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Genetic Analysis of *mecA* Gene of Methicillin-Resistant *Staphylococcus aureus* from Human, Canine and Feline Origins

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INTRODUCTION

Staphylococci are commonly implicated in bacterial infections among both human and animal populations, resulting in a significant burden of hospital-acquired and community-acquired infections annually. Methicillin-resistant *S. aureus* (MRSA) is a serious and urgent challenge for the global healthcare system (Hasmukharay *et al.*, 2023). Numerous causes, including rising antibiotic resistance and poor immunization, can be implicated to the serious clinical and economic consequences of staphylococcal infections (Asante *et al.*, 2020).

Staphylococcus aureus is responsible for a diverse range of clinical manifestations in both human and animal hosts (Tong *et al.*, 2015; Becker *et al.* 2017), *S. aureus* and other species belonging to genus *Staphylococcus* are considered significant public health pathogens due to their ability to rapidly acquire a diverse range of genes associated with resistance (Lakhundi and Zhang, 2018). Coagulase-negative staphylococci and MRSA are the predominant etiological agents responsible for healthcare-associated infections, specifically MR-CoNS (Becker *et al.*, 2020).

Staphylococcus aureus can synthesize a variety of extracellular proteins and polysaccharides that have been implicated in its pathogenicity. While the potential of antibodies to neutralize staphylococcal enzymes and toxins is recognized, the availability of vaccines remains limited. The majority of antibiotics utilized for the treatment of infections have already undergone evolu-

Abstract

The aim of the present study was to determine the genetic relatedness between Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from pet animals and human. In that context, 561 swabs were collected from dogs (n=238) and cats (n=323). In addition, 30 MRSA positive isolates from Qasr Elainy hospital, from different patients were also included. The collected samples were subjected for bacteriological examination for isolation of *S. aureus*. The recovered *S. aureus* isolates that were resistant to oxacillin and cefoxitin were tested for the presence of *mecA*, *mecC*, *spa*, and *nuc* genes by PCR, followed by a sequence analysis of the *mecA* gene in seven selected isolates. Out of 238 swab samples collected from dogs and 323 swab samples collected from cats, 13 (5.46%) and 8 (2.48%) isolates respectively were confirmed to be MRSA. In addition, the 30 human isolates were all categorized as MRSA. The *mecA* gene was successfully identified in all isolates from dogs, cats and human, while *spa* was harbored by 13, 4 and 30 isolates from dogs, cats and human respectively. Furthermore 13, 4 and 23 isolates. The phylogenetic analysis of the *mecA* gene sequence in seven selected MRSA isolates demonstrated a high degree of similarity among isolates originating from dogs, cats, and humans. This study confirms the zoonotic spread of *S. aureus* and suggests that dogs and cats are probably contributing to the spread of MRSA to human.

KEYWORDS MRSA, mecA, mecC, spa, nuc, Staphylococcus aureus

tionary changes resulting in resistance. However, it is noteworthy that vancomycin remains the sole antibiotic currently in use that has not yet developed resistance against multiple strains of bacteria that commonly infect patients in hospital settings (Foster, 1996; Tong *et al.*, 2015).

The mecA gene is responsible for the production of an altered form of penicillin-binding protein (PBP2a) that exhibits reduced affinity towards β-lactam drugs, resulting in the development of methicillin resistance. The emergence of methicillin resistance in strains of S. aureus has played a significant role in the progression of multidrug resistance in the field of human medicine, dating back to the early 1960s (Barber, 1961). PBP2a possesses the capability to facilitate the transpeptidation reaction that is essential for the cross-linking of peptidoglycan, thereby enabling the synthesis of the cell wall even in the presence of elevated concentrations of β-lactams. These β-lactams would otherwise impede the activity of the native PBPs. According to data from various countries, there have been reported associations between methicillin-resistant S. aureus (MRSA) infections and a range of health conditions in companion animals, with a particular focus on dogs. Otitis, pyoderma, pyogenic endocarditis, suppurative pneumonia, osteomyelitis, septic arthritis, wound infections, and urinary tract infections are some of these ailments.

The proximity of individuals and their cohabiting pets were found to elevate the likelihood of MRSA transmission between them (Bramble *et al.*, 2010), Human-pet interaction has been

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identified as a notable risk factor contributing to the colonization or transmission of MRSA (Khairullah *et al.*, 2023). Moreover, there is evidence suggesting a correlation between MRSA derived from pets and the occurrence of illnesses in pet owners, as well as the reverse scenario. This implies that MRSA colonization poses a potential health risk for both humans and animals (Gómez-Sanz *et al.*, 2013; Lozano *et al.*, 2017).

The objective of this study was to assess the potential role of companion animals (dogs and cats) in the epidemiology of MRSA zoonosis within the community. Additionally, to determine whether this phenomenon should be regarded as an emerging disease in pets, with potential implications for public health. To achieve this, the study compared the genetic makeup of *mec*A gene in MRSA strains found in companion animals and humans.

MATERIALS AND METHODS

Ethics Statement

The Institutional Animals Care and Use Committee (ARC-IA-CUC) at Agricultural research center, (No. ARC-AHRI-2335), approved the study protocols, following animal welfare guidelines.

Sampling

Between September 2021 and June 2022, a total of 561 swabs were collected from nasal, oral, ear, and wound sites during clinical assessments conducted at multiple veterinary clinics located in Cairo, Giza, and Dakahlia. These swabs were obtained from 238 dogs and 323 cats, which were categorized based on their clinical condition as either exhibiting symptoms of illness such as respiratory issues, skin infections, and ear infections, or apparently healthy (Table 1). Additionally, 30 positive MRSA isolates were obtained from human subjects at Qasr Elainy hospital. All of the swabs were received by the laboratory of the Military hospital in Cairo.

Table	1	Number	and	type	of the	collected	samples
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Species	Type of samples	Number of samples				
	Nasal swabs	59				
Dee	Oral swabs	36				
Dog	Ear swabs	69				
	Wound swabs	74				
Total Dogs		238				
	Nasal swabs	76				
Cat	Oral swabs	43				
Cat	Ear swabs	92				
	Wound swabs	112				
Total Cats	323					
Total Dogs and Cats	561					

Isolation and identification of S. aureus (Forbes et al., 2007)

All the swabs were promptly streaked onto Mannitol Salt Agar media (MSA, Oxoid Ltd, Hampshire, UK). Subsequently, they were incubated at a temperature of 37°C for a duration of 24 hours. The suspected colonies (yellow-colored colonies on MSA) were then subjected to gram staining, Gram-positive cocci that were irregularly arranged in clusters were then tested for their catalase activity, haemolytic activity on 5% sheep blood agar as well as coagulase test was performed utilizing lyophilized rabbit plasma (BD, MD, USA). The suspected colonies were then confirmed using a commercially available latex agglutination test known as Staphaurex (Remel, Lonex, Kans).

Antimicrobial susceptibility of MRSA isolates

Antimicrobial susceptibility of S. aureus was performed by using Kirby-Bauer test on Mueller-Hinton agar (Oxoid, UK). The isolated S. aureus was initially suspended in sterile Mueller-Hinton broth (Oxoid, UK) and subsequently calibrated to a 0.5 McFarland standard. Subsequently, the bacterial isolates obtained from the broth were spread onto Mueller-Hinton agar plates (Oxoid, UK) and the antimicrobial discs including, penicillin (10 U), cefoxitin (30 µg), gentamicin (30 µg), cefotaxime (30 µg), tetracycline (30 μg), erythromycin (15 μg), amoxicillin/clavulanate (10 μg), clindamycin (2 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (1.25 + 23.75 μ g), norfloxacin (10 μ g), and oxacillin (1 μ g) were placed onto the surface of the inoculated plates. Then, the plates were incubated at 37°C for a maximum duration of 24 hours. The diameter of the inhibition zone for each isolate was measured and compared to the antibiotic susceptibility breakpoints outlined by the Clinical and Laboratory Standards Institute (CLSI, 2018). The antimicrobial agents selected for this study are those that are commonly prescribed for the treatment of staphylococcal infections in both human and veterinary medicine. For phenotypic detection of MRSA, cefoxitin (30 µg) disc (Oxoid, UK) was utilized to assess its efficacy against the isolated S. aureus. According to Magiorakos et al. (2012), multidrug-resistant S. aureus (MDRSA) refers to strains of S. aureus that exhibit resistance to a minimum of one antibiotic from three or more antimicrobial classes.

Polymerase Chain Reaction (PCR) for the identification of MRSA isolates

Further investigation was conducted on the S. aureus isolates that exhibited resistance to oxacillin and cefoxitin. This investigation involved the use of PCR for detection of mecA, mecC, spa, and nuc genes. MRSA strain (ATCC #43300) was used as a control positive in PCR reaction. Subsequently, genomic DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) in accordance with the instructions provided by the manufacturer. All oxacillin resistant isolates were subjected to PCR amplification along with positive and negative control samples. The primers used in the reaction for mecA were forward (5'GTAGAAATGACTGAACGTCCGATAA-3') and reverse (5'CCAAT-TCCACATTGTTTCGGTCTAA-3') (McClure et al., 2006), while for spa were forward (5'TCAACAAAGAACAACAAAATGC-3') and reverse (5'GCTTTCGGTGCTTGAGATTC-3') (Wada et al., 2010). For mecC were forward (5' GCTCCTAATGCTAATGCA-3') and reverse (5' TA-AGCAATAATGACTACC-3') (Cuny et al., 2011), and for nuc were forward (5' ATATGTATGGCAATCGTTTCAAT-3') and reverse (5'GTA-AATGCACTTGCTTCAGGAC-3') (Gao et al., 2011). The temperature profile specified below was employed for this purpose. In this study, a thermocycler was employed to carry out a total of 35 cycles, with each cycle consisting of a 5-minute denaturation step at 94°C, followed by a 30 second annealing step at an increasing temperature ranging from 50 to 55°C, and finally a 7 to 10-minute extension step at 72°C. The visualization of the PCR product on a 1.5% agarose gel was conducted by employing ethidium bromide staining and a UV transilluminator, as described by Sambrook et al. (1989).

Alignment and phylogenetic comparison of mecA in MRSA from different sources

The purified PCR products were subjected to sequencing on an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA) using the Bigdye Terminator V 3.1 cycle sequencing kit (Perkin-Elmer/Applied Biosystems, Foster City, CA), Cat. No. 4336817, in both the forward and reverse directions. To determine the sequence identity to GenBank accessions, an initial analysis using the Basic Local Alignment Search Tool (BLAST®) (Altschul *et al.*, 1990) was conducted. The procedural guidelines provided by the manufacturer were adhered to in order to carry out the sequence reaction.

Phylogenetic analyses were conducted using three different methods: maximum likelihood, neighbor joining, and maximum parsimony. These analyses were performed using the software programs MEGA6 (Tamura *et al.*, 2013) and Pairwise, which is based in Madison, Wisconsin, USA. The purpose of these analyses was to compare the sequences. In addition, the CLUSTAL W multiple sequence alignment program and version 12.1 of the MegAlign module from the Lasergene DNAStar software were used.

RESULTS

Microbial and Antimicrobial Susceptibility Testing of the Collected Staphylococci.

The results revealed growth of yellow colonies on Mannitol Salt Agar medium as shown in Fig. 1a. While Fig. 1b shows hemolytic activity of these colonies on sheep blood agar. Gram staining revealed clusters of Gram-positive cocci that resembled grapes, while catalase and coagulase tests were both positive as shown in Fig. 1 c,d. These groups were regarded as *S. aureus*. For the phenotypic identification of MRSA, isolated *S. aureus* was tested for its susceptibility against different antibiotics including oxacillin and cefoxitin by disc diffusion method as shown in Fig. 1 e,f. Oxacillin and cefoxitin resistant isolates were phenotypically classified as MRSA. Out of 238 swab samples collected from dogs, 71 (29.8%) *S. aureus* isolates were identified of them, 13 (5.46%) isolates were confirmed to be MRSA. While, out of 323 swab samples collected from cats, 52 (16%) *S. aureus* isolates were identified, of them 8 isolates were resistance to oxacillin and confirmed as MRSA. In addition, the 30 human isolates were all oxacillin resistant and categorized as MRSA (Table 2).

Antimicrobial susceptibility testing results

All MRSA isolates were found to be MDR as illustrated in Table 3. The highest resistance was observed against oxacillin, cefoxitin, penicillin and norfloxacin (100%) followed by amoxicillin/clavulanate and tetracycline (88.3% and 84.3% respectively). Resistance against erythromycin was detected in 82.3% of MRSA isolates. While the lowest resistance was shown against cefotaxime and ciprofloxacin (9.8% and 17.7% respectively).

Confirmation of MRSA by PCR targeting selected genes.

In order to identify the presence of *mecA*, *mecC*, *spa*, and *nuc* genes, a PCR assay was performed on *S. aureus* bacteria that exhibited phenotypic resistance to oxacillin and cefoxitin. The findings are presented in Table 4. The *mecA* gene was successfully identified in 13 (100%), 8 (100%) and 30 (100%) isolates from dogs, cats and human respectively, while *spa* was harbored by 13 (100%), 4 (50%) and 30 (100%), 4 (50%) and 23 (76.6%) isolates from dogs, cats and human were positive for the *nuc* gene. While *mecC* was not identified from all examined isolates as shown in Fig. 2.

The present study employed phylogenetic analysis to examine the evolutionary relationships between MRSA from different species. As depicted in the phylogenetic tree presented in Fig. 3 and 4, the examination of the *mecA* gene sequence in the seven selected isolates demonstrated a high degree of similarity

Table 2. Prevalence of MRSA in the samples.

Species (No.)	Number of S. aureus	S. aureus %	Number of MRSA	MRSA%
Dog (238)	71	29.8	13	5.46
Cat (323)	52	16	8	2.48
Human (30)	30	N/A	30	N/A

Table 3. Antibiotic Sensitivity of MRSA isolates.

T1-4	A	Dias Dataman	Number of isolates %								
	Antimicrobiais	Disc Potency -	R	Ι	S						
	Penicillin	10 U	51(100)	0(0)	0(0)						
	Amoxicillin/clavulanate	10 µg	45(88.3)	6(11.7)	0(0)						
	Oxacillin	1 µg	51(100)	0(0)	0(0)						
	Erythromycin	15 µg	42(82.3)	9(17.7)	0(0)						
	Cefoxitin	30 µg	51(100)	0(0)	0(0)						
	Tetracycline	30 µg	43(84.3)	8(15.7)	0(0)						
MKSA (n=51)	Cefotaxime	30 µg	5(9.8)	41(80.4)	5(9.8)						
	Norfloxacin	10 µg	51(100)	0(0)	0(0)						
	Clindamycin	2 µg	14(27.4)	0(0)	37(72.6)						
	Trimethoprim/sulfamethoxazole	25 µg	26(50.9)	12(23.5)	13(25.4)						
	Gentamicin	30 µg	17(33.3)	6(11.8)	28(54.9)						
	Ciprofloxacin	5 µg	9(17.7)	5(9.8)	37(72.6)						

among isolates originating from dogs, cats, and humans. Specifically, there was a 100% similarity observed between isolates of the same species, while isolates from different species and humans exhibited a 99% similarity. The sequences generated for this study were submitted to the GenBank database and assigned accession numbers, as indicated in Table 5.

DISCUSSION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a substantial worldwide public health issue in both human and veterinary medicine. *S. aureus* is Gram-positive bacteria that still infect both people and animals, especially in individuals who are hospitalized, resulting in skin and soft tissue



Fig. 1. Bacteriological Identification and Antimicrobial Susceptibility of Positive Staphylococci. A) Shows growth of *S. aureus* on mannitol salt agar medium (yellow). B) Shows hemolytic activity of *S. aureus* on sheep blood agar. C) Shows catalase test among the examined *S. aureus* isolates. D) Shows coagulase test among the examined *S. aureus* isolates. E) Shows susceptibility of *S. aureus* isolates against different antibiotics including oxacillin and cefoxitin by disc diffusion method.



Fig. 2. Confirmation of MRSA by PCR targeting selected genes. A) Agar gel electrophoresis for identification of *mec*A gene: lane L: DNA marker, lane P: positive control, lane N: negative control and lanes 1-10: MRSA isolates. B) Agar gel electrophoresis for identification of *mec*C gene: lane L: DNA marker, lane P: positive control, lane N: negative control and lanes 1-10: MRSA isolates. C) Agar gel electrophoresis for identification of *spa* gene: lane L: DNA marker, lane P: positive control, lane N: negative control and lanes 1-10: MRSA isolates. D) Agar gel electrophoresis for identification of *nuc* gene: lane L: DNA marker, lane P: positive control, lane N: negative control and lanes 1-10: MRSA isolates. D) Agar gel electrophoresis for identification of *nuc* gene: lane L: DNA marker, lane P: positive control, lane N: negative control and lanes 1-10: MRSA isolates. D) Agar gel electrophoresis for identification of *nuc* gene: lane L: DNA marker, lane P: positive control, lane N: negative control and lanes 1-10: MRSA isolates. D) Agar gel electrophoresis for identification of *nuc* gene: lane L: DNA marker, lane P: positive control, lane N: negative control and lanes 1-10: MRSA isolates.

Table 4. Confirmation of MRSA by PCR targeting selected genes.

		+ve Re	esults	
Isolate Origin (No.)	mecA	spa	mecC	пис
Dog (13)	13 (100%)	13 (100%)	0 (0%)	13 (100%)
Cat (8)	8 (100%)	4 (50%)	0 (0%)	4 (50%)
Human (30)	30 (100%)	30 (100%)	0 (0%)	23 (76.6%)

infections (Joshi *et al.*, 2011). Researchers have postulated that canines and felines may function as reservoirs for MRSA transmission to humans, thus establishing MRSA as a noteworthy zoonotic pathogen. In this study, a total of 561 swabs were procured from canines and felines during clinical evaluations conducted at various veterinary clinics in Egypt. These animals were selected based on their clinical condition, which encompassed both those exhibiting signs of illness and apparently healthy. Additionally, 30 MRSA isolates were obtained from humans at Qasr Elainy Hospital. The identification of *S. aureus* and the determination of the antibiotic profile of MRSA isolates were conducted at the Military

Hospital in Cairo. All *S. aureus* isolates that exhibited resistance to oxacillin and cefoxitin underwent PCR to detect the presence of *mecA*, *mecC*, *spa*, and *nuc* genes. Afterwards, the sequences of the *mecA* gene were analyzed. The findings are in line with the occurrence of *S. aureus* zoonotic infections and indicate that these domesticated animals are likely playing a role in the dissemination of MRSA zoonotic infections within the local community. Based on the present findings, which align with the research conducted by El-Jakee *et al.* (2013), it can be concluded that there is complete similarity among isolates obtained from dogs, cats, and humans.

Table 5. Accession number and data base of the isolates in the study.

Accession number	Host	Type of Sample	Date of Isolation
OP651951	Human	Wound	May-22
OP651952	Human	Blood	Mar-22
OP651953	Human	Wound	Jun-22
OP651954	Dog	Wound	Oct-21
OP651954	Dog	Wound	Feb-22
OP651956	Cat	Wound	Dec-21
OP651957	Cat	Wound	Apr-22



Fig. 3. Genetic tree interpretation based on mecA gene sequences used for methicillin-resistant Staphylococcus aureus genetic changes according to host and regions.

_			_		_	_			_	_	_	Per	cent Id	entity	_	_	_								_	_	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		
1		99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.4	99.4	99.4	100.0	99.0	99.0	99.0	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.0	1	LR027873 S. aureus BPH2070
2	0.3		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.7	99.7	99.4	99.4	99.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.4	2	AP019545 S. aureus KG-22
3	0.3	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.7	99.7	99.4	99.4	99.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.4	3	AP019542 S. aureus KG-03
4	0.3	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.7	99.7	99.4	99.4	99.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.4	4	CP031537 S. aureus WCH-SK2
5	0.3	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	99.7	99.7	99.7	99.7	99.4	99.4	99.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.4	5	MF185206 S. aureus IS431
6	0.3	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	99.7	99.7	99.7	99.7	99.4	99.4	99.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.4	6	CP019945 S. aureus BA01611
7	0.3	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	99.7	99.7	99.7	99.7	99.4	99.4	99.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.4	7	KX232516 S. aureus 5sau489
8	0.3	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	99.7	99.7	99.7	99.7	99.4	99.4	99.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.4	8	CP014441 S. aureus USA300-SU
9	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	99.7	99.7	99.7	99.7	99.4	99.4	99.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.4	9	AP017377 S. aureus OC8
10	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		99.7	99.7	99.7	99.7	99.4	99.4	99.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.4	10	AP017320 S. aureus MI
11	0.7	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3		99.7	99.4	99.4	99.0	99.4	99.0	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.0	11	MH798864 S. aureus MRSA 4154
12	0.7	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3		99.4	99.4	99.0	99.7	99.0	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.0	12	FN433596 S. aureus TW20
13	0.7	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.7	0.7		99.4	99.0	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.0	13	EF692631 S. aureus CH10
14	0.0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.7	0.7	0.7		99.0	99.0	99.0	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.0	14	EF692630 S. aureus K704540
15	1.0	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	1.0	1.0	1.0	1.0		98.7	98.7	99.4	99.4	99.4	99.4	99.4	99.4	99.4	98.7	15	MK034955 S. aureus Ht1
16	1.0	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.3	0.3	1.0	1.3		99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	98.7	16	JQ582124 S. aureus 4977
17	1.0	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	1.0	1.0	0.3	1.0	1.3	0.7		99.4	99.4	99.4	99.4	99.4	99.4	99.4	98.7	17	LR822060 S. aureus P3.1
18	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.3	0.7	0.7	0.7		100.0	100.0	100.0	100.0	100.0	100.0	99.4	18	OP651951 S. aureus EgM1
19	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.3	0.7	0.7	0.7	0.0		100.0	100.0	100.0	100.0	100.0	99.4	19	OP651952 S. aureus EgM2
20	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.3	0.7	0.7	0.7	0.0	0.0		100.0	100.0	100.0	100.0	99.4	20	OP651953 S. aureus EgM3
21	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.3	0.7	0.7	0.7	0.0	0.0	0.0		100.0	100.0	100.0	99.4	21	OP651954 S. aureus EgM4
22	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.3	0.7	0.7	0.7	0.0	0.0	0.0	0.0		100.0	100.0	99.4	22	OP651955 S. aureus EgM5
23	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.3	0.7	0.7	0.7	0.0	0.0	0.0	0.0	0.0		100.0	99.4	23	OP651956 S. aureus EgM6
24	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.3	0.7	0.7	0.7	0.0	0.0	0.0	0.0	0.0	0.0		99.4	24	OP651957 S. aureus EgM7
25	1.0	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	1.0	1.0	1.0	1.0	1.3	1.3	1.3	0.7	0.7	0.7	0.7	0.7	0.7	0.7		25	NG_047941 S. capitis CSLA5
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		

Fig. 4. Identity and diversity between isolates.

The similarity percentages between isolates of the same species were observed to be 100%, while the similarity percentages between isolates of different species and humans were found to be 99%.

S. aureus, specifically MRSA frequently exhibits diverse patterns of antibiotic resistance. *S. aureus*, a significant pathogen associated with both healthcare facilities and the general population, is responsible for a diverse array of infectious diseases (El-Jakee *et al.*, 2013).

According to Hasmukharay *et al.* (2023), out of the total sample size of 275 individuals diagnosed with MRSA bacteremia, 139 individuals, accounting for 50.5% of the sample, were found to be below the age of 65. According to the aforementioned study, it was determined that elderly individuals exhibited a threefold higher likelihood of mortality due to MRSA bacteremia in comparison to their younger counterparts. Moreover, El-Jakee *et al.* (2013) assert that *S. aureus* food poisoning holds considerable economic significance as a prevalent food-borne illness on a global scale.

In our study, a prevalence of approximately 5.5% was observed among canine samples, with 13 positive MRSA results. While 8 MRSA isolates were attributed to cats, resulting in a prevalence of 2.5%. Unfortunately, due to the unavailability of suitable samples, it was not possible to estimate the incidence of humans in this study. The prevalence observed in this study is comparatively lower than the prevalence reported by El-Jakee *et al.* (2008) which illustrated within the course of the investigation, the prevalence of staphylococci among a total of 409 samples in both diseased humans and animals among. Human samples had the greatest staphylococci isolation rate (36%) followed by samples from dogs (28%) cattle (24.8%), rations (14.7%), and chicken (12%).

In our study, all MRSA isolates were found to be MDR. The highest resistance was observed against oxacillin, cefoxitin, penicillin and norfloxacin (100%) followed by amoxicillin/clavulanate and tetracycline (88.3% and 84.3% respectively). Resistance against erythromycin was detected in 82.3% of MRSA isolates. While the lowest resistance was shown against cefotaxime and ciprofloxacin (9.8% and 17.7% respectively). In a study conducted by El-Jakee et al. (2010), it was observed that the S. aureus isolates exhibited a high level of resistance to methicillin, followed by oxytetracycline, ampicillin, and sulfamethoxazole-trimethoprim. Additionally, the isolates displayed varying degrees of resistance to amoxicillin, ofloxacin, clindamycin, and erythromycin. Conversely, a significant proportion of the examined isolates, specifically 95%, demonstrated sensitivity to vancomycin, while 85% exhibited sensitivity to cefotaxime. Furthermore, 80% of the isolates were found to be sensitive to amoxicillin, clavulanic acid, and cefoperazone. According to Mahran et al. (2020), all S. aureus isolates obtained from samples of both human and camel origin exhibited susceptibility to vancomycin. Additionally, it was observed that all Staphylococcus aureus isolates derived from human abscesses were identified as methicillin-resistant S. aureus (MRSA). El-Jakee et al. (2011) reported that a significant majority (95%) of the S. aureus strains obtained from bovine and human samples exhibited susceptibility to vancomycin. However, it is worth noting that one isolate from a human source demonstrated intermediate resistance.

In this study, PCR assay was employed to detect the presence of the *mecA*, *spa*, *mecC*, and *nuc* genes in all *S. aureus* strains that exhibited phenotypic resistance to oxacillin and cefoxitin. The findings indicated that every MRSA isolate harbored the *mecA* gene which is responsible for the methicillin resistance in staphylococci.

Regarding *nuc* gene in our study, 13 (100%), 4 (50%) and 23 (76,6%) isolates from dogs, cats and human respectively were positive for the *nuc* gene. Despite the identification of *S. aureus* can be achieved through the search for the *nuc* gene, the MRSA isolate exhibited a partial deletion of the *nuc* gene, resulting in a false-negative result for both PCR and MRSA testing. According to Hoegh *et al.* (2014), there was a potential diagnostic issue

identified in *S. aureus* isolates that exhibited negative results in PCR tests targeting the *nuc* gene. The inadequacy of relying solely on the identification of *nuc* genes may be attributed to the presence of certain inconsistencies between the results obtained from phenotypic and genotypic methods in detecting *S. aureus* strains (Sahebnasagh *et al.*, 2014). The presence of the *nuc* gene has been observed in a significant proportion of *S. aureus* isolates, although there have been reports of isolates lacking this gene as well (Van Leeuwen *et al.*, 2008; Xu *et al.*, 2015). Moreover, it has been observed that the *nuc* gene is found in both coagulase-positive (CPS) and coagulase-negative (CNS) strains of various *Staphylococcus* species, as reported by Andrade *et al.* (2021).

In the study conducted by Mahran *et al.* (2020), it was observed that all strains of *S. aureus* examined exhibited amplification of the *nuc* gene, which spans 279 base pairs.

Additionally, the examination of the *mec*A gene sequence of the seven chosen isolates and reference isolates from a gene bank through phylogenetic analysis demonstrated that all the isolates being investigated are indistinguishable, as evidenced by the phylogenetic tree. The DNA products of this gene, which were amplified using PCR exhibited concordance with the findings reported by Mahran *et al.* (2020). This finding provides insight into the potential involvement of these domesticated animals in the transmission of MRSA zoonosis within the community in agreement with the study conducted by Khairullah *et al.* (2023) which revealed that cats and dogs harboring MRSA clones and exhibited characteristics that were closely related to those found in nearby human populations.

CONCLUSION

This study presents significant findings regarding the prevailing antibiotic resistance, specifically methicillin resistance, among a collection of *S. aureus* isolates obtained from samples of both pets and humans in Egypt. The study confirmed that the selected isolates of MRSA from different species animal (dog and cat) also MRSA positive confirmed isolates from human are highly identical that may be role of these pet animals in the epidemiology of MRSA zoonosis in the community.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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