Virulence genes distributed among *Staphylococcus aureus* causing wound infections and their correlation to antibiotic resistance

Asia Helmi Rasmi1, Eman Farouk Ahmed2*, Abdou Mohammed Abdullah Darwish3 and Gamal Fadl Mahmoud Gad4

**Abstract**

**Background:** *Staphylococcus aureus* causes many human infections, including wound infections, and its pathogenicity is mainly influenced by several virulence factors.

**Aim:** This study aimed to detect virulence genes (*hla*, *sea*, *icaA*, and *fnbA*) in *S. aureus* isolated from different wound infections among Egyptian patients admitted to Minia University Hospital. This study also aimed to investigate the prevalence of these genes in methicillin-resistant *S. aureus* (MRSA), methicillin-susceptible *S. aureus* (MSSA), and vancomycin-resistant *S. aureus* isolates and the resistance and sensitivity to different antibiotic classes.

**Methods:** A cross-sectional study was carried out from November 2019 to September 2021. Standard biochemical and microbiological tests revealed 59 *S. aureus* isolates. The Kirby-Bauer disc diffusion method was used to determine antibiotic susceptibility. DNA was extracted using a DNA extraction kit, and polymerase chain reaction was used to amplify all genes.

**Results:** A total of 59 *S. aureus* isolates were detected from 51 wound samples. MRSA isolates accounted for 91.5%, whereas MSSA isolates accounted for 8.5%. The multidrug resistance (MDR) percentage in *S. aureus* isolates was 54.2%. *S. aureus* showed high sensitivity pattern against vancomycin, linezolid, and chloramphenicol. However, a high resistance pattern was observed against oxacillin and piperacillin. *sea* was the most predominant gene (72.9%), followed by *icaA* (49.2%), *hla* (37.3%), and *fnbA* (13.6%). *sea* was the commonest virulence gene among MRSA isolates (72.2%), and a significant difference in the distribution of *icaA* was found. However, *sea* and *icaA* were the commonest genes among MSSA isolates (79.9%). The highest distribution of *sea* was found among ciprofloxacin-resistant isolates (95.2%).

**Conclusion:** The incidence of infections caused by MDR *S. aureus* significantly increased with MRSA prevalence. *sea* is the most predominant virulence factor among antibiotic-resistant strains with a significant correlation to piperacillin, gentamicin, and levofloxacin.

**Keywords:** *S. aureus*, Wound infections, Virulence genes, Antibiotic resistance

---

**Introduction**

The fundamental goal of the skin is to keep microbial populations on its surface under control and prevent diseases from colonizing the underlying tissue [1]. A
wound is a disruption in the skin’s protective action [2]. *Staphylococcus aureus* is the most frequent opportunistic bacteria, causing many superficial and life-threatening infections [3]. It can cause various disorders, including skin and soft tissue infections (SSTIs), invasive infections, and toxin-mediated disorders [4]. Since it produces several virulence factors and acquires multidrug resistance (MDR) to various antibacterial agents, it is a major infectious agent in communities and hospitals [5].
S. aureus has an incredible ability to develop resistance rapidly. Environmental factors and cell membrane disruption or DNA damage can influence the fast development of antibiotic resistance [6]. More than 90% of S. aureus is resistant to penicillin, which remains a global issue [7]. Methicillin-resistant S. aureus (MRSA) is a common inhabitant of a large part of the healthy population and can cause a wide range of illnesses, from minor skin infections to life-threatening diseases [8]. The MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [9, 10]. The MDR of S. aureus strains has been linked to longer hospital stays, higher mortality rates, and concomitant costs [11].

The presence of many virulence factors, such as surface proteins, biofilms, exoenzymes, exotoxins, and exfoliative toxins, is linked to the ability of S. aureus to cause different infections. All these factors allow bacteria to attach to tissues, causing pathogenesis, and to penetrate the immune system, causing toxicity [12]. One of the virulence factors of S. aureus is a cytolytic, pore-forming toxin, such as α-hemolysin, which is involved in the pathogenesis of S. aureus [13]. Many S. aureus strains, particularly MRSA, release one or more distinct staphylococcal exotoxins, including staphylococcal enterotoxins [14], the most important pathogenic components belonging to the superantigen family [15].

The ability of the microorganism to successfully persist within the hospital and community and several cell wall-associated adhesive molecules, such as fnb (encoding fibronectin-binding protein) is responsible for the possibility of severe animal and human diseases [16, 17]. The ability of S. aureus to build biofilms is linked to the antimicrobial resistance mechanism. Invasion isolates are more likely to form biofilm than healthy individual carriage isolates [18]. The polysaccharide intercellular adhesin (PIA) is the most important component of biofilm [19, 20]. The N-acetylgalcosamyl transferase enzyme responsible for PIA synthesis is known to be encoded by icaA [21].

S. aureus persists and spreads by acquiring antibiotic resistance genes. Identification of S. aureus virulence genes is important for evaluation of disease development. This study focused on S. aureus virulence genes and to detect their correlation to antimicrobial resistance patterns.

**Materials and methods**

**Study area, design, and population**

A cross-sectional study was carried out from November 2019 to September 2021 in Minia University Hospital (Minia, Egypt). Wound samples were collected from the Department of Plastic and Reconstructive Surgery. The samples were properly labeled, indicating the source, sex, and age of the patient. Ethical clearance for the study was granted by Minia University Hospital.

**Collection of wound pus samples**

Bacterial samples were collected from patients having wound infections present on admission to the outpatient clinic and cultured onto nutrient agar, mannitol salt agar, and DNase agar. All media were produced by Oxoid.

---

**Table 1** The list of primers sequences

| Virulence genes            | Primer Sequence | References |
|----------------------------|-----------------|------------|
| Hemolysin A (hla)          | F: CTG ATT ACT TTC AAG GAA ATT CGA TTG R: CTT TCC AGC CTA CTT TTT TAT CAG T | [48]        |
|                            |                 |            |
| Staphylococcal enterotoxin A (sea) | F: TTG GAA ACG GTT AAA ACG AA R: GAA CCT TCC CAT CAA AAA CA | [49]        |
| Intracellular adhesion-A (icaA) | F: GAT TAT GTA A TG TGC TTG GA R: ACT ACT GCT GCC TTA ATA AT | [50]        |
| Fibronectin binding protein-A (fnbA) | F: GCC GAG ATC AAA GAC AA R: CCA TCT ATA GCT GTG TGG | [51]        |

**Table 2** Conditions of PCR products

| Gene | Initial Denaturation | Denaturation | Annealing | Extension | Final Extension | Cycles | Product size (bp) |
|------|----------------------|--------------|-----------|-----------|-----------------|--------|------------------|
| hla  | 95 °C for 5 min      | 95 °C for 50 s | 58 °C for 30 s | 72 °C for 1 min | 72 °C for 10 min | 40     | 209 bp           |
| sea  | 95 °C for 5 min      | 95 °C for 1 min | 55 °C for 45 s | 72 °C for 1 min | 72 °C for 10 min | 40     | 120 bp           |
| icaA | 95 °C for 5 min      | 95 °C for 1 min | 50 °C for 1 min | 72 °C for 1.5 min | 72 °C for 5 min | 40     | 770 bp           |
| fnbA | 95 °C for 5 min      | 95 °C for 1 min | 47 °C for 1 min | 72 °C for 1.5 min | 72 °C for 5 min | 40     | 1279 bp          |
(England) and prepared according to the manufacturer’s instructions. The cultures were incubated at 37 °C for 24 h to be examined the next day.

Isolation and identification of wound bacterial isolates
The primary identification of bacterial isolates was based on colonial appearance, pigmentation, morphology, Gram staining, and biochemical characteristics. The biochemical tests applied were the standard catalase test, coagulase (tube and slide) test, and DNase test (Fig. 1). For the extended storage of bacterial isolates, preservation in 20% glycerol vials at −70 °C was carried out.

Antibiotic sensitivity testing
Antimicrobial sensitivity was determined by the Kirby-Bauer agar disc diffusion method according to the Clinical Laboratory Standard Institute (CLSI; 2018). Antibiotic discs were used with the following drug concentrations: linezolid (30 μg), tetracycline (30 μg), chloramphenicol (30 μg), rifampin (5 μg), piperacillin (100 μg), amoxicillin/clavulanic acid (30 μg), ampicillin/sulbactam (20 μg), levofloxacin (5 μg), gentamycin (10 μg), vancomycin (30 μg), oxacillin (1 μg), and ciprofloxacin (5 μg) were applied onto Müller-Hinton agar (Himedia). The plates were aerobically incubated at 37 °C for 24 h, and the diameter of the inhibition zones was measured (in mm). The results were compared to that of the CLSI.

DNA extraction and detection of virulence genes
DNA was extracted using a DNA extraction kit (Qiagen, Germany), and the procedures were carried out according to the manufacturer’s instructions. The oligonucleotide primers used in this study were for the detection of genes encoding α-hemolysin (hla), staphylococcal enterotoxin A (sea), intracellular adhesion A (icaA), and fibronectin-binding protein A (Fnha). Table 1 lists the primer sequences (Metabione, Germany) of this study, and Table 2 presents the conditions of the polymerase chain reaction (PCR) products. The PCR products were resolved by electrophoresis on 1% agarose gel, and electrophoresis was carried out at a constant current of 50 mA for 30 min. DNA bands were visualized by ethidium bromide staining and ultraviolet transillumination light. The size of the fragments was determined by comparing their migration to a 100 bp ladder as a standard.

Statistical analysis
Statistical analyses were performed using χ² using SPSS version 16 (SPSS, Inc., Chicago, IL, USA). A χ² test was used to test the association between S. aureus virulence genes and participant’s gender and age as well as with the antibiotic resistance profile. Similarly, the association between the antibiotic resistance profile with participant’s gender and age groups was detected. The results were considered statistically significant when P ≤ 0.05.

Results
Prevalence of S. aureus isolates according to gender, age, and sample source
A total of 59 S. aureus isolates were detected from 51 different wound samples. The incidence of S. aureus was much higher in males [n = 36 (70.6%)] than in females [n = 15 (29.4%)]. Patients were classified into different age groups from 1 month to 60 years (mean ± standard deviation, 28.98 ± 16.95). The highest prevalence of S. aureus was observed in the age group between 1 and 20 years (45.1%), followed by patients in the age group between 41 and 60 years (29.4%) and finally patients in the age group from 21 to 40 years (25.5%). Figure 2 shows that the highest number of samples was from accidental wounds, such as animal bites, occupational injuries, a sharp tool, or car accidents (n = 38; 74.5%), followed by seven samples of burn infection (burning agents, such as flame, scald, electrical, boiled water, and chemical reagent; 13.7%). Three samples were from surgical wounds (5.9%) and three samples were from ulcers and abscess discharge (5.9%).

Antimicrobial sensitivity testing
Table 3 shows that MRSA isolates accounted for 91.5%, whereas methicillin-susceptible S. aureus (MSSA) isolates accounted for 8.5%. S. aureus had low resistance to chloramphenicol (10.2%), vancomycin (13.5%), and linezolid (16.9%). Moderate (intermediate) resistance was recorded against gentamycin (33.9%), levofloxacin and ciprofloxacin (both 35.6%), rifampin (37.3%), and tetracycline (62.7%). High resistance was observed against oxacillin, amoxicillin/clavulanic acid, ampicillin/sulbactam (all 91.5%), and piperacillin (100%). The MDR in S. aureus isolates was 54.2%. S. aureus had R0 = 0%, R1 = 20.3%, R2 = 23.7%, R3 = 16.9%, R4 = 13.6%, R5 = 16.9%,
Table 3  Antibiotic sensitivity profile of S. aureus isolates

| Antibiotic               | Concentration µg/disc | Sensitive No. (%) | Intermediate No. (%) | Resistant No. (%) | Company                  |
|--------------------------|-----------------------|-------------------|----------------------|------------------|--------------------------|
| Linezolid                | 30                    | 49 (83.1%)        | 0 (0%)               | 10 (16.9%)       | Bioanalyse limited -Turkey|
| Tetracycline             | 30                    | 16 (27.1%)        | 6 (10.2%)            | 37 (62.7%)       | Himedia, India            |
| Chloramphenicol          | 30                    | 42 (71.2%)        | 11 (18.6%)           | 6 (10.2%)        | Bioanalyse limited -Turkey|
| Rifampin                 | 5                     | 34 (57.6%)        | 3 (5.1%)             | 22 (37.3%)       | Bioanalyse limited -Turkey|
| Piperacillin             | 100                   | 5 (8.5%)          | 0 (0%)               | 54 (91%)         | Sigma, USA                |
| Gentamycin               | 10                    | 29 (49.2%)        | 10 (16.9%)           | 20 (33.9%)       | Himedia, India            |
| Amoxicillin / Sulbactam  | 20 (10/10 µg)         | 5 (8.5%)          | 0 (0%)               | 54 (91.5%)       | Bioanalyse limited -Turkey|
| Oxacillin                | 1                     | 5 (8.5%)          | 0 (0%)               | 54 (91.5%)       | Sigma, USA                |
| Levofloxacin             | 5                     | 33 (55.9%)        | 5 (8.5%)             | 21 (35.6%)       | Himedia, India            |
| Ciprofloxacin            | 5                     | 31 (52.2%)        | 7 (11.9%)            | 21 (35.6%)       | Bioanalyse limited -Turkey|
| Amoxicillin/ Clavulanic  | 30 (20/10 µg)         | 5 (8.5%)          | 0 (0%)               | 54 (91.5%)       | Bioanalyse limited -Turkey|
| Vancomycin               | 30                    | 51 (86.4%)        | 0 (0%)               | 8 (13.5%)        | Sigma, USA                |

R6 = 1.7%, R7 = 3.4%, and R8 = 1.7% (R0 represents the number of isolates sensitive to all antimicrobial classes tested, whereas R = 1, 2, 3, 4, 5, 6, 7, and 8 represent isolates resistant to 1, 2, 3, 4, 5, 6, 7, and 8 antibiotic classes, respectively). No statistically significant difference was detected between the resistance profile of tested antibiotics and participant’s gender or age group (p > 0.05) (Tables 4 and 5).

Detection of virulence genes

To test the virulence genes of the isolates in this study, hla, sea, icaA, and fnbA were detected by PCR amplification. Table 6 shows that sea was the most predominant in 72.9% of the isolates. icaA was found in 49.2% of the isolates, followed by hla (37.3%) and fnbA (13.6% of the isolates). Amplicon sizes of 209, 120, 770, and 1279 bp were considered positive for the presence of hla, sea, icaA, and fnbA, respectively. Figure 3 shows hla, sea, icaA, and fnbA PCR amplification products among S. aureus isolates, respectively. sea was the most predominant virulence gene among MRSA and vancomycin-resistant S. aureus (VRSA) isolates (72.2% and 62.5%, respectively). However, sea and icaA were the commonest genes among MSSA isolates (80%; Table 6).

A significant correlation was observed between virulence genes (hla, sea and icaA) and patients age groups (P < 0.05). While no statistically significant difference was detected between the tested virulence genes and participant’s gender (Table 7).

sea was the commonest virulence gene among antibiotic-resistant and antibiotic-sensitive isolates, followed by icaA, hla, and fnbA. The highest distribution of sea was among the ciprofloxacin (95.2%)-, gentamicin (89.9%)-, and tetracycline (75.7%)-resistant isolates. At the same time, the highest distribution of sea was among oxacillin (79.9%)-, linezolid (75.5%)-, and rifampin (73.5%)-resistant isolates (Table 8).

A statistically significant correlation (P < 0.05) was detected between the presence and absence of hla and sea and piperacillin, gentamicin, and levofloxacin resistance and sensitivity. However, a significant difference in the distribution of icaA was found among β-lactam-resistant and β-lactam-sensitive isolates. fnbA was significantly associated with piperacillin and ciprofloxacin resistance and sensitivity (Table 8). Table 9 shows that sea and icaA had the highest coexistence (40.7%), followed by sea and hla (21.9%).

Discussion

S. aureus is the commonest pathogenic bacteria found in different wound specimens [22, 23]. Muluye et al. [24] stated that the prevalence of S. aureus in males and females was 38.1% and 28.7%, respectively. The first result was much lower than in this study, whereas the second was similar to this study. Patients were classified into different age groups from 1 month to 60 years. The highest prevalence of S. aureus (45.1%) and examined virulence genes were observed in the age group between 1 and 20 years. In the same time, there is a significant association between tested virulence genes and patients age groups. Torpy et al. [25] stated that the high prevalence of S. aureus in the age group between 1 and 20 years was because most young males (< 20 years) in the country have traditionally worked in occupations such as agriculture, construction, transportation, and industries, all of which are likely to expose them to trauma and different wound infections.

In this study, the highest prevalence of S. aureus was found in trauma and accidental wound infections, similar to other studies [26, 27], suggesting that the rate of S.
aureus isolates in open wound infection was 76.9%, similar to these findings. The predominant isolate S. aureus was sensitive to vancomycin (100%) [28], supporting the findings that considered vancomycin as one of the drugs with high susceptibility pattern against S. aureus. The same study revealed that S. aureus showed a high level of resistance to penicillin and oxacillin (84.6% and 76.9%, respectively).

### Table 4 Correlation between antibiotic resistance profile and patients' gender

| Antibiotic     | Resistance profile | p*     |
|----------------|--------------------|--------|
|                | Sensitive | Intermediate | Resistant |
| Linezolid      | Male       | 5       | 31      | 0       | 0.111 |
|                | Female     | 5       | 10      | 0       |        |
| Tetracycline   | Male       | 25      | 7       | 4       | 0.888 |
|                | Female     | 11      | 3       | 1       |        |
| Chloramphenicol| Male       | 6       | 25      | 5       | 0.626 |
|                | Female     | 1       | 12      | 2       |        |
| Rifampin       | Male       | 14      | 19      | 2       | 0.995 |
|                | Female     | 6       | 8       | 1       |        |
| Piperacillin   | Male       | 36      | 0       | 0       | N/A   |
|                | Female     | 15      | 0       | 0       |        |
| Gentamicin     | Male       | 15      | 17      | 4       | 0.321 |
|                | Female     | 4       | 7       | 4       |        |
| Amoxicillin/Sulbactam | Male | 34      | 2       | 0       | 0.878 |
|                | Female     | 14      | 1       | 0       |        |
| Oxacillin      | Male       | 34      | 2       | 0       | 0.878 |
|                | Female     | 14      | 1       | 0       |        |
| Levofloxacin   | Male       | 16      | 19      | 1       | 0.579 |
|                | Female     | 5       | 10      | 0       |        |
| Ciprofloxacin  | Male       | 15      | 18      | 3       | 0.780 |
|                | Female     | 15      | 8       | 2       |        |
| Amoxicillin/Clavulanic acid | Male | 34      | 2       | 0       | 0.878 |
|                | Female     | 14      | 1       | 0       |        |
| Vancomycin     | Male       | 5       | 31      | 0       | 0.585 |
|                | Female     | 3       | 12      | 0       |        |

*p*Chi-square test; P-value was set to 0.05; N/A: not applicable

### Table 5 Correlation between antibiotic resistance profile and patients age groups

| Antibiotic     | Resistance profile | p*     |
|----------------|--------------------|--------|
|                | Sensitive | Intermediate | Resistant |
| Linezolid      | 1 to 20    | 4       | 19      | 0       | 0.595 |
|                | 21 to 40   | 4       | 12      | 0       |        |
|                | 41 to 60   | 4       | 10      | 0       |        |
| Tetracycline   | 1 to 20    | 18      | 4       | 1       | 0.617 |
|                | 21 to 40   | 8       | 4       | 2       |        |
|                | 41 to 60   | 10      | 2       | 2       |        |
| Chloramphenicol| 1 to 20    | 2       | 19      | 2       | 0.618 |
|                | 21 to 40   | 3       | 9       | 2       |        |
|                | 41 to 60   | 2       | 9       | 3       |        |
| Rifampin       | 1 to 20    | 12      | 9       | 1       | 0.395 |
|                | 21 to 40   | 3       | 10      | 1       |        |
|                | 41 to 60   | 5       | 8       | 1       |        |
| Piperacillin   | 1 to 20    | 23      | 0       | 0       | N/A   |
|                | 21 to 40   | 14      | 0       | 0       |        |
|                | 41 to 60   | 14      | 0       | 0       |        |
| Gentamicin     | 1 to 20    | 10      | 10      | 3       | 0.851 |
|                | 21 to 40   | 4       | 8       | 2       |        |
|                | 41 to 60   | 5       | 6       | 3       |        |
| Amoxicillin/Sulbactam | 1 to 20 | 22      | 1       | 0       | 0.915 |
|                | 21 to 40   | 13      | 1       | 0       |        |
|                | 41 to 60   | 13      | 1       | 0       |        |
| Oxacillin      | 1 to 20    | 22      | 1       | 0       | 0.915 |
|                | 21 to 40   | 13      | 1       | 0       |        |
|                | 41 to 60   | 13      | 1       | 0       |        |
| Levofloxacin   | 1 to 20    | 11      | 12      | 0       | 0.450 |
|                | 21 to 40   | 4       | 9       | 1       |        |
|                | 41 to 60   | 0       | 8       | 0       |        |
| Ciprofloxacin  | 1 to 20    | 11      | 11      | 1       | 0.439 |
|                | 21 to 40   | 5       | 8       | 1       |        |
|                | 41 to 60   | 4       | 7       | 3       |        |
| Amoxicillin/Clavulanic acid | 1 to 20 | 22      | 1       | 0       | 0.915 |
|                | 21 to 40   | 13      | 1       | 0       |        |
|                | 41 to 60   | 13      | 1       | 0       |        |
| Vancomycin     | 1 to 20    | 4       | 19      | 0       | 0.556 |
|                | 21 to 40   | 1       | 13      | 0       |        |
|                | 41 to 60   | 3       | 11      | 0       |        |

*p*Chi-square test; P-value was set to 0.05; N/A: not applicable
Although these results were lower, they supported this study. They considered piperacillin and oxacillin as drugs with high resistance patterns against *S. aureus* along with ampicillin/sulbactam and amoxicillin/clavulanic acid.

Linezolid is an efficient antibiotic for treating *S. aureus* infections among four burn centers [23], in agreement with this study. The sensitivity rate of chloramphenicol against *S. aureus* was 71.2%, similar to another study [29] that showed a 68.4% sensitivity rate for chloramphenicol. The notable sensitivity of *S. aureus* to vancomycin, linezolid, and chloramphenicol could be linked to a lower use of these antibiotics due to their shortage availability in the market, high costs, and toxic side effects [30].

Many studies showed a high MRSA prevalence in wound infections [31] and reported a high rate of MRSA and VRSA (44.6% and 61.5%, respectively). The finding for MRSA was lower than in this study, whereas the findings for VRSA were much higher. Moreover, the MRSA results in this study disagreed with Bessa et al. [22], who suggested that 21.8% of *S. aureus* was resistant to oxacillin. This study also revealed a remarkable increase in MRSA compared to a previous study by Ahmed et al. [32], who reported a 24% MRSA prevalence in the same hospital 10 years ago. This raised the alarm about the escalating and noticeable increase in MRSA prevalence in Egypt. The increase of MRSA in wound infections has contributed to high treatment costs and longer hospital stays, which have major implications for infection management, particularly in developing countries. These findings contribute to a worrying situation in the Minia Government regarding MRSA expansion. The necessity for more detailed molecular epidemiologic surveillance studies on MRSA and VRSA in the next years is critical.

The MDR of *S. aureus* isolates was 54.2%, similar to other studies [33, 34], which reported 54.9% and 47.9%, respectively. However, another study [28] stated that *S. aureus* showed 94.8%, higher than this study.

Table 6 Frequencies of virulence genes among MRSA, MSSA and VRSA strains

| Virulence genes | Total No. = 59 (%) | MRSA No. = 54 (%) | MSSA No. = 5 (%) | VRSA No. = 8 (%) |
|-----------------|-------------------|------------------|-----------------|-----------------|
| *hla*           | 22 (7.3)          | 20 (36.9)        | 2 (39.9)        | 3 (37.5)        |
| *sea*           | 43 (72.9)         | 39 (72.2)        | 4 (79.9)        | 5 (62.5)        |
| *icaA*          | 29 (49.2)         | 25 (46.3)        | 4 (79.9)        | 4 (49.9)        |
| *fnbA*          | 8 (13.6)          | 6 (11.1)         | 2 (39.9)        | 0 (0)           |

![Fig. 3](image-url) Detection of amplification product of different virulence genes: A *hla* gene by PCR; lane 1: negative control, lane 2: positive control and lanes 3 to 10: positive PCR products (209 bp); B *sea* gene by PCR; lane 1: positive control, lane 2: negative control and lanes 3 to 11: positive PCR products (120 bp); C *icaA* gene by PCR; lane 1: positive control, lane 2: negative control and lanes 3 to 10: positive PCR products (770 bp); D *fnbA* gene by PCR; lanes 1 to 7: positive PCR products (1279 bp), lane 8: positive control and lane 9: negative control.
activity of commonly used antibiotics, such as amoxicillin/clavulanic acid, ampicillin/sulbactam, oxacillin, and piperacillin, may be due to increased consumption of a particular class of antibiotics, resulting in resistance due to mutation(s) at drug target sites or the disruption of drug accumulation in the cytoplasm caused by cell wall rearrangement [31–36]. As a result, they are no longer effective in treating wound infections.

The incidence of some major virulence indicators of *S. aureus* in wound specimens was examined in this study. This study concentrated on a small number of genes linked to *S. aureus* pathogenicity. These genes (*hla*, *sea*, *icaA*, and *fnbA*) were chosen because they were the most frequent in aggressive isolates. These targeted genes spread across the isolates after PCR amplification. Furthermore, the bulk of the isolates demonstrated a wide range of gene combinations, indicating that the study sample has a level of genetic diversity.

Antimicrobial resistance and virulence factor genes showed significant relationships in this study. This finding could be explained by the proximity location of the resistance gene to the virulence gene [31, 37].

The predominant virulence and inducible resistance genes in MRSA and MSSA isolates were related to *sea* [38, 39]. All previous studies supported this study because *sea* is the commonest among MRSA and MSSA isolates. Cavalcante et al. [40] reported that the prevalence of *sea* in *S. aureus* isolates collected from infected skin lesions of atopic dermatitis children was 76.4% in total *S. aureus* isolates, 73.9% in MRSA isolates, and 78.1% in MSSA isolates, in agreement with this study. Li et al. [41] reported that the frequency of *sea* in *S. aureus* isolates from SSTIs in children was 0%, which was totally opposite to this study.

PCR investigation revealed that *hla* was found in 30.5% of 85 *S. aureus* isolated from various clinical sources [42], close to the present findings. The prevalence of *icaA* in MRSA was 60.3% [43], which was slightly higher than the present data. The prevalence of *fnbA* was 4.9% and 19.9% in MRSA and MSSA strains, respectively [44]. The prevalence of *fnbA* in MRSA was close to this study, whereas *fnbA* in MSSA was much lower than in the present data. Another study [45] suggested that the incidence of *fnbA* in wound swabs was 28.8%, which was slightly higher than the present results. The prevalence of *fnbA* and *icaA* in burn units was 2.9% and 44.9%, respectively [46]. *fnbA* was slightly lower than in this study, whereas the percentage of *icaA* was similar to the present data. The incidence of *fnbA* in MRSA and MSSA strains was 15.5% and 36.9%, respectively. However, the incidence of *icaA* in MRSA and MSSA was 84.5% and 78.3%, respectively [38]. The percentages of *fnbA* were similar to this study. However, the percentage of *icaA* was much higher in MRSA but was similar to the present data in MSSA. The prevalence of *sea* was 11.8% in amoxicillin/clavulanic acid and oxacillin susceptibility samples, 9.2% in rifampin, 0% in penicillin, 88.2% in chloramphenicol, and 100% in vancomycin [47]. The first three percentages were much lower than in this study. However, the percentages of chloramphenicol and vancomycin were slightly higher than in this study. The percentage of the coexistence of *sea* and *hla* was 36.9% [17], which was slightly higher than in this study.

The limitation of this study was the inability to detect more virulence genes and express the chosen virulence factors by molecular typing of the isolates (Additional file 1, Additional file 2).

**Conclusions**

Within the limitations of the current study, it can be concluded that the challenging, increasingly difficult, and widespread bacterial resistance to antibiotics has developed, the incidence of infections caused by MDR

| Gender | *hla* (+) | P* | *sea* (+) | P* | *fnbA* (+) | P* | *icaA* (+) | P* |
|--------|----------|----|----------|----|-----------|----|-----------|----|
| Male   | 16       | 20 | 0.770    |    | 29        | 7  | 0.125     |    |
| Female | 6        | 9  | 9        | 6  | 2         | 13 | 6         | 9  |
| Age group |       |    |          |    |           |    |           |    |
| 1 to 20 | 10       | 1  | 0.002    |    | 20        | 3  | 0.006     |    |
| 21 to 40 | 6        | 8  | 12       | 2  | 2         | 12 | 6         | 8  |
| 41 to 60 | 6        | 8  | 6        | 8  | 1         | 13 | 4         | 10 |

The bold values indicate a statistically significant difference. The alpha level of significance was set to 0.05.

*Chi-square test; P-value was set to 0.05*
Table 8  Correlation between *S. aureus* virulence genes and antibiotic resistance

| Antibiotic    | hla R | hla S | p*   | sea R | sea S | p*   | icaA R | icaA S | p*       | fnbA R | fnbA S | p*   |
|---------------|-------|-------|------|-------|-------|------|--------|--------|----------|--------|--------|------|
| Linzolid      |       |       |      |       |       |      |        |        |          |        |        |      |
| Positive gene | 4 (39.9%) | 18 (36.7%) | 0.846 | 5 (50%) | 37 (75.5%) | 0.105 | 4 (39.9%) | 25 (51%) | 0.525  | 0 (0%) | 8 (16.3%) | 0.169 |
| Negative gene | 6 (59.9%) | 31 (63.3%) |      | 5 (50%) | 12 (24.5%) |      | 6 (59.9%) | 24 (48.9%) |      | 10 (100%) | 41 (83.7%) |      |
| Tetracycline  |       |       |      |       |       |      |        |        |          |        |        |      |
| Positive gene | 14 (37.8%) | 5 (31.3%) | 0.646 | 28 (75.7%) | 11 (68.8%) | 0.6 | 21 (56.8%) | 6 (37.5%) | 0.198  | 4 (10.8%) | 2 (12.5) | 0.602 |
| Negative gene | 23 (62.2%) | 11 (38.8%) |      | 9 (24.3%) | 5 (31.3%) |      | 16 (43.2%) | 10 (62.5%) |      | 33 (89.2%) | 14 (87.5%) |      |
| Chloramphenicol |       |       |      |       |       |      |        |        |          |        |        |      |
| Positive gene | 2 (33.3%) | 14 (33.3%) | 1 | 4 (66.7%) | 30 (71.4%) | 0.81 | 3 (49.9%) | 20 (47.6%) | 0.913  | 0 (0%) | 5 (12%) | 0.372 |
| Negative gene | 4 (66.7%) | 28 (66.7%) |      | 2 (33.3%) | 12 (28.6%) |      | 3 (49.9%) | 22 (52.4%) |      | 6 (100%) | 37 (88.1%) |      |
| Rifampin      |       |       |      |       |       |      |        |        |          |        |        |      |
| Positive gene | 6 (27.3%) | 15 (44.1%) | 0.203 | 16 (72.7%) | 25 (73.5%) | 0.947 | 11 (49.9%) | 17 (49.9%) | 1 | 3 (13.6%) | 5 (14.7%) | 0.911 |
| Negative gene | 16 (72.7%) | 19 (55.9%) |      | 6 (27.3%) | 9 (26.5%) |      | 11 (49.9%) | 17 (49.9%) |      | 19 (86.4%) | 29 (85.3%) |      |
| Piperacillin  |       |       |      |       |       |      |        |        |          |        |        |      |
| Positive gene | 22 (37.3%) | 0 (0%) | < 0.0001 | 43 (72.9%) | 0 (0%) | < 0.0001 | 29 (49.2%) | 0 (0%) | < 0.0001 | 8 (13.6%) | 0 (0%) | < 0.0001 |
| Negative gene | 37 (62.7%) | 0 (0%) |      | 16 (27.1%) | 0 (0%) |      | 30 (50.4%) | 0 (0%) |      | 51 (86.4%) | 0 (0%) |      |
| Gentamycin    |       |       |      |       |       |      |        |        |          |        |        |      |
| Positive gene | 3 (14.9%) | 15 (51.7%) | 0.005 | 18 (89.9%) | 18 (62.1%) | 0.03 | 12 (59.9%) | 13 (44.8%) | 0.296  | 3 (14.9%) | 5 (17.2%) | 0.835 |
| Negative gene | 17 (84.9%) | 14 (48.3%) |      | 2 (9.9%) | 11 (37.9%) |      | 8 (39.9%) | 16 (55.2%) |      | 17 (84.9%) | 17 (58.6%) |      |
| Ampicillin/ Sulbactam |       |       |      |       |       |      |        |        |          |        |        |      |
| Positive gene | 20 (36.9%) | 2 (39.9%) | 0.496 | 39 (72.2%) | 4 (79.9%) | 0.708 | 25 (46.3%) | 4 (79.9%) | 0.044  | 6 (11.1%) | 2 (39.9%) | 0.071 |
| Negative gene | 34 (62.9%) | 3 (59.9%) |      | 15 (27.8%) | 1 (19.9%) |      | 19 (35.2%) | 1 (19.9%) |      | 48 (88.9%) | 3 (59.9%) |      |
| Oxacillin     |       |       |      |       |       |      |        |        |          |        |        |      |
| Positive gene | 20 (36.9%) | 2 (39.9%) | 0.896 | 39 (72.2%) | 4 (79.9%) | 0.708 | 25 (46.3%) | 4 (79.9%) | 0.044  | 6 (11.1%) | 2 (39.9%) | 0.071 |
| Negative gene | 34 (62.9%) | 3 (59.9%) |      | 15 (27.8%) | 1 (19.9%) |      | 19 (35.2%) | 1 (19.9%) |      | 48 (88.9%) | 3 (59.9%) |      |
| Levofloxacin  |       |       |      |       |       |      |        |        |          |        |        |      |
| Positive gene | 4 (18.9%) | 17 (51.5%) | 0.017 | 19 (90.5%) | 21 (63.6%) | 0.028 | 10 (48%) | 16 (48.5%) | 0.951  | 2 (9.5%) | 5 (15.2%) | 0.548 |
| Negative gene | 16 (80.9%) | 16 (48.5%) |      | 2 (9.5%) | 12 (36.4%) |      | 11 (52.3%) | 17 (51.5%) |      | 19 (90.5%) | 28 (84.9%) |      |
| Ciprofloxacin |       |       |      |       |       |      |        |        |          |        |        |      |
| Positive gene | 5 (23.8%) | 13 (41.9%) | 0.178 | 20 (55.2%) | 20 (64.5%) | 0.01 | 11 (52.4%) | 14 (45.2%) | 0.609  | 4 (18.9%) | 4 (12.9%) | < 0.0001 |
| Negative gene | 16 (76.2%) | 18 (58.1%) |      | 1 (4.8%) | 11 (35.5%) |      | 10 (47.6%) | 17 (54.8%) |      | 17 (80.9%) | 27 (87.1%) |      |
| Amoxicillin/ Clavulanic |       |       |      |       |       |      |        |        |          |        |        |      |
| Positive gene | 20 (36.9%) | 2 (39.9%) | 0.896 | 39 (72.2%) | 4 (79.9%) | 0.708 | 25 (46.3%) | 4 (79.9%) | 0.044  | 6 (11.1%) | 2 (39.9%) | 0.071 |
| Negative gene | 34 (62.9%) | 3 (59.9%) |      | 15 (27.8%) | 1 (19.9%) |      | 19 (35.2%) | 1 (19.9%) |      | 48 (88.9%) | 3 (59.9%) |      |

The bold values indicate a statistically significant difference. The alpha level of significance was set to 0.05

* Chi-square test

P-values was set to 0.05
S. aureus has increased. The prevalence of CA-MRSA was high among patients with various wound infections. Bacterial resistance profile was the least against vancomycin and linezolid effective antibiotics. The correlation between CA-MRSA strain virulence genes distribution and antibiotic resistance profile showed high incidence of sea and icaA genes. All virulence genes were significantly distributed among piperacillin resistant isolates. β-lactam resistant isolates showed a significant correlation with IcaA virulence gene. After the emergence of high percentage of sea among ciprofloxacin resistant isolates, we expect that more genes will appear in future studies regarding S. aureus virulence genes. Therefore, the spread of bacterial resistance must be monitored in hospitals by using antibacterial agents properly to avoid more complications and to keep the empirical medications as effective as they are.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12879-022-07624-8.

Additional file 1: Figure S1. Detection of amplification product of hla gene by PCR; lane 1: negative control, lane 2: positive control and lanes 3 to 10: positive PCR products (209bp). Figure S2. Detection of amplification product of sea gene by PCR; lane 1: positive control, lane 2: negative control and lanes 3 to 11: positive PCR products (120bp). Figure S3. Detection of amplification product of fnbA gene by PCR; lane 1: negative control, lane 2: positive control and lanes 3 to 10: positive PCR products (209bp). Figure S4. Detection of amplification product of fnbA gene by PCR; lanes 1 to 7: positive PCR products (1279bp), lane 8: positive control and lane 9: negative control.

Acknowledgements
We are grateful to the staff of the Plastic and Reconstructive Surgery Department for collecting the specimens used in this study and to Associate Professor Ahmad Abdel Hamid Elheeny, Pediatric and Community Dentistry Department, Faculty of Dentistry, Minia University for making the statistical analysis of this study.

Table 9 Coexistence of virulence genes among S. aureus isolates

| Virulence genes | Distribution No. (%) |
|-----------------|----------------------|
| hla + sea       | 13 (21.96%)          |
| hla + sea + fnbA| 4 (6.8%)             |
| sea + fnbA      | 8 (13.6%)            |
| hla + fnbA      | 4 (6.8%)             |
| icaA + hla      | 11 (18.6%)           |
| icaA + sea      | 24 (40.7%)           |
| icaA + fnbA     | 5 (8.5%)             |
| hla + sea + icaA+ fnbA | 3 (5.1%) |

Author contributions
HAR was responsible for the study concept and design, data analysis, and interpretation and the drafting, critical revision, and final approval of the manuscript and agreed to be accountable for all aspects of the work, ensuring integrity and accuracy. AEF, DAM, and GGF were responsible for the study concept and design, data acquisition, and the drafting, critical revision, and agreed to be accountable for all aspects of the work, ensuring integrity and accuracy. All authors read and approved the final manuscript.

Funding
Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB). The research is self-funded with no external financial sources.

Availability of data and materials
The data sets generated and/or analyzed during this study are not publicly available due to privacy but are available from the corresponding author (A.E.F) on reasonable request.

Declarations
Ethics approval and consent to participate
The study purpose and the procedures of taking the samples, clinical steps, and expected benefits were inclusively explained to the patients who signed the required informed consent to use the samples in the study. Before obtaining the samples, written informed consent was signed by each patient and/or caregiver. Ethical standards were granted by the Ethical Committee of Minia University Hospital. Ethics approval and consent to participate in all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
1 Microbiology and Immunology Department, Faculty of Pharmacy, Deraya University, Minia, Egypt. 2 Microbiology and Immunology Department, Faculty of Pharmacy, Sohag University, Sohag, Egypt. 3 Plastic and Reconstructive Surgery Department, Faculty of Medicine, Minia University, Minia, Egypt. 4 Microbiology and Immunology Department, Faculty of Pharmacy, Minia University, Minia, Egypt.

Received: 20 April 2022   Accepted: 13 July 2022

Published: 28 July 2022

References
1. Ndip RN, Takang A, Echakachi CM, Malongue A, Akoachere J, Ndip LM, Luma HN. In-vitro antimicrobial activity of selected honeys on clinical isolates of Helicobacter pylori. Afr Health Sci. 2007;7(4):228–32.
2. Leaper D, Harding K. Wounds: biology and management. Oxford: Oxford University Press; 1998.
3. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3):603–61.
4. Morell EA, Balkin DM. Methicillin-resistant Staphylococcus aureus: a per-vasive pathogen highlights the need for new antimicrobial development. Yale J Biol Med. 2010;83(3):223–33.
5. Gauliard E, Ouellette SP, Rueden KJ, Ladant D. Characterization of interactions between inclusion membrane proteins from Chlamydia trachomatis. Front Cell Infect Microbiol. 2015;5:13.
6. McCallum N, Berger-Bachi B, Senn MM. Regulation of antibiotic resistance in *Staphylococcus aureus*. Int J Med Microbiol. 2010;300(2–3):118–29.

7. Bagdonas R, Tamelis A, Rimdelka R. *Staphylococcus aureus* infection in the surgery of burns. Medicina. 2003;39(11):1078–81.

8. Lewis K. Riddle of biofilm resistance. Antimicrob Agents Chemother. 2001;45(4):999–1007.

9. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljestrand B, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(6):E1–8.

10. Wolfensohn A, Kuster SP, Marchesi M, Zbinden R, Hornbach M. The effect of varying multidrug-resistance (MDR) definitions on rates of MDR gram-negative rods. Antimicrob Resist Infect Control. 2019;8:193.

11. Rauber JM, Carneiro M, Arnhold GH, Zanotto MB, Wapppler PR, Baggiotto B, Valim AR, d'Azzevedo PA. Multidrug-resistant *Staphylococcus spp* and its impact on patient outcome. Am J Infect Control. 2016;44(1):e261–3.

12. Costa AP, Batistão DW, Ribas RM, Sousa AM, Pereira MQ, Botelho CM. *Staphylococcus aureus* virulence factors and disease. In: Mendez-Vilas A, editor. Microbial pathogens and strategies for combating them: science, technology and education, vol. 1. Badajoz: Formatex; 2013. p. 702–10.

13. Labandeira-Rey M, Couzon F, Boisset S, Brown EL, Bes M, Benito Y. Barbu EM, Vazquez V, Höök M, Etienne J. *Staphylococcus aureus* Panton-Valentine Leukocidin causes necrotizing pneumonia. Science. 2007;315(5815):1130–3.

14. Llewelyn M, Cohen J. Superantigens: microbial agents that corrupt immunity. Lancet Infect Dis. 2002;2(3):156–62.

15. Pinchuk IV, Beswick EJ, Reyes VE. Staphylococcal enterotoxins. Toxins. 2015;7:2177–97.

16. Rahimi F, Katouli M, Karimi S. Biofilm production among methicillin resistant *Staphylococcus aureus* isolated from burn patients. Microb Pathog. 2016;97:34–7.

17. Motallebi M, Jabalameli F, Asadollahi K, Taherikalani M, Emaneini M. *Staphylococcus aureus* infection and its impact on patients after surgical interventions. Surg Infect. 2014;15(4):404–11.

18. Rasmi et al. BMC Infectious Diseases 2022, 22(1):652

19. Shittu A, Kolawole D, Oyedepo E. A study of wound infections in two health institutions in Ile-Ife. Nigeria Afr J Biomed Res. 2002. https://doi.org/10.4314/ajbr.v5i3.53994.

20. Sidaghat H, Narimani T, Esfahani BN, Mobasherizadeh S, Havaei SA. Comparison of the prevalence of microbial surface components recognized adhesin matrix molecules (MSCRAMMs) among *Staphylococcus aureus* isolates in a burn unit with non-burning units. Iran J Public Health. 2015;44(3):275–81.

21. Sabouni F, Mahmoudi S, Bahador A, Pourakbari B, Sadeghi RH, Ashtiani MTH, Nikmanesh B, Mamishi S. Virulence factors of *Staphylococcus aureus*...
isolates in an Iranian referral children's hospital. Osong Public Health Res Perspect. 2014;5(2):96–100.

48. Li X, Fang F, Zhao J, Lou N, Li C, Huang T, Li Y. Molecular characteristics and virulence gene profiles of Staphylococcus aureus causing bloodstream infection. Braz J Infect Dis. 2019;22:487–94.

49. Johnson WM, Tyler S, Ewan E, Ashton F, Pollard D, Rozeef K. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in Staphylococcus aureus by the polymerase chain reaction. J Clin Microbiol. 1991;29(3):426–30.

50. Peacock SJ, Moore CE, Justice A, Kantzanou M, Story L, Mackie K, O'Neill G, Day NP. Virulent combinations of adhesin and toxin genes in natural populations of Staphylococcus aureus. Infect Immun. 2002;70(9):4987–96.

51. Nashev D, Toshkova K, Salasia SI, Hassan AA, Lämmler C, Zschöck M. Distribution of virulence genes of Staphylococcus aureus isolated from stable nasal carriers. FEMS Microbiol Lett. 2004;233(1):45–52.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.