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Original article

Comprehensive analysis of MRSA in ICU settings: Antibiogram, molecular characterization, risk assessment, and infection control strategies in Menoufia University Hospitals

Rasha Galal Mostafa[1], Mabrouk Mahmoud Ghonaim[1], Sahar Ali Mohamed Ali[1], Alyaa Ibrahim Eliwa*[1], Esraa El-Sayed El-Mahdy[1]

1. *Department of Medical Microbiology and Immunology, Faculty of Medicine, Menoufia University, Shebin al Kom, Egypt*

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ABSTRACT

Background: *Staphylococcus aureus*, particularly MRSA, poses significant challenges due to antibiotic resistance and multi-drug-resistant strains, complicating treatment in high-risk environments like ICUs. **Objective**: The study aimed to identify MRSA molecular types, assess prevalence among healthcare workers and patients, evaluate PVL gene presence, and evaluate infection control protocols' effectiveness in ICUs at Menoufia University Hospitals. **Methods**: A cross-sectional study was conducted from May 2022 to August 2023, involving 180 ICU patients and 69 HCWs. Clinical specimens were analyzed using microbiological and molecular techniques, including PCR for mecA and PVL gene detection, SCCmec typing, and PCR-RFLP of the coagulase gene. Antimicrobial susceptibility testing was performed using the Vitek-2 Compact system. Infection control compliance was evaluated through observational studies and risk assessments. **Results**: Results revealed 81 *Staphylococcus aureus* isolates (63 MRSA and 18 MSSA), with 17.5% of MRSA and 5.6% of MSSA strains harboring PVL genes. The Vitek-2 Compact system demonstrated high diagnostic accuracy (98.4% sensitivity, 94.4% specificity). MRSA strains exhibited high resistance to benzylpenicillin and fusidic acid but remained susceptible to tigecycline, linezolid, and teicoplanin. SCCmec typing showed that hospital-acquired MRSA (HA-MRSA) strains were mostly types I, II, and III, while community-acquired MRSA (CA-MRSA) strains were mainly types IV and V. CA-MRSA strains had a high PVL-positivity rate (71.4%), indicating increased virulence. Improved hand hygiene and personal protective equipment compliance were associated with reduced MRSA infection rates. **Conclusion:** Continuous surveillance and strict infection control measures are crucial for addressing MRSA prevalence, with Vitek-2 Compact system recommended for accurate detection and PVL screening.

Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium responsible for a wide range of infections, both in community and hospital settings. The rise of multi-drug-resistant strains like MRSA (Methicillin-Resistant *Staphylococcus aureus*) complicates treatment. MRSA contains the mecA gene, which provides resistance to multiple antibiotics by encoding the penicillin-binding protein 2a (PBP-2a) with a lower affinity for beta-lactams [1].

MRSA infections affect both hospitalized and healthy individuals, with virulence influenced by the patient's immune response and bacterial

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* *Corresponding author:* Alyaa Ibrahim Abo Eliwa
E-mail address: *alyaaeliwa@gmail.com*

virulence factors like Panton-Valentine leukocidin (PVL). PVL, found in community-acquired MRSA, is a leukotoxin that lyses white blood cells and is associated with severe skin infections and pneumonia [2].

MRSA poses a serious threat in intensive care units (ICUs) globally, particularly in Egypt, where it accounts for approximately 40% of ICU-acquired infections [1]. This rising prevalence, coupled with antibiotic resistance, leads to longer hospital stays, increased costs, and higher mortality rates. These factors highlight the urgent need for improved infection control measures and targeted interventions in Egyptian ICUs [2].

To control *S. aureus* infections, molecular techniques for typing the bacteria are crucial. While pulsed-field gel electrophoresis and multilocus sequence typing are effective but expensive, PCR-based methods such as PCR-RFLP are more accessible and provide high accuracy. Targeting genes like **coa** helps in differentiating *S. aureus* strains [3]. This study aims to characterize MRSA strains and assess infection control practices in Menoufia University's ICUs.

Methodology

Study design and patients:

The study, conducted at the Medical Microbiology and Immunology Department, Faculty of Medicine, Menoufia University between May 2022 and August 2023, included two groups:

- **Group I**: 180 ICU inpatients (111 males and 69 females) who developed hospital-acquired infections 48 hours after admission.
- **Group II**: 69 healthcare workers (HCWs) from the ICUs (52 females and 17 males), including 21 physicians, 36 nurses, and 12 workers.
- Participants were selected based on predefined inclusion criteria to ensure a representative sample of the population under study. ICU patients were randomly selected to avoid selection bias, ensuring a diverse representation of different age groups, medical conditions, and treatment histories. Healthcare workers were selected from various departments within the ICU to provide a comprehensive understanding of MRSA transmission risk. The random selection process aimed to minimize bias, allowing for more generalizable results across different ICU settings.

Demographic, clinical, and laboratory data were collected from both groups, and clinical specimens were obtained. Written informed consent was gathered from all participants, and the study protocol was approved by the Ethical Committee of the Faculty of Medicine, Menoufia University.

Data collection by self-administered questionnaires

The questionnaire for healthcare workers (HCWs) assessed MRSA risk factors, including age, sex, occupation, ICU work, previous MRSA carriage, recent hospitalization, and skin lesion history. For ICU patients, infection risk factors for *S. aureus* were analyzed, focusing on age, sex, ICU stay, catheterization, intubation, mechanical ventilation, and underlying conditions like liver disease, diabetes, heart disease, and renal failure.

Sample collection and *S. aureus* identification

A total of 138 nasal and hand swabs from 69 healthcare workers (HCWs) and 180 clinical specimens from 180 patients were collected and processed. The samples were cultivated on mannitol salt agar and blood agar, incubated at 37°C for 24 hours, and *S. aureus* was identified using standard protocols and confirmed by the Vitek-2 system. The isolated strains were stored in nutrient broth with glycerol at -80°C for further study.

DNA extraction

Genomic DNA was extracted using Thermo Scientific Gene JETTM Genomic DNA Purification Kit, according to the manufacturer's instructions.

Detection of the *S. aureus* -specific *femA* and *pvl* genes

Multiplex PCR assay targets the *femA* gene as a marker of *S. aureus* and the PV genes which is shown in **Table 1**. The optimized reaction conditions were adjusted as by Duarte and Hermínia [4]. Multiplex PCR produced separate bands, corresponding to their respective molecular sizes that were shown on 1.5% agarose gel ethidium bromide stained **(Figure 1).**

The confirmed *S. aureus* isolates were subjected to the following:

- **Antimicrobial susceptibility testing (AST):** It was done using Vitek-2 compact system with AST card (P592). The minimal inhibitory concentrations (MICs) were assessed &

interpreted based on the guidelines outlined by the CLSI, 2023.

- **Phenotypic detection of MRSA strains:** It was performed using both cefoxitin disk diffusion screening method & by interpreting the MICs for cefoxitin reported by the Vitek-2 compact system.
- **Methicillin resistance was further confirmed** by the detection of the *mecA* gene and further SCC mec typing by multiplex PCR using the Thermo Scientific Gene JETTM Genomic DNA Purification Kit:
  - The multiplex-PCR assay used **mecA** gene primers along with 8 pairs of primers for SCCmec types I, II, III, IVa, IVb, IVe, Id, and V **(Table 1).**
  - The amplification process was carried out in a thermal cycler with an initial denaturation at 94°C for 5 minutes, followed by 10 cycles at 94°C for 45 seconds, 65°C for 45 seconds, and 72°C for 1.5 minutes, and then 25 additional cycles at 94°C, 55°C, and 72°C for the same durations. The process ended with a final extension at 72°C for 10 minutes. The PCR products were separated using 2% agarose gel electrophoresis and stained with ethidium bromide at a concentration of 0.5 g/mL **(Figure 1).**

PCR-restriction fragment length polymorphism (RFLP) typing of MRSA:

MRSA strains were typed using coagulase gene polymorphism. The 3' end region of the coagulase gene was amplified via PCR, following the method described by Lawrence et al. [5]. The primers used for amplification were **COAG-1** (5'CGAGACCAAGATTCAACAAG3') and **COAG-2** (5'AAAGAAAACCACTCACATCA3'). After amplification, 15 µL of the PCR products were digested with 6 IU of the restriction endonuclease **AluI** (Fermentas, Sunderland, UK) for 15 minutes. The resulting restriction fragments were separated using 2% agarose gel electrophoresis **(Figure 1).**

Infection control studies

- Studying the compliance to hand hygiene and other infection control measures among HCWs in ICUs was done using the list documented by WHO for hand hygiene compliance and the list documented by supreme council of university hospitals to reflect the baseline compliance for infection control measures compliance was done.
- Analysis of all health care worker practices related to ICUs during educational and training condensed sessions was conducted by infection control team in the ICU.
- Feedback of the assessed hand hygiene and the infection control compliance rates were analyzed according to each checklist and correlated to the incidence of new hospital-acquired MRSA infection cases and also, to the outcome of ICUs patients in each visit.
- Risk assessment scoring: different risk factors are calculated using risk assessment tool: In this study, a **Relative Risk (RR) assessment tool** was utilized to assess infection control practices and the risk of MRSA transmission across various ICU departments. The RR was calculated by dividing the incidence of improper infection control measures in a specific ICU by the incidence across all ICUs. A **RR > 1** indicates an increased risk of infection in that ICU, while **RR < 1** suggests a protective effect, reducing the infection risk.

The risk assessment process involved evaluating three main components:

Probability of occurrence:

0: none 1: rare, 2: possible, 3: permissible, 4: expected

Severity / level of failure:

1: little medical risk, 2: medium medical risk, 3: long period of stay, 4: sever loss, 5: danger to life.

Organizational preparedness:

1: strong, 2: good, 3: average, 4: weak, 5: no thing

Each component was assigned a score, and the risk level was categorized as follows:

- **1–4**: Low Risk
- **5–9**: Moderate Risk
- **10–14**: High Risk

This comprehensive tool allowed for a precise evaluation of the risk associated with MRSA transmission and helped identify departments requiring targeted infection control interventions. Departments classified as moderate or high risk were prioritized for immediate corrective actions.

REUSULTS

- Eighty-one *S. aureus* strains were isolated (56 from infected patients admitted to different ICUs & 25 isolates from HCWs) with 17.5% of MRSA and 5.6% of MSSA

strains harboring the PVL genes. The highest isolation rate of *S. aureus* strains was from HCWs nasal swabs (21%) followed by both blood & sputum (each 17.3%), ascetic fluid & wound (11.1% for each) and finally HCWs hand swabs (9.9%). **Table 2**

- Among the 81 isolated *S. aureus* strains, 63-tested positive for the *mecA* gene *via* PCR, confirming them as MRSA, while 18 tested negative and were categorized as Methicillin sensitive *S. aureus* (MSSA). The cefoxitin disk method correctly identified 53 out of 63 *mecA*-positive isolates, with a sensitivity, specificity, and diagnostic accuracy of 84.1%, 77.8%, and 82.7%, respectively. In comparison, the Vitek-2 Compact system identified 62 out of 63 *mecA*-positive isolates, achieving sensitivity, specificity, and diagnostic accuracy of 98.4%, 94.4%, and 97.5%, respectively. **Table 3.**

There was a significant difference between MRSA-infected and non-infected patients regarding invasive procedures, close contact with MRSA cases, antibiotic use, long hospital stays, liver conditions, and hospitalizations longer than 15 days. Other risk factors included skin trauma, sharing unclean equipment, surgery, surgical site infections, hemodialysis, and diabetes mellitus. However, no significant association was found for hypertension, chemotherapy, or radiotherapy.

- There was a significant difference between MRSA and non-MRSA carriers regarding the presence of rhinitis. However, no significant difference was detected regarding skin and soft tissue infections, antibiotic use, previous contact with a MRSA carrier at home, occupation in a foreign country, contact with farm and domestic animals, and contact with raw meat within the last 12 hours.

- Using the Vitek-2 compact system, *S. aureus* isolates exhibited high resistance rates to benzylpenicillin (93.8%), fusidic acid (82.7%), and oxacillin (77.8%). In contrast, the isolates showed high susceptibility to tigecycline (100%), linezolid (96.3%), and teicoplanin (81.5%). These results highlight the varying levels of resistance and susceptibility among the tested antibiotics, demonstrating the challenge of treating MRSA infections with conventional drugs. **Table 4**
- Significant differences were observed between MRSA and MSSA strains, particularly with fusidic acid and oxacillin. Additional antibiotics, such as rifampicin, vancomycin, teicoplanin, clindamycin, erythromycin, moxifloxacin, and benzylpenicillin, also showed significant differences in resistance rates between the two groups. However, no significant differences were detected for tetracycline, ciprofloxacin, and gentamicin, indicating that these antibiotics had similar effectiveness against both MRSA and MSSA strains. **Table 4**
- Further analysis revealed significant differences between healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) strains in terms of resistance to trimethoprim/sulfamethoxazole, teicoplanin, erythromycin, and ciprofloxacin. Meanwhile, no significant differences were observed for tetracycline, linezolid, and gentamicin between the two groups. These findings underscore the variability in resistance patterns across different MRSA strains and the importance of tailored infection control strategies. **Table 4**
- The isolated MRSA strains were categorized into HA-MRSA (47 isolates) and CA-MRSA (14 isolates), with two isolates being non-typeable. HA-MRSA strains were classified into three SCCmec types: type I (15 isolates), type II (8 isolates), and type III (24 isolates), while CA-MRSA strains were classified into two SCCmec types: type IV (9 isolates) and type V (5 isolates). This categorization was based on the molecular identification of SCCmec types, where HA-MRSA is typically associated with types I, II, and III, and CA-MRSA with types IV and V. **Table 5**
- The PCR amplification and restriction analysis of the coagulase gene revealed seven distinct patterns among the 63

MRSA isolates, with three isolates showing non-typeable patterns. HA-MRSA isolates displayed six distinct RFLP patterns, with pattern 2 being the most common (20 isolates, 42.6%), followed by patterns 3 and 7 (9 isolates; 19.1% and 8 isolates; 17.0%, respectively). CA-MRSA isolates also showed six RFLP patterns, with pattern 2 being the most frequent (5 isolates, 35.7%) and pattern 7 being the second most common (3 isolates, 21.4%). **Table 5**

Infection control

- Studies showed that there has been a noticeable and clear improvement in the performance of different ICUs in adhering to standard infection control procedures, hand hygiene and personal protective equipment in conjunction with our continuous medical training **(Figure 2).**The highest hand hygiene (HH) moment was found to be after exposure to body fluids (98%), after touching a patient (90%), and after touching a patient's surroundings (85%). Nurses had the highest HH compliance rate at 85% (612 of 720), followed by physicians at 79% (401 of 508). Cleaning staff had the lowest HH compliance rate at 55% (330 of 601). Common barriers to HH compliance included lack of time (90%), lack of facilities (85%), wearing gloves (50%), and skin reactions (45%). **(Figure 3).**
- Regarding personal protective equipment (PPE), nurses had the highest compliance rate at 92%, followed by physicians at 88%. Cleaning staff had the lowest PPE compliance rate at 63%. The performance of wearing gloves and surgical masks was good, with nearly all participants wearing a mask when working within three feet of a patient and most not touching the outside of the mask during use. However, there was a lower compliance rate for wearing goggles and face shields, particularly during open suctioning of patients with artificial airways. One major breakdown in PPE compliance was not wearing gowns with sleeves during procedures that generated splashes or sprays of blood, body fluids, secretions, or excretions. Common barriers to PPE compliance included shortages of PPE (92%), lack of time (75%), and skin irritation (80%) **(Figure 4).**
- A significant correlation was found between the infection control measures, hand hygiene measures, and PPE measures in ICUs and the occurrence of MRSA infections. There was also a strong positive correlation between these measures and recovery rates in ICUs **(Figure 5).** Using a risk assessment tool, all risks related to the spread of MRSA infection and colonization were assessed for different ICUs, including the new emergency ICU, old emergency ICU, anesthesia ICU, chest ICU, NICU and PICU. **Table 6** shows that admission to the new emergency ICU, old emergency ICU and anesthesia ICU increased the risk of improper infection control measures, HH and PPE non-compliance. Moreover, it shows that the admission to these ICUs increases risk of MRSA infection & MRSA colonization, with relative risk> 1 and risk scores exceeding 8 (moderate to high risk level).

Table 1. The used primers

Primer	Sequence of Oligonucleotide	Amplicon size (bp)	Specificity
femA-1F **femA-2R**	(5’ – AAAAAAGCACATAACAAGCG – 3’) (5’ – GATAAAGAAGAAACCAGCAG – 3’)	132bp	femA
Luk-PV-1F **Luk-PV-2R**	(5’-ATCATTAGGTAAAATGTCTGGACATGATCA-3’) (5’-GCATCAAGTGTATTGGATAGCAAAAGC-3’)	310 bp	PVL
MecA147-1F **MecA147-2R**	(5’-GTG AAG ATA TAC CAA GTG ATT-3’) (5’-ATG CGC TAT AGA TTG AAA GGA T-3’)	147	*mecA*
Type I-1F **Type I-2R**	(5’-GCTTTAAAGAGTGTCGTTACAGG-3’) (5’-GTTCTCTCATAGTATGACGTCC-3’)	613	SCC*mec* I
Type II-1F **Type II-2R**	(5’-CGTTGAAGATGATGAAGCG-3’) (5’-CGAAATCAATGGTTAATGGACC-3’)	398	SCC*mec* II
Type III-1F **Type III-2R**	(5’-CCATATTGTGTACGATGCG-3’) (5’-CCTTAGTTGTCGTAACAGATCG-3’)	280	SCC*mec* III
Type IVa-1F **Type IVa-2R**	(5’-GCCTTATTCGAAGAAACCG-3’) (5’-CTACTCTTCTGAAAAGCGTCG-3’)	776	SCC*mec* IVa
Type IVb-1F **Type IVb-2R**	(5’-TCTGGAATTACTTCAGCTGC-3’) (5’-AAACAATATTGCTCTCCCTC-3’)	493	SCC*mec* IVb
Type IVc-1F **Type IVc-2R**	(5’-ACAATATTTGTATTATCGGAGAGC-3’) (5’-TTGGTATGAGGTATTGCTGG-3’)	200	SCC*mec* IVc
Type IVd-1F **Type IVd-2R**	(5’-CTCAAAATACGGACCCCAATACA-3’) (5’-TGCTCCAGTAATTGCTAAAG-3’)	881	SCC*mec* IVd
Type V-1F **Type V-2R**	(5’-GAACATTGTTACTTAAATGAGCG-3’) (5’-TGAAAGTTGTACCCTTGACACC-3’)	325	SCC*mec* V
CoaG-F **CoaG-R**	(5’CGAGACCAAGATTCAACAAG3’) (5’AAAGAAAACCACTCACATCA3’)	648-810 bp	*Coa*

Table 2. Distribution of *Staphylococcus aureus* clinical isolates among the different sample types.

Sample	ICU									Total
	Anesthesia ICU	**Burn ICU**	**CCU**	**Chest ICU**	**Internal medicine ICU**	**NICU**	**PICU**	**Stroke ICU**	**Tropical ICU**	
Patients’ clinical samples (n= 56)										
Ascetic Fluid	3 (13.6%)	-	-	-	-	-	-	-	6 (66.7%)	**9 (11.1%)**
Blood	4 (18.2%)	-	2(50.0%)	1 (11.1%)	2 (28.6%)	2 (18.2%)	3 (33.3%)	-	-	**14 (17.3%)**
Pus	1 (4.5%)	5 (71.4%)	-	-	-	-	-	-	-	**6 (7.4%)**
Sputum	4 (18.2%)	-	-	4 (44.4%)	1 (14.3%)	3 (27.3%)	2 (22.2%)	-	-	**14 (17.3%)**
Urine	2 (9.1%)	-	1 (25.0%)	-	1 (14.3%)	-	-	-	-	**4 (4.9%)**
Wound	2 (9.1%)	2 (28.6%)	-	-	3 (42.9%)	1 (9.1%)	1 (11.1%)	-	-	**9 (11.1%)**
HCWs colonization samples (n= 25)										
Hand	4 18.2%	-	-	2 (22.2%)	-	1 (9.1%)	-	1 (33.3%)	-	**8 (9.9%)**
Nasal	2 9.1%	-	1 (25.0%)	2 (22.2%)	-	4 (36.4%)	3 (33.3%)	2 (66.7%)	3 (33.3%)	**17 (21.0%)**
Total	**22(27.2%)**	**7(8.6%)**	**4(4.9%)**	**9(11.1%)**	**7(8.6%)**	**11(13.6%)**	**9(11.1%)**	**3(3.7%)**	**9(11.1%)**	**81(100%)**

Table 3. Accuracy of cefoxitin test and VITEK2 in relation to PCR mec A

	PCR mec A				χ^2 (P-value)	Kappa agreement	Accuracy	Sensitivity	Specificity	PPV	NPV
	Positive (N= 63)		Negative (N= 18)								
	N	%	N	%							
Cefoxitin test:					25.73 (<0.001) HS	0.553	82.7%	84.1%	77.8%	93%	58.3%
Positive	53	84.1	4	22.2							
Negative	10	15.9	14	77.8							
VITEK2 test:					#69.84 (<0.001) HS	0.929	97.5%	98.4%	94.4%	98.4%	94.4%
Positive	62	98.4	1	5.6							
Negative	1	1.6	17	94.4							

Table 4. Antibiotic sensitivity and resistance patterns (N=81) of the isolated *Staph. aureus*, MRSA and MSSA .

	Staph aureus (N=81)	MRSA (N=63)		MSSA (N=18)		χ^2	P-value	Hospital acquired MRSA (N=47)		Community acquired MRSA (N=14)		Fisher's Exact test	P-value
		N	%	N	%			N	%	N	%		
Benzylpenicillin			1.6	4	22.2	FE 10.29	**0.008 (S)**	-	-	1	7.1	3.41	0.230
Sensitive	5 (6.2%)	1	98.4	14	77.8			47	100.0	13	92.9		
Resistant	76 (93.8%)	62											
Oxacillin						FE 81.00	**<0.001 (HS)**	47	100.0	14	100.0	-	-
Sensitive	18 (22.2%)	-	-	18	100.0								
Resistant	63 (77.8%)	63	100.0	-	-								
Gentamicin												χ^2	
Sensitive	37 (45.7%)	26	41.3	11	61.1	2.22	0.136	19	40.4	7	50.0	0.40	0.525
Resistant	44 (54.3%)	37	58.7	7	38.9			28	59.6	7	50.0		
Ciprofloxacin												χ^2	
Sensitive	32 (39.5%)	22	34.9	10	55.6	2.49	0.114	13	27.7	9	64.3	6.28	**0.024 (S)**
Resistant	49 (60.5%)	41	65.1	8	44.4			34	72.3	5	35.7		
Moxifloxacin	28 (34.6%)												
Sensitive	53 (65.4%)	18	28.6	10	55.6	4.51	**0.034 (S)**	14	29.8	4	28.6	0.01	1.000
Resistant		45	71.4	8	44.4			33	70.2	10	71.4		
Erythromycin	23 (28.4%)												
Sensitive	58 (71.6%)	14	22.2	9	50.0	5.31	**0.021 (S)**	7	14.9	7	50.0	7.52	**0.011 (S)**
Resistant		49	77.8	9	50.0			40	85.1	7	50.0		
Clindamycin	43 (53.1%)											χ^2	
Sensitive	38 (46.9%)	29	46.0	14	77.8	5.67	**0.017 (S)**	21	44.7	8	57.1	0.67	0.412
Resistant		34	54.0	4	22.2			26	55.3	6	42.9		
Linezolid	78 (96.3%)					FE 0.89	1.000						
Sensitive	3 (3.7%)	60	95.2	18	100.0			46	97.9	13	92.9	0.86	0.409
Resistant		3	4.8	-	-			1	2.1	1	7.1		
Teicoplanin	66 (81.5%)					FE 5.26	**0.018 (S)**						
Sensitive	15 (18.5%)	48	76.2	18	100.0			32	68.1	14	100.0	5.93	**0.014 (S)**
Resistant		15	23.8	-	-			15	31.9	-	-		
Vancomycin						FE 5.70	**0.017 (S)**						
Sensitive	65 (80.5%)	47	74.6	18	100.0			33	70.2	12	85.7	1.34	0.318
Resistant	16 (19.8%)	16	25.4	-	-			14	29.8	2	14.3		
Tetracycline	52 (64.2%)											χ^2	
Sensitive	29 (35.8%)	38	60.3	14	77.8	1.86	0.173	26	55.3	10	71.4	1.16	0.282
Resistant		25	39.7	4	22.2			21	44.7	4	28.6		
Tigecycline:	81(100%)	63	100.0	18	100.	-	-	47	100.0	14	100.0	-	-
Sensitive													
Fusidic acid						FE 23.71	**<0.001 (HS)**						
Sensitive	14 (17.3%)	4	6.3	10	55.6			4	8.5	-	-	1.28	0.565
Resistant	67 (82.7%)	59	93.7	8	44.4			43	91.5	14	100.0		

Rifampicin Sensitive Resistant	48 (59.3%) 33 (40.7%)	31 32	49.2 50.8	17 1	94.4 5.6	11.87	**0.001 (S)**	23 24	48.9 51.1	8 6	57.1 42.9	χ^2 0.29	0.590
Trimethoprim/ sulfamethoxazole Sensitive Resistant	55(67.9%) 26(32.1%)	43 20	68.3 31.7	12 6	66.7 33.3	0.02	0.899	28 19	59.6 40.4	14 -	100.0 -	8.22	**0.003 (S)**

Table 5.: Distribution of the 63 MRSA clinical isolates according to Scc mec genotyping and Coagulase-RFLP profile

			SCC mec typing						**Total**
			Hospital- acquired MRSA (n=47)			**Community-acquired MRSA (n=14)**		**non-typable (n=2)**	
			I	**II**	**III**	**IV**	**V**		
Coagulase-RFLP profile (in bp)	**(1) 324, 405**	**N**	-	1	3	1	-	2	7
		%	-	12.5%	12.5%	11.1%	-	100.0%	11.1%
	(2) 81, 567	**N**	6	2	12	2	3	-	25
		%	40.0%	25.0%	50.0%	22.2%	60.0%	-	39.7%
	(3) 243, 486	**N**	3	3	3	1	-	-	10
		%	20.0%	37.5%	12.5%	11.1%	-	-	15.9%
	(4) 81, 146, 178, 340	**N**	1	-	-	-	-	-	1
		%	6.7%	-	-	-	-	-	1.6%
	(5) 81, 243, 405	**N**	-	1	2	2	-	-	5
		%	-	12.5%	8.3%	22.2%	-	-	7.9%
	(6) 162, 230, 324	**N**	-	-	-	1	-	-	1
		%	-	-	-	11.1%	-	-	1.6%
	(7) 81, 230, 480	**N**	4	1	3	1	2	-	11
		%	26.7%	12.5%	12.5%	11.1%	40.0%	-	17.5%
	None	**N**	1	-	1	1	-	-	3
		%	6.7%	-	4.2%	11.1%	-	-	4.8%
Total		**N**	15	8	24	9	5	2	63
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Table 6. Risk assessment of the risks related to the spread of MRSA infection and colonization

Risk assessment of Hand hygiene measures non-compliance in ICUs					
Department	**RR**	**Probability of occurrence**	**Severity / level of failure**	**Organizational preparedness**	**Risk level (Total)**
Chest ICU	**0.7**	**2**	**1**	**1**	**4(low)**
Cardiothoracic ICU	**0.8**	**1**	**1**	**2**	**4(low))**
PICU ICU	**0.5**	**1**	**1**	**1**	**3(low)**
NICU ICU	**0.7**	**1**	**2**	**1**	**4(low)**
Old emergency ICU	**1.0**	**3**	**2**	**3**	**8(moderate)**
New emergency ICU	**2.3**	**4**	**3**	**3**	**10(high)**
Anesthesia ICU	**1.0**	**3**	**3**	**2**	**8(moderate)**
Risk assessment of PPE measures non-compliance in ICUs					
Chest ICU	**0.8**	**1**	**2**	**1**	**4(low)**
Cardiothoracic ICU	**0.8**	**1**	**2**	**1**	**4(low))**
PICU ICU	**0.5**	**1**	**1**	**2**	**4(low)**
NICU ICU	**0.7**	**1**	**2**	**1**	**4(low)**
Old emergency ICU	**1.0**	**2**	**3**	**3**	**8(moderate)**
New emergency ICU	**2.4**	**4**	**4**	**3**	**11(**high)
Anesthesia ICU	**1.0**	**3**	**3**	**2**	**8(moderate)**
Risk assessment of MRSA infection in ICUs					
Chest ICU	**1.1**	**3**	**3**	**4**	**10(**high)
Cardiothoracic ICU	**0.3**	**1**	**2**	**1**	**4(low)**
PICU ICU	**0.9**	**1**	**2**	**1**	**4(low)**
NICU ICU	**0.7**	**1**	**2**	**1**	**4(low)**
Old emergency ICU	**1.0**	**2**	**3**	**3**	**8(moderate)**
New emergency ICU	**1.6**	**3**	**3**	**4**	**10(**high)
Anesthesia ICU	**1.5**	**3**	**3**	**4**	**10(**high)
Risk assessment of MRSA colonization in all departments					
Chest ICU	**1.3**	**3**	**2**	**3**	**8(moderate)**
Cardiothoracic ICU	**0.0**	**1**	**1**	**1**	**3(low)**
PICU ICU	**1.1**	**3**	**3**	**2**	**8(moderate)**
NICU ICU	**1.1**	**3**	**3**	**2**	**8(moderate)**
Old emergency ICU	**1.0**	**3**	**2**	**3**	**8(moderate)**
New emergency ICU	**1.3**	**3**	**3**	**4**	**10(high)**
Anesthesia ICU	**1.1**	**3**	**3**	**4**	**10(**high)

RR: relative risk: a measure of risk assessment equals incidence of improper infection control measures in one ICUdivided by incidence of improper infection control measures in all ICUs. If RR > 1: It means that admission to this department increases risk of infection, while RR < 1 means that admission to this department decreases risk of infection (protective).

Probability of occurrence:

0: none 1: rare, 2: possible 3: permissible, 4: expected

Severity / level of failure:

1: little medical risk, 2: medium medical risk, 3: long period of stay, 4: sever loss, 5: danger to life.

Organizational preparedness:

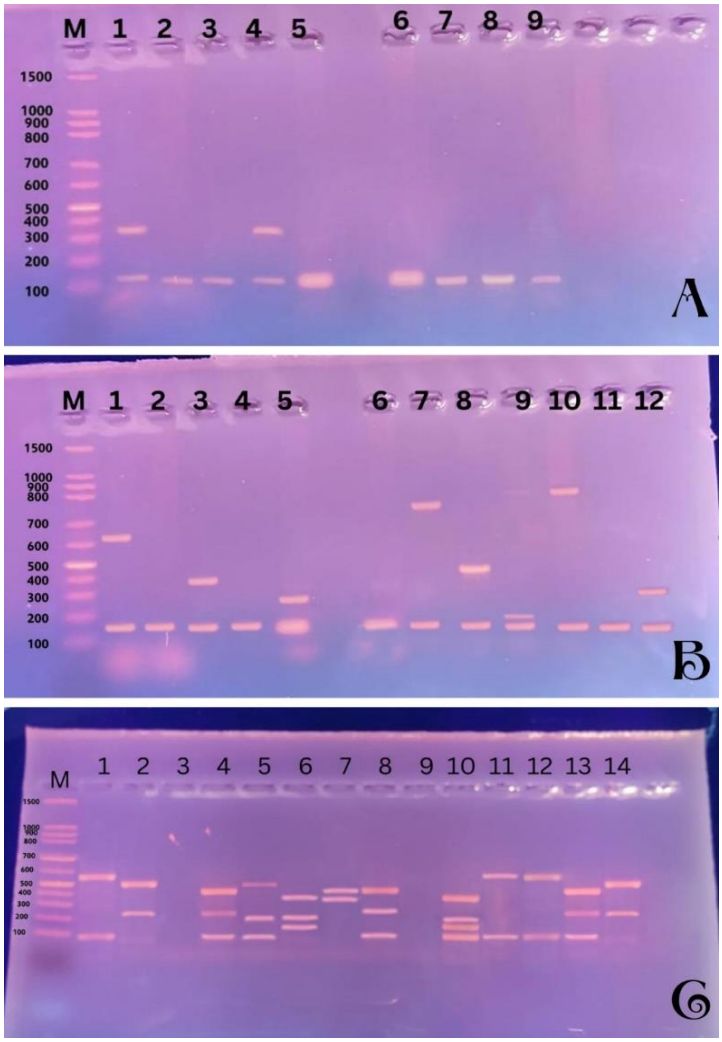
1: strong, 2: good, 3: average 4: weak 5: no thing

Category:

From 1-4, the risk level is low

From 5 – 9 the degree of risk is moderate

Figure 1. Gel electrophoresis results from the multiplex PCR products.



In panel A, the Staphylococcus aureus-specific FemA and PVL genes are displayed. Lane M contains the 100 bp DNA ladder, while lanes 1 through 9 show the presence of the FemA gene at 132 bp. Notably, lanes 1 and 4 also exhibit the PVL gene at 310 bp, indicating PVL positivity, while the remaining lanes (2, 3, 5, 6, 7, 8, 9) are PVL-negative with only the FemA gene visible at 132 bp.

Panel B presents the results for SCCmec types I, II, III, IVa, IVb, IVc, IVd, and V, alongside the mecA gene. Lane M again represents the 100-bp DNA ladder. All lanes display the mecA gene at 147 bp, confirming MRSA identification. Specific SCCmec types are visible in distinct lanes: Type I (lane 1, 613 bp), Type II (lane 3, 398 bp), Type III (lane 5, 280 bp), Type IVa (lane 7, 776 bp), Type IVb (lane 8, 493 bp), Type IVc (lane 9, 200 bp), Type IVd (lane 10, 881 bp), and Type V (lane 12, 325 bp). Lanes 2, 4, 6, and 11 contain non-typable MRSA.

Lastly, panel C shows the results from Coa RFLP typing. Lane M displays the 100-bp DNA ladder. Various banding patterns are seen in the remaining lanes: lanes 1, 11, and 12 show bands at 81 and 567 bp; lanes 2 and 14 at 243 and 486 bp; lanes 4 and 13 at 81, 243, and 405 bp; lane 5 at 81, 230, and 480 bp; lane 6 at 162, 230, and 324 bp; lane 7 at 324 and 405 bp; lane 8 at 81, 243, and 405 bp; and lane 10 at 81, 146, 178, and 340 bp

Figure 2. Compliance to infection control hand hygiene and PPE measures in the studied ICUs from June 2022 to June 2023.

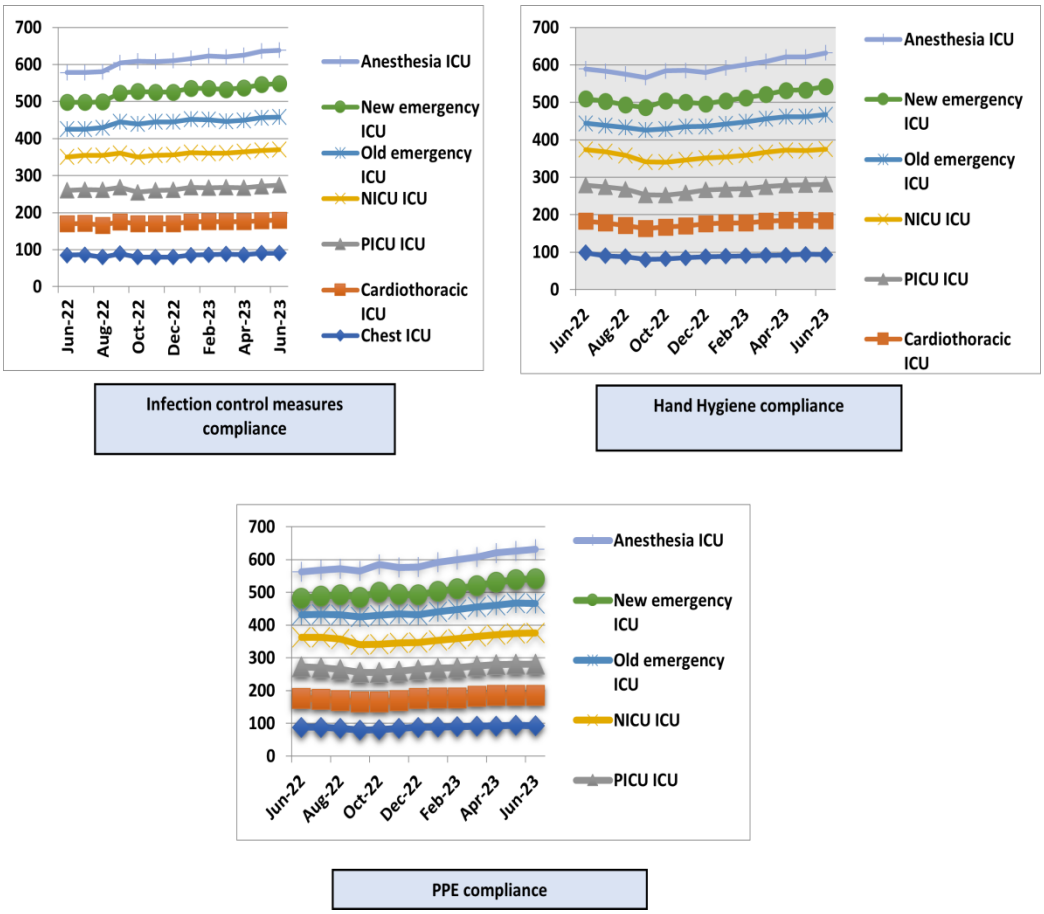


Figure 3.: The studied parameters of HH measures in ICU

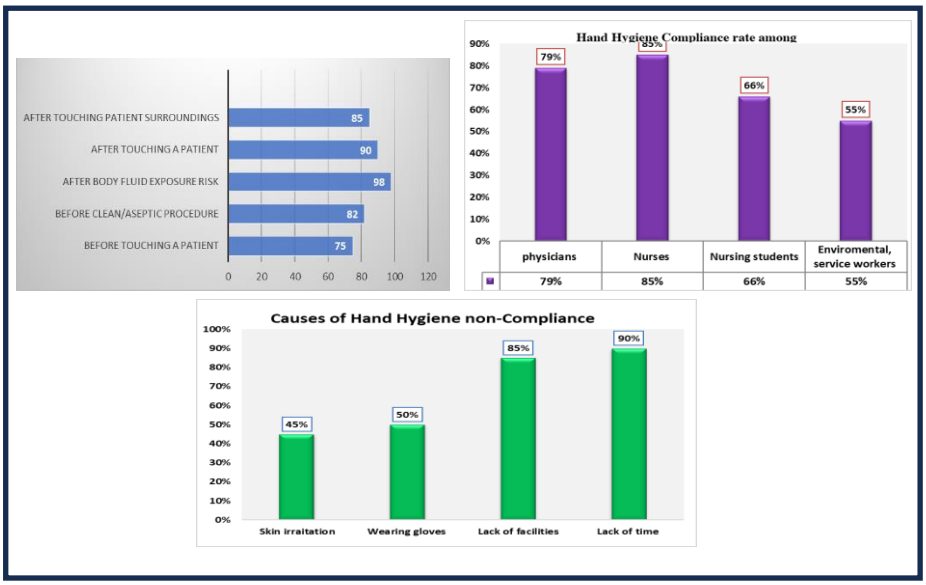


Figure 4. PPE measures in the studied ICUs.

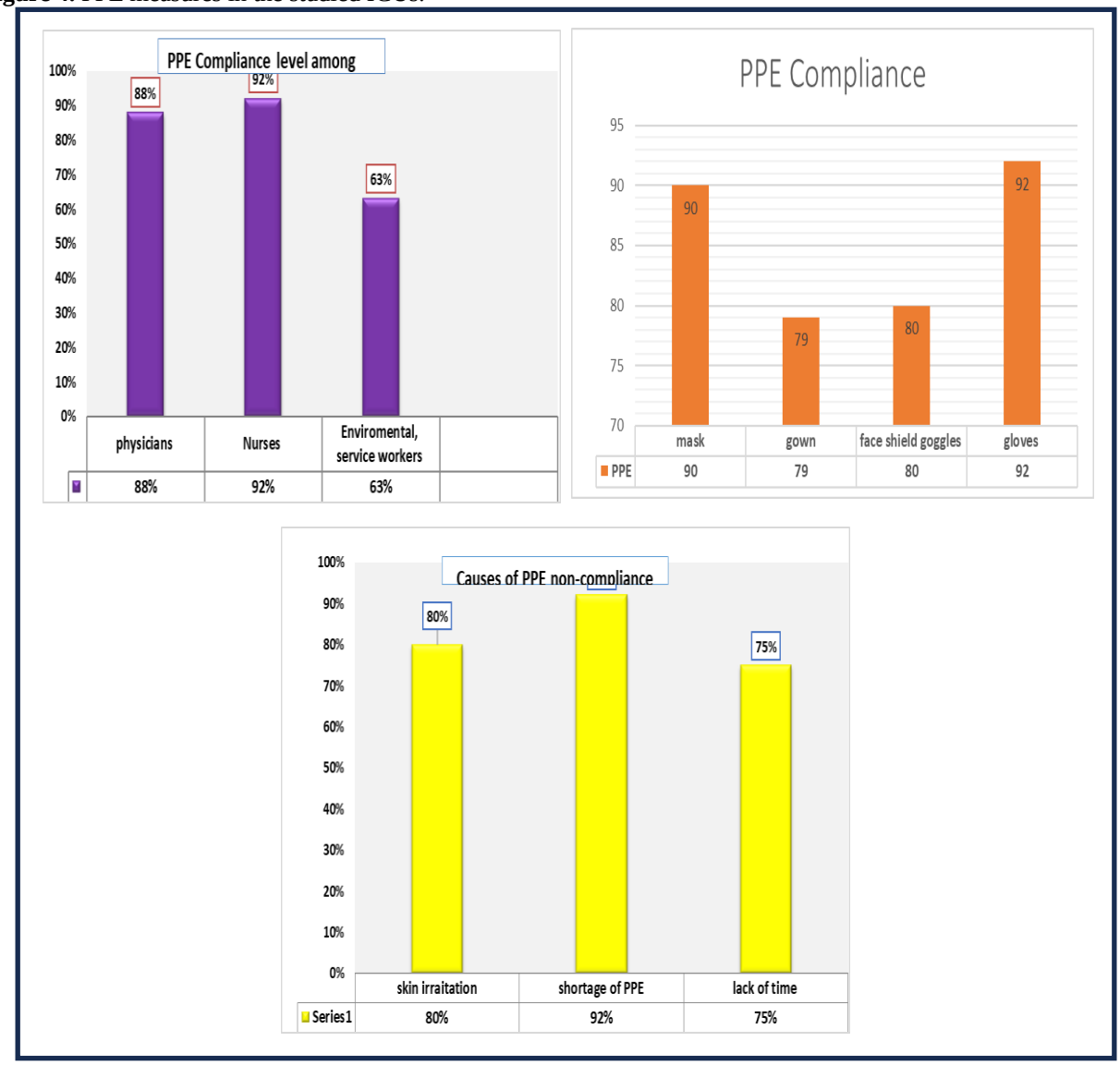
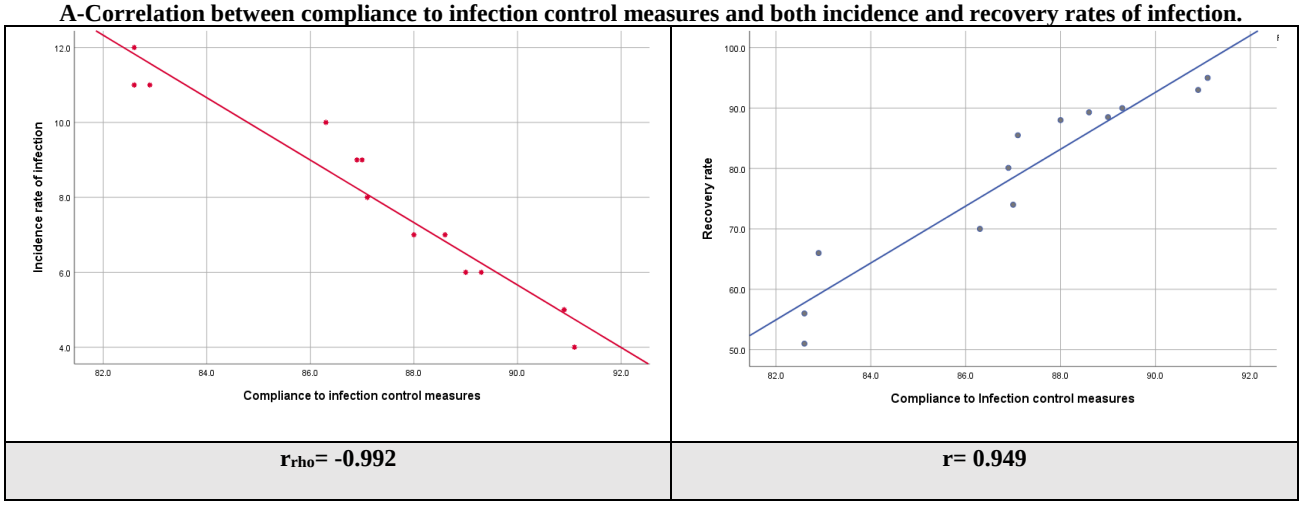


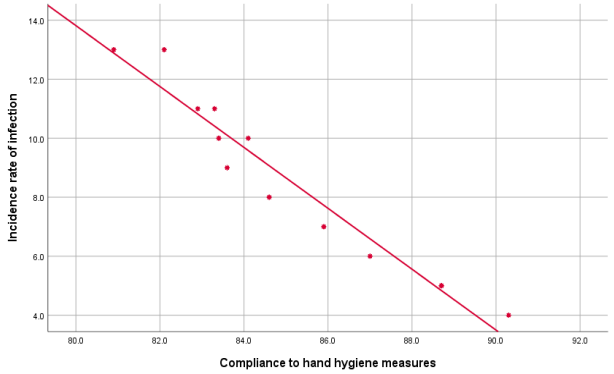
Figure 5.: Correlation between compliance to infection control , HH and PPE measures to both incidence and recovery rates of infection.

A-Correlation between compliance to infection control measures and both incidence and recovery rates of infection.

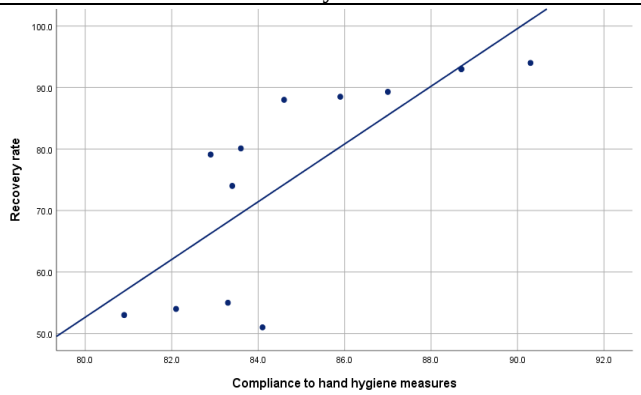


r_{rho}= -0.992	r= 0.949

B-Correlation between compliance to HH measures and both incidence and recovery rates of infection.

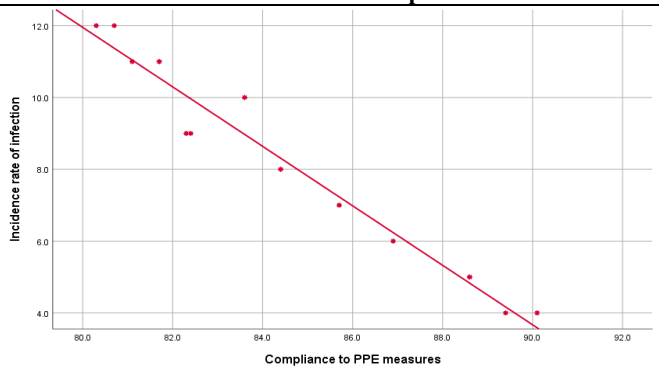


r_{rho}= -0.988

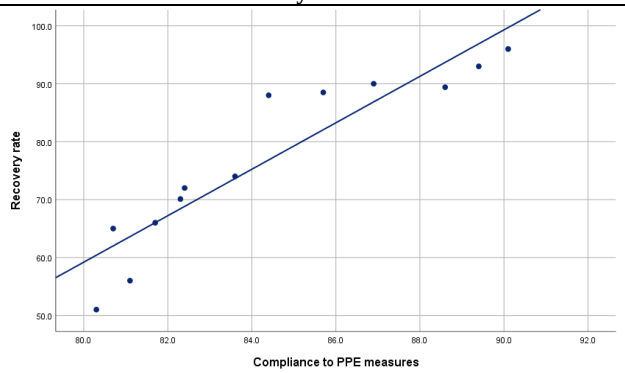


r= 0.788

C-Correlation between compliance to PPE measures and both incidence and recovery rates of infection.



r_{rho}= -0.978



r= 0.923

Discussion

MRSA is an increasing problem, and its burden continues to rise in healthcare facilities. Rising colonization rates lead to increased infection rates in hospitals [6]. Our study identified 81 *S. aureus* isolates, with 63 (77.8%) classified as MRSA. This prevalence was consistent with that reported in similar clinical settings [7, 8] . However, Other studies reported lower rates (44.6%), (24%), and (18%) [9, 10, 11]. Moreover, It was reported that some European countries have achieved very low rates of MRSA through national surveillance efforts[12]. This study included 69 sampled HCWs, among whom the prevalence of MRSA was 36.2% (25 out of 69). This finding closely aligns with other studies that reported MRSA frequencies of 33% and 37.2% among HCWs, respectively [13,14] . In contrast, it was observed a significantly lower MRSA carriage rate of 5% among HCWs [15]. These variations in MRSA prevalence may be attributed to differences in infection control measures implemented across hospitals, frequency of antibiotic usage, the methodology and sensitivity of MRSA detection, as well as the characteristics of the study populations [6]

Accurate detection of MRSA is crucial for selecting the appropriate antimicrobial treatment and preventing its spread. MRSA can be identified through either phenotypic methods or PCR, but due to the high cost and technical requirements of PCR, it is not feasible for routine use in most laboratories. Therefore, the use of a highly sensitive and cost-effective phenotypic method is essential for detecting MRSA [16]. Among the studied 81 isolates of *S. aureus* , 63 (77.8%) tested positive for the *mecA* gene by PCR and were classified as MRSA, while 18 isolates were negative for the *mecA* gene and were classified as MSSA. This result is consistent with the findings of Madhavan et al. [17], who reported a prevalence of MRSA of 72% and MSSA of 28%. However, our results differ from those of Deniz et al. [18] and Aziz & Hassan, [19] who found a higher presence of *mecA* gene in all *S.*

aureus isolates, at rates of 98% and 89.9%, respectively. The observed differences in MRSA prevalence between our study and previous studies, such as those by Deniz et al. and Aziz & Hassan, could be attributed to regional variations in infection control practices and antibiotic usage, as well as differences in the sensitivity of detection methods used in each study.

The cefoxitin disk method was able to identify 53 out of 63 *mecA*-positive isolates, yielding sensitivity, specificity, and diagnostic accuracy rates of 84.1%, 77.8%, and 82.7%, respectively. This result is in line with other previous studies [20, 21, 22]. In contrast, the Vitek-2 compact system identified 62 out of 63 *mecA*-positive isolates, resulting in sensitivity, specificity, and diagnostic accuracy rates of 98.4%, 94.4%, and 97.5%, respectively. The vitek-2 system exhibited higher diagnostic performance compared to the cefoxitin disk method. A notable advantage of the vitek-2 system over the disk diffusion test is its faster incubation time [16].

CA-MRSA isolates are typically susceptible to most non-β-lactam antimicrobial drugs compared to HA-MRSA, but there has been a recent emergence of multidrug-resistant CA-MRSA, which poses a serious public health problem [6]. In our study, the isolated CA-MRSA showed sensitivity to most non-β-lactam antibiotics except for tetracycline (28.6% resistant) and gentamicin (50% resistant), which are commonly used in community settings. Resistance to these drugs is often acquired through plasmids or transposons carrying resistance genes [6]. A previous study in Egypt found similar resistance patterns [23], while a study in Tunisia reported resistance to gentamicin and tetracycline [24].

Invasive devices, prolonged hospital stays, and chronic diseases were significantly linked to hospital-acquired MRSA infections ($p < 0.001$), aligning with previous Egyptian studies [25], which cited device use, poor hygiene, and overcrowding as contributing factors.

Regarding the antibiotic resistance patterns, our results showed that all MRSA isolates were highly resistant to various antibiotics. Specifically, 25.4% (16/63) of the isolates were resistant to vancomycin. In contrast, all isolates were sensitive to tigecycline. Our findings are consistent with those of Awad et al. [16] and Abbasian et al. [26], who reported similar antibiogram results. However, Lee et al. [27] found lower resistance rates to different antibiotics. Notably, our results agree with Tiewsoh and Dias [28], who found that MRSA isolates exhibited significantly higher resistance compared to MSSA isolates. The differences in antibiotic resistance findings between the studies may be due to variations in geographical regions, study populations, methodologies, temporal factors, and sample sizes.

In this study, *SCCmec* typing by PCR revealed that the most common types (in both patients and healthcare workers) were III and I, while types II and V were less prevalent. Our findings indicate that hospital-acquired MRSA (47/63) were more common than community-acquired MRSA (16/63). This is consistent with the results of Awad et al. [16], and Abbasian et al. [26]. Therefore, it is likely that the studied patients acquired the infection from the hospital environment and/or healthcare workers. The typing of SCC using Zhang et al.'s protocol [29] showed that 9/14 (64.3%) of CA-MRSA strains were *SCCmec* type IV, and 5/14 (35.7%) were type V. Two (3%) of all MRSA strains were untypable, which can be designated as possibly new SCCmec types. Different results were reported in a study from egypt [6], which found that 44.4% of CA-MRSA strains were *SCCmec* type V, while 27.7% % were type IV, and 27.7% were untypable.

Our study found that 14.8% of *S. aureus* isolates harbored *PVL* genes, a result that is similar to Hussein et al., [30] who reported that 16% of medical staff and 21% of community individuals harbored PVL genes. On the other hand, results were lower than the findings of Darboe et al. [31] (61.4%) and Samsudin et al. [32] (4.4%). *PVL* genes are commonly associated with CA-MRSA and are considered a stable marker for it in many studies. Our study found that 71.4% of the isolated CA-MRSA strains were PVL-positive, which is higher than previous Egyptian studies that reported 19.04% and 33.33% [23,33]. Other studies have also reported varying rates of PVL positivity in CA-MRSA strains, including 9.8% in China [34] and 79% in Tunisia [24]. The differences in the prevalence of PVL genes observed in our study compared to previous research could be attributed to regional variations in MRSA strains and the differing methodologies used to detect PVL genes. Additionally, variations in healthcare practices, community exposure, and sample populations might

also contribute to the differences in PVL positivity rates across studies. Our study suggests that PVL can be a useful marker for CA-MRSA infections, as it was detected in more than 70% of isolates.

Pulsed-field gel electrophoresis (PFGE) is considered the most discriminatory and reliable method for typing *S. aureus* , but it is complex and time-consuming. In our laboratory, we used PCR-RFLP typing of the coagulase gene (*coa*) as an alternative approach. This method involves amplifying the 3' end coding region of the *coa* gene and then digesting it with restriction enzymes to generate specific patterns. Our results showed that PCR-RFLP typing of the coa gene produced different patterns among the 81 *S. aureus* isolates. We detected 7 RFLP patterns in MRSA and 6 RFLP patterns in MSSA. Our findings are consistent with those of Kobayashi et al., [35] who reported that MRSA and MSSA were classified into 6 and 12 RFLP patterns, respectively, with 5 patterns detected frequently in both groups. Walker et al. [36] also found that AluI digestion of coa gene PCR products from 356 MRSA strains yielded 13 different RFLP patterns. However, our results differ from those of Lawrence et al., [5] who isolated MRSA strains from various hospitals and found that most of the strains had a unique RFLP pattern when analyzed by coagulase gene typing. Our study highlights the value of PCR-RFLP typing as a reliable method for differentiating MRSA and MSSA strains.

Health care-associated infections remain a significant issue in ICUs, with hand hygiene (HH) being the most effective measure to prevent hospital-acquired infections. In this study, most healthcare workers (HCWs) adhered to HH guidelines, with higher compliance rates compared to resource-limited settings like Iran (6.4%), Ethiopia (16.5%), Nigeria (16.7%), and Indonesia (19.5%) [37]. HH compliance was lower before patient contact than after, consistent with a meta-analysis showing 21% compliance before contact and 47% after. Additionally, low compliance was observed after contact with patient surroundings, similar to findings in London. Variations in compliance rates across studies are attributed to differences in resources, training, culture, policies, workload, and perceived risk among HCWs [37].

In our evaluation, hand hygiene (HH) compliance was higher among nurses (85%) compared to physicians (79%), aligning with previous studies [37]. However, compliance among cleaning staff was significantly low at 55%, likely due to insufficient infection control training. After training, HH compliance improved, and infection rates decreased. The overall HCWs' compliance rose from 80.2% to 90.3%, with significant improvements in infection control measures in ICUs. Similar studies have also shown enhanced adherence after training [37, 38].

Improper use of personal protective equipment (PPE) can increase the risk of healthcare-associated infections among HCWs due to self-contamination. PPE compliance was high at 81%, consistent with studies during SARS and H1N1 outbreaks , although other studies reported lower adherence [39]. Nurses showed better compliance than other HCWs, as seen in previous research [40]. Factors influencing PPE compliance included PPE availability and institutional policies, along with organizational challenges such as high work pressure and limited time for patient care [39]. In this study, barriers to PPE compliance included PPE shortages (92%), lack of time (75%), and skin irritation (80%). Poor access to PPE, improper sizing, and unavailability were identified as key factors leading to inconsistent use.

Our findings highlight the urgent need for improved infection control practices in ICUs, particularly in managing MRSA. Key recommendations include enhancing hygiene protocols, implementing antibiotic stewardship programs to reduce unnecessary antibiotic use, and conducting routine surveillance of MRSA cases. These interventions, along with ongoing education for healthcare staff, can help curb MRSA transmission and improve patient outcomes in critical care settings.

In conclusion,

The study found a high prevalence of MRSA, with 77.8% of *S. aureus* strains being methicillin-resistant. The Vitek-2 system was more accurate for detection than the cefoxitin disk method. Key risk factors for MRSA included invasive procedures, prolonged hospital stays, and diabetes, while hypertension and chemotherapy were not significant. MRSA strains showed resistance to many antibiotics, but all were susceptible to trimethoprim/sulfamethoxazole, teicoplanin, tigecycline, and linezolid. Rapid molecular typing and PVL screening are recommended to control spread. Despite improved infection control, PPE shortages remain a challenge.

Strict infection control protocols are critical, especially in ICUs.

Practical Recommendations:

To improve ICU infection control, stricter hand hygiene, PPE use, and routine MRSA screening for high-risk patients and healthcare workers are recommended. Implementing antibiotic stewardship programs is crucial to reduce resistance. Future research should assess the effectiveness of these measures and explore genetic factors contributing to MRSA resistance and virulence.

1. Ethical Approval

The study was approved by the Local Ethics Committee of Faulty of Medicine, Menoufa University (IRB: 12/2021 MICR 28). the study was conducted following good clinical practice and the Declaration of Helsinki.

2. Consent

All the studied cases signed a written informed consent before enrollment in the study

3. Conflicts of Interest

The authors declare that they have no conflicts of interest.

4. Funding: no funding

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