Microbes and Infectious Diseases 2025; 6(3): 3028-3040

Microbes and Infectious Diseases

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

Original article

Identification of characteristics of *Staphylococcus aureus* isolates from Suez Canal University Hospitals in Ismailia, Egypt

Christine E. Tawfeek, Wedad M. Nageeb, Anwar A. Heiba, Nermine El maraghy, Sally Khattab*

Microbiology and Immunology department, Faculty of medicine, Suez Canal University, Ismailia, Egypt.

ARTICLE INFO

Article history:
Received 15 October 2024
Received in revised form 26 November 2024
Accepted 28 November 2024

Keywords:
Multidrug resistance
Agr dysfunction
Methicillin-resistant
Staphylococcus aureus
Hospital acquired MRSA
Community acquired MRSA.

ABSTRACT

Background: *Staphylococcus aureus* is one of the most common pathogens causing hospital and community-associated infections worldwide. The prevalence of methicillin resistant *S. aureus* (MRSA) has become a global threat to public health. Only a few agents are available nowadays to treat MRSA infections. Accessory gene regulator dysfunction has been associated with MRSA and multi drug resistance phenotypes. Our study objectives were to Identify phenotypic characteristics and antibiotic susceptibility profile of *S. aureus* isolates from Suez Canal University hospitals and to study the relation between *Agr* functionality and resistance to various antibiotics. **Methods:** *Staphylococcus aureus* isolates were collected from different patients admitted to Suez Canal University Hospitals, identified by conventional methods and antibiotic susceptibility was performed using Kirby-Bauer disk diffusion method. Inducible clindamycin test was performed. *Agr* functionality was determined using colony spreading test. **Results:** The study included 100 *S. aureus* isolates obtained from 195 patients, The highest isolation was from intensive care units (ICUs) 31% followed by outpatients 24%. MRSA isolates represented 96% of all isolates. The highest antibiotic resistance rate was to cefoxitin (96%) followed by gentamicin (71%) and erythromycin (51%). Twelve isolates (12%) had inducible clindamycin resistance. *Agr* dysfunction was detected in 36% of all isolates. Most MDR isolates (52.8%) had dysfunctional *Agr*. **Conclusion:** A worrisome situation of a rising number of MRSA and MDR *S. aureus* isolates was detected in SCUHs. Also, we have detected the presence of an increasing number of *Agr* dysfunctional *S. aureus* isolates which was associated with multidrug resistance.

Introduction

Staphylococcus aureus is a part of microflora of skin and mucous membranes of healthy individuals**.** Methicillin-resistant *S. aureus* (MRSA) was described in 1961 and since that time, it has spread worldwide [1]. CDC considers MRSA a "serious threat." In healthcare facilities, MRSA can cause severe problems including bloodstream infections, pneumonia, surgical site infections, sepsis and even death [2]. It represents the only bacterial pathogen other than *Mycobacterium tuberculosis* with number of deaths that consistently exceeds one million annually [3].

MRSA isolates are classified into community acquired (CA-MRSA) or hospital acquired (HA-MRSA). All infections occurred with

DOI: 10.21608/MID.2024.328599.2291
* *Corresponding author:* Sally Khattab
E-mail address: sally.maged@med.suez.edu.eg

any MRSA isolate earlier than 48 hours of hospitalisation, are considered as CA-MRSA. The HA-MRSA referred to any MRSA which was isolated from a patient after 48 hours of hospitalisation or from a patient with a history of hospitalisation for surgery or dialysis, or of a residence in a long-term care facility within 1 year of the MRSA culture date [4]. In Egypt, reports described HA-MRSA prevalence rate of 50-82% and CA-MRSA prevalence rate of 19-47% [5]. In the Suez Canal area, *S. aureus* infections represent about 25% of all hospital-acquired infections and MRSA represent about 75% of *S. aureus* isolates [6].

Only a few agents are still available nowadays to treat MRSA infections. While glycopeptides remain the mainstay of treatment for MRSA infections, there are limited options for oral therapy. These include clindamycin, trimethoprim-sulfamethoxazole, tetracycline, fourth-generation fluoroquinolone and linezolid. Unfortunately, resistance to these agents is being increasingly reported globally [7].

The accessory gene regulatory (*Agr*) system is one of the most important quorum-sensing operons in *S. aureus* biology. *Agr* regulates staphylococcal virulence factors and other accessory gene functions. Evidence indicated that *Agr* dysfunction exists extensively in healthcare settings (13–82%) and associated with persistent bacteraemia, chronic course of infections and excessive mortality among severely ill patients [8, 9]. Studies have linked *mecA* expression in MRSA strains with alterations in *Agr* functionality [10]. The alteration of *Agr* function can result in decreased activity of various antibiotic agents against *S. aureus*. Dysfunctional *Agr* of *S. aureus* isolates cause increased resistance to beta-lactam antibiotics, daptomycin, clindamycin and levofloxacin [8, 11]. In Egypt, there is no sufficient data about prevalence of *Agr* dysfunction among *S. aureus* isolates and its relation to methicillin resistance and MDR.

The current study was designed to identify phenotypic characteristics and antibiotic susceptibility profile of *S. aureus* isolates from Suez Canal University hospitals (SCUH) and to study the relation between *Agr* functionality and resistance to various antibiotics.

Subjects and methods

A cross-sectional descriptive study was conducted with probability simple random sampling technique to identify different characteristics of *S. aureus* isolates from SCUH. Clinical specimens were collected from different sites under aseptic precautions from different departments (Intensive care units- outpatient clinics- orthopedic department- surgery departments) in the hospitals. The specimens' workup was held in Medical Microbiology and Immunology department laboratory, Faculty of Medicine, Suez Canal University. The study was conducted from July 2022 to November 2023. All male and female patients of all age groups admitted and attended in different departments in SCUH were considered as a target population.

The sample size calculation was at least 83 *S. aureus* isolates according to the following equation [12]:

$$n = \left[\frac{Z_{\propto/2}}{E}\right]^2 * P(1-P)$$

(n = sample size, Zα/2 = 1.96, P = The prevalence of *S. aureus* infections =25.3% [6], E = Margin of error = 10%. So, sample size equals 73 *S.aureus* samples with 10% as drop-out, total sample equals 83 *S. aureus* samples).

One hundred *S. aureus* isolates were identified from 195 clinical samples after full microbiologic workup.

Methods

Collection & processing of specimens

Respiratory, blood and urine specimens, skin or wound swabs, bone aspirates and pus discharge were collected and processed under aseptic precautions [13,14].

Microbiologic identification of *S. aureus*

Gram positive cocci arranged in clusters, appeared on blood agar as yellow, round, moderate-sized colonies surrounded by a clear zone and appeared as yellow colonies on mannitol salt agar (Himedia, Mumbai, India) were further identified by biochemical reactions and showed catalase and coagulase tests positive results [15].

Antibiotic susceptibility testing

The *S. aureus* isolates were tested by disk diffusion method (Kirby-Bauer) using Muller-Hinton agar (Himedia, Mumbai, India): A swab dipped in a cell suspension of isolate in sterile saline

adjusted to the turbidity of 0.5 McFarland standard was used to inoculate the surface of the agar plates [16]. The following antibiotic disks were used: cefoxitin disk (FOX 30µg, Oxoid), doxycycline (DO 30µg, Oxoid), levofloxacin (LEV 5µg, Oxoid), linezolid (LZD 30µg, Oxoid), gentamycin (CN 10µg, Oxoid), trimethoprim- sulfamethoxazole (SXT 25µg, Oxoid), erythromycin (E 15 µg, Oxoid), and clindamycin (DA 2 µg, Oxoid). Nitrofurantoin disc (F 300 µg, Oxoid) was added to isolates obtained from urine samples. Inhibition zones were interpreted according to the CLSI interpretive break points [16]. Choosing antibiotics was based upon representing different antibiotic groups. Regarding linezolid, nowadays there is an abuse in its usage in our area for its ease of administration (present in oral form) and giving good results in treating MRSA infections, although WHO consider it as a reserve group, so we wanted to assess its resistance condition in our hospital.

Identification of MRSA strains

According to CLSI guidelines for cefoxitin, it was considered sensitive if inhibition zone was ≥22 mm and resistant ≤21 mm [16]**.** The MRSA isolates were classified into CA-MRSA or HA-MRSA, based on the history of the patient [4]**.**

Detection of inducible clindamycin resistance by D test method

The erythromycin disk is placed 15 mM to 26 mM (edge to edge) from the clindamycin disk in a standard disk diffusion test. Following incubation, formation of a flattening shape of the inhibition zone ≥ 21 mm (D shape) around the clindamycin disk indicated that erythromycin has induced clindamycin resistance [16].

Phenotypic assessment of *Agr* activity

Colony spreading assay

Tryptic soy broth supplemented with 0.24% agar was autoclaved at 121 ℃ for 15 min. Sterile medium (20 ml) was poured into a petri dish (80 mm diameter), and the plates were dried for 20 mins. Bacterial overnight culture (2 µl) was spotted onto the center of the plates and dried for 10 min. The plates were covered and incubated overnight at 37 ℃ to allow the bacteria to spread. Tryptic soy soft agar plates (0.24%) were used to investigate the spreading activity of all *S. aureus* isolates. After overnight incubation of the plates at 37 °C, the spreading zones were examined, and pictures were taken [17].

Statistical analysis was performed by using the SPSS 22 software statistical package. Continuous data were summarized by mean, standard deviation and range**.** While qualitative data were summarized by frequencies. The chi-square and Fisher**'**s exact tests were used.

Ethical Approval: The study was approved by the Research Ethics Committee, Faculty of Medicine, Suez Canal University (Research 4823#).

Consent to Participate: Written informed consents were obtained from all patients participating in the study before specimen collection. Written informed consent was obtained from the parents or guardians of children respondents.

Results

The study included 100 *S. aureus* isolates, collected from 195 patients admitted and attended in different departments in SCUH in Ismailia. Fifty-one% of patients were females. The mean of age was 45.02. Forty six percent had chronic diseases. *S. aureus* isolates were obtained from the intensive care unit (ICU), outpatients, orthopaedic wards, surgical wards, paediatric ICU, neonatal ICU, internal medicine wards and critical care unit with percentages of 31%, 24%, 13%, 11%, 8%, 7%, 4% and 2% respectively. *S. aureus* isolates were obtained from blood, pus, sputum, bone aspirates, urine, skin swabs, endotracheal aspirates and peritoneal fluid specimens with percentages of 30%, 26%, 14%, 14%, 11%, 2%, 2% and 1% respectively.

Among the 100 *S. aureus* isolates, the highest antibiotic resistance was detected for cefoxitin (96%), while the least was for linezolid (3%) (**Figure 1)**. For urinary pathogens (n=11) the resistance to nitrofurantoin was 66.6%**.** Forty percent of all *S. aureus* isolates were MDR (resistant to more than three groups of antibiotics), most commonly (β- lactam drugs- macrolides-gentamicin and doxycycline).

Ninety-six percent of the *S. aureus* isolates were MRSA where 50% of them were CA-MRSA and 50% HA-MRSA. The highest rate of MRSA was from patients having sepsis & laboratory confirmed blood stream infections (LCBSI) (28%). Ninety three percent (92.9) % and 66.7% of sepsis and pneumonia infections respectively were hospital acquired. All patients having non catheter associated UTI or abscesses acquired their infections from the community **(Table 1).**

LCBSI= laboratory confirmed blood stream infections**. UTI=** urinary tract infection**. SSI =**

surgical site infection**. VAP=** ventilator associated pneumonia**. CAUTI=** catheter associated urinary tract infection. **CAI**= Community acquired infection. **HAI**=Hospital acquired infection.

The inducible clindamycin resistance was seen among 12 (12.5%) of the total MRSA isolates.

Formation of a flattening shape (D shape) around the clindamycin disk is shown in **figure (2),** which indicated that erythromycin has induced clindamycin resistance.

Differences in antibiotic susceptibility between CA-MRSA isolates and HA-MRSA isolates were detected. Resistance to gentamicin, erythromycin, clindamycin, trimethoprim-sulfamethoxazole and linezolid were higher among HA-MRSA isolates while resistance to doxycycline was higher in CA-MRSA isolates. All linezolid resistant isolates were HA-MRSA. Multi-drug resistance was higher among HA-MRSA isolates (60%) **(Figure 3)**. The relation between multi-drug resistance and source of infection of MRSA isolates wasn't statistically significant as $p>0.05$**.** Seventeen percent (8/48) of HA-MRSA exhibited inducible clindamycin resistance and 8.3% (4/48) of CA-MRSA isolates showed positive inducible clindamycin resistance.

By doing colony spreading test for the100 *S. aureus* isolates, 64% had functional *Agr* while 36% had dysfunctional *Agr* **(Figure 4)**.

Highest number of isolates with dysfunctional *Agr* (13 isolates) were found in sepsis and LCBSI patients followed by osteomyelitis (6 isolates). *Agr* dysfunctional isolates obtained from non-catheter associated UTI patients (66.7%) were higher than *Agr* functional isolates, while *Agr* dysfunctional isolates obtained from patients with sepsis, pneumonia, abscesses and superficial skin infections (43.3%, 41.1%, and 21.1%, respectively) were lower than *Agr* functional isolates. All isolates obtained from patients with SSIs had functional *Agr*. Statistically significant relation was found between abscesses and *Agr* functionality *(p value <0.05)* **(Supplementary 1).** Dysfunctional *Agr* isolates (37.5%) were higher among CA-MRSA isolates than hospital acquired isolates (35.4%).

From all MRSA isolates, 36.4% (35 isolates out of 96) had dysfunctional *Agr*. The relation between methicillin resistance and *Agr* functionality wasn't statistically significant (*p* value >0.05). Nineteen isolates (52.8%) with dysfunctional *Agr* were MDR. The relation between multi-drug resistance and *Agr* functionality in *S. aureus* isolates was statistically significant as *p* value =0.05 (**Table 2).**

Statistically significant relation was found between *Agr* dysfunction and resistance to erythromycin, clindamycin and levofloxacin (*p* value <0.05), as shown in **table (3).**

Table 1. Frequency distribution of MRSA isolates regarding the type of infection and the relationship between the type and source of infection (N = 96).

Type of infection	Percentage	CAI		HAI	
		No.	%	No.	%
Sepsis & LCBSI	28%	2	7.1%	26	92.9%
Abscess	19%	19	100%	0	0%
Osteomyelitis	11%	10	91%	1	9%
Pneumonia	12%	4	33.3%	8	66.7%
Non-Catheter-associated UTI	9%	9	100%	0	0%
SSI	7%	0	0%	7	100%
VAP	4%	0	0%	4	4%
CAUTI	2%	1	50%	1	50%
Septic arthritis	2%	1	50%	1	50%
Stye	1%	1	100%	0	0%
Ascites	1%	1	100%	0	0%

Table 2. The relation between *Agr* functionality, multi-drug and methicillin-resistance *in S. aureus* isolates.

Variable	Methicillin Resistance			MDR		
	MSSA (n=4)	**MRSA (n=96)**	**p-value**	**Positive (n=40)**	**Negative *(n=60)***	***P* value**
Functional *Agr* (n=64)	3 isolates (4.7%)	61 isolates (95.3%)	0.64*	21 isolates (33%)	43 isolates (67%)	0.05**
Dysfunctional *Agr* (n=36)	1 isolate (2.8%)	35 isolates (97.2%)		19 isolates (52.8%)	17 isolates (47.2%)	

- * Not Statistically significant — Fisher's exact test used.
- **statistically significant as p =0.05 — Chi square test used.

Table 3. The relation between antibiotic resistance and *Agr* functionality *in S. aureus* isolates (N= 100).

Variable	Functional *Agr* (N=64)		Dysfunctional *Agr* (N=36)		Total	*P value*
	N	**%**	**N**	**%**		
CN	46	71.9	25	69.5	71	0.887
E	27	42.2	24	66.7	51	0.019*
CD	22	34.4	22	61.1	44	0.010*
DO	18	28.1	10	27.8	28	0.970
LEV	14	21.9	14	38.9	28	0.05*
SXT	6	9.4	5	13.9	11	0.518

- *****Statistically significant as p<0.05. — Chi square test used.

CN= Gentamicin, **E**= Erythromycin, **CD**= Clindamycin, **DO**= Doxycycline, **LEV=** Levofloxacin, **SXT =** Trimethoprim-sulfamethoxazole**, LZD=** Linezolid

Figure 1. Frequency distribution of the studied *S. aureus* isolates according to their antibiotic susceptibility (N =100)

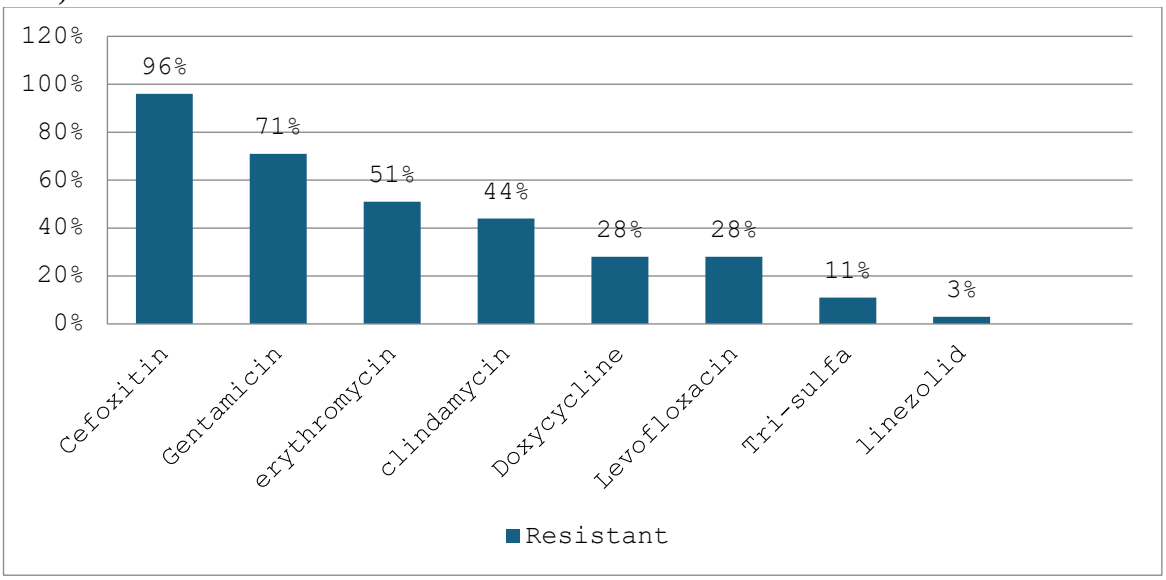


Figure 2. Inducible clindamycin resistant (D-test) positive showing MRSA in MHA media. (DA: Clindamycin; E: Erythromycin; MHA: Muller Hinton agar; MRSA: Methicillin-resistant *S. aureus*).

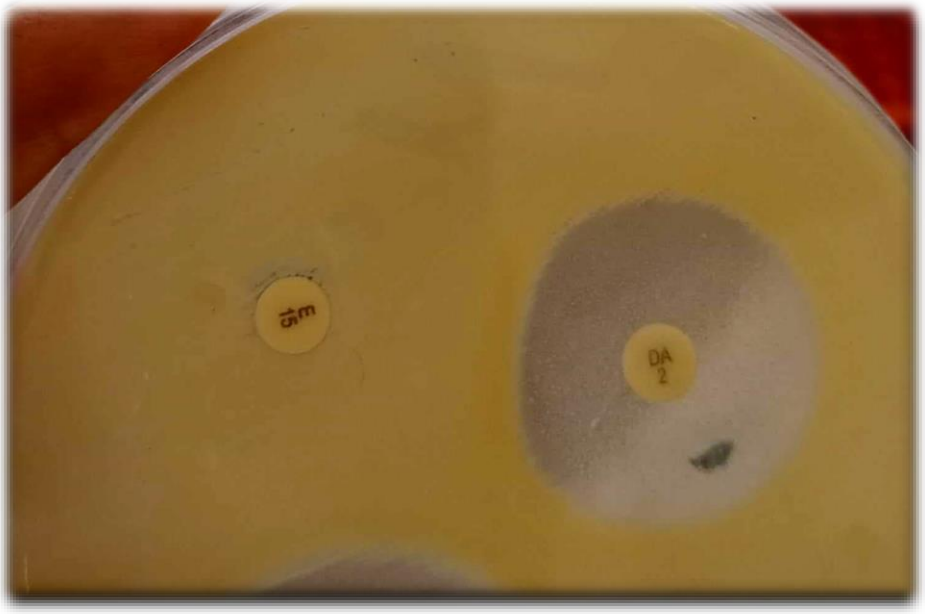


Figure 3. Comparison between HA-MRSA and CA-MRSA according to antibiotic resistance.

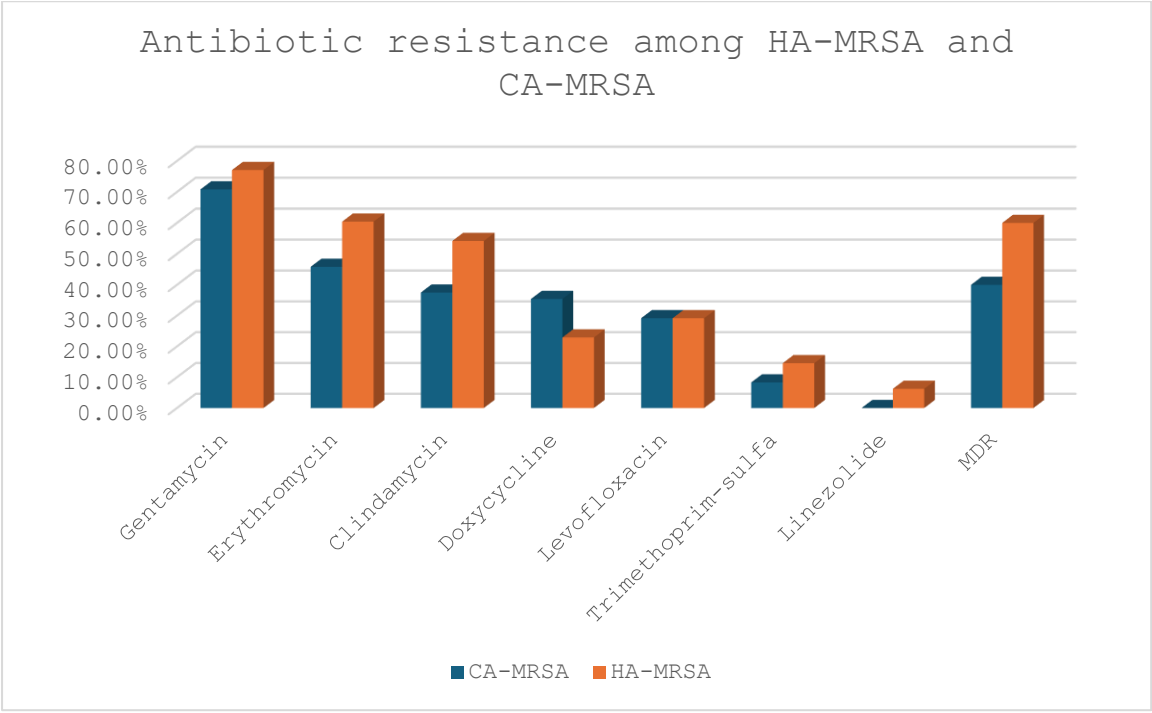
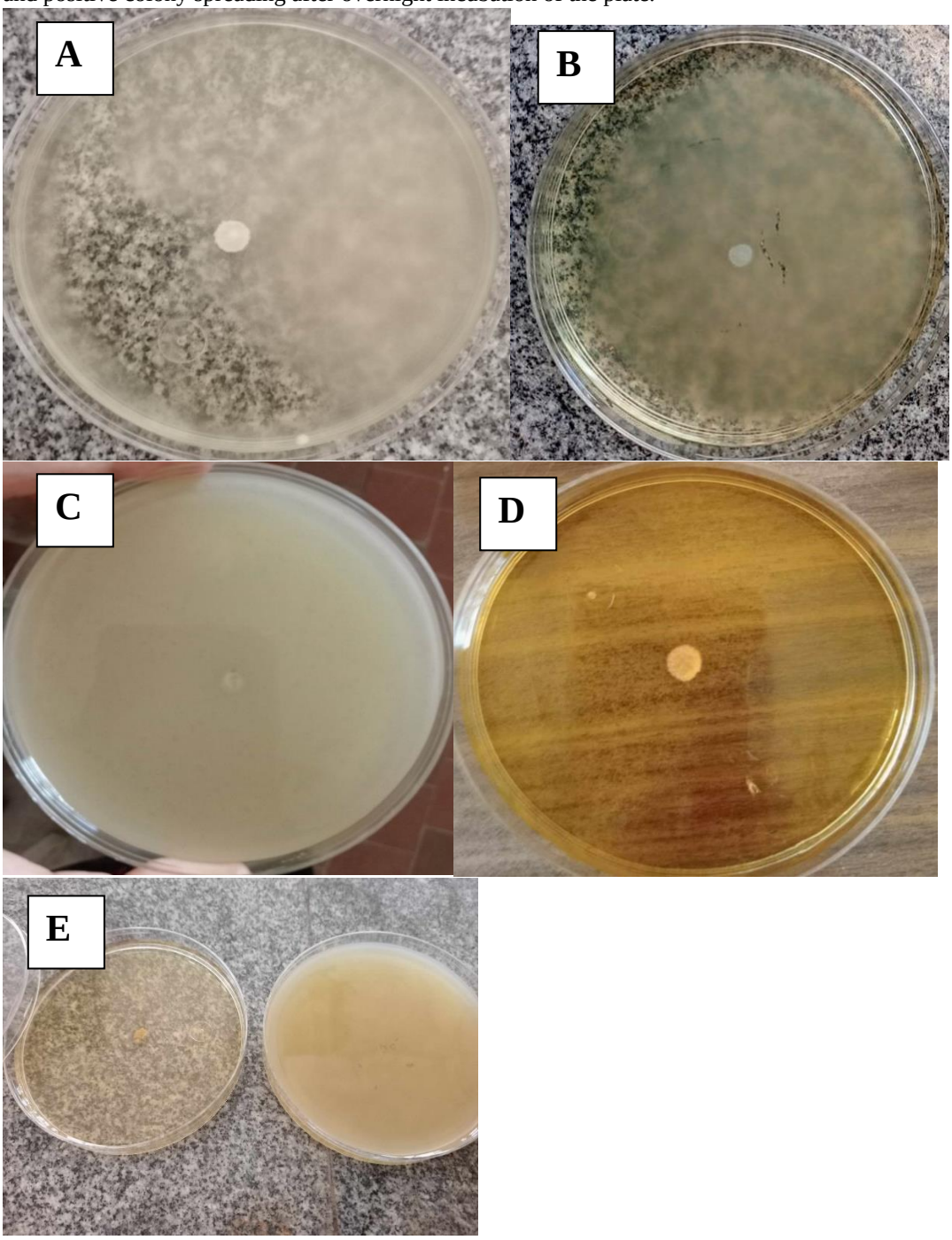


Figure 4. Phenotypic assessment of accessory gene regulator (*Agr*) activity using colony spreading test. Figure 4 shows different pictures for colony spreading on tryptic soy broth supplemented with 0.24% agar. **A, B,** and **C** pictures show positive colony spreading while **D** shows negative colony spreading and **E** shows both negative and positive colony spreading after overnight incubation of the plate.



Discussion

Staphylococcus aureus isolates cause various infections ranging from benign skin and soft tissue infections to life -threatening systemic diseases**.** *S. aureus* developed resistance to different antibacterial agents via various mechanisms [18]. The *Agr* system is one of the most important operons in *S. aureus* that is associated with MRSA and resistance to different antibiotics [19].

In our study, antibiotic susceptibility testing was done for 100 *S. aureus* isolates. MRSA were detected in 96% of all isolates. Similar results were found in a study held by Metwally and Aamir who found that 97% of *S. aureus* isolates were MRSA [20]. Other studies in Egyptian hospitals

revealed lower prevalence rates as they detected 81%, 75% and 27.6% MRSA isolates [6, 21, 22]. The high prevalence of MRSA in our hospital may be attributed to the lack of strictly applied antibiotic policy as increased taking of antibiotics can decrease the normal flora found on the body, giving MRSA an advantage. Also, increased nasal colonization of MRSA among the staff and poor infection control measures can be main causes in this high prevalence. Moreover, the empirical use of antibiotics by the publics which leads to the selection of resistant mutant strains and environmental factors such as crowdedness, poor housing conditions, and lack of personal hygiene can help in transmission of MRSA.

The present study showed that 50% of MRSA infections were HA-MRSA, while other 50% were CA-MRSA. Similar results were found by a study conducted in Ugunda in which 50% of isolated MRSA were CA-MRSA and 50% were HA-MRSA [23]**.** This may be explained as many of the studied isolates (48/96 isolates) were obtained from patients admitted in ICU, PICU, NICU and CCU (31%, 8%, 7% and 2% respectively). Many patients included in the study had chronic diseases made them more prone to Staphylococcal infections and their complications.

In the current study, the highest resistance rate was to cefoxitin (96%) followed by gentamicin (71%) which is in line with **Fahim *et al*.** [24], who reported 73% gentamicin resistance. Resistance to gentamycin was higher among HA-MRSA (77%) isolates than CA-MRSA isolates (69%). These results were similar to the study conducted by **Pan *et al.,*** that showed higher resistance to gentamicin among HA-MRSA isolates (86.7%) than CA-MRSA isolates (13.3%) [25]. Lower results were reported by **Hassan *et al*.** study who detected gentamicin resistance in 61% of HA-MRSA isolates [26]. This means that resistance to gentamicin is increasing over years.

Regarding erythromycin and clindamycin, 51% and 44% respectively of all *S. aureus* isolates in the present study were resistant to them. Resistance to erythromycin and clindamycin were also higher among HA-MRSA isolates (60.4%, 54% respectively) than CA-MRSA isolates. Higher results were recorded by **Mahfouz *et al*.** regarding erythromycin (57.1%) and lower regarding clindamycin (38.5%). In the same study, the prevalence of inducible clindamycin resistance was 15.8%, which is higher than the prevalence detected in our study (12.5%) [27]. Another study which was also conducted in Ismailia, Egypt, detected erythromycin and clindamycin resistance in 54.5% and 68 38.6% of isolates respectively. Inducible clindamycin resistance was detected in 24% of isolates which is higher than our study [28]. The increase in clindamycin resistance and decrease in inducible clindamycin resistance in our study may be explained that constitutive resistance was present as 32/44 clindamycin resistant isolates were also resistant to erythromycin (72%) and didn't show inducible clindamycin resistance.

The present study showed low resistance levels to doxycycline, levofloxacin, and trimethoprim-sulfamethoxazole (28%, 28% and 11% respectively). These results are similar to the results reported by **AlFeky *et al*.** study which detected a resistance rate of 25.3% and 11.2% to levofloxacin and trimethoprim-sulfamethoxazole respectively [21] and similar to **ElSayed *et al.*** study [29]. The low resistance rate to levofloxacin may be due to the infrequent use of this drug in the hospitals of study while the low rate of resistance to trimethoprim-sulfamethoxazole may be as this drug is only available in oral dosage form which make it unsuitable for hospitalized patients, especially in ICUs patients.

The current study showed that there were 3 isolates (3%) resistant to linezolid and all were HA-MRSA. This rate is higher than the rate reported by **Abdelwahab *et al.*** who demonstrated 100% susceptibility rate to linezolid [30] and is in line with **AbdAlhafiz *et al.*** and **Khan *et al.*** studies which showed 95.5 % and 96% susceptibility rate to linezolid, respectively [31,32] and lower than rate of resistance (92%) detected in **Alfeky *et al.*** study [21]. In an earlier study conducted in SCUH, there was no resistance to linezolid detected among *S. aureus* isolates [6], so presence of resistant isolates detected in the current study is a matter of concern. This may be due to increased consumption of linezolid in the hospitals studied and absence of strict antibiotic policies there.

In the current study, MDR isolates represented 40% of all tested isolates, the most commonly resistant antibiotics were β-lactam drugs, gentamicin, erythromycin, clindamycin, and doxycycline. This is lower than reported by **Alfeky *et al*.** study in which MDR isolates represented 79% of tested isolates, yet they are similar regarding the

most commonly resistant drugs as they reported that β-lactams, aminoglycosides, and macrolides were the most common [21]. Our results were in line with **Kot *et al.*,** they reported that resistance to erythromycin and clindamycin was the most common resistance pattern among MDR MRSA isolates [33]. According to the present study, 60% of MDR MRSA isolates were obtained from hospital acquired infections and this result was in line with **Henderson *et al.*** study [34].

According to the current study, 64% of all isolates had functional *Agr* while 36% had dysfunctional *Agr*. This finding is consistent with the prevalence reported by **Yang *et al.*** [9] and higher than **Abdulgader *et al.*** as they observed *Agr* dysfunctionality in 13% of isolates [35]. Furthermore, the results were also higher than detected by earlier studies from Brazil and China in which *Agr* dysfunction was observed in 4.7% and 3.7 %of MRSA isolates respectively [19, 9]. On the other side, higher results of *Agr* dysfunction reported by **Rezk *et al.*** as they reported that 84% of isolates had dysfunctional *Agr* [36]. The differences between the current results, compared to other studies may be due to the differences in sample size, types of infection and source of infection.

According to the present study, sepsis was the most common type of infection from which isolates with dysfunctional *Agr* were obtained (36%) followed by osteomyelitis (19.5%). These findings were consistent with **Yang *et al.*, Young *et al.*,** and **Hachani *et al.*** studies who documented that dysfunctional *Agr* isolates were higher in HA blood stream infections [9, 10, 37]. This may be because *Agr* dysfunction can leads to increased intracellular persistence of bacteraemia isolates during infection. Statistically significant relation was found between abscesses, surgical site infections (SSIs) and *Agr* functionality *(p value <0.05)* which means that *S. aureus* causing abscesses and SSIs often has functional *Agr*. This was in line with Fischer *et al.* who found that 70% of *S. aureus* isolates obtained from abscesses had functional *Agr*. Dysfunctional *Agr* isolates were higher among CA-MRSA isolates than hospital acquired isolates [38].

Sixty seven percent of isolates with dysfunctional *Agr* were resistant to erythromycin, 61 % were resistant to clindamycin, and about 40% of these isolates were resistant to levofloxacin showing a statistically significant difference (*p* value <0.05). Moreover, 53% of isolates with dysfunctional *Agr* were MDR and the relation between MDR and *Agr* functionality was statistically significant ($p = 0.05$). This is in line with results reported by **Rossato *et al.*** and **Yang *et al.*** who showed that 90% of *Agr* dysfunctional isolates exhibited multidrug resistance [9, 19]. A study held by **He *et al.*** has concluded that *Agr*-dysfunctional mutants has leaded to enhanced resistance to clindamycin and levofloxacin antibiotics [11]. **Song *et al.*** stated that *Agr* dysfunction causes resistance to different antibiotics via several changes including constant expression of *mecA*, fatty acid metabolism, and biofilm thickening [39]. **Rezk *et al.*** also found that the dysfunction of the *Agr* locus leaded the bacteria to form abundant biofilms which can leads to more antibiotic resistance [36]. According to a meta-analysis conducted by **Lee *et al.*** they found that alteration of *Agr* function can result in decreased activity of various antibiotic agents against *S. aureus* like vancomycin, beta-lactam antibiotics and daptomycin [8]. As *Agr* dysfunctional isolates in our study were found to be related to multidrug resistance and healthcare associated infections, combining approaches like improvement of infection control practices with the using of anti-*Agr treatment* could reduce the spread of *S. aureus* resistant isolates. Additional studies are required to explore the feasibility of *Agr* system as a biomarker for the outcomes of patients with *S. aureus* infections and as a new target for antimicrobial agents.

In the current study, *Agr* dysfunctionality was not associated with MRSA (*p* value >0.05), this finding was consistent with **Abdulgader *et al.*** study [35]. This could be explained that *Agr* dysfunctionality has been reported to be more frequent amongst isolates carrying certain SCC*mec* types. Many molecular epidemiology studies reported that SCC*mec* types I–III are more commonly associated with *Agr* dysfunction than SCC*mec* type IV/ V [8]. However, SCC*mec* types of isolated MRSA isolates weren't identified in this study. Moreover, most of studied isolates were MRSA, so we could not identify the occurrence of *Agr* dysfunction among MSSA.

Limitations of the study

Our sample size was not large enough to detect a significant relation between *Agr* dysfunction and resistance to different antibiotic groups. The MRSA isolates were not tested for their susceptibility to other antibiotics such as vancomycin, tigecycline, and rifampicin, which can

open a new gate for treating MRSA infections, so we recommend further studies about these antibiotics. Molecular studies such as SCC*mec* types, genetic mutations causing antibiotic resistance and *Agr* dysfunction are recommended to be studied in future research.

Conclusion

A worrisome situation of a rising number of MRSA and MDR *S. aureus* isolates was detected in SCUHs. Also, we have detected the presence of an increasing number of *Agr* dysfunctional *S. aureus* isolates which was associated with multidrug resistance. Antibiotic stewardship programme and proper infection control practices should be strictly applied in SCUHs to prevent the transmission of MRSA and MDR *S. aureus* isolates

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Authors contributions

Anwar A. Heiba and Nermine El maraghy were contributed to the conception, design, material preparation and revision of the final manuscript. Christine E. Tawfeek, Wedad M. Nageeb and Sally Khattab contributed to material preparation, data collection, data analysis, study design and writing the final manuscript. All authors revised and approved the final manuscript.

Data availability

All authors claim that all data and materials are available within the article.

References

1- **Lee AS, De Lencastre H, Garau J, Kluytmans, J, Malhotra-Kumar S, Peschel A, Harbarth S.** Methicillin-resistant Staphylococcus aureus. Nature reviews Disease primers 2018; 4(1):1-23. doi: 10.1038/nrdp.2018.33

2- **Centre for Disease Control and prevention (CDC).** Clinical Overview of Methicillin-resistant Staphylococcus aureus (MRSA) in Healthcare Settings. Available at https://www.cdc.gov/mrsa/hcp/clinical-overview/index.html. Accessed April 12, 2024.

3- **Piewngam P, Otto M.** Staphylococcus aureus colonisation and strategies for decolonisation. The Lancet Microbe 2024; 5(6); 606-618.

4- **Vysakh PR, Jeya M.** A comparative analysis of community acquired and hospital acquired methicillin resistant Staphylococcus aureus. Journal of clinical and diagnostic research: JCDR 2013; 7(7): 1339.

5- **Abouelfetouh A.** The status of methicillin resistance among Egyptian Staphylococcus aureus isolates: an overview. Infectious Disorders-Drug Targets 2017; 17(1), 67-69.

6- **El Sweify M, Raheel A, Aboul-Atta H, El-Hadidy G, Hessam W.** Identification of community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA) causing hospital-acquired infections in Suez Canal University Hospitals, Egypt by detection of its major virulence determinants. Microbes and Infectious Diseases 2021; 2(4): 715-724.

7- **Xu J, Pang L, Ma XX, Hu J, Tian Y, Yang YL, Sun DD.** Phenotypic and molecular characterisation of Staphylococcus aureus with reduced vancomycin susceptibility derivated in vitro. Open Medicine 2018; 13(1): 475-486.

8- **Lee SO, Lee S, Lee JE, Song KH, Kang CK, Wi YM, et al.** Dysfunctional accessory gene regulator (Agr) as a prognostic factor in invasive Staphylococcus aureus infection: a systematic review and meta-analysis. Scientific reports 2020; 10(1): 20697.

9- **Yang X, Dong F, Qian S, Wang L, Liu Y, Yao K, et al.** Accessory gene regulator (agr) dysfunction was unusual in Staphylococcus aureus isolated from Chinese children. BMC microbiology 2019; 19:1-2.

10- **Young BC, Wu CH, Charlesworth J, Earle S, Price JR, Gordon NC, et al.** Antimicrobial

resistance determinants are associated with Staphylococcus aureus bacteraemia and adaptation to the healthcare environment: a bacterial genome-wide association study. Microbial Genomics 2021; 7(11): 000700.

11- **He L, Zhang F, Jian Y, Lv H, Hamushan M, Liu J, et al.** Key role of quorum-sensing mutations in the development of Staphylococcus aureus clinical device-associated infection. Clinical and translational medicine 2022; 12(4). doi: 10.1002/ctm2.801

12- **Dawson B, Trapp RG.** Basic & clinical biostatistics 4th Edition, McGraw-Hill Professional, New York. 2004; 438-438.

13- **Forbes BA, Sahm DF, Weissfeld AS.** Study Guide for Bailey and Scott's Diagnostic Microbiology-E-Book. Elsevier Health Sciences 2021.

14- **Mahon CR, Lehman DC.** Textbook of Diagnostic Microbiology-E-Book: Textbook of Diagnostic Microbiology-E-Book. Elsevier Health Sciences 2022.

15- **Ghayyib AA, Ahmed IA, Ahmed HK.** Isolation, Molecular Identification, and Antimicrobial Susceptibility Testing of Staphylococcus aureus Isolates. HIV Nursing 2022; 22(2): 278-283.

16- **Clinical and Laboratory Standards Institute (CLSI).** Performance Standards for Antimicrobial Susceptibility Testing: Informational Supplement M 100. 33rd ed. USA: Wayne, PA. 2023.

17- **Liu CC, Lin MH.** Involvement of Heme in Colony Spreading of Staphylococcus aureus. Frontiers in Microbiology 2020; 11: 170.

18- **Taylor TA, Unakal CG.** Staphylococcus aureus Infection. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441868. Accessed April 2024.

19- **Rossato AM, Primon-Barros M, Dias CA, d'Azevedo PA.** Vancomycin MIC and Agr dysfunction in invasive MRSA infections in southern Brazil. Braz. J. Microbiol 2020; 51: 1819–1823. doi:10.1007/s42770-020-00384-0.

20- **Metwally W, Aamir R.** Prevalence and antimicrobial resistance patterns of nosocomial pathogens causing Surgical Site Infections in an Egyptian University hospital. Microbes and Infectious Diseases 2020; 1(3):226-237.

21- **Alfeky AE, Tawfick MM, Ashour MS, El-Moghazy.** High prevalence of multi-drug resistant methicillin-resistant staphylococcus aureus in tertiary Egyptian Hospitals. The Journal of Infection in Developing Countries 2022; 16(05): 795-806.

22- **Allam AE, Fakhr AE, Mahmoud ME, El-Korashi LA.** Staphylococcus aureus nasal colonization among health care workers at an Egyptian tertiary care hospital. Microbes and Infectious Diseases 2021; 2(1): 108-118.

23- **Kateete DP, Bwanga F, Seni J, Mayanja R, Kigozi E, Mujuni B, et al.** CA-MRSA and HA-MRSA coexist in community and hospital settings in Uganda. Antimicrobial Resistance & Infection Control 2019; 8(1): 1-9.

24- **Fahim NAE.** Prevalence and antimicrobial susceptibility profile of multidrug-resistant bacteria among intensive care units' patients at Ain Shams University Hospitals in Egypt-a retrospective study. Journal of the Egyptian Public Health Association 2021; 96: 1-10.

25- **Pan SC, Wang JT, Lauderdale TL, Ko WC, Chen YS, Liu JW, et al.** Epidemiology and staphylococcal cassette chromosome mec typing of methicillin-resistant Staphylococcus aureus isolates in Taiwan: A multicenter study.

Journal of the Formosan Medical Association 2014; 113(7):409-416.

26- **Hassan RM, Elanany MG, Mostafa MM, Yousef RHA, Salem ST.** Whole genome characterization of methicillin resistant Staphylococcus aureus in an Egyptian Tertiary Care Hospital. Journal of Microbiology, Immunology and Infection 2023; 56(4): 802-814. doi: 10.1016/j.jmii.2023.04.005.

27- **Mahfouz AA, Said HS, Elfeky SM, Shaaban MI.** Inhibition of erythromycin and erythromycin-induced resistance among Staphylococcus aureus clinical isolates. Antibiotics 2023; 12(3): 503.

28- **Kishk RM, Anani MM, Nemr NA, Soliman NM, Fouad MM.** Inducible clindamycin resistance in clinical isolates of staphylococcus aureus in Suez Canal University Hospital, Ismailia, Egypt. J. Infect. Dev. Ctries 2020; 14 (11): 1281-1287. doi: 10.3855/jidc.12250.

29- **ElSayed N, Ashour M, Amine AEK.** Vancomycin resistance among Staphylococcus aureus isolates in a rural setting, Egypt. Germs 2018; 8(3):134.

30- **Abdelwahab MA, Amer WH, Elsharawy D, Elkolaly RM, Helal RA, El Malla DA, et al.** Phenotypic and genotypic characterization of methicillin resistance in Staphylococci isolated from an Egyptian University Hospital. Pathogens 2023; 12(4): p.556.

31- **AbdAlhafiz AI, Elleboudy NS, Aboshanab KM, Aboulwafa MM, Hassouna NA.** Phenotypic and genotypic characterization of linezolid resistance and the effect of antibiotic combinations on methicillin-resistant Staphylococcus aureus clinical isolates. Annals of Clinical Microbiology and Antimicrobials 2023; 22: 23.

32- **Khan AA, Ali A, Tharmalingam N, Mylonakis E, Zahra R.** First report of mecC gene in clinical methicillin resistant S. aureus (MRSA) from tertiary care hospital Islamabad, Pakistan. Journal of Infection and Public Health 2020; 13(10): 1501-1507.

33- **Kot B, Wierzchowska K, Piechota M, Grużewska A.** Antimicrobial resistance patterns in methicillin-resistant Staphylococcus aureus from patients hospitalized during 2015–2017 in hospitals in Poland. Medical Principles and Practice 2020; 29(1): 61-68.

34- **Henderson A, Nimmo GR**. Control of healthcare-and community-associated MRSA: recent progress and persisting challenges. British medical bulletin 2018; 125(1):25-41.

35- **Abdulgader SM, Van Rijswijk A, Whitelaw A, Newton-Foot M.** The association between pathogen factors and clinical outcomes in patients with Staphylococcus aureus bacteraemia in a tertiary hospital, Cape Town. International Journal of Infectious Diseases 2020; 91: 111-118.

36- **Rezk S, Alqabbasi OS, Ghazal A, El Sherbini E, Metwally DES.** Association between accessory gene regulator alleles, Agr functionality and biofilm formation in MRSA and MSSA isolated from clinical and nasal carrier specimens. Microbes and Infectious Diseases 2023; 4(2):.459-467.

37- **Hachani A, Giulieri SG, Guérillot R, Walsh CJ, Herisse M, Soe YM, et al.** A high-throughput cytotoxicity screening platform reveals Agr-independent mutations in bacteraemia-associated Staphylococcus aureus that promote intracellular persistence. Elife 2023; 12: 84778.

38- **Fischer J, Lee JC, Peters G, Kahl BC.** A capsular clinical Staphylococcus aureus isolates lack Agr function. Clinical Microbiology and Infection 2014; 20(7): 414-417.

39- **Song HS, Choi TR, Han YH, Park YL, Park JY, Yang SY, et al.** Increased resistance of a methicillin-resistant Staphylococcus aureus Δ agr mutant with modified control in fatty acid metabolism. AMB Express 2020; 10: 64.

Tawfeek CE, Nageeb WM, Heiba AA, El maraghy N, Khattab S. Identification of characteristics of *Staphylococcus aureus* isolates from Suez Canal University Hospitals in Ismailia, Egypt. Microbes Infect Dis 2025; 6(3): 3028-3040.