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Genetic Expression of *Mec*A Gene in Methicillin Resistant *Staphyococcus aureus* (MRSA) Strains of Animal and Human Samples

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Abstract It is important to understand the zoonosis ,incidence and the relation between *Staphylococcus aureus* infection especially Methicillin Resistant *Staphylococcus aureus* (MRSA) in human and animal in Egypt and its public health hazards.Samples from human and animal origin suspected to have *Staphylococcus aureus* infections were collected from Al Fayoum, Giza, BeniSuef and Cairo Governorates under complete aseptic conditions. Isolation and identification of *Staphylococcus aureus* using standard methods, antibiogramtesting to select the multidrug resistance strains and detection of ***mec*A** gene and ***hlg*** gene in multidrug resistance strains by polymerase chain reaction (PCR) were done. Results showed that, 91.9% of the human nasal swabs were positive for *Staphylococcus aureus* while only 8% of them revealed non *Staphylococcus aureus* isolates. All animal samples were positive for *Staphylococcus aureus* except 21 cases of poultry chronic respiratory disease (CRD) where all of them have microorganisms other than Staphylococcus 38 (66.6%) of total 57 human nasal swabs were Methicillin Resistant *Staphylococcus aureus* (MRSA)and only 19 samples (33.3%) were non-Methicillin Resistant Staphylococcus aureus (MRSA). All sheep abscess pus and bumble foot samples were MRSA. While, 7 (77.7%) out of 9 mastitic milk samples were Methicillin Resistant *Staphyococcus aureus* (MRSA) and only 2 (22.2%) of these 9 samples were non-Methicillin Resistant *Staphyococcus aureus* (MRSA). All Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates either from human or animal origin were ***mec*** A gene positive by PCR. On the other hand, only 80% of non-Methicillin Resistant *Staphyococcus aureus* (MRSA) isolates of human origin were positive for ***mec*A** gene by (PCR). The highest percent of ***hlg*** gene PCR positive results were represented in Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates of animal origin 80%. Methicillin Resistant *Staphylococcus aureus* (MRSA)isolates of human origin with ***hlg*** gene PCR were 60% which is higher than those non-Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates of human origin which was 40%.Four*Staphylococcus aureus* isolates of human origin were non-Methicillin Resistant *Staphylococcus aureus* (MRSA) by disc diffusion and they were positive for ***mec* A** by PCR. The PCR results and the results of disc diffusion method were correlated in 11 isolates out of 15 animal and human isolates. The strains isolated from human and animal that showed haemolysis on sheep blood agar were positive for ***hlg*** gene by PCR.

Keywords MRSA, PCR, ***mec*A** gene, Human, Animals

1. Introduction

S. aureus could cause mastitis in cows, sheep and goats, leading to severe economic losses worldwide [1, 2].

MRSA has been found to colonize live stock including pigs, cattle and poultry. Since many of the MRSA clonallineages identified in livestock were un-Common for MRSA isolates found until then in human hosts, the term ‘‘livestock-associated MRSA’’ (LA-MRSA) has been introduced to distinguish these MRSA from classical human hospital-acquired (HA-MRSA) or community-associated MRSA (CA-MRSA) [3].

A part from having pathogenic versatility, *S. aureus* can adapt rapidly to the selective pressure of antibiotics, with the emergence and spread of methicillin-resistant *S.aureus* (MRSA) isolates being a relevant example. MRSA was first described in 1961, the year in which methicillin was marketed [4], and actually most of the nosocomial *S. aureus* infections are caused by methicillin-resistant *S.aureus* strains [5], which have become a widely recognized cause of morbidity and mortality throughout the world [6]. *S. aureus* becomes methicillin resistant by the acquisition of the ***mec*A** gene which encodes a penicillin binding protein (PBP2a)

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with a low affinity for β-lactamas. The strains producing PBP2a are resistant to all β-lactams [7]. Thus, MRSA strains resistant to quinolones or multiresistant to other antibiotics have been emerging, leaving a limited choice for their control [8].

So, the aim of this work is the characterization of MRSA strains from human and animal phynotypically and genotypically. Hemolysin gene was used as an indicator for *Stapylcoccus aureus* by PCR and ***mec*** A gene was used as an indicator for MRSA.

2. Methods

Sample collection

This study was carried on 100 samples from human and animal (poultry, cow and sheep) source. The samples were collected through 2013-2014. They were collected from Al Fayoum, Giza, BeniSuef and Cairo Governerates. Human samples were from nasal swabs of respiratory infected patients, bumble foot swabs, caseated material from poultry CRD. Mastitic milk samples and sheep swab from pus of abscess were collected.

- Isolation of *S. aureus* was done according to standard methods [9].
- Identification of isolated *Staphylococci* was done according to standard methods [9].
- Sensitivity of isolated *S. aureus* was done according to standard methods [9].

From the 100 isolates including human and animal samples, representative samples will be exposed to PCR for ***mec*A** gene and ***hlg*** gene presence confirmation in the central laboratory for veterinary quality control on poultry production in Dokki.

Extraction of DNA was done according to QIAamp DNA mini kit instructions .Preparation of PCR Master Mix for PCR according to Emerald Amp GT PCR mastermix (Takara) Code No. RR310Akit. Cycling conditions of ***mec*A** primers during PCR was done according to standard methods [10]. Cycling conditions of ***hlg*** primers during PCR was performed according to standard methods [11]. Agarose gel electrophoreses was done according to standard methods [12].

3. Results

S.aureus isolated from different animal samples are higher than in human samples. While *Staphylococcus spp*. other than *S.aureus* are higher in human samples than in animal samples. That's to say, 91.9% of the human nasal swabs were positive for *S.aureus* while only 8% of them reveled non *S.aureus* isolates and all animal samples were *S.aureus* except for the 21 case of poultry CRD where all of them were negative. It was also found that MRSA isolates were higher in animal samples than in human samples. non-MRSA isolates were higher in human samples than in animal samples.38 (66.6%) of total 57 human nasal swabs were MRSA and only19 samples (33.3%) were non-MRSA. All sheep abscess pus and bumble foot samples were MRSA. While, 7 (77.7%) out of, 9 mastitic milk samples were MRSA and only, 2 (22.2%) of these 9 samples were non-MRSA.

All *S. aureus* isolates either from animal or human origin were found to be haemolytic on sheep blood agar.

Table (1) shows that all MRSA isolates either from human or animal origin were ***mec*A** gene positive by PCR. On the other hand, only 80% of non-MRSA isolates of human origin were positive for ***mec*A** gene by PCR. Photo (1) illustrated a 391 bp for ***mec*** Agene in positive samples from animal and human. Sample in lane 4 was negative for ***mec*A** gene by PCR.

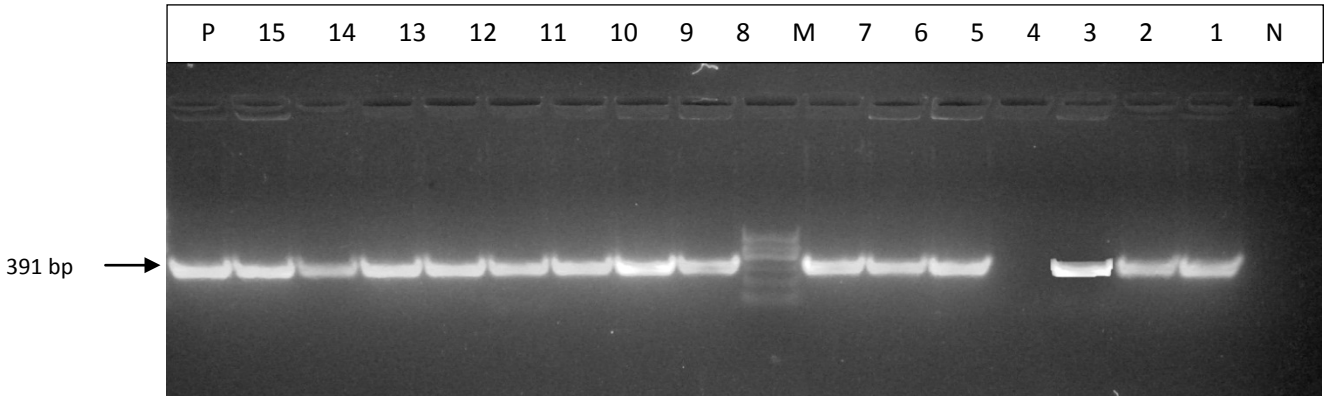
Table **(2)** showed that the highest percent of ***hlg*** gene PCR positive results were represented in MRSA isolates of animal origin 80%. MRSA isolates of human origin with ***hlg*** gene PCR were 60% while, the percent of non-MRSA isolates of human origin which was 40%. Photo(2) Illustrated a 937 bp for ***hlg*** gene in MRSA strains from animal and human. Positive samples for ***hlg*** gene were from MRSA isolated from animals; lane 5,6 and 10 and from human lane 1,3 and 13. Positive samples from non-MRSA isolates from human were clear in lane 7 and lane 12.

Table (1). Incidence of *mec*A gene from MRSA and non-MRSA isolates of animal and human samples by PCR

Types of isolates	Total No.of examined samples	Positive ***mec*A** gene		Negative ***mec*A** gene	
		No.	%	No.	%
Human MRSA	5	5	100	0.0	0
Human non-MRSA	5	4	80	1.0	20
Animal MRSA Cow mastitic milk	5	5	100	0.0	0

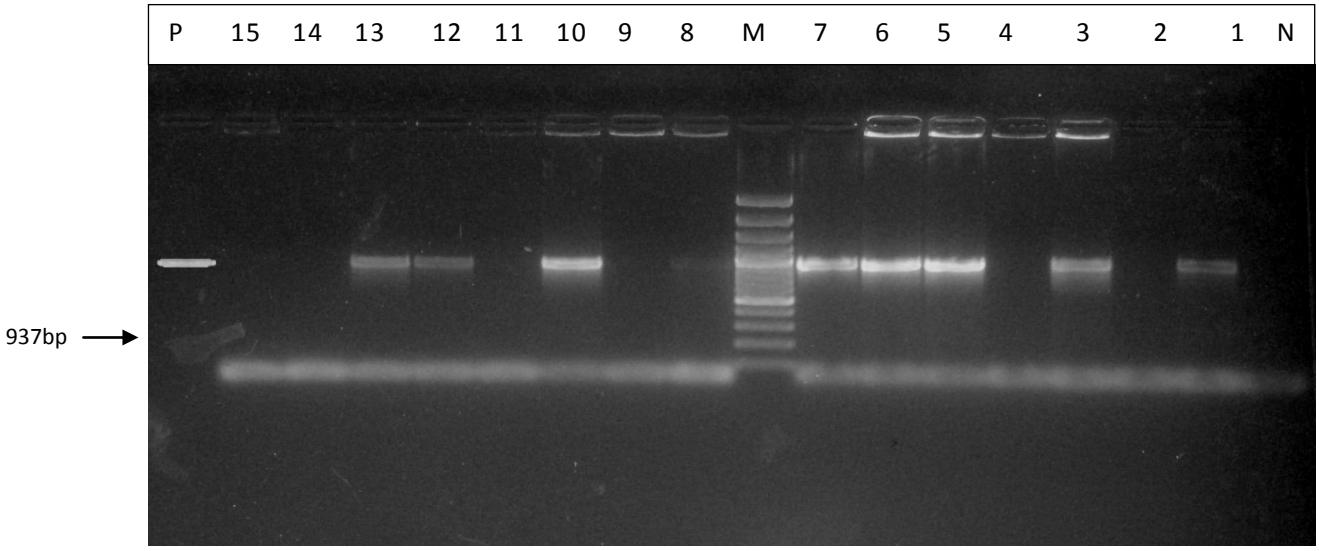
Table (2). Incidence of *hlg* gene in MRSA and non-MRSA isolates among animal and human samples by PCR

Types of isolates	Total No. of examined samples	Positive ***hlg*** gene		Negative ***hlg*** gene	
		No.	%	No.	%
Human (MRSA)	5	3	60	2	40
Human (non-MRSA)	5	2	40	3	60
Animal (MRSA) Cow mastitic milk	5	4	80	1	20



M: Marker (100-600 bpladder). P: Positive control for ***mec*A** gene (*S.aureus* (MRSA)). N: Negative control for ***mec*A** gene (*E.coli*). Lanes (1-3, 13 & 14): Human MRSA isolates were positive for ***mec*A** gene. Lane (4): Human MRSA isolate was negative for ***mec*A** gene. Lanes (5-6, 8, 10 &15): Animal MRSA isolates were positive for ***mec*A** gene. Lanes (7, 9, 11 & 12): Human non-MRSA isolates were positive for ***mec*A** gene

Photo (1). Electrophoretic pattern of ***mec*A** gene in MRSA strains isolated from animal and human samples



M: Marker (100-3000 bp ladder). P: Positive control for ***hlg*** gene (*S.aureus* (MRSA)). N: Negative control for ***hlg*** gene (*E. coli*). Lanes (1, 3 & 13): Human MRSA isolates were positive for ***hlg*** gene. Lanes (2 & 14): Human MRSA isolates were negative for ***hlg*** gene. Lanes (4, 9 & 11): Human non-MRSA isolates were negative for ***hlg*** gene. Lanes (5, 6 & 10): Animal MRSA isolates were positive for ***hlg*** gene. Lanes (7 & 12): Human non-MRSA isolates was positive for ***hlg*** gene. Lanes (8 & 15): Animal MRSA isolate negative for ***hlg*** gene.

Photo (2). Electrophoretic pattern of ***hlg***gene of MRSA isolated from animal and human samples

On comparison between the result of methicillin resistance by disc diffusion method and the presence of ***mec*A** gene among MRSA and non-MRSA isolates, 4 *S.aureus* isolates of human origin were non-MRSA by disc diffusion and they were positive for ***mec* A** by PCR. The PCR results and the results of disc diffusion method were correlated in 11 isolates out of 15 animal and human isolates.

On comparison between the result of haemolysis on sheep blood agar and the presence of ***hlg*** gene among MRSA and non-MRSA isolates. All the strains isolated from human and animal that showed haemolysis on sheep blood agar were positive for ***hlg*** gene by PCR.

Results of PCR for the detection of ***mec* A** gene and ***hlg***gene among MRSA and non-MRSA strains isolated from animal and human isolates, there were 5 MRSA isolates positive ***mec*A** gene and in the same time negative for ***hlg*** gene. One MRSA isolate from animal was +ve***mec*A** gene and –ve for ***hlg*** gene. Two MRSA isolates from human were +ve***mec*A** gene and –ve for ***hlg*** gene. Two non-MRSA isolates from human were +ve***mec*A** gene and –ve for ***hlg*** gene. Seven MRSA isolates (4 animal and 3 human) were positive for both genes. While, 2non-MRSA isolates were positive for both genes.

4. Discussion

The primary habitat of *S. aureus* is in the nasal passage on the skin and hair of human and warm-blooded animals. The transmission of the organisms may occur through skin lesions, contaminated food, including milk and other animal products [13]. *S. aureus* also could cause mastitis in cows, sheep and goats, leading to severe economic losses worldwide [2]. In humans, this bacteria causes food poisoning, toxic shock and variety of pyogenic infections [14].

Concerning the human samples included in our study, 57(91.9%) isolates out of 62 *Staphylococcus* isolates were *S. aureus* while only 5 (8%) isolates were non *S. aureus.* These results disagreed with **Habeeb *et al.*** [15] who detected *S.aureus* in 90 (18.4%) of 489 students carried *S.aureus*.

S. aureus was isolated from nasal swabs of 102 (40.8%) out of the 250 volunteers in Brazil [16]. In northern Pakistan, the nasal colonization of *S. Aureus* was documented in 86 out of 360 students (24%) [17]. In the United States from 2003-2004, the *S. aureus* carriage rate in the civilian non-institutionalized population according to the National Health and Nutrition Examination Survey was 28.6% [18]. *S.aureus* was isolatedin a low percentin India as the nasal carriage rate of *S. aureus* was 13% and it was only 12.6% in Sudan [19], and **Onanuga and Temedie** [20] found that 40 (33.3%) *S. aureus* strains were isolated from the nasal swabs screened in Nigeria.

Resistance to several drugs was determined by plating on trypticase soy agar containing antibiotics. After 24h incubation, growth of more than two colonies was determined as resistance. In addition, resistance to meticillin was detected on oxacillin resistance screening agar (Mueller–Hinton agar+oxacillin) and was confirmed by screening forpenicillin-binding protein 2a (Slidex MRSA detection; Denka Seiken) [21]. In the present study, 57 Human nasal swabs showed 38 isolates (66.6%) of MRSA strains. This result disagreed with **Habeeb *et al.*** [15] who found that only 10 (2.04%) of the students were found to be MRSA carrier and he exceeded that the nasal carriage of MRSA among the *S. aureus* isolates was 11.1% [15]. In Palestine in 2011, it was found that the nasal carriage of MRSA among the students was 2.5% [17]. In 2010, a total of 322 university students in Taiwan were screened and 2.2%, of them harboured MRSA [22]. In Pakistan, from 2007 to 2008, MRSA isolated from nasal swabs from anterior nares was 1.5% [23]. In 2001-2002, it was reported that national MRSA colonization prevalence in USA was 0.8% [24].

There have been few investigations of MRSA in poultry. Two reports describe MRSA isolation from healthy and sick chickens [25, 26], but there are limited prevalence and incidence data. Two recent studies reported isolation of sequence type 398 (ST398) from healthy chickens [27, 28] and a third, involving characterized isolates from infected ultry, reported the predominance of a common human epidemic clone (clonal complex 5) [29].

To determine whether MRSA is present in poultry, 50 laying hens and 75 broiler chickens were examined. MRSA was found in some broiler chickens but no laying hens. In all samples, spa type t 1456 was found [30].

Youssef and Hamed [31] performed a study as they confirmed that to the best of our knowledge Staphylococcosis caused by *S. aureus* impacts on chicken broilers. Its public health hazards have not been illustrated in Egypt. As their study aimed to estimate the incidence, antibiotic resistance profile and zoonotic implications of *S. aureus* (MRSA) related arthritis in some broiler farms where they took samples from birds with clinical findings of depression and inability to stand, arthritis with swelling and local worming at hock and stifle joints and necropsy showed whitish to yellowish exudates at affected joints were collected from 20 broiler farms at finishing ages (>30 days) located at Ismailia governorate, Egypt. Swabs from joint exudates were tested for *S. aureus* on the basis of cultural and biochemical properties and confirmed by PCR amplification of 16S rRNA gene. Results showed that, 13/20 (65%) farms, 110/200 (55%) arthritic birds, 7/60 (11.7%) apparently health and 7/20 (35%) litter samples were positive for *S. aureus*. Coagulase positive strains were isolated from 11 (65%), 93(46.5%), 5 (8.3%) and 6 (30%). The *in vitro* antibiotic sensitivity test revealed that 58.2% of the isolates were completely resistant, 35.3% were moderately sensitive and 6.5% were highly sensitive to 17 different antibiotic discs. Complete antibiotic resistance to methecillin and cloxacillin, oxytetracycline and Sulbactin-Ampicillin were observed in all isolates.

In farm workers, 14 (31.1%) of 45 were *S.aureus* carriers. All human isolates were multi-drug resistant (MRSA) strains and none of the workers had skin affection. In conclusion, MRSA infections were prevalent among broilers at the finishing ages; it was a potential cause of economic losses by arthritis and posing a health hazard of zoonotic transmission to human contacts and consumers [31].

In the present study, we could detect *S. aureus* isolates among 15 animal samples out of 100 examined samples, out of which 9 samples were mastitic milk due to *S. aureus* infection. The rest of animal isolates were 3 isolates from sheep abscess and 3 isolates from bumble foot of poultry. The rest of the 21 isolates from poultry CRD were microorganism other than *Staphylococcus.* From the isolated *S.aureus*,13 isolates from animal samples represented by 86.6% out of which 3 isolates (100%) from bumble foot of chicken and 3 isolates (100%) were from sheep pus from abscess were methicillin resistant by using disk diffusion technique.

Concerning animal samples, all sheep abscess pus and bumble foot samples were MRSA. While, 7(77.7%) out of 9 mastitic milk samples were MRSA and only 2(22.2%) of these 9 samples were non–MRSA isolates. *S. aureus*is one of the most important bacterial pathogens in bovine mastitis, a disease that causes significant economical losses in the milk industry; thus, *S. aureus* in general and MRSA in particular have been the focus of several studies in dairy cattle. **Devriese and Hommez** [32], were the first to report MRSA

in bovine mastitis milk comes from Belgium where in **1972 where** isolated strains that, using biotyping methods, appeared to be of human origin.

On the other hand, in the present study, 7(77.8) MRSA isolates out of total 9 *S.aureus* isolates were from cow mastitic milk. While, only 2 (22.2%) *S.aureus* isolates showed non-MRSA isolates. A study in the Republic of Korea was performed and isolated a small proportion (0.4%) of MRSA among 3047 bacterial isolates from bovine mastitis milk [33]. Alsoin Switzerl and in Japan, MRSA was isolated only from 1.4% and 1.10% of mastitic milk samples, respectively [34, 35]. Regarding human samples, 57 isolates out of 62 examined samples were suspected MRSA by using disc diffusion technique where our results agreed with **Rushdy *et al.*** [36] who isolated out of 200bacterial isolates tested, 83 (41.5%) were confirmed as *S. aureus* of which 51 (61.45%) were oxacillin resistant (ORSA). Out of 51 isolates 26 had single resistance (oxacillin resistance), while 25 had double resistance (oxacillin & methicillin) resistance (MRSA/ORSA).

The pathogen city of *S. aureus*, is related to the production of a wide variety ofexoproteins, including alpha and beta haemolysins which contributes to its ability to cause diseases in many mammalian species [37]. The incidence of haemolytic *S.aureus* isolated from animal and human samples in the present study was 100% of *S.aureus* isolates were haemolytic on sheep blood agar. It was found that characterization of haemolysinphenotypically based on haemolysis pattern of *Staphylococcus aureus*on sheep blood agar plate revealed only an alpha haemolysis pattern (18%), beta haemolysis (27%) and gammahaemolysis (54%) [38]. Meticillin resistance is conferred by carriage of the ***mec*A** gene [39],which is carried by a mobile exogenous genetic element known as the staphylococcal cassette chromosome ***mec*(SCC*mec*)** [40].

In the present study resistance to methicillin was determined by the methicillin disk susceptibility test and confirmed by ***mec*A** by PCR [41]. All MRSA isolates in the present either from human or animal origin were ***mec*A** gene positive by PCR. While, 80% of non-MRSA isolates of human origin were ***mec*A** gene positive by PCR. It is interesting to note that 4/5 human non-MRSA isolates were positive for ***mec* A** gene by PCR. While, all 5 human MRSA isolates were positive for ***mec* A** gene by PCR and the same was found concerning the 5 animal MRSA isolates. The **mecA** gene which lies in the **SCCmec** resistance island [42], were found to be carried by 95% of the isolates that display a phenotype of methicillin resistance and was detected in all multiresistant S. aureus isolates which agrees with the results in the present study [43].

Habeeb *et al.* [15] found that PCR data showed that all isolates of MRSA typed in his study were positive for ***mec* A** gene where he verified genetic resistance to methicillin by PCR for detection of ***mec*A** gene, which was in agreement with the present study results. Regarding the incidence of ***hlg*** gene in MRSA and non-MRSA isolates among animal and human samples by PCR were represented in MRSA isolates of human origin with ***hlg*** gene PCR positive results by60% which is higher than those non-MRSA isolates of human origin which was 40%. On the other hand, these results showed that the highest percent of ***hlg*** gene PCR positive results were represented in MRSA isolates of animal origin (80%).

On comparison between the results of haemolysis on sheep blood agar and the presence of ***hlg*** gene among MRSA and non-MRSA isolates. We found that, all strains showed haemolysis on blood agar from human and animal were positive for ***hlg*** by PCR. While, those which did not show haemolysis on sheep blood agar, did not possess ***hlg*** gene by PCR. Amplification of the gene encoding haemolysin of *S. aureus* with specific primers showed ***hla*** genes with percentage of 81,81 and ***hla*** combined with ***hlb*** genes in the percentage of 18,18 [38].

While, on comparison between the results of Methicillin resistance and the presence of ***mec*A** gene among MRSA and non-MRSA isolates. It was clear that human no. 7 (non-MRSA) isolate which showed sensitivity to Oxacilline bacteriologically reveled positive PCR for ***mec* A** gene presence. Also, Human no. 9, Human no.11 and Human no.12 (non-MRSA) isolates showed the same variance. On the other hand, the rest 11 isolates out of total 15 isolates gave identical Oxacilline sensitivity PCR result concerning ***mec*A** gene presence either by sensitivity or resistance to those results of traditional disc diffusion test. As a result, there is differences between disc diffusion oxacilline resistance results and PCR results for the ***mec* A** gene.

In contrast to the present study results, ***mec*A** gene was present in all isolates recovered by **Wielders *et al.*** [43] and which was resistant to four or more antibiotic. Moreover, this multiresistance was displayed by the most prevalent and geographically widespread MRSA types (types I, IIa, IIb, and IIIb), which together represented 99% of the ***mec*A** population in Europe. Of the European isolates that appeared to be susceptibletomethicillin in the phenotypic test, 10% nevertheless contained ***mec* A**, and some of these were multiresistant. In contrast, 5% of the phenotypically methicillin- resistant isolates did not carry ***mec*A** and displayed low-level resistance (>8μg /ml). **Rushdy *et al.*** [36] provided an evidence for the presence of ***mec*A** gene at 533 bp in all methicillin resistant isolates. This is supported by the work of **Murakami *et al.*** [21], **Perez-Roth *et al.*** [44] and **Japoni *et al.*** [45] who all detected a DNA fragment of 533 bp in all ***mec*A** gene in positive methicillin resistant *S. aureus*, which was absent in susceptible strains, which all disagreed with results of the present study. That's because the used primers are different from the one used in the present study [21, 44, 45].

In the present study, PCR for the detection of ***mec*A** gene and ***hlg*** gene among MRSA and non-MRSA strains isolated from animal and human samples was performed. There were 5 MRSA isolates positive ***mec*A** gene and in the same time negative for ***hlg*** gene.

In conclusion, virulent drug resistant MRSA was isolated from human and animals. Current data strongly suggest that

MRSA can move between people and animals in households, farms and hospitals whether the individuals are colonized or infected. Farther research is needed to understand the frequency of this cross-species transmission and its risk to animal and human populations. Strict hygienic and preventive measures are needed among animals and human populations and during food processing to avoid colonization of MRSA isolates.

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