j.Egypt.net.med.Assoc 81, no 1. 185– 198 (2021)

NASAL CARRIAGE OF METHICILLIN-RESISTANT *Staphylococcus aureus* AMONG APPARENTLY HEALTHY HUMAN, CATTLE AND SHEEP: WITH OR WITHOUT THE PRESENCE OF *mecA* GENE

By

Basma Hassanin[1],Wagih Armanious[2], Rehab Elhelw[2]

[1]Veterinarian at General Organization For veterinary Services Al Doqi, Giza District

[2]Microbiology Department, Faculty of Veterinary Medicine, Cairo University, Giza.

ABSTRACT

The objectives of the study were to check the occurrence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and the molecular characteristics of the isolates within the nasal carriage of healthy human, cattle and sheep.

One hundred and fifty animal samples (100 from sheep, 50 from cattle) and 100 samples from humans in contact with animals were collected in the study. Human and animal nasal swabs samples were collected at Basateen slaughter house. The isolation and identification of *S. aureus* isolates were applied according to traditional biochemical tests. Disc diffusion test was performed to record resistance and MRSA isolates. PCR was done for detection of *nuc*, and *mecA genes*.

Only 60 out of 250 nasal swab samples produced *S. aureus* (24%), and 10 of which were MRSA (16.6%). All *S. aureus* isolates were sensitive to vancomycin and resistant to oxacillin, cefoxitin and erythromycin.Two isolates from sheep nasal swabs were intermediate resistant to cefoxitin and ofloxacin (50%). All isolates were *nuc* gene positive, while two out of the 10 MRSA isolates (20 %) were *mecA* negative, whereas all, the methicillin sensitive *S. aureus* (MSSA) were *mecA* negative.

Therefore *S. aureus* and MRSA from sheep, cattle and human are considered a potential risk for zoonotic transmission, this study drew attention to the credibility of the *mecA* gene and its usefulness in the detection of all MRSA strains without referring to the traditional methods.

Keywords:

Nasal carriage, MRSA, *S. aureus*, human sheep and cattle, *mecA* and *nuc* genes.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is still a major problem in the medical institutions around the world **(Lee *et al*., 2018)**.*Staphylococcus aureus* is the most common bacterial cause of life-threatening infections, including sepsis, deep abscesses, pneumonia, osteomyelitis, and endocarditis **(CDC 2014 and Tong *et al*., 2015)**. *S. aureus* is an important pathogen of human foodborne diseases and mastitis of milk ruminants **Painter *et al.*, 2013 and CDC 2014).**

S. aureus has a unique ability to quickly adapt to anti-bacterial agents and has developed resistance to methicillin and penicillin and more recently to daptomycin and linezolid which is a growing problem **(Pantosti *et al*.,2012)**.MRSA is resistant to many antibacterial drugs especially methicillin, tetracycline and cephalosporin **(EFSA,2009).** What is important about strains is that in addition to being resistant to methicillin most strains are also resistant to other beta lactam antibiotics,with the exceptionof glycopeptides antibiotics**(Moses*etal*., 2013).** The resistance of *S. aureus* to methicillin is encoded by *mecA* and *mecC* genes. MRSA that carries the staphylococcal cassette chromosome (SCCmec) are resistant to beta-lactam antimicrobials. Although both *mecA* and *mecC* show resistance to cefoxitin, *mecC* is sensitive and *mecA* is resistant to oxacillin **(Kim *et al*., 2012; Cartwright *et al*., 2013)**. MRSA is the leading cause of mastitis in ruminants worldwide **(Pilla *et al*., 2012; Guimaraes *et al*., 2017).** In the case of sub clinical mastitis, MRSA does not change the organoleptic characteristics of milk,so it can be transmitted to humans through milk and dairy products. Several studies have reported zoonotic transmission between humans and ruminants **(Feßler *et al*., 2010; Vanderhaeghen *et al*., 2010a, b; Spohr *et al*., 2011).**

The rate of nasal carriage of *S. aureus* strains is varying from 16.8% to 90% worldwide in human **(Askarian *et al*.,2009 and Kluytmans *et al*.,1997**) Although several studies have reported the prevalence of MRSA nasal carriage among patients **(Prates *et al*., 2010).**

The purpose of this study was to determine the prevalence of MRSA and detection of *mec*A gene in apparently healthy nasal passages of sheep,cattle and human,and the *in vitro* antibiotic susceptibility pattern of MRSA.

MATERIAL AND METHODS

Samples:

Total number of 250 samples of sheep (*n*=100), cattle (*n*=50) and human (*n*=100) in contact with these animals (Table 1) were collected from Bassatin slaughter house in Maadi, Cairo in the period between October 2017 and December 2018.

Sterile wet cotton swabs were inserted into the nostrils one by one, with a depth of about 1 cm, and twisted five times. The collected swabs were placed into Stuart transport medium and immediately transferred to the laboratory.

Table (1): Sources and number of the collected samples.

Source of samples	Number of samples
Human	**100**
Sheep	**100**
Cattle	**50**
Total	**250**

Isolation and Identification of *Staphylococcus aureus:*

Each swab was cultured in brain- heart infusion broth (Oxoid, Hampshire, UK) and incubated at 37°C for $24^{h.}$ Two loopfuls from each broth were plated on mannitol salt agar (Oxoid, Hampshire, UK) and 5% sheep blood agar (Oxoid Ltd., Hampshire, UK) and incubated aerobically at 37 °C for 24^{h} . The typical *Staphylococcus* species. Colonies were further examined by Gram staining and traditional biochemical methods according to Quinn **(Quinn *et al*. 2002**) then the antimicrobial resistance was applied.

The Kirby-Bauer disc diffusion method was done to determine the antibiotic susceptibility profiles of the isolates. After incubating overnight on Mueller-Hinton agar at 37°C (Oxoid Ltd., Hampshire, UK), the zones of inhibition were determined, and the interpretation was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines **(CLSI, 2016).** *S. aureus* isolates were tested against the following different antibiotics**:** chloramphenicol (CHL) (30 µg/ disc), clindamycin (CLI) (2 µg/disc), erythromycin (ERY) (15 µg/disc), linezolid (LZ) (30 µg/disc), Ofloxacin (OFX) (5 µg/disc), cefoxitin (FOX) (30 µg/disc), oxacillin (OXA) (1 µg/disc), trimethoprim-sulfamethoxazole (SXT) (23.75 µg/disc) and VAN (30 µg/disc). The discs were purchased from Oxoid Ltd. (Hampshire, UK).

Molecular charachterization:

DNA extraction:

In order to extract DNA from bacteria, the boiling method was performed. Briefly, the bacterial colonies were inserted into a sterile microtube filled with 1 ml distilled water. Then the suspension was boiled for 5 minutes at 100°C and frozen for 5 minutes, Boiling was repeated for 5 minutes followed by -for 10 minutes at 3,000 (rpm).The supernatant containing DNA was used as template for PCR amplification.

MRSA and MSSA isolates were subjected for the detection of *nuc*, *mecA* genes using the primers depicted in (Table 2).

PCR:

PCR conditions were done according to **(Al-Soud, 2019)** for *nuc* gene detection and **(Tiwari and Sen, 2006**) for *mecA* gene detection.

The amplification products were identified by electrophoresis in a 1.5% agarose gel (Sigma, Darmstadt, Germany) stained with 1 μg/ml of ethidium bromide (Sigma,Darmstadt, Germany) in 1x TAE buffer for 30 min before being visualized under UV light and photographed.

Table (2): primer sequences of *nuc* and *mecA* genes of *S. aureus*.

Gene	Primer	Seqence (5' to 3')	Amplicon size (bps)	Reference
mecA	**Forward**	**5'-CTTCCACATACCATCTTC-3'**	**310 bp**	**(Tiwari &Sen, 2006)**
	Reverse	**5'-CTTGTAGTTGTCGGGTTT-3'**		
nuc	**Forward**	**5'-GCGATTGATGGTGATACGGTT-3'**	**279 bp**	**(Al-Soud, 2019)**
	Reverse	**5'-CAAGCCTTGACGAACTAAAGC -3'**		

RESULTS

Occurrence of *S. aureus*:

Sixty out of 100 human nasal swab samples (60%), 6 out of 100 sheep nasal samples (6%) and 4 out of 50 cattle nasal swab samples (8%) were positive for staphylococci.

As illustrated in (Table 3), Staphylococci isolates were 70 (28%) of the total examined samples.

Of the 250 nasal swabs examined, *S. aureus* was isolated from human 53/100 (53%), from sheep 4/100 (4%), and from cattle 3/50 (6%) samples (Table 3).

Table (3): Prevalence of *S. aureus* isolates among the samples.

Samples	Number of samples	Staphylococci isolates		*S. aureus* isolates to the total samples	
		No.	%	No.	%
Sheep nasal swabs	100	6	6	4	4
Cattle nasal swabs	50	4	8	3	6
Human nasal swabs	100	60	60	53	53
Total	250	70	28	60	24

%: was calculated according to the total number of examined samples.

Antimicrobial sensitivity of *S. aureus* isolates:

It from (Table 4) all *S. aureus* isolated from sheep, cattle and human nostrils were sensitive to vancomycin (100%) and resistant to oxacillin. of *S. aureus* isolated from sheep 100 % and 75% are resistant to oxacillin and cefoxitin, respectively and 100% sensitive to trimethoprim-sulfamethoxazole,linezolid,erythromycin,clindamycin, chloramphenicol while it was sensitive to ofloxacin in a percentage of 75%. of *S. aureus* isolated from cattle nasal swabs 100% were resistance to oxacillin and 33% were resistance to ofloxacin.while 100% weresensitive to vancomycin, trimethoprim-sulfamethoxazole, cefoxitin, linezolid, erythromycin, chloramphenicol, clindamycin. The sensitivity to ofloxacin was 66.6% concerning *S. aureus* isolated from human nasal swabs.Then showed a resistance pattern 100% for erythromycin, cefoxitin and oxacillin and the sensitivity for chloramphenicol, linezolid, trimethoprim-sulfamethoxazole and vancomycin was in 100%.

Table (5) illustrates that 10 out of 60 *S. aureus* isolates (16.6%) were resistant to oxacillin, cefoxitin and erythromycin.The occurrence of MRSA was 50,25 and 13.2% among *S. aureus* isolated from sheep, cattle and human nasal swabs respectively.

Table (4): Antimicrobial sensitivity testing of *S. aureus* isolates.

Antimicrobial discs									
CHL	**CLI**	**ERY**	**LZ**	**OFX**	**FOX**	**OXA**	**SXT**	**VAN**	
Sheep nasal swab (4 isolates)									
(4)100%	**(4)100%**	**(4)100%**	**(4)100%**	**(3)75%**	**(0) 0%**	**(0)0%**	**(4) 100%**	**(4)100%**	**S**
0%	**0%**	**0%**	**0%**	**0%**	**(3)75%**	**(4) 100%**	**0%**	**0%**	**R**
0%	**0%**	**0%**	**0%**	**(1) 25%**	**(1)25%**	**0%**	**0%**	**0%**	**I**
Cattle nasal swab (3 isolates)									
(3)100%	**(3)100%**	**(3)100%**	**(3)100%**	**(2) 66.6%**	**(3) 100%**	**(0) 0%**	**(3)100%**	**(3)100%**	**S**
0%	**0%**	**0%**	**0.00%**	**(1).33.3%**	**0.00%**	**3(100) %**	**0.00%**	**0.00%**	**R**
0%	**0%**	**0%**	**0.00%**	**0.00%**	**0.00%**	**0%**	**0%**	**0.00%**	**I**
Human Nasal swabs (53 isolates)									
(53)100%	**(25) 47%**	**0%**	**(53)100%**	**(25) 47%**	**0%**	**(0) %**	**(53)100%**	**100%**	**S**
0%	**(26)49%**	**100%**	**0%**	**(26) 49%**	**(53)100%**	**(53)100 %**	**0%**	**0%**	**R**
0%	**0%**	**0%**	**0.00%**	**0%**	**0%**	**0%**	**0%**	**0%**	**I**

CHL: **chloramphenicol,** ***CLI:*** **clindamycin, ERY: erythromycin,** ***LZ*****: linezolid,** ***OFX:*** **Ofloxacin,** ***FOX:*** **cefoxitin,** ***OXA*****: oxacillin,** ***SXT*****: trimethoprim,** ***VAN*****: vancomycin.**

Table (5): Prevalence of MRSA among *S. aureus* isolates.

Source of isolates	Number of *S. aureus* isolates	MRSA isolates	
		No	%*
Sheep nasal swab	**4**	**2**	**50**
cattle nasal swab	**3**	**1**	**33.3**
Human nasal swab	**53**	**7**	**13.2**
Total	**60**	**10**	**16.6**

*** %was calculated according to the number of positive *Staphylococcus* spp. isolates**

Detection of *nuc* and *mecA* genes among the *S.aureus* isolates:

nuc gene was detected in 100 % of the isolates, Fig. (1) shows the agarose gel electrophoresis of (PCR) of the strains containing *nuc*A genes,From 10 MRSA isolates, 8 were positive for *mecA* gene, Fig. (2).

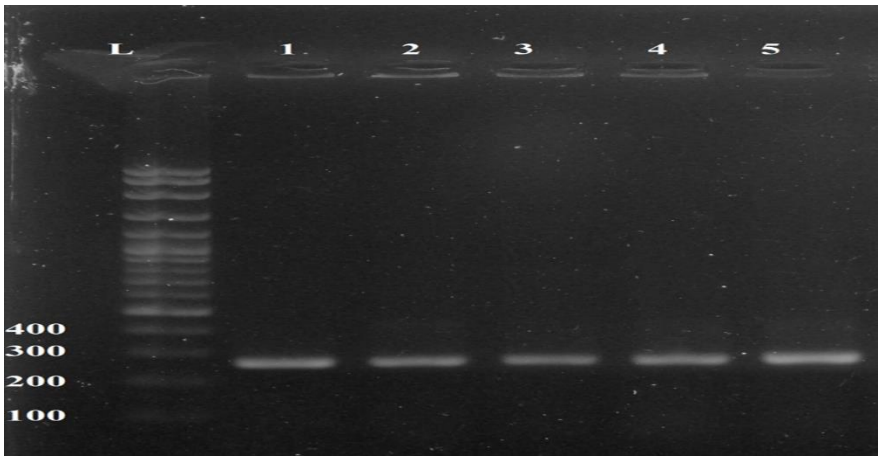

Fig. (1): Amplicon of *nuc* gene; lane 100 bp L molecular size ladder; lane 1: positive control; lanes 2 to 5: positive samples as indicated by the 279 bp PCR products.

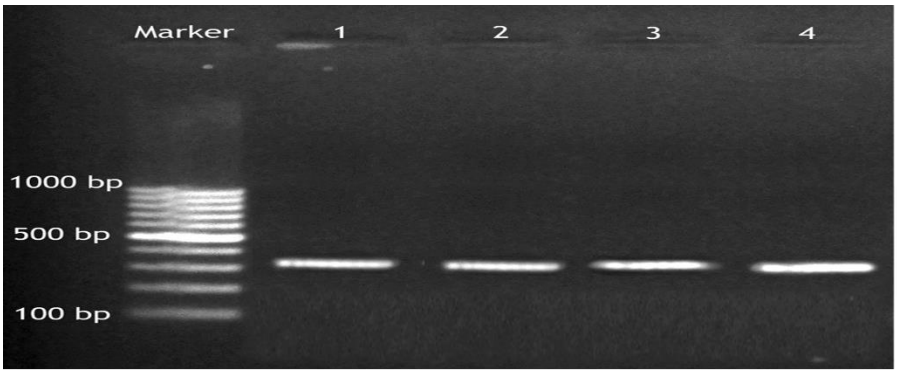

Fig. (2): Amplicon of *mecA* gene; lane L: 100 bp molecular size ladder; lane 1: positive control; lanes 2 to 4: positive samples as indicated by the 310 bp PCR products.

DISCUSSION

Nowadays, emergence of antibiotic - resistant bacteria, especially MRSA is not only a universal public health challenge but also an emerging veterinary concern throughout the world **(Kluytmans *et al.*, 1997**). After the introduction of ß-lactam antimicrobials, the prevalence of MRSA infections and colonization in food-producing animals has gradually increased over time. **(Rahman *et al*., 2018; El-Deeb *et al*., 2018).**

The present study indicated a relatively low prevalence of *S. aureus* nasal carriage (6%) in healthy cattle. This finding agrees with earlier reports from Norway and Sweden that evaluated potential sources of *S. aureus* in dairy herds **(Mork *et al*., 2005; Capurro, 2010**). However, our investigations showed low prevalence of *S. aureus* nasal carriage in healthy sheep such a result disagrees with those previously done Norwegian sheep **(Vautor *et al*., 2005; Mork *et al*., 2012).**

The overall occurrence rate of MRSA among examined animals was in 2% sheep and cattle. The isolation rate in this study was lower than those recorded by **Nemeghaire *et al*., (2014**) **and Alzohairy, (2011)** as the later examined healthy bovines and the prevalence were 28.9%, 15.5% in healthy sheep and cow respectively. On the other hands, our result was nearly similar to that reported after examining cattle and calves in Switzerland 0.3% and 1% **(Huber *et al*.,2010).** On the contrary, all *S. aureus* isolates in the investigated apparently healthy animal from Tunisia **(Gharsa *et al*., 2015**) and China **(Zhou *et al*., 2017)**were methicillin - susceptible *S. aureus* (MSSA). The rate of MRSA nasal carriage in healthy individuals in this study is (13.2%) is higher than the rate reported by **Benslama *et al*.,(2011)** in Tunisia (0.24%). It is interesting to remark that, the MRSA-positive person was a veterinarian who worked with farm animals. Studies performed in other countries also showed low nasal carriage of MRSA among healthy populations **(Bloomfield *et al*., 2007)**, although this prevalence seems to be higher among people in contact with farm animals **(Loeffler *et al*., 2010; Van *et al*., 2010).** The present study concluded that 3 out of the 7 *S. aureus* isolates were found to be MRSA strains (42.8%) isolated from animal samples.

MRSA is probably the best example of a prevalent and important multidrug resistant bacterium that has successfully transitioned from an almost exclusively nosocomial setting to being widespread in the community **(Duin *et al*., 2016)**.

Lower rates were recorded in healthy human, where 7 out of 53 *S. aureus* isolates were found to be MRSA strains (13.2%), so the total number of MRSA isolates was 10 out of 60 isolated from all, the examined samples in the present study (16.4%). It was such a lower rate that recorded by **Nsofor *et al*., (2016)** which was 38.5%. the present study, 7 *S. aureus* isolated from animal samples were examined by the antimicrobial sensitivity test, and revealed high sensitivity against vancomycin (100%).The finding does not agree with that of **Negash (2015)** who reported that,all animal isolates were found susceptible to gentamycin (100%) in addition to vancomycin.The animal isolates were sensitive to timethoprim+sulfamethoxazole, linezolid,

erythromycin, clindamycin and chloramphenicol. Similarly, a higher resistance of oxacillin (100%) and cefoxitin (75%) were reported by **(Nagash 2015).** On the other hand, we tested 53 *S. aureus* isolates from the healthy human for antimicrobial sensitivityandtheyshowedhigh sensitivity to vancomycin (100%), timethoprim + sulfamethoxazole (100%), linezolid (100%), chloramphenicol (100%), whereas the isolates were highly resistant to oxacillin, cefoxitin and erythromycin. The results obtained in this study reflect the extent of misuse of antibiotics in the treatment of bacterial infections in both animals and human, which may lead to antibiotic resistance. As confirmed by **Frieden (2013)**, the animal, the environment and the infected persons play a vital role in the spread of bacteria. The differences in the sample size and geographical variations may lead to the discrepancy in the prevalence of MRSA.

The prescence of *mecA gene* which encodes a modified penicillin-binding protein (pBp), i.e., PBP2a is a useful molecular marker of ß-lactam resistance in *Staphylococci* **(Mulligan *et al*., 1993; Pinho *et al*., 2001).** Hence, PCR amplification of the *mecA* gene had been used in this study for specific identification of MRSA among the oxacillin-resistant *S. aureus* isolates and the same was carried out by **Choi *et al*., (2003) and Kalhor *et al*., (2012).** Existence of *mecA* gene is the major proof for the recognition of MRSA isolate. This was approved by numerous studies: in Egypt **(Hafez *et al*., 2009)**, in Japan **(Hotta *et al*., 1999)**, in Spain **(Del-Valle*et al*.,1999)**,inEngland **(Hartman and Tomasz 1984)(Wongwanich *et al*.,2000).** The absence of *mec*A gene within resistant staphylococcal isolates that are phenotypically MRSA suggest a possibility of hyperproduction of β-lactamase as a cause of the phenomenon **(Olayinka *et al*., 2009).** Recently Ba and colleagues mentioned specific variations in different amino acids present in protein binding proteins cascade (PBPs 1, 2, and 3) which may be the base of resistance **(Ba *et al*., 2014)**. These variations were found to include three amino acid substitutions which were identical and were present in PBPs 1, 2, and 3. Moreover, the same amino acid was found to have two other different substitutions in PBP1. Both the identical and different amino acid substitutions were detected in isolates from different multilocus types **(Ba,X *et al*.,2014).** These outcomes provided perfect evidence that there are mechanisms other than the presence of *mec*A gene responsible for beta-lactam resistance of MRSA and that molecular methods alone are not sufficient for definite characterization of MRSA isolates.

CONCLUSION

Our findings show that, the nares of healthy ruminant may represent a reservoir for MRSA, This highlights the need for further extensive research to devise appropriate control and prevention strategies. In addition, the absence of *mecA* gene in a considerable percentage of MRSA isolates requires finding alternative genetic methods for detection of MRSA.

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