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Enterobacterial Repetitive Intergenic Consensus- PCR of Methicillin-resistant *Staphylococcus aureus* Isolated from Pet Animals

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the potential pathogens in pet animals and has public health hazards worldwide. This project aimed to investigate the frequency of MRSA in pet animals and to assess the antimicrobial susceptibility of the recovered strains as well as to determine the isolates genetic relatedness using Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR. Out of the 270 swab (nostril, mouth, and abscess swabs) samples collected from veterinary clinics and shelters in Mansoura city, Egypt, 64 (23.7%) *S. aureus* isolates (49/224; 21.8 % from cats and 15/46; 32.6% from dogs) were identified. Among them, 40.6% (26 /64) were confirmed to be MRSA and 59.4% (38/64) were identified as MSSA. Antimicrobial susceptibility test results showed the highest resistance rates of MRSA isolates to penicillin (100%), oxacillin (100%), followed by amoxicillin-clavulanic acid (92%), cefotaxime (92.4%), kanamycin, streptomycin, tetracycline (84.6%; 22/26 each), and lower resistance to vancomycin (38.5%). Furthermore, MSSA isolates showed moderate resistance to amoxicillin-clavulanic acid (52.6%), followed by sulfamethoxazole trimethoprime (26.3%). Multi drug resistant (MDR) was found in all MRSA isolates (100%; 26/26) and the most identified antimicrobial resistance patterns was P, AMC, OX, CTX, TE, S, K. A total of 26 identified MRSA strains were divided into 22 ERIC-PCR groupings (A-V) that were categorized into two clusters, ERIC cluster I and ERIC cluster II. Among them, the most common ERIC type (11.5%) was ERIC A. The significance of multidrug resistance MRSA to public health needs continuous testing of antimicrobial medications against MRSA isolates. Also, ERIC PCR demonstrate promising typing that might be conveniently employed on a regular basis to study the genotypic alterations of MRSA, particularly in pets.

KEYWORDS
Methicillin-resistant *Staphylococcus aureus*, MRSA, ERIC PCR

INTRODUCTION

There are numerous host animals, including as cats, dogs, pigs, cattle, poultry, and horses, can become colonized by and infected with *S. aureus*, including MRSA. With animals serving as a possible source for the generation of novel MRSA clones in humans, this has zoonotic effects in addition to veterinary significance (Cuny *et al*., 2010). The reports of human infections and the colonization of companion animals have shown that animals have the potential to function as a source of MRSA transmission. MRSA is gaining more attention in the community, and surveillance is advised, including rates of transmission in healthy cats and dogs (Duquette and Nuttall *et al*., 2004).

Methicillin resistance in *S. aureus* is mediated by PBP2a, a penicillin-binding protein with low affinity for β-lactam antibiotics. It is encoded by the *mec*A gene, which is found on the staphylococcal chromosomal cassette *mec* (SCCmec), a large mobile genetic element. At least five different forms of SCCmec have been described in up to this day (Strommenger *et al*., 2006). Additionally, MRSA has been discovered wildlife, pest animals, and companion animals (Abdullahi *et al*., 2021; Pletinckx, *et al*., 2013). In general, these species' MRSA strains are different from livestock and production animal strains (Haag *et al*., 2019). Numerous studies revealed that the majority of MRSA strains seen in companion animals are of human origin and spread through close contact between animal owners and their pets (Van Duijkeren *et al*., 2004; Baptiste *et al*., 2005). These strains can also be passed on to people. However, there are few studies on the incidence and features of MRSA infection in non-farm animals in Arab nations (Tarazi *et al*., 2015; Elmoslemany *et al*., 2021). MRSA infection rates in pets ranged from 5.3% to 25% (Tarazi *et al*., 2015; Elnageh *et al*., 2021)

Staphylococci isolated from cats and dogs show resistance patterns that are comparable to those of human staphylococci, as shown by studies on antibacterial susceptibility in companion animals, and the frequency with which certain antibiotics are used has an impact on the development of resistance (Malik *et al*., 2005). Particularly since the *mec*A gene was discovered to be in charge of beta-lactam antibiotic resistance, including methicillin, penicillin, oxacillin, and cefoxitin. This was confirmed to be the case with methicillin-resistant *S. aureus*. MRSA is a severe public health concern because it cannot be treated effectively with several medications where antibiotic beta lactam activity is rare (Lee *et al*., 2016). Furthermore, substantial discrepancies in MRSA identification at the phenotypic and genotypic levels pose an additional risk toward pathogenesis of this bacteria (Aqib *et*

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al., 2018).

Tracking the spread of microorganisms requires accurate identification and typing. The "gold standard" for MRSA typing is PFGE analysis of genomic macro-restriction fragments. (Murchan *et al*., 2003), Due to their reducability across laboratories, DNA sequence-based methods gained increased appeal (Harmsen *et al*., 2003). Single-locus DNA sequencing of repeat areas of the coa and spa genes was one of the approaches used. Spa typing is based on the sequence of the protein A gene's polymorphic X region, which is found in all strains of *S. aureus* (Hallin *et al*., 2009). Additionally, PCR-based techniques like multi-locus sequence typing (MLST) and enterobacterial repetitive intergenic consensus (ERIC-PCR) are commonly employed. The two methods are frequently employed since they are both easy and very simple, ERIC-PCR has the extra benefit of being relatively cost effective (Vázquez-Sánchez *et al*., 2012). In the ERIC-PCR method, a DNA fingerprint is produced by amplifying repeated DNA fragments using a single primer. This strategy enables the monitoring of the propagation of certain strains, could speed up the analysis of transmission, and aid in infection prevention efforts (Güler *et al*., 2011). Internal sequence snippets from seven housekeeping genes are typically used in MLST to characterize bacterial strains, which is based on single nucleotide variation (Maiden *et al*., 1998; Enright *et al*., 2000). Because of the fluctuating expression of the appropriate molecular marker, phenotypic investigations are unreliable, although molecular typing technologies such as enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) (Ye *et al*., 2012) are suitable and sensitive in detecting harmful pathogens origin.

To date, no dedicated research has concentrated on genetic characterizationof MRSA isolated from pet animals by ERIC-PCR in Egypt. Therefore, the goals of our research were to detect prevalence of methicillin resistant *S. aureus*, antibiotic resistance patterns, and characterize MRSA by ERIC-PCR and determine the possible relationship between ERIC-PCR fingerprinting and patterns of antibiotic resistance.

MATERIALS AND METHODS

Ethical approval

This study was ethically approved by Research Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt (Code No: M/108).

Sample collection and preparation

Two hundred and seventy swab samples involving; 68 nostrils, 178 mouth, and 24 abscess swabs; from cats (n=224) and dogs (n=46) were obtained from various veterinary clinic and shelters of pet animals in Mansoura city, Egypt during the period from September 2021 to March 2022. Animals, that were seen as outpatients or kenneled, were sampled regardless of the condition they were suffering from or associated medication. None of the animals were being treated for confirmed MRSA infections. Specimens were collected from the subjects in the following manner: a sterile moistened swab was inserted into each nostril or in mouth in turn to a depth of approximately 1 cm and rolled over the sample area (for at least 5 s in dogs and 2 s in cats). All samples were placed in sterile bags, closed tightly, labeled appropriately, and immediately transported in an insulated ice box to the laboratory without delay for bacteriological examination.

Bacteriological analysis

Each swab sample was immersed in 10 mL of Tryptone Soya Broth (TSB, Oxoid, UK) containing 10% sodium chloride and incubated at 37°C for 48 h for staphylococci selective enrichment. A loop of the incubated broth was then streaked onto the surface of Baird Parker media (Oxoid, UK) for isolation of total staphylococci. Typical jet black colonies surrounded by a clear halo zone were selected from the plates and purified ontoTrypticase Soya Agar (TSA, Oxoid, UK). Presumptive staphylococcal colonies had been subjected to Gram staining and standard biochemical assays, such as catalase, oxidase, coagulase, and DNase tests (Boerlin *et al*., 2003; De Freitas Guimarães *et al*., 2013). All isolates were kept in 30% glycerol solution at -20°C for further examination.

Antimicrobial susceptibility testing

Isolates of *S. aureus* were investigated for their antibiotic susceptibility to ten antimicrobial agents using disc diffusion method on Mueller-Hinton agar (Oxoid) in accordance with guidelines of Clinical and Laboratory Standards (CLSI 2014). The following antimicrobial agents (Oxoid) were tested: β-lactams (amoxicillin-clavulonic acid 30µg, oxacillin 15µg and penicillin 10IU), cephalosporines (cefotaxime 30µg), aminoglycosides (streptomycin 10µg and kanamycin 30 µg), fluoroquinolones (ciprofloxacin 5µg), tetracycline (tetracycline 30 µg), glycopeptide (vancomycin 30µg), and sulfonamides (sulfamethoxazole-trimethoprim 25µg). The outcomes were interpreted as susceptible, intermediate, or resistant according to the breakpoints of CLSI (2018). The isolated organisms showed resistance to three or more antimicrobial classes were recorded as multiple antimicrobial resistant (MAR) strains (Waters *et al*., 2011). A multi-antimicrobial resistance index (MARI) was determined by dividing each isolate's total number of resistances to antimicrobials by the total number of antimicrobials tested (Krumperman *et al*., 1983).

Molecular assays

The DNA template was obtained from overnight culture of TSB (Oxoid, UK) by boiling bacterial colonies suspended in sterile water for 20 min (Zhang *et al*. 2005). PCR-amplification of the *nuc*

Table 1. Oligonucleotide primers sequence and PCR cycling conditions for PCRs.

Target gene	Primer direction and sequence	Extent of amplified product	Reference
nuc	F: GTGCTGGCATATGTATGGCAATTG R: CTGAATCAGCGTTGTCTTCGCTCCAA	270 bp	Sallam *et al*. (2015)
*fem*A	F: AAA AAA GCA CAT AAC AAG CG R: GAT AAA GAA GAA ACG AGC AGA	132 bp	Teixeira *et al.* (2014)
*mec*A	F: AAAATCGATGGTAAAGGTTGGC R: AGTTCTGGAGTACCGGATTTGC	533bp	Pournajaf *et al*. (2014)
ERIC	ERIC1R: ATG TAA GCT CCT GGG GAT TCA C ERIC2: AAG TAA GTG ACT GGG GTG AGC G		Arslan *et al*. (2016)

gene (*S. aureus* species-specific determinants), *mec*A, and *fem*A genes (methicillin resistance determinants) were performed in Applied Biosystem, 2720 Thermal Cycler (USA). Primer sequences and PCR conditions were summarized in Table 1. The reaction mixture (25 µL) contained 12.5 µL 2X PCR master mix (Promega, Madison, USA), 1µL 20-pmol of each primer (Metabion, Germany), 5µL template DNA, and 5.5 µL PCR grade water. PCR products were analyzed using agarose gel electrophoresis on 1.5% agarose gel stained with ethidium bromide and then visualized using Gel Documentation (cleaver scientific ltd UV gel documentation system, USA). Identified *S. aureus* strain obtained from Faculty of Veterinary Medicine, Mansoura was utilized as positive control and nuclease free water as negative control.

ERIC-PCR Fingerprinting

Genotyping of MRSA strains was studied utilizing the Enterobacteria Repetitive Intergenic Consensus (ERIC) Polymerase Chain Reaction (PCR) fingerprinting assay in accordance with Arslan *et al.* (2016). Briefly, PCR reactions were prepared in a total volume of 25 µL, 12.5 µL of 2× PCR master mix (Promega, Madison, USA), 1 µL of each primer (Metabion, Germany), 4.5 µL PCR-grade water, and 6 µL DNA template were combined. PCR conditions were applied as the following: initial denaturation at 95°C for 7 min followed by 30 cycles of denaturation 94°C/1 min, annealing 52°C/1 min, extension at 65°C/8 min, and a final extension at 65°C/16 min. The amplification products were separated by gel electrophoresis in 2% agarose gels, stained with ethidium bromide, and photographed on a UV transilluminator. The PCR patterns were visually compared, and each pattern was assigned a binary value (either 0 or 1) indicating individual band's absence or presence. The dendrogram was created using the unweighted pair group approach with arithmetic average (UPGMA) and Ward's hierarchical clustering routine. Cluster analysis and dendrogram construction were presented with SPSS, version 22 (IBM 2013) (Hunter, 1990). Similarity index (Jaccard/ Tanimoto Coefficient and number of intersecting elements) among all samples was analyzed by the online tool (https://planetcalc.com/1664/).

Statistical analysis

All data collected was entered into a Microsoft Excel spreadsheet and analyzed using the Statistical Package for Social Sciences, version 16. To determine the prevalence, descriptive statistics such as percentages and frequency distribution have been used.

RESULTS

Methicillin-resistant S. aureus (MRSA) prevalence in pets

Out of the 270 swab samples, 64 (23.7%) isolates (49/224; 21.8 % from cats and 15/46; 32.6% from dogs) were detected as *S. aureus* based on growth pattern and gray to jet-black colonies surrounded by clear zone formation on Baird Parker medium (Table 1). *S. aureus* isolates include 30 (65.2%) isolates from mouth, 20 (43.5%) isolates from nostril and 14 (31.2%) isolates from abscess have been recovered. By gram staining, *S. aureus* appeared as Gram- positive small round cocci and most commonly as grape-like clusters. Biochemically, *S. aureus* showed positive results with catalase, coagulase and oxidase tests. All biochemically identified *S. aureus* isolates were confirmed by PCR targeting *nuc* gene which was identified in all suspected isolates yielding 270 bp PCR products (Figure 1) Remarkably, MRSA has been identified in 9.6% (26 /270) of the total examined samples based on amplification of *mec*A gene and *fem*A from the isolated *S. aureus* by PCR (Figure 2, 3), while other isolates of *S. aureus* were considered as MSSA (n=38; 14.07%). Out of the 26 confirmed MRSA isolates, 20 (40,8%) were isolated from cats and 6 (40%) from dogs.

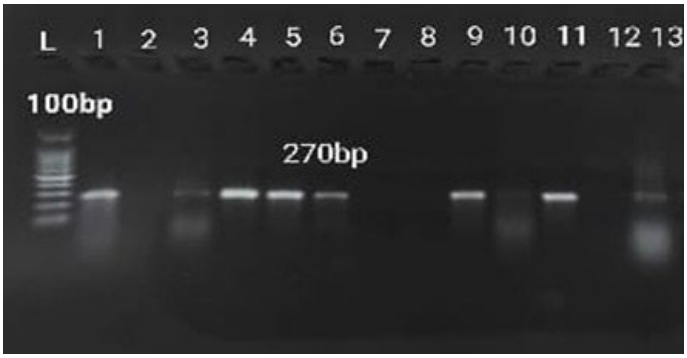

Fig. 1. Agarose gel electrophoresis showing amplification of *nuc* gene (Lane 4,5, 6 ,9,11 positive at 270 bp. lane 1 positive control).

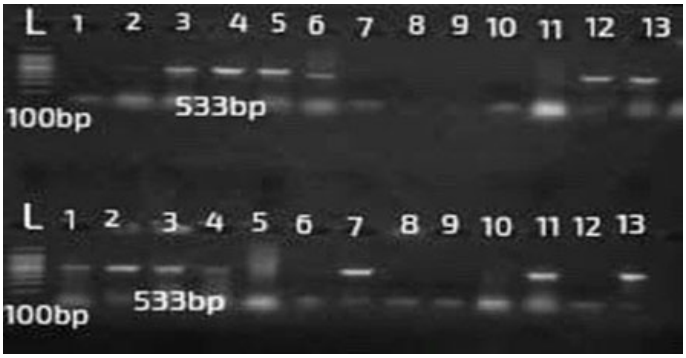

Fig. 2. Agarose gel electrophoresis showing amplification of *mec*Agene (positive at 533 bp. Lane 13 control positive).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test was performed on all *S. aureus* isolates (n=64) identified as MRSA (n=26) and MSSA (n=38) in this study. Variable resistance patterns were displayed against the used antibiotics (Table 3). MRSA isolates showed an absolute resistance to penicillin (100%; 26/26), oxacillin (100%; 26/26), followed by amoxicillin-clavulanic acid (92%; 24/26), cefotaxime (92.4%), kanamycin, streptomycin, tetracycline (84.6%;22/26 each), ciprofloxacin (76.92%; 20/26), and sulfamethoxazole-trimethoprim (69.23%; 18/26) and lower resistance to vancomycin (38.46%; 10/26). Moreover, MSSA isolates showed moderate

Table 2. Prevalence of MRSA and other staphylococcal species in pet animals.

Animals	*S. aureus*		Other staphylococcal species	Total staphylococcal species
	MRSA	MSSA		
Cats (n=224)	20 (8.9%)	29 (12.9%)	73(32.5%)	122 (54.46%)
Dogs (n=46)	6 (13.04%)	9(19.5%)	11(23.9%)	26 (56.5%)
Total (n =270)	26 (9.6%)	38 (14.07%)	84(31.3%)	148 (54.8 %)

resistance to amoxicillin-clavulanic acid (52.6%; 20/38) followed by sulfamethoxazole trimethoprime (26.3%; 10/38), ciprofloxacin, tetracycline (21.1%; 8/38). The majority of the MSSA isolates in the study were susceptible to vancomycin (84.6%; 32/38), streptomycin (73.7%; 28/38) and cefotaxime (84.6%; 32/38). Multi drug resistant (MDR) was found in all MRSA isolates (100%; 26/26) and the most identified antimicrobial resistance patterns was P, AMC, OX, CTX, TE, S, K (Table 4). Furthermore, antibiotypes identified in this study had MAR index values ranged from 0.6 to 1.0 (Table 4).

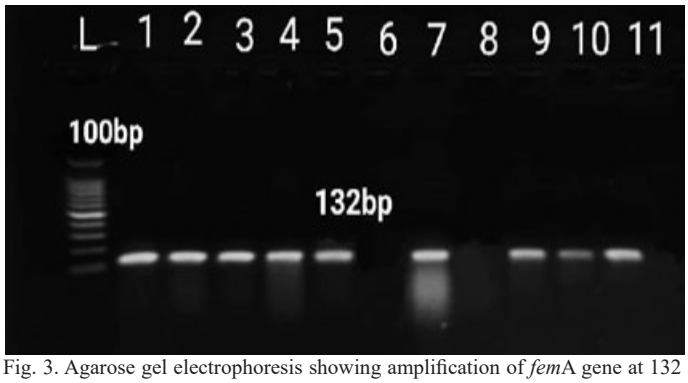

Fig. 3. Agarose gel electrophoresis showing amplification of *fem*A gene at 132 bp. Lane 1 control positive, lane 2,3,4,5,7,9,10,11 positive samples.

ERIC Typing

The MRSA isolates collection (n= 26) were genotyped by ERIC-PCR molecular typing. The amplified DNA fragments in the acquired profiles (13 bands) size ranged from 100 to 900 bp. These Band Pattern information (Figures 4, 5) indicate that, shows the ERIC-PCR dendrogram. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was used to create this image,

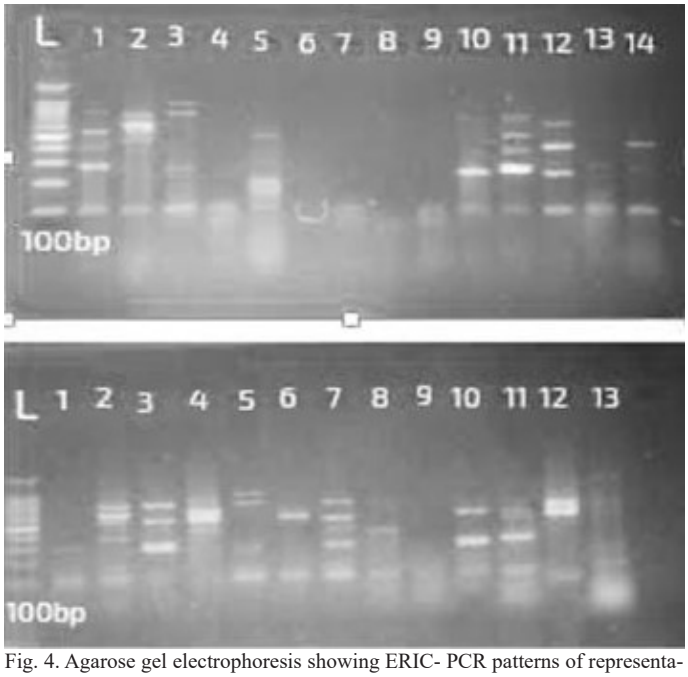

Fig. 4. Agarose gel electrophoresis showing ERIC- PCR patterns of representative *S. aureus* isolates.

Table 3. Antimicrobial susceptibility testing of MSSA and MRSA.

Antibiotics	Antimicrobial Classes	MSSA			MRSA		
		Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive
Penicillin	β -Lactam	13 (34.2%)	5 (13.15%)	20 (52.6%)	26 (100%)	0	0
Amoxicillin-clavulanic acid	β -Lactam	20 (52.6%)	4 (10.52%)	14 (36.8%)	24 (92.4%)	0	2 (7.6%)
Oxacillin	β -Lactam	0	0	38 (100%)	26 (100%)	0	0
Vancomycin	Glycopeptide	6 (15.8%)	0	32 (84.6%)	10 (38.5%)	0	16 (61.5%)
Kanamycin	Aminoglycosides	5 (13.15%)	18 (47.4%)	15 (39.4%)	22 (84.6%)	4 (15.4%)	0
Streptomycin	Aminoglycosides	6 (15.8%)	4 (10.52%)	28 (73.7%)	22 (84.6%)	0	4 (15.4%)
Ciproflaxacin	Quinolones	8 (21.1%)	12 (31.6%)	18 (47.4%)	20 (76.9%)	0	6 (23.1%)
Tetracycline	Quinolones	8 (21.1%)	4 (10.5%)	26 (68.4%)	22 (84.6%)	2 (7.6%)	2 (7.6%)
Sulfamethoxazole Trimethoprim	Potentiated Sulfonamides	10 (26.3%)	0	28 (73.7%)	18 (69.2%)	8 (30.8%)	0
Cefotaxime	Cephalosporin	0	6 (15.8%)	32 (84.6%)	24 (92.4%)	2 (7.7%)	0

Table 4. Antimicrobial resistance profiles of MRSA (n=26) isolated from cats and dogs.

ID	Antibiotic resistance pattern	Noof isolates	Percentage of resistant isolates (%)	No of resistance antibiotic	MARI
1	K, CTX, OX, P, AMC, CIP	2	7.6	6	0.6
2	K, OX, P, S, T.E, AMC, CIP	2	7.6	7	0.7
3	CTX, OX, P, S, T.E, AMC	2	7.6	7	0.7
4	CTX, OX, SXT, P, S, AMC, CIP	2	7.6	7	0.7
5	K, CTX, OX, SXT, P, S, T.E, AMC	4	15.3	8	0.8
6	K, CTX, OX, P, S, T.E, AMC, AMC	2	7.6	8	0.8
7	K, CTX, OX, P, VA, SXT, T.E, AMC, CIP	2	7.6	9	0.9
8	K, CTX, OX, SXT, P, S, T.E, AMC, CIP	2	7.6	9	0.9
9	K, CTX, OX, VA, SXT, P, S, T.E, AMC, CIP	10	30.7	10	1

P: Pencillin; k: Kanamycin, AMC: Amoxicillin-clavulanic acid, OX: Oxacillin, SXT: Sulfamethoxazole Trimethoprim; CIP: Ciproflaxacin; TE : tetracycline; VA: Vancomycin; CTX: Cefotaxime; S: Sterptomycin .

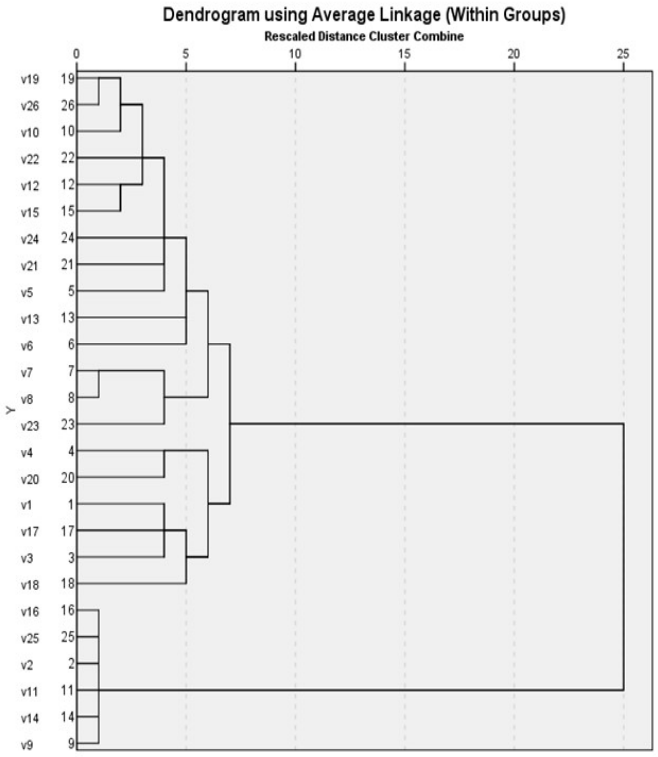

Fig. 5. Dendrogram shows relatedness between ERIC -PCR band patterns of 26 Methicillin-resistant *S. aureus* strains isolated from pet animals.

which was then displayed with Figtree demonstrated that MRSA isolates obtained from pet animals were genetically diverse and heterogeneous. ERIC PCR pattern generated 22 distinct patterns of MRSA isolates (A-V) that were categorized into two clusters, ERIC cluster I and ERIC cluster II. ERIC cluster I contained 76.9% of the isolates (20 strains) that were designated alphabetically (A-P), while only six strains (23.1%) were belonged to ERIC cluster II (ERIC Q-V). The MRSA isolates (12 and 15), (19, 26 and 10), (16, 25, 2, 11, 14 and 9), (7 and 8) showed substantial similarity, indicating that those isolates are part of a clonal lineage, according to the dendrogram that was constructed. ERIC A was the most common ERIC type (Table 5) (11.5%, 3), followed by ERIC C and I (7.7%, 2 each) in cat samples.

DISCUSSION

S. aureus causes a variety of diseases in both animals and humans, from minor skin infections to life-threatening conditions including septicaemia, toxic shock, endocarditis, and pneumonia (Werckenthin *et al*., 2001; Algammal *et al*., 2020). An increase in the incidence of methicillin resistance in *S. aureus* isolates during the last four decades has resulted in increased morbidity and mortality and length of hospital stay and represents a significant load on healthcare services (Diederen *et al*., 2006). This study detected 23.7% *S. aureus* isolates (21.8 % from cats and 32.6% from dogs), of them 40.6% `was classified as MRSA (40,8% from cats and 40 % from dogs) and 59.4% were identified as MSSA in pet animal samples. Previous studies reported similar incidence rate of *S. aureus* (20.5%, 24%, and 25%) in pet animals (Findik *et al*., 2009; Reddy *et al*., 2016; Lilenbaum *et al*., 2000). It was consistent with the findings of Shoaib *et al*. (2020) who confirmed *mec*A gene in 33.91% dogs and 30.43% cats in Pakistan and by Ibira *et al*. (2023) from dogs (23,73%) in Nigeria. However, lower

Table 5. ERIC-PCR, and antibiotic resistance of MRSA isolated from pet animals (n=26).

Animal	Specimen	Sample	ERIC-PCR Type	Antibiotic Pattern	Antibiotic Resistant Gene
Dog	Nasal Swap	19	A	K, CTX, OX, SXT, P, S, TE	*mec*A, *fem*A
Cat	Nasal Swab	26	A	K, CTX, OX, P, S, T.E, AMC, Cip	*mec*A *fem*A
Cat	Abscess	22	B	K, OX, VA, SXT, S, AMC, CIP, P, CTX, TE	*mec*A
Cat	Mouth Swab	12	C	K, OX, VA, SXT, S, AMC, CIP, P, CTX, TE	*mec*A
Cat	Abscess	15	C	OX, CTX, P, SXT, S, AMC, TE.	*mec*A ,*fem*A
Cat	Abscess	24	D	K, OX, VA, SXT, S, AMC, CIP, P, CTX, TE	*mec*A
Dog	Nasal Swab	21	E	K, OX, Ctx, SXT, S, P, AMC, Cip	*mec*A, *fem*A
Cat	Mouth Swab	5	F	P, K, OX, Ctx, AMC, CIP	*mec*A
Cat	Mouth Swab	6	H	OX, SXT, S, AMC, CIP, P, CTX	*mec*A
Cat	Nasal Swab	7	I	K, OX, VA, SXT, S, AMC, CPS, P, CTX, TE	*mec*A
Dog	Mouth Swab	8	I	K, CTX, OX, VA, SXT, P, TE, AMC, CIP.	*mec*A , *fem*A
Cat	Abscess	4	K	K, CXT, VA, SXT, OX, S, P, AMC, C.P.S, TE	*mec*A
Cat	Abscess	20	L	K, CXT, SXT, OX, S, AMC, C.P.S, TE, P.	*mec*A
Cat	Mouth Swab	1	M	K, OX, P, S, TE, AMC, CIP	*mec*A, *fem*A
Cat	Mouth Swab	17	N	K, CTX, OX, P, S, T.E, AMC, CIP	*mec*A, *fem*A
Cat	Abcess	3	O	K, CXT, VA, SXT, OX, S, P, AMC, C.P.S, TE	*mec*A
Cat	Mouth Swab	18	P	K, CTX, OX, SXT, S, P, AMC, CIP, VA, TE	*mec*A
Cat	Mouth Swab	16	Q	P, K, OX, Ctx, AMC, CIP	*mec* A
Cat	Mouth Swab	25	R	K , OX, P, S, TE, AMC, CIP	*mec*A, *fem*A
Cat	Abscess	2	S	OX, CTX, P, SXT, S, AMC, TE	*mec*A , *fem*A
Cat	Abcess	11	T	K, CXT, SXT, OX, S, AMC, C.P.S, TE,P.	*mec*A
Cat	Mouth Swab	14	U	K, OX, VA, SXT, S, AMC, CIP , P, CTX, TE	*mec*A
Cat	Mouth Swab	9	V	OX, SXT, S, AMC, CIP , P, CTX	*mec* A
Dog	Nasal Swab	10	A	K, OX,CTX, SXT, S, P, AMC, CIP	*mec* A, *fem* A
Dog	Nasal Swab	13	G	K, CTX, OX, VA , SXT, P, TE, AMC, CIP	*mec* A , *fem* A
Dog	Nasal Swab	23	J	K, CTX, OX, SXT, P, S, TE	*mec* A, *fem* A

prevalence of MRSA isolates were detected by Anette Loeffler *et al.* (2005) who detected 17.9% MRSA among staff and kennelled dogs in London. Also, previous research detected 7.8% MRSA isolates from household dogs in Jordan (Tarazi *et al*., 2015). Additionally, Habibullah *et al.* (2017) identified a higher prevalence of *S. aureus* (40.86%) with low prevalence of MRSA isolates in dogs (4.91%) and cats (3.13%) in Bangladesh. Kanagarajah *et al.* (2017) confirmed MRSA in canines (11.66%) and felines (7.70%) in Malaysia. The variance in prevalence is most likely due to unsanitary conditions in the research area and geographical diversity.

Improper and overuse of antimicrobials pose significant risks to human and animal health because of the advent of drug-resistant *S. aureus* strains among which MRSA has a significant public health concern (Akindolire *et al*., 2015). The isolated MRSA in this study displayed higher resistance ranges from 76%–100% to penicillin, oxacillin, amoxicillin-clavulanic acid, cefotaxime, kanamycin, streptomycin, tetracycline, and ciprofloxacin. A high antimicrobial resistant of MRSA isolates from pet animals; dogs and cats; were reported in many previous studies worldwide (Abdel-Moein *et al*., 2012; Reddy *et al*., 2016; Ibira *et al*., 2023). Our isolates exhibited lower antimicrobial resistance to ciprofloxacin, vancomycin, sulfamethoxazole/trimethoprim, which was congruent with other previous studies worldwide (Abdel-Moein *et al*., 2012; Habibullah *et al*., 2017). Such resistant antibiotics are commonly used antibiotics in treating staphylococcal infections in this region. Further, the high resistance to β-lactam antibiotics could be explained by their widespread and uncontrolled usage of these antibiotics by veterinary personnel and dog owners. All of our tested isolates had a MAR index value greater than 0.6 is indication of a high-risk source of contamination and an environment where antibiotics are often utilized.

A streamlined typing method for many various organisms is ERIC-PCR. This typing technique is a good choice because it is reliable and doesn't call for any disease-specific reagents. The MRSA isolates in in this study were categorized using ERIC typing into 22 distinct patterns that were categorized into two clusters, ERIC cluster I and ERIC cluster II. The ERIC-PCR study found that MRSA isolates from pets were genetically varied and heterogeneous. Heterogeneity was shown by the diverse origins of the strains (nasal swabs, mouth swabs, and abscess). This result is congruent with the findings of Abdollahi *et al.* (2014) who claimed that the ERIC-PCR profiles enabled the classification of 90 isolates into 75 ERIC types, and that the majority of the isolates showed distinct patterns, indicating that the rate of resistance strains transmission were relatively low. A 46 distinct ERIC types were identified for the 50 MDR-MRSA isolates were also reported by a previous Egyptian study (Alfeky *et al*., 2022). Additionally, Arslan *et al.* (2016) reported a 64 genotypes of 98 *S. aureus* strains. A clear association between ERIC-PCR and resistance patterns in some strains suggests that their combination may provide important and trustworthy MRSA strain information for tracking origins. Furthermore, antibiotic susceptibility varied among strains with the same genotype. For example, antibiotic resistance patterns and antibiotic resistance genes differed between isolates with a similar ERIC C type (n =12, 15). Antibiotic resistance patterns and antibiotic resistance genes were also diverse across the strains (n = 7, 8) of the same ERIC I type.

CONCLUSION

MRSA infections continue to be a significant issue in Egyptian society. According to the current study, more than 40,6 % of pets harbor MRSA. MRSA isolates in vitro were resistant to a variety of antibiotic classes, including β -lactams and aminoglycosides. The highest resistance rate among MRSA isolates was to penicillin, oxacillin, amoxicillin-clavulanic acid, cefotaxime and the lower resistance was to vancomycin. The study discovered a varied reaction to antibiotic susceptibilities as well as changeable potential risk factors so, studies should be performed frequently to evaluate MRSA isolate epidemiology and their antibiotic susceptibility profiles for efficient MRSA infection control and management.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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