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Prevalence of enterotoxin genes (SEA to SEE) and antibacterial resistant pattern of *Staphylococcus aureus* isolated from clinical specimens in Assiut city of Egypt

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Abstract

Background: Infections in communities and hospitals are mostly caused by *Staphylococcus aureus* strains. This study aimed to determine the prevalence of five genes (*SEA, SEB, SEC, SED* and *SEE*) encoding staphylococcal enterotoxins in *S. aureus* isolates from various clinical specimens, as well as to assess the relationship of these isolates with antibiotic susceptibility. Traditional PCR was used to detect enterotoxin genes, and the ability of isolates expressing these genes was determined using Q.RT-PCR.

Results: Overall; 61.3% ($n=46$) of the samples were positive for *S. aureus* out of 75 clinical specimens, including urine, abscess, wounds, and nasal swabs. The prevalence of antibiotic resistance showed *S. aureus* isolates were resistant to Nalidixic acid, Ampicillin and Amoxicillin (100%), Cefuroxime (94%), Ceftriaxone (89%), Ciprofloxacin (87%), Erythromycin and Ceftaxime (85%), Cephalexin and Clarithromycin (83%), Cefaclor (81%), Gentamicin (74%), Ofloxacin (72%), Chloramphenicol(59%), Amoxicillin/Clavulanic acid (54%), while all isolates sensitive to Imipinem (100%). By employing specific PCR, about 39.1% of isolates were harbored enterotoxin genes, enterotoxin A was the most predominant toxin in 32.6% of isolates, enterotoxin B with 4.3% of isolates and enterotoxin A and B were detected jointly in 2.1% of isolates, while enterotoxin C, D and E weren't detected in any isolate.

Conclusion: This study revealed a high prevalence of *S. aureus* among clinical specimens. The isolates were also multidrug resistant to several tested antibiotics. Enterotoxin A was the most prevalent gene among isolates. The presence of antibiotic resistance and enterotoxin genes may facilitate the spread of *S. aureus* strains and pose a potential threat to public health.

Keywords: *Staphylococcus aureus*, Antibiotic resistance, Enterotoxin, Real-time PCR

Background

Staphylococcus aureus is a commensal and opportunistic human pathogen that can be found all over the world [1]. It causes a variety of clinical infections, including impetigo, furunculosis, and abscesses on the skin and soft tissues, as well as systemic infections, including pneumonia, bacteremia, endocarditis, and toxin-mediated diseases [2]. It is one of the most common pathogens linked to nosocomial infections in hospitals [3]. *S. aureus* cause a large amount of morbidity and mortality in developing countries as opposed to other infectious diseases like malaria, tuberculosis, and HIV infections [4]. The fast expansion of drug resistance as well as prominent virulence factors, surface proteins, metabolites and enzymes have all contributed to *S. aureus* clinical significance [5]. Large numbers of toxins, including hemolysin (α, β, γ, δ) and leukocidin (PVL, Luk E/D) are the most commonly

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associated virulence factors with these microorganisms [6]. Other virulence factors that cause enterocolitis, scalded skin syndrome, and toxic shock include heat-stable staphylococcal enterotoxins (SEs), exfoliative toxins (ETA and ETB), the toxin of toxic shock syndrome-1(TSST-1) [7, 8]. *S. aureus* is a concern not only because of its widespread distribution and pathogenicity but also because of its ability to resist antimicrobials [9]. In addition to drug resistance, monitoring of S. aureus strains and determination of susceptibility patterns are critical [10]. Staphylococcal enterotoxins have been identified as etiologic agents of human food poisoning and as active immunologic superantigens that induce non-specific T cell proliferation [11]. Enterotoxins are emetic toxins linked to a broad family of pyrogenic exotoxins produced by staphylococci and streptococci, they are active in concentrations ranging from nanograms to micrograms and are resistant to heat and low pH, also have proteolytic enzymes allowing them to remain active in the digestive tract after ingestion [7]. There are 23 staphylococcal superantigens that have been described, enterotoxins A to E, G to J, R to T, and staphylococcal enterotoxin-like toxins (SE1) K, Q, U, and X (SElK-SElQ, SE1U-SElX) [12–17]. Polymerase chain reaction (PCR), DNA probes, and reverse-transcription RT-PCR were used to detect enterotoxins and their activity in *S. aureus* strains [18]. So the aim of this research is to use PCR to detect enterotoxins genes in *S. aureus*, assess their distribution through clinical sources, and quantify their prevalence. Furthermore, we looked for any possible connection between enterotoxin prevalence and expression on the one side and antimicrobial susceptibility on the other.

Methods

Collection of samples

The present descriptive study was carried out over a six-month period, between January and July 2015, the research was approved by the ethics committee of the Botany and Microbiology department- the College of Science (Al-Azhar University, Assiut). A total of 75 clinical specimens were collected under aseptic conditions from patients admitted to Assiut University hospital—Faculty of Medicine, Political hospital, Al-Eman hospital, and El-Shmla hospital in Assiut governorate, Egypt. All clinical specimens collected from diverse infections were received from both genders and all age groups. Urine from the infected urinary tract ($n=29$; 38.6%), Pus swabs from an abscess ($n=20$; 26.6%), Swabs from septic wounds ($n=15$; 20%), and Nasal swabs from cases with respiratory symptoms ($n=11$; 14.6%). The samples obtained from patients were transported directly to the laboratory in an icebox within 4 h of collection and were immediately processed according to standard microbiological procedures. The sampling swabs were inoculated into a 5 ml *Staphylococcus* enrichment broth medium [19], blended briefly as necessary, and incubated at 37 °C for 24 h.

Prevalence and characterization of *S. aureus* isolates

The bacterial culture was streaked on mannitol salt agar (MSA) (Oxoid, Basingstoke, Hampshire, England), with a loopful of enrichment broth and incubated aerobically at 37 °C for 24 to 48 h. Suspected colonies were picked up, sub-cultured once on MSA and twice on 5% defibrinated sheep blood agar medium (Oxoid, Basingstoke, Hampshire, England). Bacterial colonies showing typical characteristics of *S. aureus* (i.e., beta-hemolytic on blood agar and colonies with golden yellow pigmentation on MSA) were subjected to subculture on to basic media, Gram stain, and biochemical tests catalase and coagulase. Catalase positive and Gram-positive bacteria appearing in the grape-like cluster was spot inoculated to DNase agar (Oxoid, Basingstoke, Hampshire, England). Inoculated DNase agar plates were incubated at 37 °C overnight and flooded with 1 N HCl (Merk, Darmstadt, Germany). Isolates that hydrolyzed DNA in DNase agar were considered *S. aureus*. Purified colonies were selected, propagated on nutrient agar slope, and preserved at 4 °C.

Antibiotics susceptibility testing

The disc diffusion method was used to determine the antibiotic susceptibility of *S. aureus* isolates on Mueller Hinton Agar (Oxoid, Basingstoke, Hampshire, England) [20]. Sixteen antibiotics were tested, Imipinem (IPM:10 μg), Nalidixic acid (NA:30 μg), Cephalexin (CN:30 μg), Chloramphenicol (CM:30 μg), Ofloxacin (OFX:5 μg), Amoxicillin (AMX:25 μg), Amoxicillin/Clavulanic acid(AMC:30 μg), Ampicillin (Amp:10 μg), Cefaclor (CEC:30 μg), Gentamycin (GM:30 μg), Erythromycin (E:15 μg), Ciprofloxacin (CIP:5 μg), Ceftriaxone (CRO:30 μg), Clarithromycin (CLR: 15 μg), Ceftaxime (CTX: 30 μg), Cefuroxime (CXM: 30 μg).

DNA extraction and detection of staphylococcal enterotoxin genes

DNA was extracted using a genomic DNA isolation kit (QIAGEN, Germany) according to the manufacturer's instructions. Specific PCR using specific primers was used to detect genes encoding enterotoxin A (*SEA*), enterotoxin B (*SEB*), enterotoxin C (*SEC*), enterotoxin D (*SED*), and enterotoxin E (*SEE*) [21–25] (Table 1). PCR reaction was conducted in the final volume 25 μl using 2.5 μl Taq polymerase buffer 10X (Promega, Madison, USA) containing a final concentration of 1 mM $MgCl_2$, 2 μl of 2.5 mM dNTPs, 1 μl DNA (50 ng), 1 μl of 10 pmol/μl each specific primer and 0.2 μl Taq polymerase (5 U/

Table 1 Oligonucleotides primers used in the study

Gene	Primer	Oligonucleotide sequence 5^{-}–3^{-}	Size of amplified products (bp)	Annealing temp. °C	References
SEA	F	GGTTATCAATGTGCGGGTG	102	52	Marsha and Betley [21]
	R	CGGCACTTTTTTCTCTTCGG			
SEB	F	GTATGGTGGTGTAACTGAGC	478	52	Jones and Khan [22]
	R	CCAAATAGTGACGAGTTAGG			
SEC	F	AGATGAAGTAGTTGATGTGTATGG	451	50	Bohach and Schlievert [23]
	R	CACACTTTTAGAATCAACCG			
SED	F	CCAATAATAGGAGAAAATAAAAG	278	50	Bayles and landolo [24]
	R	ATTGGTATTTTTTTTCGTTC			
SEE	F	AGGTTTTTTCACAGGTCATCC	209	50	Couch et al. [25]
	R	CTTTTTTTTCTTCGGTCAATC			
16 s rRNA	F	AGAGTTTGATCMTGGCTCAG	1500	55	Turner et al. [27]
	R	AACTGGAGGAAGGTGGGAT			

µl). The PCR program started with an initial denaturation of 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at (50 or 52 °C) for 30 s and extension at 72 °C for 1 min. Additional extension at 72 °C for 10 min was done. Aliquots of amplified products were loaded in 1.5% agarose gel, visualized, and photographed using a gel documentation system (Syngene, USA).

RNA extraction and expression of staphylococcal enterotoxin genes

Total RNA was extracted using the RNeasy Mini Kit according to the manufacturer's instructions (QIAGEN, Germany). The first strand of cDNA synthesis was performed in a total reaction volume of 25 µl. The reaction mixture contained 2.5 µl of 5X Reverse Transcriptase buffer with 1 mM $MgCl_2$ (Fermentas, USA), 2.5 µl of 2.5 mM dNTPs, 0.5 µl of oligo dT primer 10 pmol/µl, 1 µl RNA (50 ng), 0.2 µl of 200 U/µl Reverse Transcriptase Enzyme in a final reaction volume up to 25 µl by RNase free water. The reverse transcriptase reaction was performed in a thermal cycler (Eppendorf, Germany) run at 42 °C for 1 h and 72 °C for 10 min.

Quantitative PCR was performed using SYBR Green PCR Master Mix (Fermentas, USA). Each reaction was performed in a 25 µl mixture, which contained 1 µl of 10 pmol/µl of each primer, 1 µl of cDNA (50 ng), 12.5 µl of 2X SYBR Green PCR Master and 9.5 µl of nuclease-free water. Samples were spin before loading in the rotor wells and each sample was run in triplicate. The amplification program proceeded at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s; annealing at 60 °C for 30 s and extension at 72 °C for 30 s, then followed by a final extension at 72 °C for 10 min. The reaction was performed using a Rotor-Gene 6000 (QIAGEN, ABI System, USA).

Real-Time Q-PCR data analysis

The relative expression ratio was quantified and calculated accurately. Accordingly, for each biological sample, the difference (Δ) in quantification cycle value (C_T) between the target ($C_{T\ (target)}$ averaged from three technical repeats) and the reference 16S rRNA was used as the reference gene ($C_{T\ (reference)}$, a fixed C_T value was used for all samples). The C_T (threshold of the cycle) value of each detected gene was determined by automated threshold analysis on ABI System [26]. The C_T value of each target gene was normalized to $C_{T\ (reference)}$ to obtain $\Delta C_{T\ (target)}$ where

$$\Delta C_{T\ (target)} = \left(C_{T\ (target)} - C_{T\ (reference)}\right),$$
$$\Delta C_{T\ (control)} = \left(C_{T\ (control)} - C_{T\ (reference)}\right)$$

The relative expression quantity of the target gene was indicated with

$$\Delta\Delta CT = \left(\Delta C_{T(target)} - \Delta C_{T(control)}\right) \text{according to } 2 - \Delta\Delta Ct \text{ algorithm}$$

Sequencing, sequence analysis, and phylogenetic tree construction of highly expression isolates

Amplified products of (16S rRNA gene and specific enterotoxin genes) for high expression isolates were purified according to (Maxim biotech INC, USA) and subjected to DNA sequencing using a forward primer

in the sequencing reaction [27]. Sequencing was performed using BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and model 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The obtained DNA nucleotide sequences were analyzed using NCBI-BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for confirming the identity of the obtained sequences. Multiple sequence alignment of our sequences and the other published ones were performed using CLUSTLAW (1.83) [28] and a phylogenetic tree was analyzed and generated using MEGA 4 [29].

Statistical analysis

Correlations between data of antibiotic resistance and prevalence of enterotoxins were statistically analyzed using the Graphpad Instat 3. Fisher's exact test was used to evaluate these correlations, where *P* value less than 0.05 was considered statistically significant.

Results

Isolation and identification of *S. aureus* isolates

In total, 46 *S. aureus* isolates were isolated from 75 patient samples (29 urine samples, 20 abscess samples, 15 wound samples, and 11 nasal swabs samples). Suspected colonies turned MSA medium yellow due to mannitol fermentation and were given a clear zone around colonies on blood agar because of β- hemolytic activity of isolates. *S. aureus* isolates were found most often in abscess swabs (15 isolates from 20 samples; 75%) and then wound swabs (9 isolates from 15 samples; 60%), urine samples (16 isolates from 29 samples; 55.1%), and lastly nasal swabs (6 isolates from 11 samples; 54.5%) (Table 2). Data are presented in Table 3 concluded that *S. aureus* isolates were positive for catalase, coagulase and, DNase, growth on crystal violet agar were purple, white and yellow colonies, also growth on Baird Parker agar medium were black and shiny with narrow white margins surrounded by clear zone, it also positive for Nitrate reduction, Methyl red test, Voges Prausker test and their ability for hydrolysis of Gelatin and Casein while negative for Indole production and Citrate utilization.

Table 3 Biochemical characterization of *S. aureus* isolates

Name of test	No. of isolates	
	No	%
Catalase	Positive for all isolates	100
Coagulase by rabbit plasma		
Slide	Positive for 25 isolates	54.4
Tube	Positive for 21 isolates	45.6
DNase agar	Positive for all isolates	100
Crystal violet agar		
Purple colonies	39 isolates	84.7
White colonies	3 isolates	6.5
Yellow colonies	4 isolates	8.6
Nitrate reduction	Positive for all isolates	100
Baird parker medium	Positive for all isolates	100
Indole production test	Negative for all isolates	100
Methyl red test	Positive for all isolates	100
Voges Prausker test	Positive for all isolates	100
Citrate utilization	Negative for all isolates	100
Gelatin hydrolysis	Positive for all isolates	100
Casein hydrolysis test	Positive for all isolates	100

Susceptibility of *S. aureus* isolates to antibiotics

The disc diffusion method was used to screen all of the bacterial isolates on Muller–Hinton agar for sixteen antibiotics. The results revealed that all isolates were sensitive to imipinem (100%), 46% of isolates sensitive to amoxicillin/clavulanic acid, 41% sensitive to chloramphenicol, 28% sensitive to ofloxacin, 26% sensitive to gentamicin, 19% sensitive to Cefaclor, 17% sensitive to cephalexin and clarithromycin, 15% sensitive to erythromycin and ceftaxime, 13% sensitive to ciprofloxacin, 11% sensitive to ceftriaxone, 6% sensitive to cefuroxime, while

Table 2 Frequency of *Staphylococcus aureus* isolates among clinical specimens

Type of specimens	No. of examined specimens	Mannitol fermentative *S. aureus* isolates		Pigments on MSA						β-hemolysis isolates	
				yellow		Lemony yellow		White			
		No	%	No	%	No	%	No	%	No	%
Urine	29	16	55.1	8	50	–	–	8	50	16	55.1
Abscess	20	15	75	8	53.3	2	13.3	5	33.3	15	75
Wounds	15	9	60	5	55.5	–	–	4	44.4	9	60
Nasal swabs	11	6	54.5	1	16. 6	–	–	5	83.3	6	54.5
Total	75	46	61.3	22	47.8	2	4.3	22	47.8	46	61.3

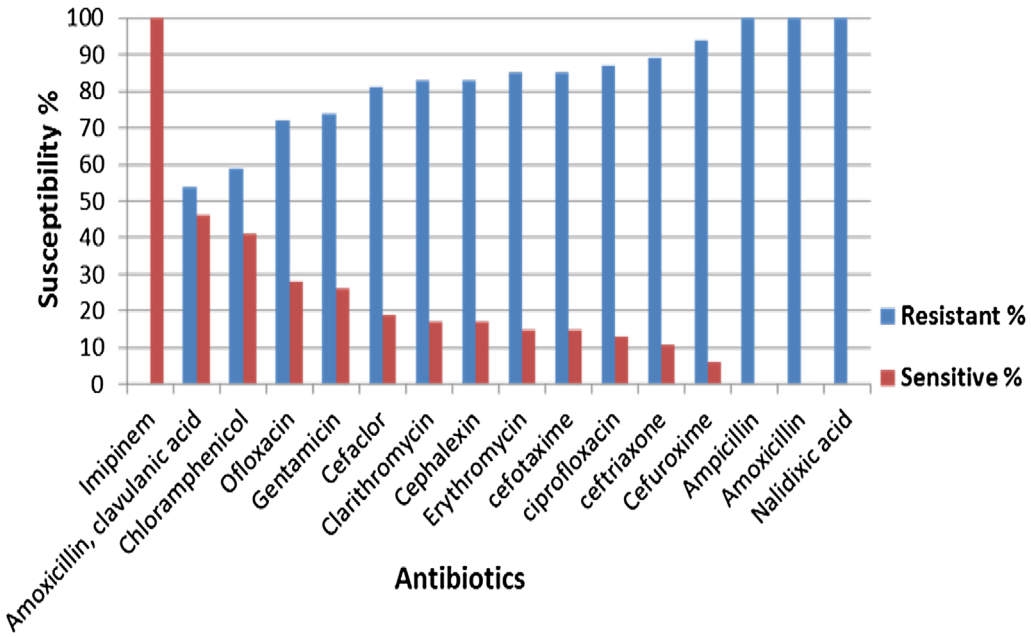

Fig. 1 Antibiotic susceptibility pattern of *S. aureus* isolate from clinical specimens

current results demonstrated all isolates resistant to nalidixic acid, ampicillin, and amoxicillin (Fig. 1).

PCR amplification of enterotoxin genes

The prevalence of different five enterotoxin genes in *S. aureus* isolates was investigated using PCR, it was reported that 15 isolates (32.6%) were positive for the *SEA* gene, 2 isolates (4.3%) were positive for the *SEB* gene, and only one isolate (2.1%) was positive for both the *SEA* and *SEB* genes (Fig. 2), *SEC*, *SED* and *SEE* genes were not found in any of the tested isolates.

Real-time PCR data analysis of *S. aureus* enterotoxins

The relative gene expression was examined of the tested harbored genes by isolation of RNA from the selected *S. aureus* isolates and gene expression was verified using a Reverse Transcription Real-time PCR, which enabled comparison of the target and reference genes, where the 16S rRNA was used as the reference gene and the

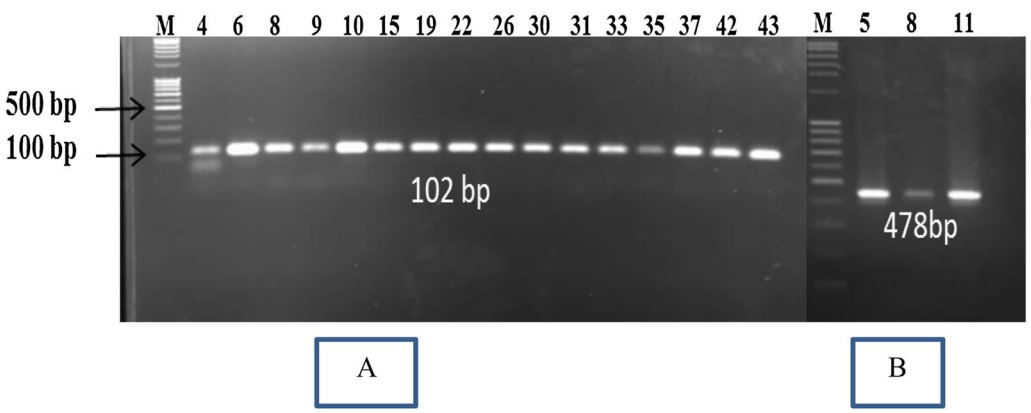

Fig. 2 The amplified products for *SEA* gene (**A**) and *SEB* gene (**B**) on 1.5% agarose gel electrophoresis which given positive with 16 isolates for *SEA* with 102 bp and positive with 3 isolates for *SEB* gene with 478 bp. **A** Lane M: DNA Ladder 100 bp (Thermo Scientific Fisher. USA), Lane 2 to Lane17: amplified products of 16 isolates for *SEA* gene. **B** Lane M: DNA Ladder 100 bp (Thermo Scientific Fisher, USA) lane 2 to Lane 4: amplified products of 3 isolates for *SEB* gene

Table 4 Distribution of enterotoxin genes pattern and relative gene expression among isolation sources

Isolate no	Specimen type	Type of enterotoxin	Gene expression level By real time PCR (%)
4	Wounds	A	0.3195
5	Abscess	B	66.00
6	Abscess	A	9.214
8	Abscess	A and B	0.002 Enterotoxin A 75.8 Enterotoxin B
9	Wounds	A	6.886
10	Abscess	A	100.00
11	Abscess	B	100.00
15	Abscess	A	15.072
19	Urine	A	14.559
22	Abscess	A	20.166
26	urine	A	4.543
30	Urine	A	0.0001
31	Urine	A	1.770
33	Nasal swabs	A	0.003
35	Wounds	A	0.0874
37	Abscess	A	22.375
42	Abscess	A	80.664
43	Abscess	A	0.0103

enterotoxin genes as the target genes. The expression of the *SEA* and *SEB* genes were expressed to variable degrees in all isolates, it was revealed that isolate no: 10 had the highest *SEA* gene expression, followed by isolate no: 42 and isolate no: 37, while isolate no: 11 had the highest *SEB* gene expression, followed by isolate no: 8 and isolate no: 5, (Table 4, Fig. 3). The current report found that MDR of the tested isolates expressed *SEA* gene with (P value = 0.01) and *SEB* gene was expressed with (P value = 0.03).

Identification of highly expression isolates and phylogenetic analysis

Completely identification of *S. aureus* isolates exhibited high level of enterotoxin A and enterotoxin B expression; 16S rRNA gene and enterotoxin genes were amplified and sequenced. The annotated sequences were deposited in GenBank under accession number MF563554 Azhar1 strain for enterotoxin A producing strain and MF563555Azhar2 strain for enterotoxin B producing strain. Sequence alignment and phylogenetic tree analysis revealed that MF563554 given the similarity of about 96% with LT677428 and LT677437 human strains isolated from the head and neck tissue in the USA, while MF563555 had similar of about 90% with HM452073 isolated from bovine mastitis in India (Fig. 4). High expression isolates for *SEA* and *SEB* genes using specific primers were amplified and sequenced. The annotated sequence was deposited in GenBank under accession number LC315607 ELBAZ1 for enterotoxin A strain and MF621929 ELBAZ2 for the enterotoxin B strain. Sequence analysis revealed that LC315607 ELBAZ1 strain given 98% similarity with KX777250 which was a local isolate in Egypt while MF621929 ELBAZ2

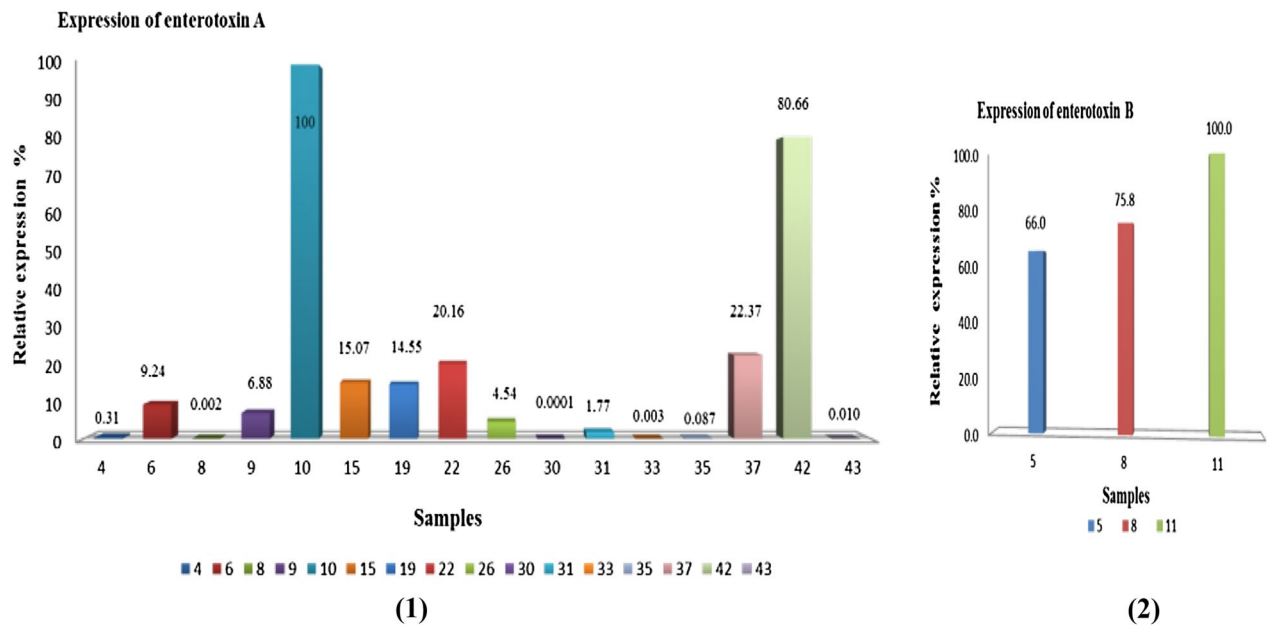

Fig. 3 Histogram for estimation the quantitative expression level of specific enterotoxin A gene (1) and for estimation the quantitative expression level of specific enterotoxin B gene (2)

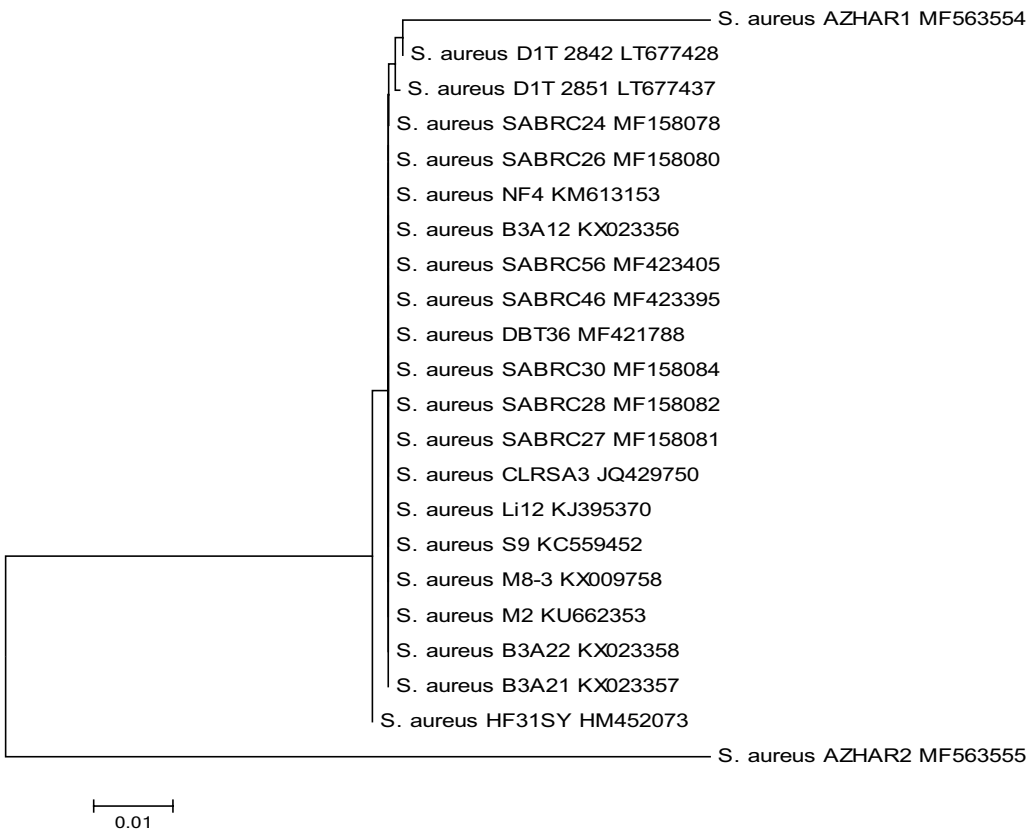

Fig. 4 Phylogenetic tree of 1500 bp of 16S rRNA gene of *S. aureus* MF563554, MF563555, compared with other strains available in GeneBank using neighbor-joining distance method

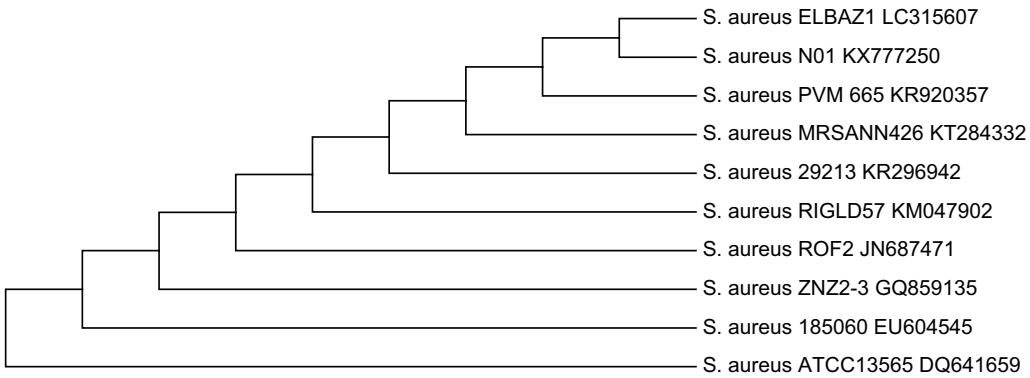

Fig. 5 The minimum evolution distance analysis phylogenetic tree of *SEA* gene encoding *S. aureus* MF563554 compared with other strains of *SEA* encoding gene available in GeneBank

strain given similar 99% with AB860415 strain isolated from Tokyo food poisoning outbreak and AB716349 isolated from a human nasal swab in Japan. The relationship between ELBAZ1 and ELBAZ2 among other standard strains available in GeneBank were assessed by constructing a neighboring-Joining tree (Figs. 5, 6).

Discussion

S. aureus is a widespread human pathogen that can be found in both hospitals and the public, it's an opportunistic pathogen that can cause several diseases in humans, both self-limiting and life-threatening [30, 31]. In the current investigation revealed high frequency of *S. aureus* in

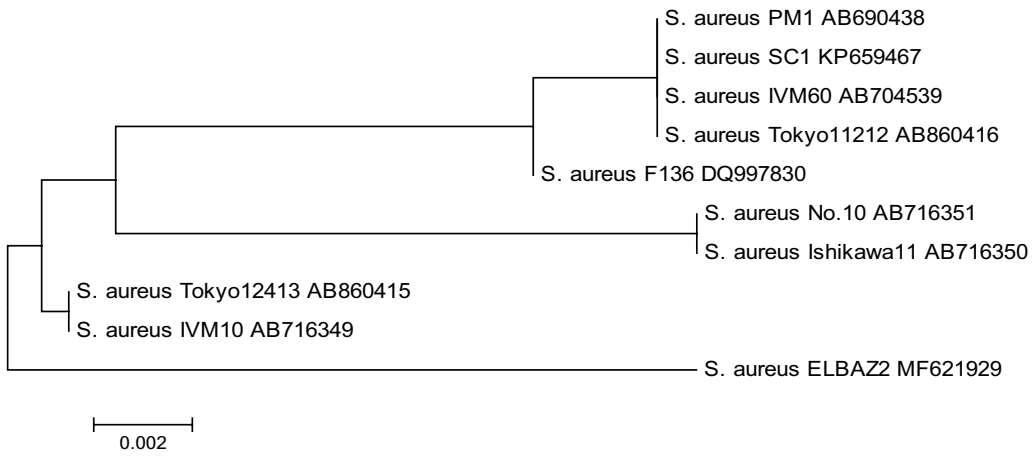

Fig. 6 The minimum evolution distance analysis phylogenetic tree of *SEB* gene encoding *S. aureus* MF563555 compared with other strains of *SEB* encoding gene available in GeneBank

(61.3%) of isolates. This is higher isolation rate than seen in earlier research (28.1% and 24.5%, respectively) [31, 32]. The higher isolation of *S. aureus* was observed especially in children and neonates and this finding consistent with report [31], it is believed that their immunity is not properly developed at this phase to cope with bacterial illness hence they are vulnerable and easily infected especially when hospitalized. The older children have also been observed to be more active than adults during their interaction with their playmates and while playing for hours, come in contact with various objects. In this process, they become a target to ubiquitous bacteria such as *S. aureus*. On MSA and blood agar media, purified *S. aureus* colonies were smooth, circular, convex, entire, and given different pigments: yellow (47.8%), white (47.8%), and lemony yellow (4.3%), the present data supported through other investigations [32–34]. *S. aureus* isolates were found to have the highest occurrence rate in abscess specimens, which is consistent with previous results [32, 33, 35]. In contrast, the highest incidence rate of *S. aureus* was showed in urine specimens [36], wounds infections [37, 38] and nasal swabs specimens [39, 40]. The high occurrence of *S. aureus* was found in abscess specimens which could be attributed to poor personal hygiene and abscess exposure, making it more susceptible to contamination and infection. In addition, some people in the study region treat their abscesses with self-medication or by employment unqualified or poorly trained quacks before seeking proper medical attention, which might account for the level of settlement by *S. aureus*. Regarding antibiotic resistance, the current results were explained, all *S. aureus* isolates were resistant to ampicillin, amoxicillin, and nalidixic acid, these findings are consistent with those published in other studies [41, 42]; this suggests that these antibiotics are no longer successful against infections caused by *S. aureus*. The other studied antibiotics showed a wide range of resistance like Cefuroxime (94%), Ceftriaxone (89%), Ciprofloxacin (87%), Ceftaxime and Erythromycin (85%), Cephalexin and Clarithromycin (83%), Cefaclor (81%), Gentamicin (74%), Ofloxacin (72%), Chloramphenicol (59%). and Amoxicillin/Clavulanic acid (54%), these data were compatible with some reports [32, 43, 44]**.** The present data were revealed significantly higher resistance to the tested antibiotics than other studies [33, 34, 45]. As a result of the high prevalence of antibiotic resistance in strains, antibiotics commonly used to treat *S. aureus* infections may not be sufficient. So, physicians must take into account the care guidelines for MRSA infections. All isolates, on the other hand, were found to be susceptible to Imipinem; this finding was consistent with prior investigations [46, 47] that found 87% and 98% of isolates to be susceptible to Imipinem, respectively. So this medication is still successful and can be used as an additional treatment choice for *S. aureus* infections in the study area. It's possible that the great prevalence of resistance to the antibiotics mentioned is attributable to their widespread usage in the treatment of human diseases. This suggests that these antibiotics are no longer effective as an empirical treatment for *S. aureus* infections in the research field. The low activity of these antibiotics could be related to earlier exposure to these drugs that would have accelerated the development of resistance. The rise in antibiotic abuse in our region, which stems from self-medication, failure to react to care, and antibiotic-sale actions, can bolster this assertion.

A long history of effective *SE* determination in epidemiology is cited in both clinical and environmental

settings [48]. Enterotoxin genes were discovered in 39.1% of the isolates. Some investigations [49–51] found lower levels of toxigenicity (36%; 43%, and 23%, respectively). However, several studies revealed greater levels of enterotoxigenicity (88%, 76.4%, and 93.5%, respectively) [46, 52, 53]. Only two distinct enterotoxin genes were revealed among five enterotoxin genes, which was consistent with the findings of a previous investigation [50]. The enterotoxin A gene was found in 32.6% of isolates, which was similar to other studies [51, 54–56] that showed roughly similar levels of enterotoxin A (41%, 42.9%, 44%, and 40%, respectively). Previous studies [46, 57, 58] reported a greater frequency of the SEA gene (66%, 65.2%, and 60.6%, respectively). In contrast, other reported data [59–61] found lower prevalence rates of the SEA gene (15%, 18.8%, and 17%). Enterotoxin B was discovered in 4.3% of testing isolates, which was corroborated by investigations [47, 60–62], which indicated that (2%, 5%, 5.1%, and 5%, respectively) of isolates carried the SEB gene. According to several investigations, the detection rate of the SEB gene was 44.3%, 38%, and 19.6%, respectively [6, 46, 51]. The SEB gene was found in 21.6% of bovine mastitis cases [63] and 24% of cutaneous infections [64]. SEA and SEB genes were discovered together in only one isolate (2.1%), which is lower than studies [46, 65], which detected SEA and SEB genes combined in 11% and 22% of isolates, respectively. SEC, SED, and SEE genes were not found in any of the examined isolates; nevertheless, additional findings revealed the existence of the SEC, SED, and SEE genes in isolates [46, 51, 56, 66].

It is well recognized that the presence of toxin genes does not imply the potential to produce toxin [46, 67]. As a result, we used the real-time PCR technique to demonstrate the ability of chosen isolates to express the examined enterotoxin genes. With 100% and 80.66%, respectively, two isolates (10 and 42) showed extraordinarily high levels of expression. Furthermore, the SEB gene expression levels were demonstrated in varied degrees in isolates (5, 8, and 11) with a percentage ranging from 66 to 100%. These findings revealed considerable heterogeneity in the expression of enterotoxins among isolates, which was consistent with prior findings [46]**.** The discrepancies in the prevalence of enterotoxin encoding genes between studies can be due to a variety of factors, including the origin of the isolates, research locations, hygiene restrictions in different countries, and assay methods. The correlation between enterotoxin and resistance to antibiotics is unknown, but the current study demonstrated a strong connection between the determined toxin pattern distribution and antibiotic resistance and that was statically significant with (P value = 0.01 and > 0.05) for *SEA* and *SEB* genes respectively, and this was consistent with prior investigations where more association between level of enterotoxins and resistance to antibiotics [46, 68, 69], although some studies suggested a negative correlation between antibiotic resistance and enterotoxin investigation [70, 71]**.** Also, one of the most important findings in the current investigation was the pigmentation of isolates; although, the relationship between pigment creation on MSA and enterotoxigencity remains unclear; however, there was a putative link between isolate pigmentation and enterotoxin generation. These findings corroborated those investigations that noticed that *SEs* genes were more correlated with antibiotic resistance and pigment development [69, 70].

Conclusion

The current research looks at the prevalence of enterotoxin genes in clinical samples, especially the *SEA* gene, which is followed by the *SEB* gene. The source of isolation was not related to the enterotoxin genes. On the other hand, the *SEA* gene was discovered to be closely linked to clinical isolates. The existence of toxin genes does not necessarily mean that the toxin can be produced. Our results suggest that assessing *S. aureus* potential to cause serious disease requires evaluating the degree of expression of certain toxins at mRNA level. Despite the fact that the genetic relationship between resistance and enterotoxins is poorly understood, evidence from our study indicates that enterotoxin and antibiotic resistance are linked in a significant way.

Abbreviations

S. aureus: *Staphylococcus aureus*; SEs: Staphylococcal enterotoxins; SEA: Staphylococcal enterotoxin A; SEB: Staphylococcal enterotoxin B; SEC: Staphylococcal enterotoxin C; SED: Staphylococcal enterotoxin D; SEE: Staphylococcal enterotoxin E; MSA: Mannitol salt agar; CT: Threshold of a cycle; Q.RT-PCR: Quantitative Real Time-PCR.

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Authors' contributions

AB, EB, UA and AA study concept and design, development of the study, data interpretation and manuscript revision and drafting; AB, EB and UA contributed to sample collection**,** phenotypic studies; AB and AA contributed reagents/materials/analysis tools and molecular studies. All the authors have read and approved of the final version of the manuscript for publication.

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Declarations

Ethics approval and consent to participate

The study protocol was approved by the ethics committee, Botany and Microbiology department- the College of Science (Al-Azhar University, Assiut) obtained the approval of the Ethics Committees and the research was

conducted following the principles of the Declaration of Helsinki but Ethical approval number: not available. All specimens were collected aseptically and transported to the microbiology laboratory, where they were immediately processed according to the standard microbiological procedures. We would like to confirm that this material is the authors' own original work, which has not been previously published elsewhere. The paper is not currently being considered for publication elsewhere. The paper reflects the authors' own research and analysis in a truthful and complete manner. The paper properly credits the meaningful contributions of co-authors and co researchers. The results are appropriately placed in the context of prior and existing research. All authors have been personally and actively involved in substantive work leading to the manuscript and will hold themselves jointly and individually responsible for its content.

Consent for publication
"Not applicable".

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Competing interests
The authors declare that they have no competing interests.

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