

Original Research Paper

# Prevalence and Antibiotic-Sensitivity of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Pyogenic Skin Affections of Animals and Human

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**Abstract:** MRSA infections commonly present as skin and soft tissue infections in man and animals. Treatment usually includes opening of abscess and drainage with or without antibiotic therapy. MRSA is usually resistant to certain antibiotics including methicillin, oxacillin, penicillin and ampicillin. Therefore, the aim of the present study was to investigate the prevalence of MRSA in man and animals with suppurative skin affections in Assiut Governorate and to test the phenotypic and genotypic antibiotic susceptibility of the isolated MRSA strains to the commonly-used antibiotics in Egyptian medical practices. MRSA were detected in 3.0% of tested sheep and goat, 27.3% of equines, 7.7% of cows and in 14.3% of tested humans in the same area. All animal and man MRSA strains were positive to *erm(A)*, *TetK* and *TetM* genes, but *erm(C)* gene could only be detected in 80% of human MRSA isolates. human and animal MRSA isolates were resistant to penicillin and ampicillin antibiotics with MIC ranging from 8 to 128 µg/ml. Sheep and cow MRSA isolates were resistant to oxytetracycline, while equine isolates were sensitive to it. About 44.4% of human isolates were resistant to oxytetracycline. All animal-derived MRSA isolates were resistant to cefixime and more than of 55% of human isolates were also resistant to the same drug. All MRSA isolates were sensitive to clindamycin except for strains isolated from cows. Enerofloxacin and ciprofloxacin were the most effective antibiotics against all MRSA isolates. None of the 16 MRSA isolates had reduced susceptibility to vancomycin with MICs laying in the 0.5-4 µg/mL range. The similarity in phenotypic and genotypic antibiotic susceptibility of MRSA isolates recovered from both humans and animal cases, despite the difference in the frequently used antibiotics in veterinary and human hospitals, suggesting the possibility of zoonotic circulation of those isolates between them.

**Keywords:** MRSA, Egypt, Zoonosis, Antibiotic Resistance Genes, Antimicrobial Sensitivity

## Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has now become a major nosocomial infection worldwide (Deurenberg and Stobbering, 2008) and a common cause

of skin and soft tissue infections (Moran *et al.*, 2006). In Egypt, the highest proportion of MRSA among invasive *Staphylococcus aureus* (*S. aureus*) strains isolated from blood cultures has been reported (Borg *et al.*, 2007; Falagas, *et al.*, 2013). Unfortunately, MRSA found its

way outside hospitals to the community leading to emerging Community-Acquired Methicillin-Resistant *S. aureus* (CA-MRSA) infections (Herold *et al.*, 1998). Animals may act as reservoir of MRSA to human (Hadjirin *et al.*, 2015). MRSA infections have become a growing concern in the veterinary field and the appearance of Livestock-Associated Methicillin-Resistant *S. aureus* (LA-MRSA) has been increasingly reported worldwide (Van Cleef *et al.*, 2010). The risk of zoonotic transmission especially for people with occupational livestock exposure is rising (Hanselman *et al.*, 2006; Vanderhaeghen *et al.*, 2010; Fessler *et al.*, 2012). Interestingly, strains originating from companion animals are often human strains in origin (Bhandari and Jhala, 2011). On the other hand, LA-MRSA are divergent from human strains may result in MRSA being an emerging zoonotic pathogen with veterinarians, cattle farmers and pet owners put at risk of acquiring the infection (Morgan, 2008). Several reports have shown that MRSA can also spread in veterinary hospital settings from humans to animals and from animals to humans and has the ability to survive in the environment (Seguin *et al.*, 1999; Duijkeren *et al.*, 2004; Weese *et al.*, 2004; 2005; 2006; Baptiste *et al.*, 2005; O'Mahony *et al.*, 2005; Leonard *et al.*, 2006). Using the genotypic and phenotypic methods, scientists found that there is no difference between human and bovine MRSA, which confirming MRSA transmission between cattle and human (Lee, 2003; Juhász-Kaszanyitzky *et al.*, 2007; Hata *et al.*, 2010).

CA-MRSA should be considered when treating skin and soft tissue infections especially in developing countries (Sobhy *et al.*, 2012). Antimicrobial testing from suppurative skin lesions are recommended to guide individual therapy (Abdel Fattah and Darwish, 2012). MRSA causes a variety of skin affections and septic wounds in man and animals, but information about the prevalence of MRSA infection causing these affections in Egypt is scarce. Assiut Governorate in Egypt consists of rural villages where animals are usually kept in very close contact to houses of their owners. Patients with skin affections from Assiut governorate are often referred to Assiut University teaching hospital dermatology unit. Assiut University veterinary teaching hospital usually treats animals with skin abscesses and infected wounds from all Assiut Governorate villages as well. Therefore, we collected animal and human samples from patients with skin affections admitted to these two hospitals, which covers Assiut Governorate villages. The study aimed to investigate the prevalence of MRSA infection in human and animal individuals with pyogenic skin lesions and wound at Assiut Governorate, Egypt. Phenotype and genotype screening the antibiotic susceptibility of the isolated MRSA strains to the commonly used antibiotics in human and veterinary Egyptian field was also undertaken.

## Materials and Methods

### *Ethical Approval*

All the participants were informed about the objectives of the study, methods, voluntary participation and the individual information was concealed. Consents were recorded following participant agreement and their rights were clearly explained to each one of them. Also, animal samples and data were obtained after agreement of their owners.

### *Patients*

#### *Human*

Samples were collected from 63 patients from the Assiut University hospital dermatology clinic with suppurative skin lesions, infected wounds and abscess during the period from 2015 to 2017.

#### *Animals*

Samples were collected from a three different animal species (66 sheep, 11 equines and 26 cows) that were referred to the Veterinary teaching hospital, Faculty of Veterinary Medicine, Assiut University during the same time period. These animals suffered from pyogenic skin affections, suppurating wounds and abscesses.

### *Demographic Data of Human Patients*

Age: from less than 2 to 40 years. Gender: 40 males and 23 females. Residence: 47 patients from overcrowded bad hygienic condition rural areas, 16 patients from urban areas. Sites of skin lesions: 26 on the hand, 13 on the leg, 11 on the abdomen and breast, 9 on the buttock and 4 on the face.

### *Samples and Bacterial Identification*

Sterile bacteriological swabs were collected from already opened skin lesions, wounds and abscesses of the diseased animals and man after disinfection of the opening with tincture iodine, while the closed abscesses were surgically incised and pus material were swabbed under septic conditions. Swabs taken from abscesses and wounds were directly inoculated into tubes containing tryptic soy broth then incubated at 37°C for 24-48 h. The incubated tubes were streaked onto blood agar base enriched with 10% citrated sheep blood and on Baired-Parker agar used for selective isolation of Staphylococci. The inoculated plates were aerobically incubated at 37°C and examined for bacterial growth after 24-48 h. The plates were examined for growth, morphologic features and hemolytic characteristics following routine guidelines (Quinn *et al.*, 1994; Carter *et al.*, 1995). Identification of the isolated bacteria was done according to colony morphology, microscopic examination of gram stained smear and biochemical examination (Collins *et al.*, 1991; Cruickshank *et al.*, 1975).

**Table 1:** Sequence of primer sets for antibiotic resistance genes and the amplicon size

Resistance phenotype	Gene	Sequences of primer sets	Amplicon Size (bp)	References
Clindamycin, erythromycin	<i>erm(A)</i>	F- AAGCGTAAACCCCTCTGA R- TTCGCAAAATCCCTTCTCAAC	190	Strommenger <i>et al.</i> (2003)
	<i>erm(C)</i>	F- AATCGTCAATTCTGCATGT R- TAATCGTGAATACGGGTTTG	299	
Tetracycline	<i>tetK</i>	F-GTAGCGACAATAGGTAATAGT R-GTAGTGACAATAAACCTCCTA	360	
	<i>tetM</i>	F-AGTGGAGCGATTACAGAA R-CATATGTCCTGGCGTGTCTA	158	

### Detection of *mecA* Gene

All isolates identified as *S. aureus* were tested for the presence of *mecA* gene using specific primers. DNAs from *S. aureus* isolates were extracted as described by using Dneasy Tissue Kit (QIAamp® DNA Mini Kit) with some modifications. The extracted DNA was used as a template for PCR amplification. PCR amplifications were performed with a pair of primers specific for *mecA* gene, synthesized from the previously published Sequences: Primer 1: 5'-AAA ATC GAT GGT AAA GGT TGG C-3', primer 2: 5'-AGT TCT GCA GTA CCG GAT TTG C-3' (Louie *et al.*, 2009). The PCR cycles consisted of initial denaturation at 96°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min and final extension at 72°C for 10 min. The PCR products were analyzed by electrophoresis in a 1.5% agarose gel containing 0.5 mg of ethidium bromide per mL. The size of the amplifications were estimated by comparison with a 1kp DNA step ladder. MRSA strain ATCC BAA-44 (KwikStik, 01055 P) was used as positive control and nuclease-free water (Promega, P119A) was used as negative control.

### Detection of Antibiotic Resistance Genes

*mecA* gene positive MRSA isolates were examined by using PCR for the presence of some antibiotic resistance genes (*erm(A)*, *erm(C)*, *tetK*, *tetM* genes) by using specific primers (Table 1). DNAs from MRSA isolates were extracted as described by using Dneasy Tissue Kit (QIAamp® DNA Mini Kit) with some modifications. The PCR cycles consisted of initial denaturation at 96°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min and final extension at 72°C for 10 min. The PCR products were analyzed by electrophoresis in a 1.5% agarose gel containing 0.5 mg of ethidium bromide per mL. The sizes of the amplification products were estimated by comparison with a 100bp DNA step ladder.

### Antimicrobials Test

*mecA* gene positive MRSA isolates were tested for their sensitivity towards the most commonly used antibiotics in the human and veterinary field in Egypt. These antibiotics included penicillin, ampicillin,

oxytetracycline, enrofloxacin, vancomycin, ciprofloxacin, clindamycin and cefixime. Minimum Inhibitory Concentrations (MIC) of antibiotics were evaluated with the broth microdilution technique in Mueller Hinton broth (MHB) with an initial inoculum of  $5 \times 10^5$  in non-treated Polystyrene microtiter plates in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2010). The MIC were interpreted as the lowest concentration of antibiotic that completely inhibited the visible growth of bacteria after 16 h. incubation of the plates at 37°C. Each agent was tested in triplicate in at least two independent experiments. The highest MIC value were reported.

### Statistical Analysis

The svy commands in Stata statistical software (release 15.1; 2017, Stata Corp LP, College Station, TX) was used to measure the impact of each factor individually on the occurrence of the disease. Odds ratio and 95% confidence intervals was calculated and a probability value (P-Value)  $p < 0.05$  was considered statistically significant.

## Results

### Occurrence of *S. aureus* in Animals and Human

*S. aureus* was isolated from 28 out of 103 (27.2%) different animal species with both open and closed skin abscess. The 28 cases with *S. aureus* included 10 out of 66 (15.2%) examined sheep, 7 out of 11 (63.6%) examined equine and 11 out of 26 (42.3%) examined cows (Table 2). Meanwhile, 63 human individuals with infected wounds after surgery or skin abscesses in different body areas (hands, legs, face, breast and buttock) were examined for *S. aureus* infection and it could be isolated by 23.8% (15 out of 63).

### Detection of *mecA* Gene

Methicillin-Resistant *S. aureus mecA* gene positive isolates (MRSA) could be detected in 3.0% of examined sheep and goat, 27.3% of examined equines and 7.7% of examined cows (Table 2). PCR product of MRSA isolates gave positive band at 533bp on the agarose gel electrophoresis (Fig. 1). MRSA isolates

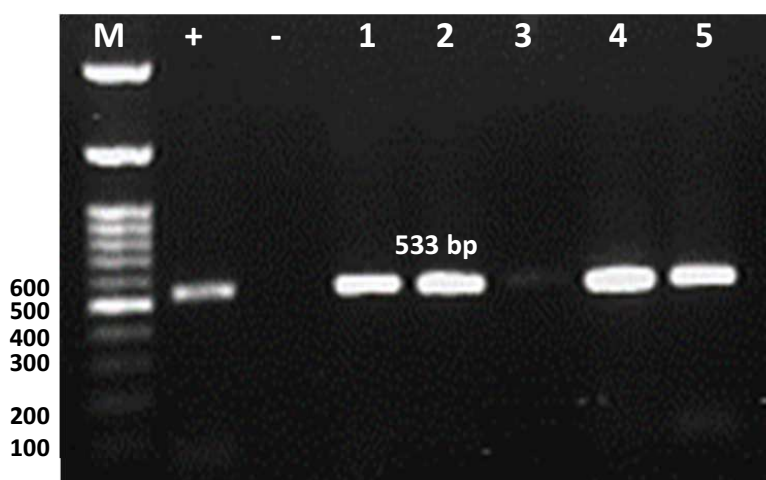
could also be detected in examined human individuals with prevalence 14.3%. The impact of some factors like gender, age and area on MRSA prevalence in humans has been evaluated (Table 3).

#### Detection of Antibiotic Resistance Genes in MRSA Isolates

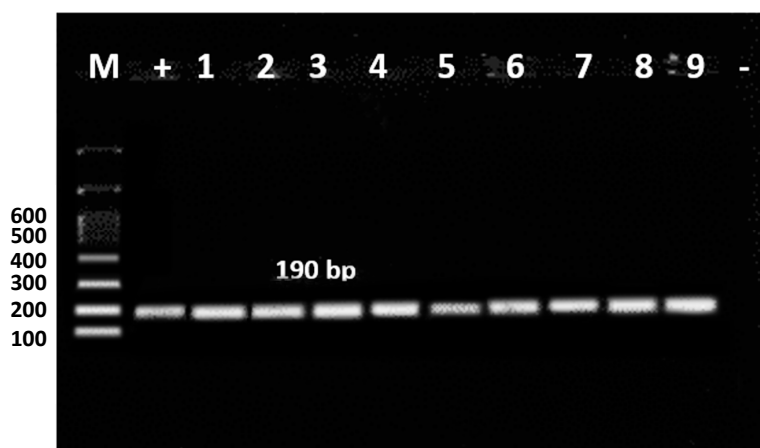
All MRSA strains isolated from animal and human individuals were positive to *erm(A)*, *TetK* and *TetM* genes (Table 4) and PCR product showed positive bands at 190 bp, 360 bp and 158 bp respectively (Fig. 2, 4 and 5). Meanwhile, *erm(C)* gene could not be detected in all animal MRSA isolates and were detected in 80% of human MRSA isolates (Table 4). PCR product showed positive band for *erm(C)* gene at 299bp (Fig. 3).

#### Antimicrobial Testing Results

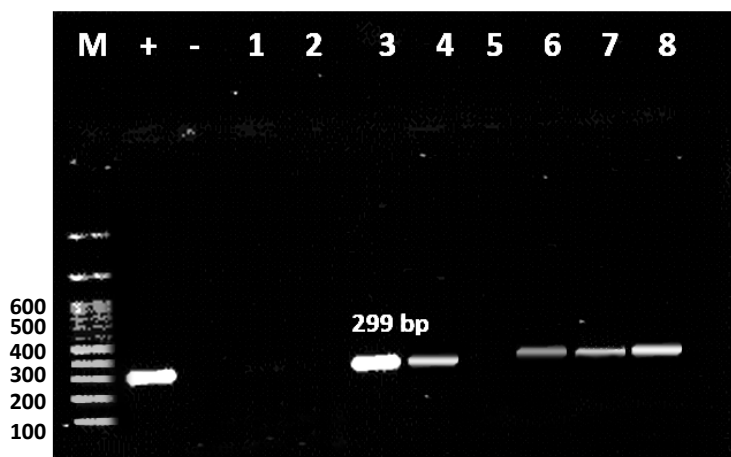
As demonstrated in Table 5, human and animal MRSA isolates were resistant to penicillin and ampicillin antibiotics with MIC ranging from 8 to 128 µg/ml. Sheep and cow MRSA isolates were resistant to oxytetracycline, while equine isolates were sensitive to it. About 44.4% of human isolates were resistant to oxytetracycline. All animal MRSA isolates were resistant to cefixime and more than of 55% of human isolates were also resistant to it. All MRSA isolates were sensitive to clindamycin except for cow's strains. Enoxifloxacin and ciprofloxacin were the most effective antibiotics against all MRSA isolates. None of the 16 MRSA isolates had reduced susceptibility to vancomycin with MICs laying in the 0.5-4 µg/ml range.



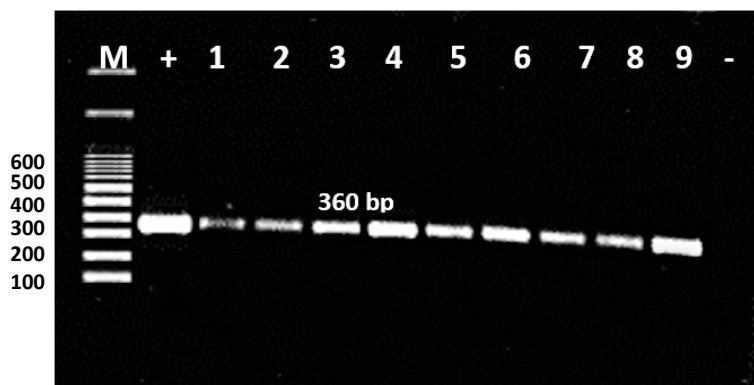
**Fig. 1:** Agarose gel electrophoresis showing PCR products of *mecA* gene positive *S. aureus* isolates from animal and human cases with skin affections (positive band at 533 bp). M= 100 bp ladder, lane (+)= positive control, lane (-)= negative control, lane (1,2,4,5)= positive samples and lane (3)= negative sample



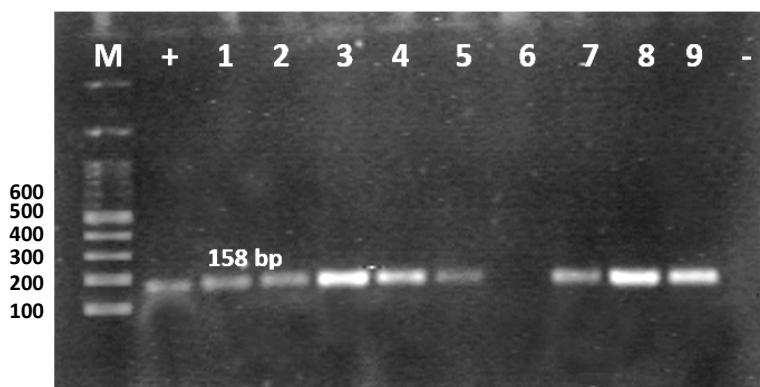
**Fig. 2:** Agarose gel electrophoresis showing PCR products of *erm(A)* gene positive *S. aureus* isolates from animal and human cases with skin affections (positive band at 190 bp). lane (+)= positive control, lane (-)= negative control, lane (1,2,3,4,5,6,7,8,9) = positive samples



**Fig. 3:** Agarose gel electrophoresis showing PCR products of *erm(C)* gene positive *S. aureus* isolates from animal and human cases with skin affections (positive band at 299 bp). lane (+)= positive control, lane (-)= negative control, lane (3,4,6,7,8)= positive samples and lane (1,2,5)= negative samples



**Fig. 4:** Agarose gel electrophoresis showing PCR products of *tetK* gene positive *S. aureus* isolates from animal and human cases with skin affections (positive band at 360 bp). lane (+)= positive control, lane (-)= negative control, lane (1-9)= positive samples



**Fig. 5:** Agarose gel electrophoresis showing PCR products of *tetM* gene positive *S. aureus* isolates from animal and human cases with skin affections (positive band at 158 bp). lane (+)= positive control, lane (-)= negative control, lane (1,2,3,4,5,7,8,9)= positive samples and lane (6)= negative sample

**Table 2:** Prevalence of MRSA among animals with skin affections

Species	No. tested	Positive <i>S. aureus</i> (%)		Odds ratio	95% CI	P Value
		MRSA (%)	MSSA (%)			
Sheep and goat	66	2 (3.0)	8 (12.1)	6.39	2.05-19.92	0.001
Equine	11	3 (27.3)	4 (36.4)			
Cow	26	2 (7.7)	9 (34.6)			
Total	103	7 (6.8)	21 (20.4)			

95% confidence intervals (95% CI), probability value (P-Value)

**Table 3:** Prevalence of MRSA among human individuals with skin affections

Factor	No. tested	Positive <i>S. aureus</i> (%)		Odds ratio	95% CI	P Value
		MRSA (%)	MSSA (%)			
Gender				0.45	0.09-2.37	0.35
Male	40	7 (17.5)	3 (7.5)			
Female	23	2 (8.7)	3 (13.0)			
Total	63	9 (14.3)	6 (9.5)			
Age (Year)				1.43	0.57-3.59	0.45
> 2 - 20	29	3 (10.3)	2 (6.9)			
20 - 40	23	4 (17.4)	4 (17.4)			
40 - 70	11	2 (18.2)	0			
Total	63	9 (14.3)	6 (9.5)			
Area				0.66	0.49-0.87	0.003
Rural	47	8 (17.0)	4 (8.5)			
Urban	16	1 (6.3)	2 (12.5)			
Total	63	9	6			

95% confidence intervals (95% CI), probability value (P-Value)

**Table 4:** Correlation between phenotypic antibiotic resistance and PCR results of antibiotic resistance genes in MRSA isolates.

Isolates	Resistance phenotype	<i>mecA</i>	<i>erm(A)</i>	<i>erm(C)</i>	<i>tetK</i>	<i>tetM</i>
Animal isolates						
Sheep and goat	PEN, OTE, CEF, AMP	+	+	-	+	+
	PEN, OTE, CEF, AMP	+	+	-	+	+
Equine	PEN, CEF, AMP	+	+	-	+	+
	PEN, CEF, AMP	+	+	-	+	+
	PEN, CEF, AMP	+	+	-	+	+
Cows	PEN, OTE, CLI, CEF, AMP	+	+	-	+	+
	PEN, OTE, CLI, CEF, AMP	+	+	-	+	+
Human isolates						
Female	PEN, OTE, AMP	+	+	+	+	+
	PEN, CEF, AMP	+	+	-	+	+
Male	PEN, AMP	+	+	-	+	+
	PEN, OTE, AMP	+	+	+	+	+
	PEN, OTE, AMP	+	+	+	+	+
	PEN, CEF, AMP	+	+	+	+	+
	PEN, CEF, AMP	+	+	+	+	+
	PEN, OTE, CEF, AMP	+	+	+	+	+
	PEN, CEF, AMP	+	+	+	+	+

PEN, penicillin; CLI, clindamycin; OTE, oxytetracycline; CEF, cefixime; AMP, ampicillin.

**Table 5:** Minimum inhibitory concentrations of the common used antibiotics in field ( $\mu\text{g/ml}$ ) against animal and human MRSA isolates

Antibiotics	MIC ( $\mu\text{g/ml}$ )															
	Animal Isolates								Human Isolates							
	Sheep isolates		Equine isolates			Cow isolates			Female isolates				Male isolates			
Penicillin	8	64	16	8	32	32	32	32	16	32	32	64	8	32	8	32
Oxytetracycline	64	64	0.25	0.125	0.25	16	16	64	0.125	0.125	64	0.125	0.25	0.25	64	0.125
Enrofloxacin	0.125	0.125	0.5	0.125	0.125	0.125	0.125	<0.03125	<0.03125	0.125	<0.03125	0.125	0.125	0.25	<0.03125	0.25
Vancomycin	0.5	1	2	1	1	4	4	1	1	1	1	1	1	1	0.5	1
Ciprofloxacin	0.5	0.25	2	0.5	0.25	0.25	0.25	0.25	0.125	0.25	0.25	0.25	0.5	0.5	0.25	0.5
Clindamycin	0.125	0.125	0.125	0.125	0.125	64	64	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Cefixime	8	8	8	8	8	8	8	1	8	1	1	1	8	8	128	8
Ampicillin	16	128	16	32	64	64	64	32	16	64	64	64	64	16	32	16

## Discussion

MRSA is one of the most problematic pathogens in veterinary and human medicine. The increasing number of MRSA cases detected in Egypt necessitates the continuous monitoring of the epidemiological phenotypic and genotypic antimicrobial susceptibility features of MRSA infections (Abd El-Hamid and Bendary, 2015). Moreover, the recovery of MRSA from various animal species increases the concern about the role of animals in MRSA infection in human (Weese, 2010). The current investigation was carried out to detect MRSA prevalence at Assiut Governorate, Egypt and to compare the phenotypic and genotypic antibiotic resistance of the isolated MRSA strains.

MRSA was first isolated from milk of mastitic cows (Devriese and Hommez, 1975), then it could be isolated from other animal species including dogs, cats, pigs, horses and poultry worldwide (Leonard and Markey, 2008). MRSA was identified in the present study from cattle with septic skin infections by 7.7%. This finding was similar to Jayaweera and Kumbukgolla (2017), who reported prevalence rate 6.2% from nasal and perineal swabs from cattle. Meanwhile, despite the high number of sheep and goat with skin abscesses subjected to this study, the estimated prevalence of MRSA was low (3.0%). This finding came in agreement with other investigations carried out on small ruminants (Giacinti *et al.*, 2017; Caruso *et al.*, 2015; Cortimiglia *et al.*, 2015) in Italy, (Ariza-Miguel *et al.*, 2014) in Spain and (Pexara *et al.*, 2005) in Greece, who reported prevalence rates ranged from 0-2%.

The highest MRSA prevalence was recorded in equine (27.3%), despite the low number of examined horses with septic wounds and skin abscesses. The difference in MRSA prevalence between equine and other animal species recorded in this study was of high significance (OR = 6.39, 95% CI = 2.05-19.92,  $P = 0.001$ ). MRSA reported as emerging pathogen in veterinary medicine especially in small animals and equine (Leonard and Markey, 2008). Wound infections and post-operative contaminations are the most common manifestation of MRSA in horses. The principle route of transmission is contaminated hands of veterinary personnel (Weese *et al.*, 2004). The emergence of MRSA as an equine pathogen implies that horses could be a community reservoir of MRSA and a source of infection to human (Weese *et al.*, 2005).

Nine out of 63 (14.3%) human patients with suppurative skin lesions were positive to MRSA infection. The prevalence of CA-MRSA in the present study agreed with Abdel-Maksoud *et al.* (2016), who recorded 11.5% prevalence of CA-MRSA in Egypt. Also, this finding was in concordance with findings reported in other studies done in Egypt (Guirguis, 2004). A previous study by Ahmed *et al.* (2014) in Egypt at

Minya Governorate, which is the geographically close to Assiut, recovered MRSA from infected skin and soft tissue sites in 31 (15%) out of 208 patients admitted to Minya University hospital. The prevalence rate of CA-MRSA in Egypt is probably due to the self-medication with antibiotics for mild bacterial or viral infections, where antibiotics usually received without prescription (Sabry *et al.*, 2014). Baddour *et al.* (2006) studied the antibiotic susceptibility pattern of MRSA isolates from several hospitals in Saudi Arabia and concluded that the inappropriate use of antibiotics is an important cause of the increasing prevalence of resistant bacteria as MRSA in developing countries.

Prevalence of MRSA in male patients was 17.5% while it was 8.7% in females. This finding meant that 77.8% of recovered MRSA isolates from human patients were from males. Despite this finding was statistically insignificant (OR = 0.45, 95%CI = 0.09-2.37,  $P = 0.35$ ), it was similar to those recorded by Baddour *et al.* (2006), Tentolouris *et al.* (2006) and Van Belkum *et al.* (1997). They recovered more than 66% of their MRSA isolates from males. The higher prevalence of MRSA in males may be attributed to the fact that exposure in males working as farmers, veterinarians, workers and milkers is greater (Baddour *et al.*, 2006). Concerning age of the examined patients, the difference in MRSA prevalence between the three age groups was statistically insignificant (OR = 1.43, 95% CI = 0.57-3.59,  $P = 0.45$ ), which indicate that infection with MRSA is not related to the age of the patient.

Most of human patients subjected to our study were originally from rural village of Assiut (47 out of 63) and 17.0% of them were positive to MRSA infection. Statistically the higher prevalence of MRSA in patients from rural areas than those from urban areas were highly significant (OR = 0.66, 95%CI = 0.49- 0.87,  $P = 0.003$ ). In rural areas, individuals usually keep their animals in the house where there is a close daily contact between these individuals and their animals. Most of animal's MRSA infections are skin and soft tissue infections (Seguin *et al.*, 1999). Close contact of human with animals gives the opportunity for horizontal transmission of MRSA between them (Morgan, 2008). Historically, MRSA infections in companion animals caused by strains resembling human strains (Rich and Roberts, 2004). Veterinary personnel may play a role as a source of MRSA infection to animals (Walther *et al.*, 2009). MRSA infected animals like cattle may act as a reservoir for transmission of infection to other animals and their human handlers (AVMA, 2014; Klevens *et al.*, 2007). Owners, farmers, veterinarians, milkers and people working at slaughter houses who meet MRSA colonized or infected animals are at risk to be colonized by MRSA (Paterson *et al.*, 2012). MRSA transmission from horses to humans was also documented especially with animals

experienced skin infections (Weese *et al.*, 2006). 10 (9.7%) of 103 tested veterinary hospital personnel in a large animal clinic were colonized with MRSA and pyogenic skin infections were recorded in 3 of them (Weese *et al.*, 2006).

Antimicrobial testing and analysis for presence of antibiotic resistance genes were employed in this study. The analyzed genes were the most frequently associated with resistance of *S. aureus* (Beninati *et al.*, 2015). All the identified MRSA strains were both phenotypically (MICs 8 -128 µg/mL) and genotypically (*mecA*-positive) resistant to penicillin and ampicillin. Lee (2003) also found that all MRSA isolates were resistant to penicillin and ampicillin. Moreover, Ahmed *et al.* (2014) found that all MRSA isolates recovered from infected surgical sites were completely resistant to β-lactams. A 100% of animal MRSA strains and more than 55% of man strains were shown to be resistant to the cephalosporin antibiotic (cefixime), meanwhile higher resistance rates reported by Vinodkumar *et al.* (2011) and lower rates were recorded by Sasikala *et al.* (2007). Previous studies, also done in Egypt, reported that β-lactams, macrolides and cephalosporins were the most prescribed antibiotics in Egyptian hospitals (Abdel-Maksoud *et al.*, 2016). Tetracycline resistance was also common, especially among cattle and sheep MRSA isolates and in 44.4% of human isolates. Meanwhile, equine isolates and the remaining 55.6% of human isolates were sensitive to tetracycline despite that all MRSA strains were positive to *tetK* and *tetM* resistance genes. El-Jakee *et al.* (2011) also studied the antimicrobial resistance in MRSA isolates from cattle and human in Egypt and found high resistance rate to penicillin followed by oxytetracycline of these isolates. Rajala-Schultz *et al.* (2004), Wang *et al.* (2008), Coelho *et al.* (2009) and Bhatt *et al.* (2011) indicated that β-lactams (penicillins) and tetracycline are widely used for intra-mammary treatment of staphylococcal bovine mastitis, therefore these drugs most frequently associated with resistance.

Due to the close structural similarity of tetracycline resistance plasmids of different staphylococcal species from man and animals, the exchange of tetracycline resistance genes between man's and animal's staphylococci is possible (Schwarz *et al.*, 1998). Tetracyclines are not commonly used in treatment of bacterial infections of horses in Egypt which explains the sensitivity of equine isolates to oxytetracycline but the present of the resistance genes of tetracycline may indicate the transmission of MRSA infection from another animal species or from human handlers (Pantosti, 2012). On the other hand, presence of tetracycline resistance genes in all human isolates even the phenotypically sensitive ones indicate the risk of transmission of these isolates from food animals where tetracycline is widely used (Lewis *et al.*, 2008). Transfer of MRSA from food animals to human is

possible, which may lead to convey of novel resistance genes (Pantosti, 2012).

Except for cow's isolates, the recovered MRSA strains in this study were sensitive to clindamycin. Clindamycin is frequently used in human patients to treat skin and soft tissue infections, because of its ability to distribute well into skin structures and to inhibit toxins production in staphylococci (Levin *et al.*, 2005; Coyle *et al.*, 2003; Stevens *et al.*, 1988). However, the possibility of inducible resistance during therapy with clindamycin is a major concern, that inducible resistance does not usually appear in susceptibility testing and molecular detection of resistance genes (*erm* genes) is necessary (Abdel Fattah and Darwish, 2012). Expression of *erm* genes may be either constitutive or inducible and staphylococci with inducible resistance against clindamycin are sensitive to the drug if no inducer is present (Steward *et al.*, 2005). This could explain the presence of *erm(A)* and *erm(C)* genes in phenotypically sensitive isolates to clindamycin.

All the 16 MRSA isolates recovered in this study were sensitive to vancomycin with MICs laying in the 0.5-4 µg/ml range. This could be explained by the fact that vancomycin use in human Egyptian out patients is not common due to lack of oral bioavailability and it is usually used in intensive care units for life-threatening conditions (Abdel Fattah and Darwish, 2012). Moreover, vancomycin is not used in veterinary Egyptian field.

MRSA isolates showed no resistance against ciprofloxacin and enrofloxacin. This finding agreed with Lee (2003) and Kumar *et al.* (2017), they found that MRSA isolates were susceptible to fluoroquinolones like ciprofloxacin and norfloxacin. Although the fluoroquinolones are not new antibiotics but its use in Egyptian field is not common like penicillines and cephalosporins. They don't affect by β-lactamase enzymes or altered by penicillin binding proteins so, it might be less likely to develop resistance (Kayser, 1985).

## Conclusion

The study documented the isolation of MRSA from clinical specimens in several animal species at Assiut Governorate and the recovery of MRSA from human individuals at the same area. This suggests that transmission between animals and humans can be of both veterinary and public health importance. Zoonotic transmission risk may be real, it should be considered when handling animals with skin and soft tissue infections especially horses. Antibiotic susceptibility of bacteria isolated from suppurative skin lesions is recommended to guide individual therapy and to limit misuse of antimicrobials. Absence of concurrent sampling of animals and their in-contact humans and the limited number of MRSA cases in the study necessitates



the further investigation on the epidemiology of MRSA infection in animals and humans in the study area.

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## Author's Contributions

**Maha I. Hamed:** Helped in collection of animal samples, shared in the practical part and wrote the manuscript.

**Sylvia O. Ahmed:** Shared in the practical part.

**Doaa S. Sayed:** Helped in collection of human samples.

**Hossam-Elden M. Hassan:** Collected human and animal samples, helped in the practical part.

## Conflict of Interest

Authors declare that there is no conflict of interests.

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