pvl* gene by PCR; lane P: positive control, lane N: negative control and all lanes are positive PCR products (433 bp) except lane 13&20.

Figure 2. Prevalence of antibiotic resistance among the studied isolates



Discussion

Virulence determinants and antimicrobial resistance among *S. aureus* clinical isolates are of great concern. *S. aureus* generates a diverse array of toxins, which significantly contribute to its pathogenic nature. The current study included 94 non-repeated *S. aureus* clinical isolates collected from different clinical samples and different departments at ASU hospital. The prevalence of MRSA detected by cefoxitin disc was high, 78.7%. This result was near that reported in Assiut (73%)

and higher than that reported in different areas in Egypt; Cairo and Zagazig (67% for each), Alexandria, Mansoura and Tanta (61%, 59% and 40%, respectively) [19]. A higher prevalence of MRSA isolates (81.2%) was revealed in two different tertiary hospitals in Cairo [20]. The difference in percentage of MRSA between studies may be due to different methods used to detect MRSA isolates (oxacillin discs, which is well-known to be less specific or *mec A* gene detection which has higher sensitivity). Moreover, different sample types may affect prevalence of MRSA as the invasive *S. aureus* isolates thought to have more resistance than non-invasive isolates.

Most MRSA isolates in this study were obtained from ICU 54.1% followed by general medicine and surgery wards 45.9% and ICU was significantly more frequent in MRSA *(p*-value <0.001). However, all isolates obtained from out-patient clinics were MSSA. The highest frequency of MRSA isolates was obtained from blood (35.1%) followed by pus (27%) then sputum and wound swabs (24.3%, 13.5%) respectively. This result was against many previous studies in Egypt, Palestine and Pakistan who reported more isolation of MRSA from wound infections followed by bloodstream infections then respiratory tract infections [20-22]. Another study from Alexandria tertiary hospital reported that highest frequency of MRSA isolates was from pus sample (60%) followed by wound swabs, blood, and sputum (13.3%, 6.7%, 3.3%) respectively [23]. In this study, results revealed that blood stream infections were the main sources of MRSA isolates and this may be due to high number of isolates obtained from ICU. The high percentage of MRSA isolates in ICU is alarm about increasing drug resistance among critical patients.

S. aureus isolates in this study showed high resistance to nearly all used antimicrobial agents with highest resistance rate among MRSA isolates. The highest resistance (100%) was reported against penicillin. Resistance rate was high among all tested beta lactams (resistance rate ranged between 93.6% and 78.7%) followed by erythromycin (58.5%), ciprofloxacin and levofloxacin (44.7% each), tetracycline, gentamycin and trimethoprim-sulfamethoxazole (39.4%, 36.2%, 34% respectively). These results are slightly different from that reported by other studies in Egypt and worldwide. In Egypt, a study conducted in Minia University Hospital, reported lower resistance rate except for piperacillin (100%) where 91.5%, 62.7%, 35.6% 33.5% of isolates were resistant to amoxicillin-clavulanate, tetracycline, ciprofloxacin, gentamycin respectively [24]. Another study conducted in Gaza strip in Palestine reported lower rates of resistance where 96.1%, 69.5% of isolates were resistant to penicillin and cefoxitin while resistance to amoxicillin-clavulanate, erythromycin, tetracycline and ciprofloxacin was 73.6%, 47.7%, 27.2%, 26.2% respectively. However, they reported that MRSA isolates had higher resistance rate than MSSA isolates [25]. A study conducted in Nepal reported lower resistance rate (91.6%, 13.5%) towards penicillin, gentamycin and higher resistance rate (63.2%, 59.1%, 39.5%) towards ciprofloxacin, erythromycin, and trimethoprim-sulfamethoxazole respectively [26]. Higher resistance rate to gentamycin 90%, 49.4%, 46.8%, 52% or 54.5% was reported by different studies [20, 23, 27-29]. While, lower resistance rates to gentamicin 21.9%, 25.5% and 32.9% was reported in other studies [30-32]. Very high resistance rate to beta lactams and to lesser extent erythromycin is usually due to unrestricted and over the counter consumption of these antibiotics leading to mutation of drug target sites. So, they are no longer effective in treating various infections. Higher rates of resistance to gentamycin, ciprofloxacin and tetracycline may be due to the frequent use of these antibiotics in ASU hospitals.

The least resistance revealed in this study was against clindamycin (18.3%), moxifloxacin (6.4%), vancomycin, linezolid and doxycycline (0.00% each). Near result detected by Monecke et al. in Alexandria [23] who stated that all *S. aureus* isolates were susceptible to vancomycin while Rasmi et al. in Minia [24] reported 13.5% resistance to vancomycin. In contrary to our results, lower resistance rate towards clindamycin (10.9%) and higher resistance (11.2%) towards vancomycin was detected in Gaza strip hospitals [25]. Higher resistance rate (39.9%, 28.8%) towards clindamycin and doxycycline was reported in Nepal [26]. Difference in antimicrobial resistance between studies confirm that resistance pattern differ between various countries and even between different hospitals in the same country. However, all studies agreed that *S. aureus* has the highest rate of resistance towards beta lactams. The highest sensitivity to vancomycin, linezolid and doxycycline even among MRSA in this study may be due to infrequent use of these antibiotics owing to their unavailability to public population, high

prices, and their toxic side effects. However, regular monitoring of vancomycin, linezolid and doxycycline resistance should be performed.

The prevalence of toxin encoding genes in this study was high in comparison to other studies. *Sea* gene was found in 93.6% as well as *hla* genes were found in 93.6% of isolates while *pvl* gene was found in 86.2% of isolates. Moreover, 79.8% of isolates have the three genes together. Presence of these three genes in the same isolate increase the virulence of the strains and clinical manifestations related to them. In addition, prevalence of *hla*, *pvl* genes were significantly more frequent in MRSA (100%, 90.5%) respectively vs (70%) in MSSA which render treatment of these strains more difficult. However, prevalence of *sea* gene was more frequent in MSSA (100%) vs (91.9%) in MRSA isolates. In Egypt, lower prevalence of *sea* and *hla* genes were detected in isolates from infected wounds (72.9%, 37% respectively) with no difference between MRSA and MSSA isolate [24]. Another studies reported *sea* and *hla* genes in only 32.6% and 30.5% of isolates from different clinical samples [33, 34]. In contrary to the present study, different studies all over the world reported a lower prevalence of *pvl* gene among MRSA isolates (30.5% in Palestine, 28% in Australia, 27.7% in Cyprus, 25% in South Africa and 13.5% in Japan) [25, 35-38]. On the other hand, Alli et al. and Khosravi et al. reported higher prevalence of the *pvl* gene in MSSA (53.3%, 33.5%) than in MRSA isolates (9.1%, 7.23%) [39, 40]. Hou et al. reported higher prevalence of *sea*, *pvl* genes among MRSA, MSSA respectively [41]. This indicates that the prevalence of toxin encoding genes differs between countries, even among diverse location in the same country and between various isolates. The results of the present study denote that prevalence of such genes show tendency to increase in Egypt with time especially among MRSA isolates. Although *sea* gene is frequently detected in foodborne MRSA strains and associated with food poisoning outbreaks [42], it is also detected in MSSA strains in high rate. MSSA strains may have high virulence despite of its low antimicrobial resistance pattern which necessitates early diagnosis and treatment of MSSA infections.

Isolates with positive *pvl*, *hla* genes had significant more frequent distribution in ICU (51.9%, 47.7%) and general ward (48.1%, 52.3%) while both genes were absent in isolates collected from outpatient clinics which confirm aggressive behavior of these isolates. Non-significant distribution among different departments was reported regarding isolates having *sea* gene. To our knowledge, one study compared prevalence of toxin genes (*hla* and *pvl*) in ICU, non-ICU and out-patient clinics. They reported non-significant high prevalence rate in ICU (44.1%, 0%) followed by non-ICU (40.8%, 1.4%) and out-patient clinics (15.4%,0%). Their results were agreed with the current study in high *hla* prevalence but antagonize current results concerning *pvl* [43]. Although *pvl* is known as a marker for community acquired MRSA [44], it showed high prevalence in ICU and general wards which suggests increasing community acquired strains in ASU hospital.

Moreover, isolates with positive *pvl*, *sea* or *hla* genes had significant more frequent distribution among blood samples (45.7%, 42.0%, 42.0%) and significant low frequent distribution among wound samples (9.9%, 13.6%, 11.4%). A different result was reported by Rasmi et al. who reported that *sea* gene (72.9%) followed by *hla* gene (37%) was predominant in isolates from wound samples [24]. In addition, numerous studies reported different predominance of *pvl* gene in different samples. Holmes et al. and Bhatta et al. stated that *pvl* gene was higher in pus samples from SSTIs followed by blood, urine, or sputum samples [45, 46]. El Aila et al. reported that the prevalence of the *pvl* gene among sputum, blood, and urine isolates was 60%, 30%, and 11%, respectively [25]. Another study claimed that wound specimens (30.4%) followed by urine specimens (27.6%) were *pvl* positive [47]. Singh-Moodley et al. reported *pvl* positivity in only 25% of MRSA isolates from blood cultures which was lower than the prevalence in our study [37].

Presence of strains carrying such genes more frequently in the ICU and blood samples denoted that these strains are highly virulent and may carry many other variants of toxin encoding genes. On the other hand, low prevalence of genes in isolates from out-patient clinics and wound swabs confirm that isolates causing mild superficial infections are low virulent.

By comparing between isolates with positive and negative genes regarding antibiotic resistance, results showed that isolates with positive *hla*, *pvl* genes had more resistance to all antibiotics except ampicillin and they had significant more frequent resistance to oxacillin followed by other beta lactams, erythromycin, ciprofloxacin and

levofloxacin. Moreover, isolates with positive *pvl* genes had significant more frequent resistance to gentamycin and trimethoprim-sulfamethoxazole. Isolates with positive *sea* gene had lower resistance rate to nearly all antibiotics. However, this difference statistically not significant. Results of this study support what reported by El Aila et al. and Bhatta et al. who stated that *pvl* positive isolates showed more resistance towards amoxicillin-clavulanic acid, ciprofloxacin, tetracycline and vancomycin [25] and towards erythromycin [46]. In addition, Rasmi et al. reported that isolates with positive *sea* or *hla* gene showed more resistance to piperacillin, gentamycin, cirofloxacin and levofloxacin which is matching with our result regarding *hla* gene and antagonize our results regarding *sea* gene [24]. The difference concerning *sea* gene may be owing to that all *sea* negative isolates in our study were MRSA which carried high resistance pattern. Isolates with *pvl* or *hla* gene are associated with resistance to a large scale of antibiotics more than isolates carrying *sea* gene which limits treatment options of such virulent strains. However, our results support the previous studies which incriminated that antimicrobial resistance and virulence genes are located in close proximity to each other [48].

Conclusion

This study reveals a high prevalence of MRSA isolates that resist most antimicrobials and carry multiple toxin encoding genes in ASU tertiary hospital especially in the ICU which is alarming. An inevitable vigorous action must be considered to limit spread of such isolates and ensure introduction of new policies for antimicrobial usage and infection control measures. Vancomycin, linezolid and doxycycline conserve their susceptibility towards MRSA isolates. Positive *pvl* isolates had the highest rate of resistance to antibiotics followed by *hla* positive isolates. Presence of either gene may indicate increased rate of resistance to antimicrobial agents. MSSA isolates may be highly virulent and carry more than one toxin encoding gene.

Recommendation

MRSA isolates must be continuously monitored and characterized in hospitals in order to facilitate the implementation of enhanced infection control measures and prevent the spread of virulent nosocomial infections. Staphylococcal infections should not be treated with β-lactam antibiotics as a primary course of empirical treatment. It is essential to screen clinical isolates for virulence genes in order to identify patients who are carrying virulent bacterial strains, thereby facilitating their isolation in hospitals.

Limitations

Limited resources of our study are enforced us to minimize sample size 94 isolates from a solitary hospital and select only three toxin encoding genes, therefore does not denote the overall prevalence of virulence strain in the region. Further investigations utilizing more samples obtained from patients admitted to various hospitals nationwide are required in order to ascertain the prevalence and distribution of virulent strains on a national scale.

Conflict of interest: None

Funding: None

References

1- Zhu Z, Hu Z, Li S, Fang R, Ono HK, Hu D. Molecular Characteristics and Pathogenicity of Staphylococcus aureus Exotoxins. Int. J. Mol. Sci 2024; 25(1): 395.

2- Van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB. Predictors of mortality in Staphylococcus aureus Bacteremia. Clin Microbiol Rev 2012; 25(2): 362–386.

3- McCaig LF, McDonald LC, Mandal S, Jernigan DB. Staphylococcus aureus-associated skin and soft tissue infections in ambulatory care. Emerg Infect Dis 2006; 12(11): 1715–1723.

4- Tam, K, & Torres VJ. Staphylococcus aureus secreted toxins and extracellular enzymes. Microbiol. Spectr 2019; 7(2): 10.

5- Wang X, Lin D, Huang Z, Zhang J, Xie W, Liu P, Jing H, Wang J. Clonality, virulence genes and antibiotic resistance of Staphylococcus aureus isolated from blood in Shandong, China. BMC Microbiology 2021; 21(1): 281.

6- Moraveji Z, Tabatabaei M, ShirzadAski H, Khoshbakht R. Characterization of hemolysins of Staphylococcus strains isolated from human and bovine, southern Iran. Iran J Vet Res 2014; 15(4): 326–330.

7- Lynch L, Shrotri M, Brown CS, Thorn Heathcock R. Is decolonization to prevent Panton–Valentine leukocidin-positive Staphylococcus aureus infection in the population effective? A systematic review. J Hosp Infect 2022; 121: 91-104.

8- Olaniyi RO, Pancotto L, Grimaldi L, Bagnoli F. Deciphering the pathological role of staphylococcal α-toxin and Panton-Valentine Leukocidin using a novel ex vivo human skin model. Front Immunol 2018; 9: 951.

9- Strau ßL, Stegger M, Akpaka PE, Alabi A, Breurec S, Coombs G, et al. Origin, evolution, and global transmission of community-acquired Staphylococcus aureus ST8. Proc Natl Acad Sci USA 2017; 114(49): E10596–E10604.

10- Boyle-Vavra S, & Daum RS. Molecular strategies of Staphylococcus aureus for resisting antibiotics. Caister Academic Press U.K 2016; 249–300.

11- Jaradat ZW, Ababneh QO, Sha'aban ST, Alkofahi AA, Assaleh D, Al Shara A. Methicillin Resistant Staphylococcus aureus and public fomites: a review. Pathog Glob Health 2020; 114(8): 426-450.

12- Urushibara N, Aung MS, Kawaguchiya M, Kobayashi N. Novel staphylococcal cassette chromosome mec (SCCmec) type XIV (5A) and a truncated SCCmec element in SCC composite islands carrying speG in ST5 MRSA in Japan. J Antimicrob Chemother 2020; 75(1) :46–50.

13- Otto M. MRSA virulence and spread. Cell Microbiol 2012; 14(10): 1513-21.

14- Kateete DP, Kimani CN, Katabazi FA, Okeng Okee MS, Nanteza A, Joloba ML, et al. Identification of Staphylococcus aureus: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Ann. Clin. Microbiol. Antimicrob 2010; 13(9): 23.

15- Clinical and Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing. CLSI 2023, Wayne, PA, USA.

16- Mohammed TA, Khalid AE, Saadabi AM. PCR Detection of Staphylococcal Enterotoxin A and B Genes in Staphylococcus aureus Isolated from Salted Fermented Fish. J Microbiol 2014; 4 (2): 51-56.

17- Fei W, Hongjun Y, Hong-bin H, Changfa W, Yundong G, Qifeng Z, et al. Study on the hemolysin phenotype and the genetype distribution of staphylococcus aureus caused bovine mastitis in Shandong dairy farms. Intern J Appl Res Vet Med 2011; 9(4).

18- Park HK, Woo SY, Jung YJ, Lee EO, Cha JE, Park HS, et al. Detection of Virulence Genes of Staphyloccus aureus and Staphylococcus epidermidis isolated from suprapubic urine from infants with fever. JBV 2008; 38(4): 189–196.

19- Azzam A, Khaled H, Mosa M, Refaey N, AlSaifi M, Elsisi S, et al. Epidemiology of clinically isolated methicillin-resistant Staphylococcus aureus (MRSA) and its susceptibility to linezolid and vancomycin in Egypt: a systematic review with meta-analysis. BMC Infect Dis 2023; 23(1): 263.

20- Alfeky AE, Tawfick MM, Ashour MS, El-Moghazy AA. High Prevalence of Multi-drug Resistant Methicillin-resistant Staphylococcus aureus in Tertiary Egyptian Hospitals. J Infect Dev Ctries 2022; 16(5): 795-806.

21- Hadyeh E, Azmi K, Seir RA, Abdellatief I, Abdeen Z. Molecular characterization of methicillin resistant Staphylococcus aureus in West Bank-Palestine. Front Public Health 2019; (7): 130.

22- Akram A, Izhar M, La C, Ghaffar H, Zafar S, Saifullah A, et al. Frequency of panton valentine leucocidin gene in Staphylococcus aureus from skin and soft tissue infections. J Ayub Med Coll Abbottabad 2020; 32(4): 487–491.

23- Monecke S, Bedewy AK, Müller E, Braun SD, Diezel C, Elsheredy A, et al. Characterisation of Methicillin-Resistant Staphylococcus aureus from Alexandria, Egypt. J Antibiot 2023; 12(1): 78.

24- Rasmi AH, Ahmed EF, Darwish AMA, Gad GFM. Virulence genes distributed among Staphylococcus aureus causing wound infections and their correlation to antibiotic resistance. BMC Infect Dis 2022; 22 (1): 652.

25- El Aila NA, Al Laham NA, Naas T. Prevalence of mec A and Panton-Valentine Leukocidin Genes in Staphylococcus aureus Clinical Isolates from Gaza Strip Hospitals. Microorganisms 2023; 11(5): 1155.

26- Adhikari P, Basyal D, Rai JR, Bharati L, Budthapa A, Gharti KP, et al. Prevalence, antimicrobial susceptibility pattern and multidrug resistance of methicillin-resistant Staphylococcus aureus isolated from clinical samples at a tertiary care teaching hospital: an observational, cross-sectional study from the Himalayan country, Nepal. BMJ Open 2023; 13: e067384.

27- Sapkota J, Sharma M, Jha B, Bhatt CP. Prevalence of Staphylococcus aureus isolated from clinical samples in a tertiary care hospital: A Descriptive cross-sectional study. J Nepal Med Assoc 2019; 57: 398–402.

28- Caboclo RM, Cavalcante FS, Iorio NL, Schuenck RP, Olendzki AN, Felix MJ, et al. Methicillin-resistant Staphylococcus aureus in Rio de Janeiro hospitals: Dissemination of the USA400/ST1 and USA800/ST5 SCCmec type IV and USA100/ST5 SCCmec type II lineages in a public institution and polyclonal presence in a private one. Am J Infect Control 2013; 41: 21–26.

29- Hassan R, Barwa R, El-Sokkary MM, Ashraf D. Virulence characteristics of methicillin resistant Staphylococcus aureus isolated from different clinical sources. N. Egypt J. Microbiol 2017; 48: 14–31.

30- Yu F, Liu Y, Lv J, Qi X, Lu C, Ding Y, et al. Antimicrobial susceptibility, virulence determinant carriage and molecular characteristics of Staphylococcus aureus isolates associated with skin and soft tissue infections. Braz J Infect Dis 2015; 19: 614–622.

31- Raut S, Bajracharya K, Adhikari J, Pant SS, Adhikari B. Prevalence of methicillin resistant Staphylococcus aureus in Lumbini Medical College and teaching hospital, Palpa, Western Nepal. BMC Res Notes 2017; 10: 187.

32- Naimi HM, Rasekh H, Noori AZ, Bahaduri MA. Determination of antimicrobial susceptibility patterns in Staphylococcus aureus strains recovered from patients at two main health facilities in Kabul, Afghanistan. BMC Infect Dis 2017; 17: 737.

33- Baz AA, Bakhiet EK, Abdul-Raouf U, Abdelkhalek A. Prevalence of enterotoxin genes (SEA to SEE) and antibacterial resistant pattern of Staphylococcus aureus isolated from clinical specimens in Assiut city of Egypt. Egypt J Med Hum Genet 2021; 22: 84.

34- El-baz R, Rizk DE, Barwa R, Hassan R. Virulence factors profile of Staphylococcus aureus isolated from different clinical sources. J Microbiol 2016; 43:126–44.

35- Dotel R, Sullivan M, Davis J, Newton P, Gilbert G. Molecular epidemiology of methicillin-resistant Staphylococcus aureus

isolates in New South Wales, Australia, 2012–2017. Infect Dis Heal 2019; 24(3): 134–40.

36- Amin D, Guler E, Baddal B. Prevalence of Panton-Valentine leukocidin in methicillin-resistant Staphylococcus aureus clinical isolates at a university hospital in Northern Cyprus: a pilot study. BMC Res Notes 2020; (13): 490.

37- Singh-Moodley A, Strasheim W, Mogokotleng R, Ismail H, Perovic O. Unconventional SCCmec types and low prevalence of the Panton-Valentine Leukocidin exotoxin in South African blood culture staphylococcus aureus surveillance isolates, 2013–2016. PLoS ONE 2019; 14(11): e0225726.

38- Funaki T, Yasuhara T, Kugawa S, Yamazaki Y, Sugano E, Nagakura Y, et al. SCCmec typing of PVL-positive community-acquired Staphylococcus aureus (CA-MRSA) at a Japanese hospital. Heliyon 2019; 5(3): e01415.

39- Alli O, Ogbolu DO, Shittu AO, Okorie AN, Akinola JO, Daniel JB. Association of virulence genes with mecA gene in Staphylococcus aureus isolates from Tertiary Hospitals in Nigeria. Indian J. Pathol. Microbiol 2015; 58: 464.

40- Khosravi AD, Hoveizavi H, Farshadzadeh Z. The prevalence of genes encoding leukocidins in Staphylococcus aureus strains resistant and sensitive to methicillin isolated from burn patients in Taleghani Hospital, Ahvaz, Iran. Burns 2012; 38: 247–251.

41- Hou Z, Xu B, Liu L, Yan R, Zhang J, Yin J, et al. Prevalence, drug resistance, molecular typing and comparative genomics analysis of MRSA strains from a tertiary A hospital in Shanxi Province, China. Front Microbiol 2023; 22(14): 1273397.

42- Lv G, Jiang R, Zhang H, Wang L, Li L., Gao W et al. Molecular characteristics of *Staphylococcus aureus* from food samples and food poisoning outbreaks in Shijiazhuang, China. Front. Microbiol 2021; (2): 652276.

43- Moazen J, Riyahi Zaniani F, Hallaj Asghar B. Characterization of Virulence Genes and Antibiotic Resistance of Methicillin-resistant Staphylococcus aureus (MRSA) and Methicillin-susceptible Staphylococcus aureus (MSSA) Isolates in Intensive Care Unit (ICU) and Non-ICU Wards. J Adv Immunopharmacol 2022; 2(2): e129037.

44- Hou Z, Liu L, Wei J, Xu B. Progress in the prevalence, classification and drug resistance mechanisms of methicillin-resistant Staphylococcus aureus. Infect. Drug Resist 2023; (16): 3271–3292.

45- Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. Staphylococcus aureus isolates carrying Panton-Valentine leucocidin genes in England and Wales: Frequency, characterization, and association with clinical disease. J. Clin.Microbiol 2005; (43): 2384–2390.

46- Bhatta DR, Cavaco LM, Nath G. Kumar K, Gaur A, Gokhale S. Association of Panton Valentine Leukocidin (PVL) genes with methicillin resistant Staphylococcus aureus (MRSA) in Western Nepal: a matter of concern for community infections (a hospital based prospective study). BMC Infect Dis 2016; (16): 199.

47- Kamarehei F, Ghaemi, E, Dadgar T. Prevalence of enterotoxin A and B genes in Staphylococcus aureus isolated from clinical samples and healthy carriers in Gorgan City, North of Iran. Indian J. Pathol. Microbiol 2013; (56): 265–268.

48- Kondo K, Kawano M, Sugai M. Distribution of Antimicrobial Resistance and Virulence Genes within the Prophage-Associated Regions in Nosocomial Pathogens. ASM Journal mSphere 2021; 6(4):10.1128.