Research Article

Antimicrobial Resistance and Virulence Genes` Profiles of *Staphylococcus aureus* in Meat and Meat Products

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Abstract | The emergence of multidrug resistance (MDR) among methicillin-resistant *Staphylococcus aureus* (MRSA) has become a significant threat worldwide. Thus, the current work was designed to elucidate the prevalence and antimicrobial susceptibility patterns of *S. aureus* among meat and meat products' samples in Egypt and to determine the virulence genes profiles of MDR biofilm-producing MRSA isolates. A total of 150 samples were obtained from bovine meat (n= 30) and meat products (n= 120) samples in Sharkia Governorate, Egypt. Conventional identification techniques detected 50 (33.3%) staphylococcal isolates (32.8%); 28 (18.7%) were classified as *S. aureus* and 22 (14.7%) were identified as coagulase-negative staphylococci (CoNS). All the examined *S. aureus* isolates were resistant to oxacillin and cefoxitin. Meanwhile, the lowest resistance patterns were detected against chloramphenicol, and ceftriaxone (28.6% each). Of note, 21 *S. aureus* isolates (75%) were MDR and they were recognized phenotypically as strong biofilm producers. The genes *icaA, sea, pvl,* and *eta* were detected in 100, 76.2, 42.9, and 33.3% MDR biofilm-producing MRSA isolates, respectively. Interestingly, 8 MDR MRSA (38.1%) were multi-virulent (harbored 3 or more virulence genes). In essence, our results displayed the role of retail meat as a possible source for the spread of multi-virulent MDR biofilm-producing MRSA in Egypt, thus more focus should be placed on continuous monitoring of antimicrobial utilization with the requirement for effective control strategies against multi-virulent MRSA.

Keywords | *Staphylococcus aureus*, MRSA, Multidrug-resistant, Biofilm producing MRSA, Virulence genes, *icaA* gene, *pvl* gene, *eta* gene, *sea* gene

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INTRODUCTION

Staphylococcus aureus (*S. aureus*), particularly methicillin-resistant *S. aureus* (MRSA), has been considered a key contributing factor in numerous illnesses worldwide including toxic shock syndrome, septicemia, pneumonia, food-poisoning outbreaks, (Lin and Peterson, 2014; Islam *et al*., 2019), in addition to subclinical and clinical mastitis in dairy animals (Abdel-Raheem *et al*., 2023).

Recently, the incidence of multidrug-resistant (MDR) bacteria has dramatically increased as a result of antimicrobials abuse, which hindered the healing process (Ammar *et al*., 2021a, b). The widespread dissemination of *S. aureus* is owing to its prospective virulence factors as well as its extraordinary capacity to withstand most antimicrobials released in recent years with the development of MDR strains (Ammar *et al*., 2016). MRSA has evolved into an aggressive endemic bacterium with adverse effects on public

health globally and the majority of these incriminated strains are MDR. A modified penicillin-binding protein (PBP)2a that is encoded by an exogenous *mecA* gene is involved in the development of MRSA (Abd El-Aziz *et al*., 2018).

Staphylococcus aureus's pathogenicity is a complicated mechanism including a wide variety of virulence attributes (Cotar *et al*., 2010), which is made up of numerous exoproteins and proteins linked to cell walls (Abd El-Hamid and Bendary, 2013). Exoproteins including exotoxins and enzymes such as coagulase, nucleases, proteases, collagenase, hyaluronidase, and lipases, are secreted by almost all strains of *S. aureus*. Plasma coagulation caused by staphylocoagulase is used to distinguish *S. aureus* from other less pathogenic staphylococci that are often known as coagulase-negative staphylococci (CoNS). *S. aureus* was also identified using nuclease (*nuc*) gene since it encodes the TNase synthesis that contains species-specific sequences (Abdel-Raheem *et al*., 2023).

Heat-stable staphylococcal enterotoxins (SEs) and two exotoxins including exfoliative toxins (ETs) and toxic shock syndrome toxins can be produced by some *S. aureus* strains (Islam *et al*., 2019). The staphylococcal scalded skin disorder is thought to be caused by the two ETs (ETA and ETB) either together or separately (Iandolo, 1989; Islam *et al*., 2019). Notably, SEs are pyrogenic toxins that play a significant role in *S. aureus* pathogenicity including evasion of host immune defense, infection of the gastrointestinal tract, colonization of the host, invasion of injured skin and mucus and they are in charge of food poisoning and certain acute staphylococcal toxemia disorders worldwide (Ballah *et al*., 2022a). There are 18 various types of SEs including SEA-SEE, SEG-SER, and SEU and more than 70% of *S. aureus* strains have been shown to secret one or more SEs (Abd El-Hamid *et al*., 2013).

Additionally, Panton-Valentine leucocidin (PVL), a bi-complex pore-making cytotoxin that destroys leukocytes and causes necrosis of tissues. It is encoded by *pvl* gene, which is a significant virulence determinant that is primarily linked to raising the severity of *S. aureus* infection as it increases its adherence to the extracellular matrix (Abd El-Hamid and Bendary, 2015).

Staphylococcus aureus infection is difficult to treat because of its adherence and invasion ability, which is facilitated by the bacteria's natural capacity to produce biofilms on abiotic and biotic surfaces (Laverty *et al*., 2013). Biofilms shield the cells from the impact of chemotherapeutic drugs as well as the host immune system (Van Acker *et al*., 2014), which increases antibiotic resistance (McCarthy *et al*., 2015). The synthesis of different proteins, which bind to one or more extracellular matrix components of the host promotes bacterial adhesion to a substratum, which in turn leads to the creation of biofilms (Otto, 2014). The intercellular adhesion ADBC (icaADBC) operon-coded enzymes, which are expressed by *icaADBC* genes control the formation and excretion of the polysaccharide intercellular adhesin, which is primarily responsible for the establishment of an actual biofilm during the bacterial accumulation phase (Abd El-Hamid *et al*., 2020).

Regarding previous researches carried out in Egypt, infections caused by MRSA strains harboring more than three virulence genes (multi-virulent strains) were identified in people, but information on MRSA infections in meat and meat products is few. This justification calls for ongoing studies into the phenotypic and genotypic identification of MRSA to identify the best antimicrobials treatment and manage MRSA infections. Thus, the present study aimed to investigate the prevalence and antimicrobial susceptibility patterns of *S. aureus* obtained from meat and meat product samples in Sharkia Governorate, Egypt as well as to investigate biofilm-producing abilities and the virulence genes profiles of MDR biofilm-producing MRSA strains.

MATERIALS AND METHODS

Sample collection

One hundred and fifty various meat specimens of bovine origin including fresh meat (n= 30) and meat products (n= 120) were randomly selected from 10 local retail outlets during the period from March 2022 to March 2023 from various localities at Sharkia Governorate, Egypt. The meat product samples consisted of luncheon, minced meat, sausage, and burger (n= 30 each). The collected samples were then as soon as possible aseptically transported to the laboratory in an icebox, where they were screened for the presence of *Staphylococcus* spp.

Microbiological analysis of *Staphylococcus* species

All collected samples were analyzed using conventional phenotypic techniques for isolation and identification of *Staphylococcus* spp. as pronounced previously (Becker *et al*., 2015). *Staphylococcus* spp. were isolated utilizing mannitol salt agar (Oxoid, Cheshire, UK). Then, to test the capacity of the obtained isolates for producing beta hemolysis and golden yellow pigments, a single suspected colony was grown onto the surface of blood agar (Oxoid, Cheshire, UK) and milk agar (Oxoid, Cheshire, UK) plates, respectively. Traditional phenotypic techniques including Gram's staining, culture characteristics, and biochemical identification tests using catalase and tube coagulase were used to identify the suspected isolates.

Identification of *Staphylococcus aureus* biofilm formation

The *in vitro* formation of biofilms was assessed phenotypically using the adhesion technique on a standard microtiter plate (Stepanović *et al*., 2000), and Congo red agar assay (Freeman *et al*., 1989), and genotypically via the recognition of *icaA* gene (Ciftci *et al*., 2009; Abd El-Hamid *et al*., 2020).

Antimicrobial susceptibility testing

All recovered *S. aureus* isolates underwent antimicrobial susceptibility testing using disc diffusion technique following the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2017). All *S. aureus* isolates were examined against 13 antimicrobial agents belonging to 9 antimicrobial classes utilizing the following antimicrobial discs (Oxoid, Cheshire, UK); ciprofloxacin (CIP, 5 µg), trimethoprim/sulfamethoxazole (SXT, 1.25 + 23.75 µg), amoxycillin/clavulanic acid (AMC, 20 + 10µg), oxacillin (OX, 1 µg), ceftriaxone (CRO, 5 µg), imipenem (IPM, 10 µg), cefoxitin (FOX, 30 µg), erythromycin (E, 15 µg), chloramphenicol (C, 30 µg), vancomycin (VA, 30 µg), clindamycin (DA, 2 µg), gentamicin (CN, 10 µg), and rifampin (RD, 5 µg).

The results were interrupted according to the CLSI guidelines and the MDR isolates were identified as isolates that exhibited resistance to at least one antimicrobial agent from three or more different antimicrobial classes (Ammar *et al*., 2021c). After that, the multiple antibiotic resistance (MAR) indices of the isolates were calculated by the the ratio between the number of antimicrobial agents to which the investigated isolates were resistant and the total number of antimicrobial agents utilized (Aljazzar *et al*., 2022).

Conventional polymerase chain reaction assays

The PCR tests carried out in this study were done on MDR MRSA isolates. The DNA was extracted via QIAamp DNA Mini Kit (Qiagen, USA) according to the directions of the manufacturer. Conventional PCR techniques were performed for identification of *nuc* and *mecA* genes of *S. aureus* and MRSA, respectively. Furthermore, four important virulence genes (*icaA, pvl, eta,* and *sea*) were also determined utilizing conventional PCR techniques. All PCR procedures were conducted, in triplicate, utilizing Emerald Amp GT PCR Master Mix (Takara, USA) according to the manufacturers` regulations. All PCR amplification methods were performed as formerly described (Brakstad *et al*., 1992; Mehrotra *et al*., 2000; Larsen *et al*., 2008; Ciftci *et al*., 2009; Niesche and Haase, 2012). The sequences of primers for the investigated genes in all PCR protocols are listed in Table 1. After that, ethidium bromide staining (Sigma-Aldrich, USA) and agarose gel electrophoresis were utilized to visualize the products of PCR assays (Sambrook *et al*., 1989). In all PCR protocols, PCR-grade water (no template DNA), and the reference strain of *S. aureus* ATCC25923 served as controls negative and positive, respectively.

Statistical analysis

The SPSS Inc. version 26 (IBM Corp., USA) was utilized for analyzing the results. The Chi-square test was utilized to examine the differences in the occurrence of *Staphylococcus* spp. from various sources and to evaluate the variations in antibiotic resistance profiles and the prevalence of virulence genes among the obtained *S. aureus* isolates from different origins. If the *p*-value was less than 0.05, it was deemed statistically significant. Graphs were generated by the GraphPad Prism software Version 8 (San Diego, CA, USA) and R program version 4.3.1 (https//www.r-project.org/).

Table 1: Sequences of oligonucleotide primers targeting five genes of *Staphylococcus aureus* and their respective amplified PCR products.

Target gene	Primer sequence (5'-3')	Amplified product (bp)	Reference
nuc	F: GCGATTGATGGTGATACGGTT	270	Brakstad *et al*., 1992
	R: AGCCAAGCCTTGACGAACTAAAGC		
mecA	F: TCCAGATTACAACTTCACCAGG	162	Niesche *et al*., 2012
	R: CCACTTCATATCTTGTAACG		
icaA	F: CCTAACTAACGAAAGGTAG	315	Ciftci *et al*., 2009
	R: AAGATATAGCGATAAGTGC		
pvl	F: GCTGGACAAAACTTCTTGGAATAT	80	Larsen *et al*., 2008
	R: GATAGGACACCAATAAATTCTGGATTG		
eta	F: GCAGGTGTTGATTTAGCATT	93	Mehrotra *et al*., 2000
	R: AGATGTCCCTATTTTTGCTG		
sea	F: GGTTATCAATGTGCGGGTGG	102	
	R: CGGCACTTTTTTCTCTTCGG		

Table 2: Prevalence of *Staphylococcus* species in the investigated meat and meat product samples.

Samples source (No)	Sample type (symbol, No)	No. of staphylococcal isolates (%) *		Total No. of staphylococcal isolates (%) *
		S. aureus	*CoNS*	
Meat (30)	Meat (M, 30)	10 (33.3)	3 (10)	13 (43.3)
Meat products (120)	Luncheon (L, 30)	3 (10)	9 (30)	12 (40)
	Minced meat (MM, 30)	6 (20)	2 (6.7)	8 (26.7)
	Sausage (S, 30)	5 (16.7)	7 (23.3)	12 (40)
	Burger (B, 30)	4 (13.3)	1 (3.3)	5 (16.7)
Total 150		28 (18.7)	22 (14.7)	50 (33.3)
p-value		0.191	0.013[a]	0.211

CoNS: coagulase-negative staphylococci. * The isolation rate was determined regarding the total number of the investigated specimens from each source. [a] indicate statistically significant variations in the prevalence of bacterial isolates between various sources; $p < 0.05$.

RESULTS AND DISCUSSION

Prevalence of *Staphylococcus* species in meat and meat products

The conventional phenotypic identification of 150 meat and meat product samples showed the presence of 50 staphylococcal isolates (33.3%) (Table 2 and Figure 1). Of all examined specimens, 28 (18.7%), and 22 (14.7%) were positive for *S. aureus*, and CoNS, respectively. *Staphylococcus* spp. were highly prevalent among meat (43.3%) and sausage and luncheon (40% each) samples.

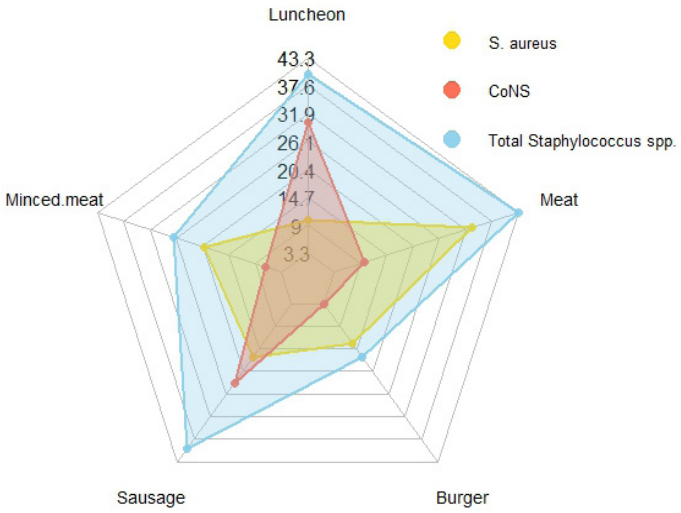

Figure 1: Prevalence of *Staphylococcus* species in the investigated meat and meat products samples. CoNS: coagulase-negative staphylococci.

Among meat samples, *S. aureus* (33.3%) was the most prevalent spp. followed by CoNS (10%). Moreover, among meat products' samples, CoNS (15.8%) was the most prevalent spp. followed by *S. aureus* (15%). Of note, *S. aureus* was highly prevalent among meat (33.3%) and minced meat (20%) samples, meanwhile CoNS was highly distributed among luncheon (30%) and sausage (23.3%) samples (Table 2 and Figure 1). Our outcomes exhibited no statistically significant variations in the incidence of *S. aureus*, and total *Staphylococcus* spp. (p =0.191 and 0.211, respectively) across various sample types. Meanwhile, there was a significant trend in the prevalence of CoNS (p =0.013) among various sample types (Table 2).

Characterization of *Staphylococcus aureus* biofilm producers

Of the 28 examined *S. aureus* isolates, 21 isolates (75%) were recognized phenotypically as strong biofilm producers based on strong adhesion to microtiter plates and strong growth onto Congo red agar. Additionally, 5 *S. aureus* isolates were weak biofilm producers, meanwhile, 2 isolates were non-biofilm producers. Of note, the highest rate of biofilm-producing *S. aureus* was detected between the strains recovered from minced meat (100%, 6 out of 6) and luncheon (100%, 3 out of 3) samples, followed by sausage (80%, 4 out of 5), and meat (70%, 7 out of 10) ones. Meanwhile, the lowest rate of biofilm-producing *S. aureus* was detected among burger (25%, 1 out of 4) samples.

Antimicrobial susceptibility testing of *Staphylococcus aureus*

Antibiogram of the recovered 28 *S. aureus* isolates against the tested 13 antimicrobial agents are displayed in Table 3 and Figure 2. Notably, our results showed that 21 *S. aureus* isolates (75%) were MDR, and all the staphylococcal isolates recovered from luncheon and minced meat samples were MDR. Additionally, all the investigated isolates were resistant to oxacillin and cefoxitin (100%). Additionally, more than half of the examined *S. aureus* isolates were resistant to erythromycin (60.7%) and trimethoprim-sulfamethoxazole (57.1%). Meanwhile, the lowest resistance patterns were detected against chloramphenicol, and ceftriaxone (28.6% each), and imipenem (32.1%) (Table 3 and Figure 2).

Of note, all *S. aureus* isolates from luncheon samples (100%) were resistant to trimethoprim-sulfamethoxazole, erythromycin, and ciprofloxacin, while half (50%) of the *S. aureus* isolates from meat samples were resistant

Table 3: Antimicrobial resistance patterns of *Staphylococcus aureus* from meat and meat product samples.

Antimicrobial class	Antimicrobial agent	No. of resistant *S. aureus* isolates from different sources (%)					p value	Total No. of resistant *S. aureus* isolates (%) (n= 28)
		Meat (n= 10)	Luncheon (n= 3)	Minced meat (n= 6)	Sausage (n=5)	Burger (n= 4)		
Quinolones	Ciprofloxacin	3 (30)	3 (100)	4 (66.7)	3 (60)	0	0.046*	13 (46.4)
Sulphonamides	Trimethoprim sulfamethoxazole	5 (50)	3 (100)	4 (66.7)	3 (60)	1 (25)	0.399	16 (57.1)
Beta-lactams	Amoxycillin clavulanic acid	3 (30)	1 (33.3)	5 (83.3)	4 (80)	1 (25)	0.122	14 (50)
	Oxacillin	10 (100)	3 (100)	6 (100)	5 (100)	4 (100)	NA	28 (100)
	Ceftriaxone	5 (50)	0	1 (16.7)	1 (20)	1 (25)	0.024*	8 (28.6)
	Imipenem	3 (30)	0	3 (50)	2 (40)	1 (25)	0.732	9 (32.1)
	Cefoxitin	10 (100)	3 (100)	6 (100)	5 (100)	4 (100)	NA	28 (100)
Macrolides	Erythromycin	5 (50)	3 (100)	4 (66.7)	4 (80)	1 (25)	0.276	17 (60.7)
Phenicols	Chloramphenicol	0	2 (66.7)	4 (66.7)	2 (40)	0	0.013*	8 (28.6)
Glycopeptides	Vancomycin	4 (40)	1 (33.3)	4 (66.7)	1 (20)	0	0.386	10 (35.7)
Lincosamide	Clindamycin	3 (30)	0	3 (50)	3 (60)	1 (25)	0.492	10 (35.7)
Aminoglycosides	Gentamicin	3 (30)	2 (66.7)	3 (50)	2 (40)	0	0.427	10 (35.7)
Ansamycins	Rifampin	4 (40)	1 (33.3)	5 (83.3)	3 (60)	2 (50)	0.542	15 (53.6)

n: number, * and ** indicate the statistically significant variations in the resistance patterns between *S. aureus* isolates from various sources to the indicated antimicrobial utilizing the Chi-square test; * $p < 0.05$, ** $p < 0.01$, NA: non-applicable.

to trimethoprim-sulfamethoxazole, erythromycin, and ceftriaxone. Moreover, most of *S. aureus* isolates from minced meat and sausage were resistant to amoxycillin-clavulanic acid (83.3 and 80%, respectively) (Table 3 and Figure 2). Furthermore, statistically significant trends were detected in the resistance rates among *S. aureus* isolates from various sources to ciprofloxacin, ceftriaxone, and chloramphenicol ($p = 0.046, 0.024$, and 0.013, respectively). On the other hand, there were no statistically significant variations ($p > 0.05$) in the resistance patterns among *S. aureus* isolates from the various sources to the other investigated antimicrobials (Table 3).

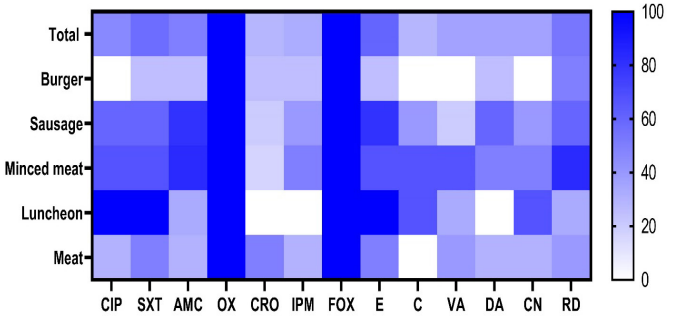

Figure 2: Frequency of resistance of *Staphylococcus aureus* isolates from meat and meat product samples to the antimicrobial agent. CIP: ciprofloxacin, SXT: trimethoprim-sulfamethoxazole, AMC: amoxycillin-clavulanic acid, OX: oxacillin, CRO: ceftriaxone, IPM: imipenem, FOX: cefoxitin; E: erythromycin, C: chloramphenicol, VA: vancomycin, DA: clindamycin, CN: gentamicin, and RD: rifampin. The antimicrobials resistance percentages are color-coded on the right of the figure.

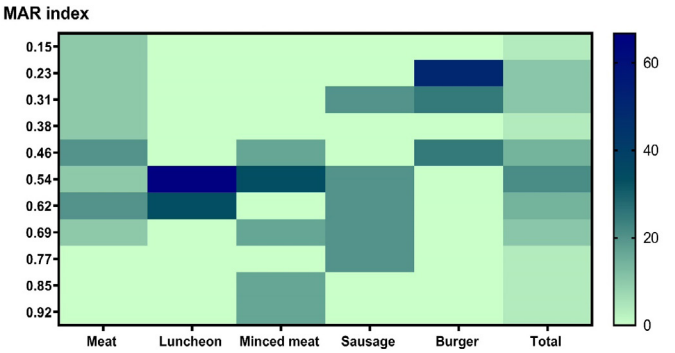

Figure 3: Multiple antibiotic resistance indices of *Staphylococcus aureus* isolates from meat and meat products samples. The antimicrobials resistance percentages are color-coded on the right of the figure.

Estimating the MAR indices revealed that 96.4% of the examined staphylococcal isolates had an index of 0.2 or greater, which suggested high-risk sources of contamination, where antibiotics are frequently used (Table 4 and Figure 3). Of note, only two *S. aureus* isolates obtained from minced meat samples had MAR indices of 0.85 and 0.92 (resistance to 11 and 12 antimicrobials) and those two isolates were resistant to eight and nine antimicrobial classes, respectively. Furthermore, only one sausage staphylococcal isolate was resistant to ten antimicrobials (Figure 4A). Interestingly, three staphylococcal isolates (10.7%) were resistant to seven antimicrobial classes and those isolates were obtained from meat, luncheon, and sausage samples (Figure 4B). There was a statistically significant variation in the resistance rates to two antimicrobial classes among *S. aureus* isolates from the various sources ($p = 0.018$).

Table 4: Multiple antibiotic resistance indices of *Staphylococcus aureus* from meat and meat product samples.

MAR index	No. of antimicrobials to which the isolates were resistant*	No. of AMC	No. of *S. aureus* from various sources (%)					Total No. of *S. aureus* isolates (%) (n=28)
			Meat (n= 10)	Luncheon (n= 3)	Minced meat (n= 6)	Sausage (n=5)	Burger (n= 4)	
0.15	2	1	1 (10)	-	-	-	-	1 (3.6)
0.23	3	1	1 (10)	-	-	-	-	1 (3.6)
		2	-	-	-	-	2 (50)	2 (7.1)
0.31	4	2	-	-	-	1 (20)	1 (25)	2 (7.1)
		3	1 (10)	-	-	-	-	1 (3.6)
0.38	5	4	1 (10)	-	-	-	-	1 (3.6)
0.46	6	2	1 (10)	-	-	-	-	1 (3.6)
		3	-	-	-	-	1 (25)	1 (3.6)
		4	1 (10)	-	1 (16.7)	-	-	2 (7.1)
0.54	7	4	1 (10)	-	-	-	-	1 (3.6)
		5	-	1 (33.3)	2 (33.3)	1 (20)	-	4 (14.3)
		6	-	1 (33.3)	-	-	-	1 (3.6)
0.62	8	5	1 (10)	-	-	-	-	1 (3.6)
		6	-	-	-	1 (20)	-	1 (3.6)
		7	1 (10)	1 (33.3)	-	-	-	2 (7.1)
0.69	9	6	1 (10)	-	1 (16.7)	-	-	2 (7.4)
		7	-	-	-	1 (20)	-	1 (3.6)
0.77	10	6	-	-	-	1 (20)	-	1 (3.6)
0.85	11	8	-	-	1 (16.7)	-	-	1 (3.6)
0.92	12	9	-	-	1 (16.7)	-	-	1 (3.6)

MAR: multiple antibiotic resistance, AMC: antimicrobial classes, n: number.

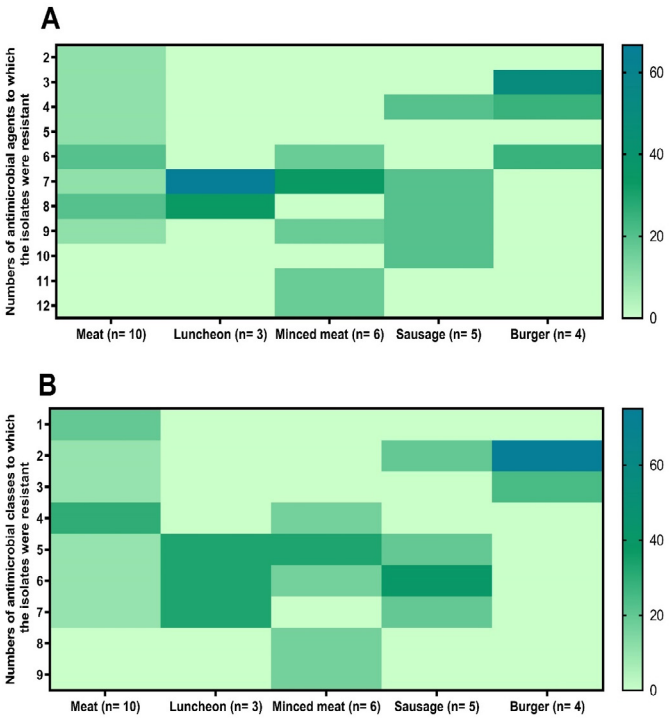

Figure 4: Antimicrobial resistance patterns of *Staphylococcus aureus* isolates from meat and meat products samples. n: number. The antimicrobials resistance percentages are color-coded on the right of the figure.

Molecular characterization of multidrug-resistant *Staphylococcus aureus* isolates from meat and meat products samples

Of the 28 recovered isolates, 21 MDR, strong biofilm-producing *S. aureus* isolates, which were resistant to oxacillin and cefoxitin antimicrobial agents were subjected to PCR amplifications of *nuc* and *mecA* genes for molecular confirmation of *S. aureus* and MRSA, respectively. All the 21 investigated isolates (100%) were identified as *S. aureus* and confirmed to be MRSA. These findings were 100% correlated with those of the phenotypic identification techniques. These 21 isolates were obtained from meat (n= 7), minced meat (n= 6), sausage (n= 4), luncheon (n= 3), and burger (n= 1) samples.

Molecular investigation of *Staphylococcus aureus* virulence-related genes

All molecularly verified MRSA isolates (n= 21) were examined for the existence of four crucial virulence genes (*icaA, pvl, sea,* and *eta*), which are crucial to the pathogenesis of *S. aureus*. Of note, all the tested biofilm-producing MRSA were positive for the presence of *icaA* gene. Of the 21 investigated isolates, 16 (76.2%), 9 (42.9%), and 7 (33.3%) were positive for the *sea, pvl,* and *eta* genes, respectively (Figures 5 and 6). Additionally, there was a

statistically significant difference in the distribution of *pvl* gene among MRSA isolates from various sources (p= 0.044), meanwhile, there were no statistically significant differences in the distribution of *sea* and *eta* genes among MRSA isolates from various sources (p= 0.926 and 0.055, respectively) (Figure 5).

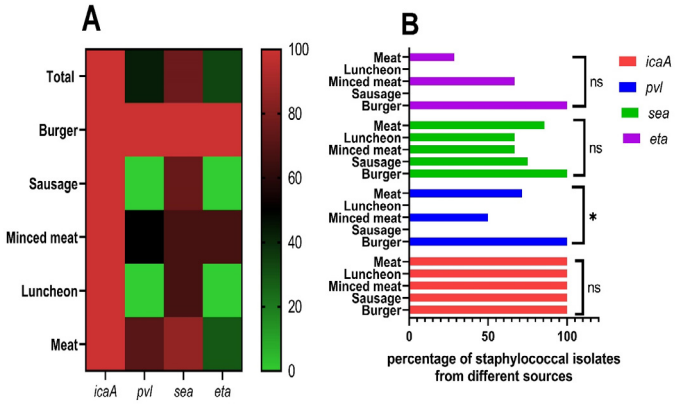

Figure 5: Distribution of virulence genes among *Staphylococcus aureus* from meat and meat product samples. (A) Heat map style, in which the percentages of virulence genes are color-coded on the right of the figure. (B) Columns style utilizing GraphPad prism, which displayed the percentages of *S. aureus* virulence-related genes from each sample type. *icaA:* intercellular adhesion A gene, *pvl:* Panton-Valentine leukocidin gene, *sea:* staphylococcal enterotoxin a gene, and *eta:* exfoliative toxin a gene.

According to the distribution of the tested virulence genes among the investigated isolates, *pvl,* and *sea* genes were more distributed among meat MRSA isolates (71.4%; 5 out of 7 and 85.7%; 6 out of 7, respectively), followed by minced meat ones (50%; 3 out of 6 and 66.7%; 4 out of 6, respectively). Meanwhile, *eta* gene was more prevalent among minced meat MRSA isolates (66.7%; 4 out of 6), followed by meat ones (28.6%; 2 out of 7) (Figure 6).

Notably, of the 21 tested isolates, five MDR MRSA (23.8%) contained the 4 investigated virulence genes and these isolates were recovered from two meat (28.6%), two minced meat (33.3%), and one burger (100%) samples. Additionally, two meat (28.6%) and one minced meat (16.7%) MRSA isolates harbored three investigated virulence genes (Figure 7A). Interestingly, eight MDR MRSA (38.1%) were multi-virulent (harbored 3 or more virulence genes). Of note, among the investigated MDR MRSA isolates, seven virulence genes profiles were displayed. The most prevalent virulence gene profile (*icaA, sea*) was present among nine MDR MRSA isolates (42.9%) (Figure 7B). There were no statistically significant variations in the distribution of virulence genes' profiles among MRSA isolates from various sources (p > 0.05) (Figure 7).

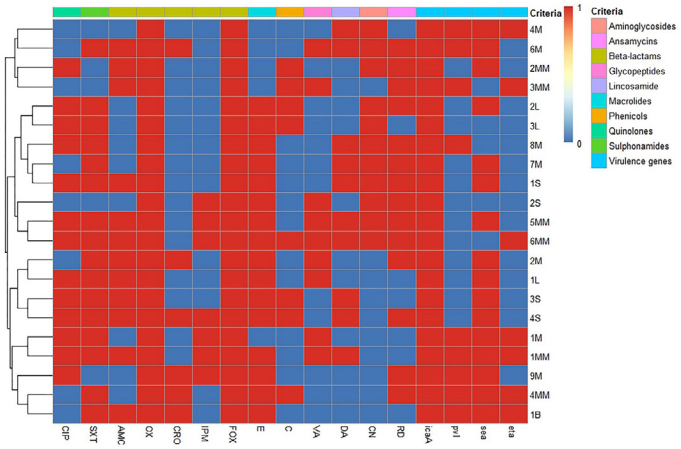

Figure 6: Heat map and hierarchical clustering of the tested *Staphylococcus aureus* isolates based on the occurrence of antimicrobial resistance, and virulence genes. In the heat map, red and blue colors represent the resistance/sensitivity to an antimicrobial agent and the presence/absence of a virulence gene. The code numbers on the left of the heat map and hierarchical clustering dendrogram represent the isolate code numbers for meat (M), luncheon (L), minced meat (MM), sausage (S), and burger (B) samples. CIP: ciprofloxacin, SXT: trimethoprim-sulfamethoxazole, AMC: amoxycillin-clavulanic acid, OX: oxacillin, CRO: ceftriaxone, IPM: imipenem, FOX: cefoxitin, E: erythromycin, C: chloramphenicol, VA: vancomycin, DA: clindamycin, CN: gentamicin, and RD: rifampin, *icaA:* intercellular adhesion A gene, *pvl:* Panton-Valentine leukocidin gene, *sea:* staphylococcal enterotoxin a gene and *eta:* exfoliative toxin a gene.

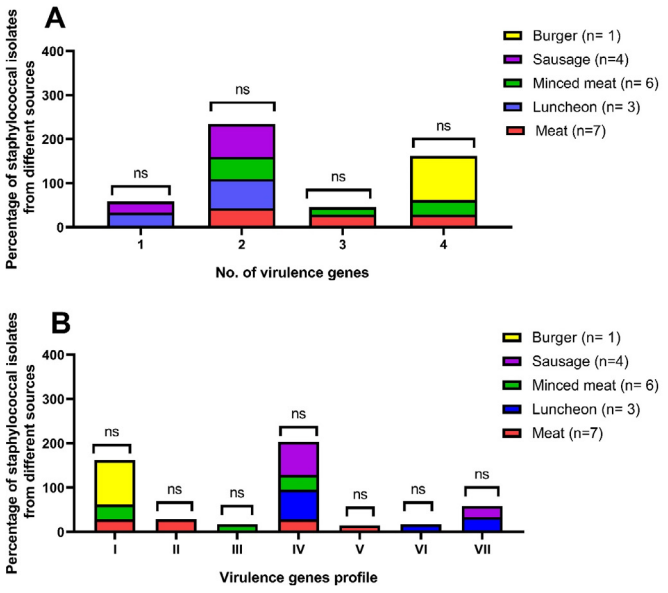

Figure 7: Distribution of the virulence genes (A) and virulence genes profiles (B) among methicillin-resistant *Staphylococcus aureus* isolates from meat and meat product samples. ns: non-significant, n: number, virulence gene profiles; I: *icaA, pvl, sea, eta*, II: *icaA, pvl, sea*, III: *icaA, pvl, eta*, IV: *icaA, sea*, V: *icaA, pvl*, VI: *icaA, eta*, and VII: *icaA*.

Staphylococcus aureus, especially MRSA, is considered one of the most challenging bacteria in human and veterinary medicine worldwide (Abd El-Hamid *et al*., 2015; Abdel-Raheem *et al*., 2023). It has been thought that the rise in the prevalence of MDR MRSA strains poses a danger to medicinal fields (Ammar *et al*., 2016). Therefore, the current work aimed to determine the prevalence and antimicrobial susceptibility patterns of *Staphylococcus* spp. in meat and meat products in Egypt in addition to investigating the virulence genes profiles of MDR biofilm-producing MRSA isolates. Our outcomes showed a high prevalence of total *Staphylococcus* spp. and CoNS (33.3 and 14.7%, respectively) in meat and meat products samples at Sharkia Governorate, Egypt. This is in complete agreement with the outcomes of a previous report conducted in Egypt (32.6 and 14.2%, respectively) (Abd El-Hamid *et al*., 2013), but it was lower than that reported in a previous study carried out in Egypt (58.1 and 27.8%, respectively) (Abd El-Aziz *et al*., 2018). In the current study, 18.7% of the examined meat and meat product samples of bovine origins were positive for *S. aureus,* which is lower than that stated in a previous study conducted in India (41.4%) (Latha *et al*., 2017). Herein, *S. aureus* was highly prevalent among meat samples (33.3%), which is lower than those reported in previous reports carried out in eastern Nepal (68%) (Bantawa *et al*., 2019), India (46.7%) (Latha *et al*., 2017), and Nigeria (46%) (Yemisi *et al*., 2011). Among our minced meat samples, 15% were positive for *S. aureus,* which is higher than those obtained in a recent study conducted in Ethiopia (6.3%) (Zerabruk *et al*., 2019). Overall, the types of analyzed specimens, isolation and identification techniques, sanitary practices, geographic area, and ambient circumstances could all affect the incidence of *Staphylococcus* spp. in different researches (Ammar *et al*., 2022).

Of note, there are differences in antibiotic resistance between and within various nations, and these differences have a direct relationship with the kind of antibiotics that are administered as well as with the regulations for utilizing antibiotic treatments (Ammar *et al*., 2021b). In light of this, all our investigated *S. aureus* isolates were resistant to oxacillin and cefoxitin (100%), which identified them phenotypically as MRSA. This result is in complete agreement with the findings of a recent research conducted in Bangladesh (Ballah *et al*., 2022a), but it was higher than those determined in prior studies carried out in India; (58.9 and 49.3%, respectively) (Parbin, 2021), and Bangladesh (30% each) (Islam *et al*., 2019). Herein, a high rate of erythromycin resistance (60.7%) was detected in the investigated *S. aureus* isolates, which was less severe than those obtained in a recent report carried out in Bangladesh (74%) (Islam *et al*., 2019), but it was higher than those detected in previous studies conducted in India (52.9%) (Yaiphathoi and Sharma, 2020), and Bangladesh (30%) (Ballah *et al*., 2022a). Alarmingly, the emergence of MDR strains is the subject of worldwide concern. In the current work, 75% of the examined *S. aureus* isolates were determined as MDR, which is higher than the outcomes of a prior report carried out in Algeria (16.7%) (Mekhloufi *et al*., 2021). The overuse of antibiotics as growth enhancers in veterinary medicine and unprescribed human and animal treatments may be the cause of the high resistance rates of *S. aureus* isolates in developing nations. Furthermore, the high rates of MRSA found in the current work are concerning because this leads to a significant issue, where antimicrobial treatment becomes constrained (Abdel-Raheem *et al*., 2023). Thus, it is necessary to regulate the use of antibiotics in both animals and humans. Moreover, it is necessary to utilize novel natural alternative antibiotics from herbal medicine (Ammar *et al*., 2021d; Hashem *et al*., 2022; Ibrahim *et al*., 2022; Abd El-Hamid *et al*., 2024).

It's interesting to note that 75% of the examined *S. aureus* isolates were strong biofilm producers based on strong adhesion to microtiter plates and strong growth onto Congo red agar. This result was higher than those of prior research conducted in Egypt (27%) (Abd El-Hamid *et al*., 2020), and Bangladesh (20%) (Ballah *et al*., 2022b). Furthermore, all the tested biofilm-producing MRSA were positive for the presence of *icaA* gene, which is in complete agreement with the findings of a prior report conducted in Egypt (Abd El-Hamid *et al*., 2020), but it was higher than those obtained in a recent study conducted in Bangladesh (71.4%) (Ballah *et al*., 2022b). Of note, among our investigated *S. aureus* isolates, *sea* gene (76.2%) was the most prevalent one, followed by *pvl* gene (42.9%), which is similar to the findings of a recent study conducted in Bangladesh, where *sea* gene (30%) was the most prevalent one, followed by *pvl* gene (15%) (Ballah *et al*., 2022a). Furthermore, all our investigated MDR MRSA harbored at least one virulence gene, which is higher than the findings of a recent study conducted in Bangladesh (35%) (Ballah *et al*., 2022a). These changes in the prevalence of the virulence gene could be attributed to the sample kinds, geographic location, and the origins of the isolates (Ammar *et al*., 2021c).

CONCLUSIONS AND RECOMMENDATIONS

Our findings displayed an alarming prevalence of MDR biofilm-producing MRSA isolates in the examined meat and meat products' samples. All the tested biofilm-producing MRSA were positive for the presence of *icaA* gene. Furthermore, *pvl* and *sea* genes were more distributed among meat MRSA isolates, followed by minced meat ones. Meanwhile, *eta* gene was more prevalent among the

minced meat MRSA isolates, followed by meat ones. Our results gave helpful insights into the prevalence of MDR biofilm-producing multi-virulent MRSA isolates among meat samples, which will help health specialists in the management of MRSA infection. Thus, to decrease the prevalence of multi-virulent MRSA strains, it is necessary to regulate the utilization of antimicrobials in animal production and to enhance sanitary control strategies during slaughter, carcass, and meat product processing. Additionally, we suggest further scientific investigations on the application of novel natural alternative antibiotics from herbal medicine with antimicrobial, anti-biofilm, and anti-virulence characteristics to control MRSA infection.

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NOVELTY STATEMENT

Our study introduced novel insights into alarming prevalence of MDR biofilm-producing multi-virulent MRSA isolates among meat samples.

AUTHOR'S CONTRIBUTION

H.F.A. Investigation, resources, review and editing, funding acquisition.
E.H.E. Validation, formal analysis, visualization, supervision.
M.I.A.E. Conceptualization, methodology, data curation, writing, original draft preparation.
A.E. Investigation, formal analysis snd visualization.

Conflict of interest

The authors have declared no conflict of interest.

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