## **Research Article**



# AntimicrobialResistanceandVirulenceGenes`Profilesof*Staphylococcus aureus* in Meat and Meat Products

## HELAL F. AL-HARTHI<sup>1\*</sup>, ESSAM H. ELDREHMY<sup>1,2</sup>, AFAF ELADL, MARWA I ABD EL-HAMID<sup>2\*</sup>

<sup>1</sup>Department of Biology, Turabah University College, Taif University, Taif, Saudi Arabia; <sup>2</sup>Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.

**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

\*Correspondence | Helal F. Al-Harthi and Marwa I Abd El-Hamid, Department of Biology, Turabah University College, Taif University, Taif, Saudi Arabia; Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt; Email: h.alharthy@tu.edu.sa, mero\_micro2006@yahoo.com Citation | Al-Harthi HF, Eldrehmy EH, Afaf E, El-Hamid MIA (2024). Antimicrobial resistance and virulence genes profiles of *Staphylococcus aureus* in meat and meat products. Adv. Anim. Vet. Sci., 12(7):1290-1300. DOI | https://dx.doi.org/10.17582/journal.aavs/204/12.7.1290.1300

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## **INTRODUCTION**

Staphylococcus aureus (S. aureus), particularly methicillinresistant 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

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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 (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 bicomplex 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

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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) operoncoded 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

#### **S**AMPLE 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.

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# **I**DENTIFICATION 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 E1-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	
пис	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 <i>et al.</i> , 2008	
	R: GATAGGACACCAATAAATTCTGGATTG			
eta	F: GCAGGTGTTGATTTAGCATT	93	Mehrotra <i>et al.</i> , 2000	
	R: AGATGTCCCTATTTTTGCTG			
sea	F: GGTTATCAATGTGCGGGTGG	102		
	R: CGGCACTTTTTTCTCTTCGG			

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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	
		S. aureus	CoNS	isolates (%) *	
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)	
<i>p</i> -value		0.191	0.013ª	0.211	

CoNS: coagulase-negative staphylococci.\* The isolation rate was determined regarding the total number of the investigated specimens from each source. <sup>a</sup> 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.* 

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*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 trimethoprimsulfamethoxazole (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	Antimicrobial agent	No. of resistant <i>S. aureus</i> isolates from different sources (%)				p value Total No. of		
class		Meat (n= 10)	Luncheon (n= 3)	Minced meat (n= 6)	Sausage (n=5)	Burger (n= 4)		resistant <i>S. aureus</i> isolates (%) (n= 28)
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 amoxycillinclavulanic 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.

ger <i>aureus</i> isolates (%) (n=28)
1 (3.6)
1 (3.6)
0) 2 (7.1)
5) 2 (7.1)
1 (3.6)
1 (3.6)
1 (3.6)
5) 1 (3.6)
2 (7.1)
1 (3.6)
4 (14.3)
1 (3.6)
1 (3.6)
1 (3.6)
2 (7.1)
2 (7.4)
1 (3.6)
1 (3.6)
1 (3.6)
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 biofilmproducing *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 biofilmproducing 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: trimethoprimsulfamethoxazole, 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*, *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 biofilmproducing 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

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(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.

#### **CONFLICT OF INTEREST**

The authors have declared no conflict of interest.

#### **REFERENCES**

- Abd El-Aziz NK, Abd El-Hamid MI, Bendary MM, El-Azazy AA, Ammar AM, Abd El-Aziz NK, Bendary MM, El-Azazy AA, Ammar AM (2018). Existence of vancomycin resistance among methicillin resistant *S. aureus* recovered from animal and human sources in Egypt. Slovenian Vet. Res., 55: 221–230.
- Abd El-Hamid MI, Bendary MM (2013). Association between agr alleles and toxin gene profiles of *S. aureus* isolates from human and animal sources in Egypt. Int. J. Adv. Res., 1: 133–144.
- Abd El-Hamid MI, Bendary MM (2015). Comparative phenotypic and genotypic discrimination of methicillin resistant and susceptible *Staphylococcus aureus* in Egypt. Cell Mol. Biol., 61: 101–112.
- Abd El-Hamid MI, El-Azzouny MM, El-Malt RMS, Elkenawy ME, Abdelwarith AA, Younis EM, Youssef W, Dawod

#### Advances in Animal and Veterinary Sciences

- RE, Elged DWAH, Habaka MAM, El-Oksh ASA, Mekawy S, Davies SJ, Ibrahim D (2024). Future impact of thymoquinone-loaded nanoemulsion in rabbits: Prospects for enhancing growth, immunity, antioxidant potential and resistance against *Pasteurella multocida*. Front. Vet. Sci., 10: 1340964. https://doi.org/10.3389/fvets.2023.1340964
- Abd El-Hamid MI, El-Naenaeey ESY, Kandeel TM, Hegazy WAHH, Mosbah RA, Nassar MS, Bakhrebah MA, Abdulaal WH, Alhakamy NA, Bendary MM, El-Naenaeey Y, Sayed E, Kandeel MT, Hegazy WAHH, Mosbah RA, Nassar MS, Bakhrebah MA, Abdulaal WH, Alhakamy NA, Bendary MM (2020). Promising antibiofilm agents: Recent breakthrough against biofilm producing methicillinresistant *Staphylococcus aureus*. Antibiotics, 9: 667. https:// doi.org/10.3390/antibiotics9100667
- Abdel-Raheem SM, Abd El-Hamid MI, Ibrahim D, El-Malt RMS, El-Ghareeb WR, Ismail HA, Al-Sultan SI, Meligy AMA, El-Tarabili RM (2023). Future scope of plant-derived bioactive compounds in the management of methicillinresistant *Staphylococcus aureus*: *In vitro* antimicrobial and antivirulence prospects to combat MRSA. Microbial Pathogen., 183: 106301. https://doi.org/10.1016/j. micpath.2023.106301
- Aljazzar A, Abd El-Hamid MI, El-Malt RMS, El-Gharreb RW, Abdel-Raheem SM, Ibrahim AM, Abdelaziz AM, Ibrahim D (2022). Prevalence and antimicrobial susceptibility of campylobacter species with particular focus on the growth promoting, immunostimulant and anti-campylobacter jejuni activities of eugenol and trans-cinnamaldehyde mixture in broiler chickens. Animals, 12: 905. https://doi.org/10.3390/ ani12070905
- Ammar AM, Attia AM, Abd El-Hamid MI, El-Shorbagy IM, Abd El-Kader SA (2016). Genetic basis of resistance waves among methicillin resistant *Staphylococcus aureus* isolates recovered from milk and meat products in Egypt. Cell. Mol. Biol. (Noisy-le-Grand, France), 62: 7–15.
- Ammar A, Abd El-Hamid MI, Hashem YM, El-Malt RMS, Mohamed HM (2021a). Mycoplasma bovis: Taxonomy, characteristics, pathogenesis and antimicrobial resistance. Zagazig Vet. J., 49: 444–461. https://doi.org/10.21608/ zvjz.2021.103834.1160
- Ammar AM, Abd El-Hamid MI, El-Malt RMS, Azab DS, Albogami S, Al-Sanea MM, Soliman WE, Ghoneim MM, Bendary MM (2021b). Molecular detection of fluoroquinolone resistance among multidrug, extensively drug, and pan-drug-resistant campylobacter species in Egypt. Antibiotics, 10: 1342. https://doi.org/10.3390/ antibiotics10111342
- Ammar AM, El-Naenaeey ESY, El-Malt RMS, El-Gedawy AA, Khalifa E, Elnahriry SS, Abd El-Hamid MI (2021c). Prevalence, antimicrobial susceptibility, virulence and genotyping of campylobacter jejuni with a special reference to the anti-virulence potential of eugenol and beta-resorcylic acid on some multi-drug resistant isolates in Egypt. Animals, 1: 10003. https://doi.org/10.3390/ani11010003
- Ammar AM, El-Naenaeey ESY, El-Hamid MIA, El-Gedawy AA, El-Malt RMS (2021d). Campylobacter as a major foodborne pathogen: A review of its characteristics, pathogenesis, antimicrobial resistance and control. J. Microbiol. Biotechnol. Food Sci., 10: 609–619. https://doi. org/10.15414/jmbfs.2021.10.4.609-619
- Ammar AM, El-Hamid MIA, Mohamed YH, Mohamed HM, Al-Khalifah DHM, Hozzein WN, Selim S, El-

#### **Advances in Animal and Veterinary Sciences**

## **OPEN OACCESS**

Neshwy WM, El-Malt RMS (2022). Prevalence and antimicrobial susceptibility of bovine mycoplasma species in Egypt. Biology, 11: 1083. https://doi.org/10.3390/biology11071083

- Ballah FM, Islam MS, Rana ML, Ullah MA, Ferdous FB, Neloy FH, Ievy S, Sobur MA, Rahman AT, Khatun MM, Rahman M, Rahman MT (2022a). Virulence determinants and methicillin resistance in biofilm-forming *Staphylococcus aureus* from various food sources in Bangladesh. Antibiotics, 11: 1666. https://doi.org/10.3390/antibiotics11111666
- Ballah FM, Islam MS, Rana ML, Ferdous FB, Ahmed R, Pramanik PK, Karmoker J, Ievy S, Sobur MA, Siddique MP, Khatun MM, Rahman M, Rahman MT (2022b). Phenotypic and genotypic detection of biofilm-forming *Staphylococcus aureus* from different food sources in Bangladesh. Biology, 11: 949. https://doi.org/10.3390/biology11070949
- Bantawa K, Sah SN, Subba Limbu D, Subba P, Ghimire A (2019). Antibiotic resistance patterns of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, *Shigella* and *Vibrio* isolated from chicken, pork, buffalo and goat meat in eastern Nepal. BMC Res. Notes, 12: 1–6. https://doi.org/10.1186/s13104-019-4798-7
- Becker K, Skov RL, Eiff C (2015). von Staphylococcus, micrococcus, and other catalase-positive cocci. Manual Clin. Microbiol., pp. 354–382. https://doi. org/10.1128/9781555817381.ch21
- Brakstad OG, Aasbakk K, Maeland JA (1992). Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. J. Clin. Microbiol., 30: 1654– 1660. https://doi.org/10.1128/jcm.30.7.1654-1660.1992
- Ciftci A, Findik A, Onuk EE, Savasan S (2009). Detection of methicillin resistance and slime factor production of *Staphylococcus aureus* in bovine mastitis. Braz. J. Microbiol., 40: 254–261. https://doi.org/10.1590/S1517-83822009000200009
- CLSI, (Clinical and Laboratory Standards Institute) (2017). Performance standards for antimicrobial susceptibility testing 27<sup>th</sup> ed. CLSI supplement M100 2017. Wayne, PA, USA, Vol. 27<sup>th</sup> ed.
- Cotar AI, Chifiriuc MC, Dinu S, Bucur M, Iordache C, Banu O, Dracea O, Larion C, Lazar V (2010). Screening of molecular virulence markers in *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains isolated from clinical infections. Int. J. Mol. Sci., 11: 5273–5291. https://doi.org/10.3390/ijms11125273
- Freeman DJ, Falkiner FR, Keane CT (1989). New method for detecting slime production by coagulase negative staphylococci. J. Clin. Pathol., 42: 872–874. https://doi. org/10.1136/jcp.42.8.872
- Hashem YM, Abd El-Hamid MI, Awad NFS, Ibrahim D, Elshater NS, El-Malt RMS, Hassan WH, Abo-Shama UH, Nassan MA, El-Bahy SM, Samy OM, El-Sharkawy RB, Algabri N, Elnahriry SS (2022). Insights into growthpromoting, anti-inflammatory, immunostimulant, and antibacterial activities of Toldin CRD as a novel phytobiotic in broiler chickens experimentally infected with *Mycoplasma gallisepticum*. Poult. Sci., 101: 102154. https://doi. org/10.1016/j.psj.2022.102154
- Iandolo JJ (1989). Genetic analysis of extracellular toxins of Staphylococcus aureus. Ann. Rev. Microbiol., 43: 375–402. https://doi.org/10.1146/annurev.mi.43.100189.002111
- Ibrahim D, Shahin SE, Alqahtani LS, Hassan Z, Althobaiti F, Albogami S, Soliman MM, El-Malt RMS, Al-Harthi HF, Alqadri N, Elabbasy MT, Abd El-Hamid MI

(2022). Exploring the interactive effects of thymol and thymoquinone: Moving towards an enhanced performance, gross margin, immunity and aeromonas sobria resistance of nile tilapia (*Oreochromis niloticus*). Animals, 12: 3034. https://doi.org/10.3390/ani12213034

- Islam MA, Parveen S, Rahman M, Huq M, Nabi A, Khan ZUM, Ahmed N, Wagenaar JA (2019). Occurrence and characterization of methicillin resistant *Staphylococcus aureus* in processed raw foods and ready-to-eat foods in an urban setting of a developing country. Front. Microbiol., 10: 421351. https://doi.org/10.3389/fmicb.2019.00503
- Larsen AR, Stegger M, Sørum M (2008). SPA typing directly from a mecA, spa and pvl multiplex PCR assay. A costeffective improvement for methicillin-resistant *Staphylococcus aureus* surveillance. Clin. Microbiol. Infect., 14: 611–614. https://doi.org/10.1111/j.1469-0691.2008.01995.x
- Latha C, Anu CJ, Ajaykumar VJ, Sunil B (2017). Prevalence of Listeria monocytogenes, Yersinia enterocolitica, Staphylococcus aureus, and Salmonella enterica, Typhimurium in meat and meat products using multiplex polymerase chain reaction. Vet. World, 10: 927. https://doi.org/10.14202/ vetworld.2017.927-931
- Laverty G, Gorman SP, Gilmore BF (2013). Biomolecular mechanisms of staphylococcal biofilm formation. Future Microbiol., 8: 509–524. https://doi.org/10.2217/fmb.13.7
- Lin YC, Peterson ML (2014). New insights into the prevention of staphylococcal infections and toxic shock syndrome. Exp. Rev. Clin. Pharmacol., 3: 753–767. https://doi.org/10.1586/ ecp.10.121
- McCarthy H, Rudkin JK, Black NS, Gallagher L, O'Neill E, O'Gara JP (2015). Methicillin resistance and the biofilm phenotype in *Staphylococcus aureus*. Front. Cell. Infect. Microbiol., 5: 125791. https://doi.org/10.3389/fcimb.2015.00001
- Mehrotra M, Wang G, Johnson WM (2000). Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J. Clin. Microbiol., 38: 1032–1035. https://doi.org/10.1128/JCM.38.3.1032-1035.2000
- Mekhloufi OA, Chieffi D, Hammoudi A, Bensefia SA, Fanelli F, Fusco V (2021). Prevalence, enterotoxigenic potential and antimicrobial resistance of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (Mrsa) isolated from algerian ready to eat foods. Toxins, 13: 835. https://doi.org/10.3390/toxins13120835
- Niesche R, Haase M (2012). Emotions and ethics: A foucauldian framework for becoming an ethical educator. Educ. Philos. Theor., 44: 276–288. https://doi.org/10.1111/j.1469-5812.2010.00655.x
- Otto M (2014). Physical stress and bacterial colonization. FEMS Microbiol. Rev., 38: 1250–1270. https://doi. org/10.1111/1574-6976.12088
- Parbin N (2021). Characterization of staphylococcal exotoxin (PVL) gene of methicillin resistant *Staphylococcus aureus* (MRSA) from foods of animal origin. Indiana J. Agric. Life Sci., 1: 6–9.
- Sambrook J, Fritsch EF, Maniatis T (1989). Molecular cloning: A laboratory manual. Cold spring harbor laboratory press, 2<sup>nd</sup> edn. Cold Spring Harbor Laboratory Press, New York.
- Stepanović S, Vuković D, Dakić I, Savić B, Švabić-Vlahović M (2000). A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J. Microbiol. Methods, 40: 175–179. https://doi.org/10.1016/S0167-7012(00)00122-6

- Van Acker H, Van Dijck P, Coenye T (2014). Molecular mechanisms of antimicrobial tolerance and resistance in bacterial and fungal biofilms. Trends Microbiol., 22: 326– 333. https://doi.org/10.1016/j.tim.2014.02.001
- Yaiphathoi S, Sharma I (2020). Characterization and phylogenetic reconstruction of *MecA* and *Pvl* genes of methicillin resistant *Staphylococcus aureus* from retail meats in north east India metagenomic insights into gut microbial communities of tribal population from Arunachal Pradesh Vie. Indian J. Natl. Sci., 10: 27661–27670.

## Advances in Animal and Veterinary Sciences

- Yemisi AO, Oyebode AT, Margaret AA, Justina JB (2011). Prevalence of arcobacter, *Escherichia coli, Staphylococcus aureus* and *Salmonella* species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. Sierra Leone J. Biomed. Res., 3: 8–12. https://doi.org/10.4314/sljbr.v3i1.66644
- Zerabruk K, Retta N, Muleta D, Tefera AT (2019). Assessment of microbiological safety and quality of minced meat and meat contact surfaces in selected butcher shops of Addis Ababa, Ethiopia. J. Food Qual., 2019. https://doi. org/10.1155/2019/3902690