Prevalence of Methicillin Resistant Staphylococcus aureus in humans and dairy cattle farms

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Abstract

The current study was carried out in Behera province for isolation, identification of methicillin resistant *Staphylococcus aureus* (MRSA) recovered from workers in human hospitals and animal farms as well as dairy cattle farms. A total of 127 nasal and hand swabs were randomly collected from workers including; health care workers (89) and farm workers (38). In addition, a total of 266 different samples were collected from dairy cattle farms including; nasal swabs of cattle (135), milk (89) and milking machine swabs (42). Isolation of MRSA on Oxacillin Resistant Screening Agar Base (ORSAB) was attempted. Finally, the recovered MRSA isolates were screened for presence of *mecA, mecC* and *PLV* genes by conventional polymerase chain reaction (PCR). The recorded results showed that the prevalence of MRSA in healthcare and farm workers was 29.2 and 36.8%, respectively. Concerning dairy cattle farms, the prevalence of MRSA was 48.14, 30.33 and 90.47 % in the examined samples of cattle nasal swabs, milk and milking machine swabs, respectively. Finally, 34 isolates of MRSA were randomly selected and screened for the presence of *mecA, PVL* and *mecC* genes by conventional PCR and it was found that *mecA* gene was detected in 16 isolates while *PVL* and *mecC* genes could not be detected. Based on the recorded results in this study, it was clear that MRSA colonized workers in both hospitals and animal farms. Also, cattle could be considered as potential source and reservoir of MRSA for humans.

Key words: MRSA, Prevalence, Antibiotic Resistance, Humans, Cattle.

1- Introduction:

Staphylococcus aureus (*S. aureus*) is a gram positive coccus, catalase positive, mainly coagulase positive, non-motile bacterium. It is found in a wide range of habitats including environmental surfaces, in nasal nares of domesticated animals like dogs, cats and horses **(Ryan and Ray, 2004).** In humans, *S. aureus* is found on the mucus membranes of nasal passages and skin. Also, it is well adaptable to antibiotic pressure, therefore it is able to colonize healthy individuals which can be a source of infections and spread among people. People with suppressed immunity are at higher risk of acquiring *S. aureus* infections **(Chambers and DeLeo, 2009).** Treatment of *S. aureus* infections has been difficult due to its ability to resist the commonly used drugs in the hospitals; methicillin was the drug of choice but has been replaced by oxacillin due to significant kidney toxicity. *S. aureus* has been known to possess antibiotic resistance genes; these genes includes *mecA* gene which leads to methicillin resistance. It is resistant to a number of antibiotics which include beta lactam antibiotics like penicillin, amoxylin and oxacillin. In the USA, half of the *S. aureus* infections are resistant to penicillin, methicillin, tetracycline and erythromycin leaving vancomycin as the only drug of choice. Animals can be colonized for prolonged periods without developing clinical signs. The large

polysaccharide capsule of the organism protects it from recognition by the immune system of the cow **(Cenci-Goga et al., 2003)**. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium that causes infections in different parts of the body. It's tougher to treat than most strains of *S. aureus.* It has been specifically isolated from various skins and wound infections including abscesses, dermatitis, severe pyoderma, postoperative wound infections and fistulas. Also, it has been found in other conditions including pneumonia, rhinitis, sinusitis, otitis, bacteremia, septic osteomyelitis, omphalo-phlebitis, metritis, mastitis (Including gangrenous mastitis) and urinary tract infection **(CDC, 2018)**. Cattle can be considered a reservoir of an emerging strain of MRSA infecting human **(Alp and Damani, 2015). Juhász-Kaszanyitzky et al. (2007)** isolated MRSA from cows with subclinical mastitis and from a person who worked with these animals and they noticed that bovine and human strains were indistinguishable by phenotyping and genotyping methods and were of a low frequency spa type. Good hygiene is an important general preventive and control measure, both in hospitals and animal healthcare environments because environmental contamination with MRSA acts as a reservoir for infection. The current work aimed to determine the prevalence of MRSA in healthcare workers and farm workers and investigate the role of dairy cattle in spreading MRSA infection to humans and molecular detection of antibiotic resistance genes of MRSA isolated during the work.

2- MATERIAL AND METHODS:

2.1. Source of samples:

2.1.1.Humans:

A total of 127 nasal and hand swabs were randomly collected from healthcare workers (89) and farm workers (38). Samples of healthcare workers were collected from those in Surgery departments in hospitals under supervision of infection control specialists. They were obtained in sterile swab from anterior nasal where swab was rotated for 4 times in each opening then placed in a sterile test tube containing nutrient broth. On arrival to the laboratory, they were incubated for only 6 hours.

2.1.2. Cattle farms:

A total of 266 different samples were collected from 3 dairy cattle farms including; nasal swabs of dairy cattle (135), milk (89) and milking machine swabs (42).

Two farms had no history of staphylococcal mastitis outbreaks and they had no history of application of lysigin vaccine while the third farm had a history of an outbreak with subclinical mastitis where about 100 cows were diagnosed to be infected with *S. aureus* after one month of lysigin vaccine administration.

Nasal swabs were collected under veterinarian supervision by rotating the swab four times in each anterior opening then placed in a sterile test tube with nutrient broth while milk samples were collected before milking and after scrubbing teat ends with a pledged of cotton moistened with betadine (iodine antiseptic) where a separate pledged of cotton was used for each teat. Also, the first streams of milk were discarded **(Schalm et al., 1971).** At the laboratory, 20 ml of each milk sample were centrifuged at 10.000 xg for a minute then 5 ml of nutrient broth were added to sediments and incubated at 37°C for 12 hours. Finally, milking machine swabs were obtained by rotating the swab for 4 times in each teat cup then placed in a sterile test tube containing nutrient broth.

2.2.Isolation of MRSA:

It was performed in the laboratory of Animal Health Research Institute (AHRI), Behera Branch. Inoculated nutrient broth was incubated at 37°C for 6 hours then a loopful of each inoculated broth was streaked onto the surface of Oxacillin resistant screening agar base (ORSAB) **(Oxoid)** plates then they were incubated aerobically at 37°C for 24-48 hours. MRSA appeared as intense blue glistering colonies in plates. All colonies were picked and preserved into aliquots of nutrient broth with glycerol for further identification.

2.3.Identification of isolated bacteria (Quinin et al., 1994):

Cellular morphology was determined by Gram staining technique where MRSA appeared as violet grasp non-motile bacteria arranged in clusters under microscope. Also, catalase test was performed and positive result was indicated by the development of gas bubbles. Finally, coagulase test was carried out where coagulated plasma was considered positive result.

2.4. Detection of the resistance genes of MRSA by PCR:

DNA extraction was performed by using the boiled-cell method **(Sambrook et al., 1989)**. The nucleotide sequence primer sets required for PCR and the expected amplification products were presented in the following table;

Target gene	Nucleotide sequence (5'to 3')	Amplicon Size (bp)	Reference
mecA	GTTGTAGTTGTCGGGTTTGGCTTCCACAT ACCATCTTCTTTAAC	300-350	**Wielders et al., (2002)**
Pvl	GCTGCACAAAACTTCTTGGAATAT AGGACACCAATAAATTCTGGATTG	85	**Stegger et al., (2012)**
Mec C	GAAAAAAAGGCTTAGAACGCCTCGAAG ATCTTTTCCGTTTTCAGC	138	**Stegger et al., (2012)**

PCR amplification:

The reaction was conducted in a total volume of 25 µl as shown in the following table;

PCR reaction mixture	Reaction volume
5x Taq Master Mix (Jena Bioscience, 5ml)	5µl
PCR grade water	12.5 µl
Forward primer (20 pmol/µl)	1.5µl
Reverse primer (20 pmol/µl)	1µl
Template DNA	5µl
Total	25µl

PCR cycling program:

PCR cycling program was performed in the thermal cycler as presented in the following table;

PCR cycle	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	Cycles	Hold
MecA	94 °C 10 min.	95 °C 1 min.	58 °C 1 min.	72 °C 1 min.	72 °C 10 min.	30	4 °C 20 min.
PVL	94 °C 10 min.	94 °C 5 min.	59 °C 1 min.	72 °C 1 min.	72 °C 10 min.	30	4 °C 20 min.
MecC	94 °C 10 min.	94 °C 5 min.	59 °C 1 min.	72 °C 1 min.	72 °C 10 min.	30	4 °C 20 min.

Detection of PCR products (Sambrook et al., 1989):

Once the PCR was completed, specific amplicons were observed under ultraviolet trans illumination, compared with the DNA ladder and photographed.

3- Results and Discussion:

MRSA has been isolated from most of animals and zoonotic cases have been reported in persons in direct contact with MRSA-colonized livestock, such as farmers, veterinarians, workers at slaughterhouses and transporters of livestock are at high risk of becoming colonized with MRSA. In turn, they may become a source of transmission to animals and other humans. Subsequent contact with household members may transfer the bacteria.

The recorded results in **Table (1) s**howed that the overall estimated prevalence of MRSA in the examined human contacts was 31.5 %. Also, it was noticed that the prevalence of MRSA healthcare workers (HCWs) and farm workers was 29.2 and 36.8%, respectively. The prevalence of MRSA in HCWs was nearly similar to that of **Rahbar et al. (2003) (**35%) and **Gurieva, (2017**) (32.8%) in Iran. On the other side, it was higher than that of **Oh et al., (2001**) in Korea who found that the confirmed rate of the transient carrier rate of MRSA in HCWs was 10.8% and that of the permanent carrier was 2.6 % and **Chen et al., (2015**) who found that the prevalence of *S. aureus* among Chinese HCWs was 21.6 % and among these 4.7% was MRSA. On contrary, it was lower than that of **Hefzy et al., (2016**) who found that the prevalence of nasal carriage of MRSA *S. aureus* among Egyptian HCWs was 58.8%. Several factors may explain the higher prevalence of MRSA among HCWs observed in this study; infection control policies were likely to be ineffective in Egypt. The prevalence of MRSA among farm workers was 36.8% that was nearly similar to that of **Elhaig and Selim, (2015**) (40%) and **Elemo et al., (2017**) (39.6%) while it was lower than that of **Abdelalla et al., (2010**) (56.52%) and **Sarkar et al., (2014**) (70%). On the other hand, it was higher than that of **Asrat et al., (2013**) (13.2%), **Khaleel et al., (2016)** (10%) and **Reshma, (2016**) (28.3%).

Table (1): Prevalence of MRSA in human contacts in relation to occupation

Occupation	No. of examined samples	Positive	
		No.	%
Healthcare workers	89	26	29.2
Farm workers	38	14	36.8
Total	127	40	31.5

Fisher's exact test: P = 0.41(Non-significant)

The close contact between dairy cows and humans, particularly in the milking parlor may create the opportunity for the transmission of bacteria between hosts. The effect of animal contact on the prevalence of MRSA showed that the group with history of animal contact (30.67%) had slightly lower prevalence than the other group with history of no animal contact (32.69%) **(Table, 2)**. The presence of MRSA in those with animal contact may be explained by the fact that most farm workers lived in farms and had their own animals so they had a contact with one or more species of animal all day and many studies had confirmed that the persistence of MRSA carriage in farmers was associated with duration of animal contact. Also, MRSA prevalence dropped during a low exposure period and this was strong evidence for a relation with animal exposure that could be clearly noticed in the current study and it could explain the higher prevalence among farm workers (36.8%) compared to HCWs (29.2%). The presence of

MRSA in farm workers is strongly animal-exposure related and it was suggested that MRSA was a poor persistent colonizer in most humans. This observation that MRSA carriage is lower after a longer period of low exposure are both in line with the hypothesis that exposure to MRSA-positive animals plays a major role in MRSA carriage in farmers and this hypothesis according to **(Graveland et al., 2011).**

Table (2): Prevalence of MRSA in human samples in relation to animal contact

Animal contact	No. of examined samples	Positive	
		No.	%
Contacts	75	23	30.67
Non-contacts	52	17	32.69
Total	127	40	31.49

Fisher's exact test: P = 0.84 (Non-significant)

MRSA reservoirs included dogs, cats, pet birds, cattle, zoo animals and marine mammals and MRSA isolates can be shared between animals and people in close contact. Prevalence of MRSA in human contacts in relation to contact with different animal species was presented in **Table (3)**. It was observed that healthcare workers with history of cattle contact scored the highest prevalence (100%) followed by those with history of pet animals' contact (27.27%) then those with history of poultry contact (20.83%) and lastly those with history of equine contact (0.0%).

Table (3): Prevalence of MRSA in human contacts in relation to contact with different animal species

Animal species	Health care workers			Animal Farms workers		
	No.	Positive	%	No.	Positive	%
Poultry	24	5	20.83	25	14	56.0
Bovine	2	2	100.0	38	14	36.84
Pets	11	3	27.27	2	0	0.00
Equine	0	0	0.00	5	2	40.0
Total	37	10	27.2	70	30	42.85

Fisher's exact test: Healthcare workers, P = 0.08; Farm workers, P = 0.32; All workers, P = 0.74 (P for all is non-significant)

On the other hand, prevalence of MRSA in farm workers in relation to contact with different animal species revealed that workers with history of poultry contact scored the highest prevalence (56%) followed by those with history of equine contact (40%) then those with history of cattle contact (36.84%) and lastly those with history of pet animals contact (0.0%). Poultry farmers could be colonized by MRSA CC398 and that explained the high prevalence in poultry contact farm workers as all of them were poultry breeder. Elevated rates of MRSA carriage were also reported in poultry slaughterhouse workers in the Netherlands, with much higher carriage rates among workers who contacted live birds that those who worked only with dead fowl **(Mulders et al., 2010)**. In contrast to livestock, transmission between people and pets seemed to be relatively infrequent. MRSA isolates in dogs and cats tend to be human hospital associated or community-associated strains, and most canine and feline infections were thought to be acquired from people **(Faires et al., 2009**) and that might describe the absence of MRSA in farm workers as they were away of hospitals to adapted hospitals or community associated strain and the

percentage of 27% for HCWs. Case reports and case series suggested that, once they become colonized, companion animals can sometimes transmit MRSA back to humans. Transmission between staff and dogs or cats has been reported in some veterinary Hospitals (**Baptiste et al., 2005).**

S. aureus is well known as a contagious mastitis pathogen that is predominantly spread during the milking process via milkers' hands, towels and milking clusters that is considered the most likely route of transmission between cows and quarters for MRSA as well **(Hoedemaker, 2001).** The presented data in **Table (4)** showed that 89 milk samples collected from three different dairy cattle farms were examined for detection of MRSA and only 27 samples were identified as MRSA (30.33%). Also, swabs from 42 cups of milking machine were examined for MRSA and 38 were identified as MRSA contaminated (90.47%) that was considered very high percentage and could transmit MRSA to milk and handlers by contact. The recorded prevalence of MRSA in milk samples (30.33%) was higher than that recorded in India by **Spohr et al., (2011)** (29%) and that recorded in Korea (3.2%), Malaysia (1.4%), and Japan (0.9%) as reported by **Li et al., (2017**). These different frequencies may be due to various animal populations studied or the implemented methodologies. Concerning milking machine, it was noticed that 38 out of 42 cups were contaminated with MRSA (90.47%) that could be considered as very high percentage and could transmit MRSA by contact to milk and handlers. There were few studies taking milking machine in concern, **Spanu et al., (2016)** in Italy tested 17 milking machines in a sheep farm and found that all were positive for presence of MRSA. In addition, cattle nasal swabs were tested and MRSA was identified in 65 (48.14%). These positive cows could be considered a potential source of MRSA to other cows and human. The potentiality of the problem may be increased with the unwise antibiotic use in veterinary field as well as human population in Egypt that might result in rising of new resistant strain which could not be treated. The obtained result was higher than that recorded by **Reshma, (2016)** (37.7%) and **Kumar et al., (2017) (**34.28%).

Table (4): Prevalence of MRSA in the examined samples of dairy cattle farms

	No. of examined samples	**Positive**	
		No.	**%**
Nasal swabs of dairy cattle	135	65	48.14
Milk	89	27	30.33
Milking machine swabs	42	38	90.47
Total	266	130	48.87

Fisher's exact test: $P < 0.001$ (significant)

MRSA includes *S. aureus* that has acquired a gene, called *mec*A, giving them resistance to methicillin and essentially to all other beta-lactam antibiotics. From the whole positive samples only 20 represented isolates were entered PCR for *mecA* detection. The *mecA* gene was detected in only 16 isolates and no *mecC*-positive MRSA was detected in the other 4 negative isolates **(Fig., 1)**

Another 10 MRSA isolates were screened for *PVL* and none of them was positive. Only 4 isolates of MRSA were screened for presence of *mecC* gene but they did not give the characteristic band indicating the absence of target gene.

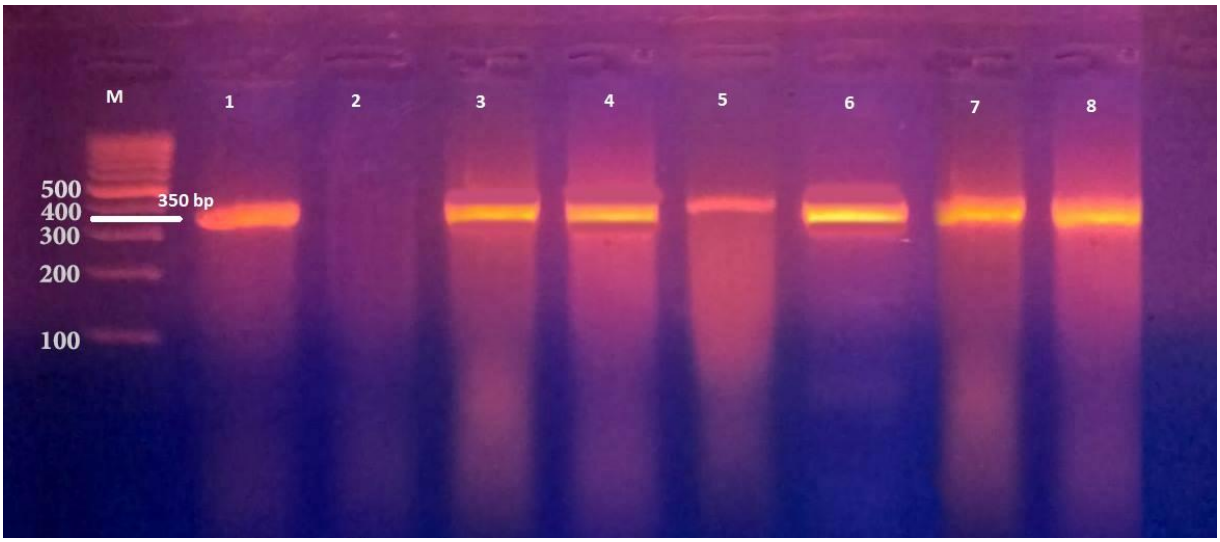

Fig. (1): Agarose gel electrophoresis of PCR products for ***mecA* gene** (350 bp) for characterization MRSA isolates

Lane M: Ladder (100 bp), **Lane 1:** Control positive**, Lane 2:** Control negative, **Lanes 3, 4, 5, 6, 7 and 8:** Positive MRSA strains for *mecA* gene.

A total of 10 MRSA isolates were screened for *PVL* and none of them was positive. It was known that most of LA-MRSA isolates were *PVL* negative and this was confirmed by the study of **Feßler et al., (2010)** in Germany who identified exclusively MRSA ST398 isolates of different spa and dru types and found that all of these isolates were *PVL* negative. Only 4 isolates of MRSA were screened for presence of *mecC* gene but they did not give the characteristic band indicating the absence of target gene. **Hefzy et al. (2016**) found that among all HCWs isolated MRSA acquired *PVL* gene only 16.7% were identified and none of *mecA* negative MRSA were positive for *mecC.*

4- Conclusion:

According to the recorded results in the current study, it is concluded that dairy cattle played a significant role in transmission of MRSA to humans as the close contact between dairy cows and humans, particularly in the milking parlor may create the opportunity for the transmission of such bacteria. Also, MRSA could be transmitted to human through contaminated milk. In addition, the higher prevalence in cattle is reflecting a continuous state of presence of various sources of infection in dairy cattle farms including; animal and human reservoirs that could transmit MRSA regularly to cattle. PCR is a good diagnostic tool for identification and detection genes responsible for occurrence of antibiotic resistance state.

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الملخص العربي

انتشار الميكروب العنقودى الذهبى المقاوم للميثيسلين فى مزارع الأبقار والإنسان

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أجريت الدراسة الحالية فى محافظة البحيرة بهدف محاولة عزل وتصنيف بكتيريا الميكروب العنقودى الذهبى المقاومة للميثيسيلن من كل من مزارع أبقار الحليب والعاملين فى مجال الرعاية الصحية والانتاج الحيوانى وعليه فقد تم عدد 127 عينة من مسحات الأنف بواقع 89 عينة من العاملين فى مجال الرعاية الصحية تم جمعها من 4 مستشفيات مختلفة وبالتحديد من أقسام الجراحة و38 عينة من عمال المزارع بشكل عشوائى . بالإضافة إلى ذلك، تم تجمع عدد 266 عينة مختلفة من مسحات الأنف من الأبقار الحلابة (135) اللبن الحليب (89) ومسحات آلة الحلب (42). تم إجراء الفحص البكتريولوجي للعينات في المعمل التابع لمعهد بحوث صحة الحيوان، فرع البحيرة. أظهرت النتائج أن معدل انتشار الميكروب العنقودى الذهبى المقاوم للميثيسيلين في عينات البشر ومزارع الأبقار بلغ 31.5 % و48.87 % على التوالي. وبدراسة تأثير طبيعة المهنة على معدل انتشار الميكروب لوحظ أنه كان أعلى فى عينات العاملين فى مزارع الحيوانات (36.8%) مقارنة بالعاملين فى مجال الرعاية الصحية (29.2%). فيما يتعلق بمزارع الأبقار الحلابة، بلغت نسبة انتشار الميكروب فى عينات مسحات الأنف المجمعة من الأبقار 48.14% بينما كانت فى عينات اللبن 30.33% وأخيرا كانت 90.37% فى عينات مسحات آلات الحلب والتي اعتبرت نسبة عالية جدا وعليه فإنه يمكن اعتبار الأبقار الإيجابية مصدرا محتملا لنقل الميكروب إلى الأبقارالأخرى وكذلك إلى الإنسان. أيضا تم فحص عدد 34 من المعزولات التى تم الحصول عليها أثناء الاختبار باستخدام تفاعل البلمرة المتسلسل لوجود الجينات المسئولة عن مقاومة المضادات الحيوية (mecA وmecC وPVl) وذلك فى المعمل التابع لقسم صحة الحيوان وألمراض المشتركة – كلية الطب البيطرى – جامعة الأسكندرية حيث بين الاختبار وجود الجين (mecA) فى عدد 16 معزولة بينما تبين عدم وجود الجينات الأخرى فى المعزولات موضع الفحص(mecC وPVl). بناء على ما سبق ينبغي اتخاذ التدابير الصحية من أجل تقليل مخاطر انتقال هذا الميكروب من خلال مزارع الأبقار الحلوب إلى العاملين فى تلك المزارع مثل تطهير آلة الحلب وأواني منتجات الألبان مع تطهير الضرع جيدا قبل الحلب، كما يجب التنبيه على العمال بضرورة استعمال المطهرات لتطهير ايديهم قبل وبعد عملية الحلب.