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Prevalence and Antibiotic Susceptibility of Methicillin Resistant *Staphylococcus aureus* in Colonized Health Care workers, Farm Workers and Dairy Cattle

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ABSTRACT

The current study focused on determination of the prevalence of Methicillin- resistant *Staphylococcus aureus* (MRSA) in health care workers (HCWs), farm workers and dairy cattle (apparently healthy and mastitic) and studied the effects of some demographic factors on the estimated prevalence. Also, the antibiotic sensitivity of the obtained isolates was investigated. A total of 127 human samples were collected including nasal and hand swabs; 89 from HCWs in four hospitals mainly from surgical department and 38 from farm workers in three different dairy cattle farms. In addition, a total of 116 samples were obtained from dairy cattle farms including; 62 nasal swabs of dairy cattle (apparently healthy and mastitic), 26 swabs from cups of milking machine and 28 milk samples. The recorded results clarified that the overall estimated prevalence of MRSA in the examined human and cattle samples was 31.5 and 52.65%, respectively. Fisher's exact test showed higher prevalence in cattle than in humans ($P < 0.001$). Concerning human samples, it was noticed that farm workers (36.8%) showed higher prevalence than healthcare workers (29.2%). Also, it was found that females (32.05%) showed higher prevalence than males (30.61%) and the age group (20 – 40 years) showed the highest prevalence (38.46%) followed by the age group (> 40 years) (23.25%). Concerning dairy cattle farms, it was estimated that the prevalence of MRSA was 53.23, 21.43 and 84.62% in the examined samples of nasal swabs, milk and milking machine swabs, respectively. In addition, the prevalence of MRSA in nasal swabs of mastitis free cattle (54.55%) was higher than in mastitis infected cattle (45.45%). On the other side, mastitic milk samples (66.67%) showed higher prevalence that milk samples of mastitis free cattle (33.33%). Finally, antibiotic sensitivity testing showed that human isolates were resistant to cefoxitin (92.5%), vancomycin (12.5%), linezolid 10 (25%) and tetracycline (42%) while those of dairy cattle farms were resistant to cefoxitin (78.7%), vancomycin (24.6%), linezolid (16.4%) and tetracycline (42.6%). On conclusion, detection of MRSA in both cattle and human samples constituted a public hazard as well as the development of antibiotic resistance.

1. INTRODUCTION

Staphylococcus aureus (*S. aureus*) is an opportunistic bacterium usually carried asymptomatically on the human body, Methicillin-resistant *Staphylococcus aureus* (MRSA) includes those strains that have acquired a gene responsible for resistance to methicillin and essentially all other beta-lactam antibiotics. MRSA was first reported in 1961; soon after methicillin was introduced into human medicine (Fitzgerald et al., 2001). Approximately 25-50% of the human population is a nasal carrier of *S. aureus*. About 20% are thought to carry one strain persistently, and up to 60% are intermittent carriers MRSA carriage rates in general population vary from less than 1% to 5%. The prevalence varies with the

geographic region (Leonard and Markey, 2008). Nasal carriage is an epidemiologic biomarker of *S. aureus* exposure associated with increased risk of infection (Safdar and Bradley, 2008). Human and animals usually got infected with the same colonized strain they had. MRSA epidemiology was changed in 2005 when a new community-acquired MRSA clone of animal origin emerged. The animal-related MRSA isolates appeared to represent a distinct clone, characterized as sequence type (ST) 398 by MLST (Huijsdens et al., 2006). Due to the low host specificity of MRSA ST398, transfer of such isolates between different animal species, but also between humans and animals, might occur in either direction (Witte, et al., 2007 and Denis, et al., 2009). The emergence in livestock causes strong increase in MRSA occurrence in humans between 2001 and 2006 in the Netherlands. Now cc-398 reported from different countries around world (Graveland, et al., 2011). LA-MRSA strains have been found mainly in pigs and veal calves (as main reservoir) but they have the capacity to colonize wide spectrum of hosts include sheep, poultry, horse, dairy and beef cattle. Pets animals can be colonized for prolonged periods without developing clinical signs Infection with MRSA result in the same syndrome as *S. aureus* which can cause wide range of suppurative infection including (skin and wound infection as sever pyoderma abscesses, dermatitis, post-operative wound , also found in other condition including, sinusitis, rhinitis, otitis, pneumonia, bacteremia, septic arthritis, mastitis including gangers mastitis and sub clinical mastitis and urinary tract infection) (Mohammed and Nigatu. 2015). On the other hand, *S. aureus* was found to be responsible for one third of mastitis cases (clinical and sub clinical) and it causes major economic loss in dairy industry worldwide (Bradely et al., 2007) and cattle can consider as a reservoir of an emerging strain of MRSA infecting human (Alb and Damani , 2015). One study reported that MRSA (mainly CC398) could be isolated from 88% of veal calf rearing units in the Netherlands, and from 28% of these calves overall. The prevalence of MRSA was less in farms with good hygiene, and calves were more likely to be colonized on larger farms MRSA CC398 was detected on almost 10% of farms with mastitis problems, and 4-7% of the cattle on infected farms was reported to be infected (Vandenbroek et al., 2009). Also, the use of antibiotics was linked to MRSA carriage. So, the aim of the current study focused on determination of the prevalence of MRSA in health care workers, farm workers and dairy cattle (apparently healthy and mastitic) and studied the effects of some demographic factors on the estimated prevalence. Also, animal colonization prior to infection was investigated.

2. MARTIAL AND METHODS:

2.1. Sampling:

This study was carried out in Beheira province for a period of 8 months from November 2016 to June 2017. A total of 127 human samples were collected including nasal and hand swabs; 89 from health care workers (HCWs) in four hospitals mainly from surgical department and 38 from farm workers in three different dairy cattle farms. Hand swabs obtained during work time were collected by rolling moistened sterile swab over the palm of hands, area between fingers, finger tips and nails (Cobeljic et al., 1996). Nasal swabs were collected by rubbing a moistened sterile swab into one naris, rotated it against the anterior nasal mucosa and repeated with the same swab in the 2nd naris (VandenBergh et al., 1999). Swabs were inserted into sterile glass tubes filled with nutrient broth and closed tightly then placed in ice box and transported to the laboratory as soon as possible to be incubated for not more 6 hours. In addition, a total of 116 samples were obtained from dairy cattle farms including; 62 nasal swabs of dairy cattle (apparently healthy and mastitic), 26 swabs from cups of milking machine and 28 milk samples.

2.2. Isolation and identification of Methicillin Resistant *Staphylococcus aureus* (MRSA):

Swabs inserted into nutrient broth tubes were incubated for 6 hours then they were inoculated and streaked on Oxacillin Resistance Screening Agar Base (ORSAB) (a selective medium for detection of MRSA) (Oxoid Basingstoke, UK). The ORSAB plates were incubated at 37 °C for 24-48 hours and then observed for growth of MRSA that appeared as intense blue glistering small round colonies. Isolates were identified as Gram positive violet non-motile cocci arranged in cluster and they were coagulase and catalase positive.

2.3. Antibiotic sensitivity testing:

It was performed by using Muller Hinton agar medium (HI media, India). Standard agar disk diffusion method was employed according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI), (2012) using commercial antibiotic disks (Oxoid). The used antibiotic discs were cefoxitin 30 µl, vancomycin 30 µl, tetracycline 30 µl ad linezolid 30 µl.

2.4. Statistical analysis

Fisher's exact test was used to assess associations between the prevalence of MRSA and the studied factors for human and cattle samples

3. RESULTS AND DISCUSSION:

Staphylococcus aureus is well known as a contagious mastitis pathogen that is predominantly spread during the milking process via milkers´ hands, towels and milking clusters (Hoedemaker, 2001). This is the most likely route of transmission between cows and quarters for MRSA as well. The prevalence of MRSA among *S. aureus* isolates was lower than 50 % in most of the African countries while in Egypt, it was as high as 52% during the period between 2003 and 2005 (Falagas et al. 2013).

The recorded results in Table (1) showed that the overall prevalence of MRSA in the examined samples of cattle (52.6%) was higher than that of human (31.49%). Nearly similar results were recorded in Egypt by El-haig and Selim, (2014) who confirmed MRSA in isolates of *S. aureus* of dairy animals (52.2 %) and animal workers (45 %). Also, it was supported by results of Daka et al., (2012) in Ethiopia. The higher prevalence in cattle may be traced back to inadequate hygienic measures applied in these farms with unwise use of antibiotics as well as inadequate personal hygiene with absence of farm workers. The occurrence of MRSA in cattle and workers in the present study has to be considered as a public health burden where resistant bacteria may spread to the community.

Table (1): Prevalence of MRSA in the examined human and cattle samples

Source of samples	No. of examined Samples	Positive		Fisher's exact test
		No.	%	
Human	127	40	31.49	P < 0.001 showing higher prevalence in cattle than in humans
Cattle	116	61	52.60	

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

Occupations	No. of examined samples	Positive		Fisher's exact test
		No.	%	
Healthcare workers (HCW)	89	26	29.2	**P = 0.41** showing higher prevalence in farm workers than HCW
Farm workers	38	14	36.8	
Total	**127**	**40**	**31.49**	

Table (3): Prevalence of MRSA in human samples in relation to gender

Gender	No. of examined samples	Positive		Fisher's exact test
		No.	%	
Males	49	15	30.61	P = 1.00 showing higher prevalence in females than in males
Females	78	25	32.05	
Total	**127**	**40**	**31.49**	

Table (4): Prevalence of MRSA in human samples in relation to age groups

Age groups	No. of examined samples	Positive		Fisher's exact test
		No.	%	
< 20 years	6	0	0.0	P = 0.05 showing higher prevalence in age group (20-40 years) followed by age group (>40 years)
20 – 40 years	78	30	38.46	
> 40 years	43	10	23.25	
Total	**127**	**40**	**31.49**	

Table (5): Prevalence of MRSA in human samples in relation to health status

Health status	No. of examined samples	Positive		Fisher's exact test
		No.	%	
Apparently healthy	93	32	34.41	P = 0.07 showed higher prevalence in autoimmune disease followed by apparently healthy then chronic disease
Chronic diseases	29	5	17.24	
Autoimmune diseases	5	3	60.00	
Total	**127**	**40**	**31.49**	

Table (6): Antibiogram pattern of MRSA obtained from human samples (n= 40 isolates)

Antibiotics	Sensitive isolates		Resistant isolates	
	No.	%	No.	%
Cefoxitin (fox)	3	7.5	37	92.5
Vancomycin (VA)	35	87.5	5	12.5
Linezolid (lz)	30	75.0	10	25.0
Tetracycline (te)	23	57.5	17	42.5
Fisher's exact test	P < 0.001 showed higher resistance of isolates to cefoxitin (92.5%) followed by tetracycline (42.5%), linezolid (25%) then vancomycin (12.5%)			

Table (7): Prevalence of MRSA in dairy cattle farms

Cattle samples	No. of examined samples	Positive		Fisher's exact test
		No.	%	
Milk	28	6	21.43	P < 0.001 showed higher prevalence in milking machine swabs followed by nasal swabs and finally milk samples
Milking machine swabs	26	22	84.62	
Nasal swabs	62	33	53.23	
Total	**116**	**61**	**52.6**	

Table (8): Prevalence of MRSA in dairy cattle samples in relation to occurrence of mastitis

Source of samples	No. of isolates	Mastitis		Mastitis Free	
		Positive	%	Positive	%
Nasal swabs	33	15	45.45	18	54.55
Milk	6	4	66.67	2	33.33
Total	**39**	**19**	**48.72**	**20**	**51.28**
Fisher's exact test		**P = 0.40**			

Table (9): Antibiogram pattern of MRSA obtained from cattle samples (n= 61 isolates)

Antibiotics	Sensitive isolates		Resistant isolates	
	No.	%	No.	%
Cefoxitin (fox)	13	21.31	48	78.69
Vancomycin (va)	46	75.41	15	24.59
Linezolid (lz)	51	83.61	10	16.39
Tetracycline (te)	35	57.38	26	42.62
Fisher's exact test	P < 0.00 showed higher resistance of isolates to cefoxitin followed by tetracycline, vancomycin then linezolid with two samples were resistant for the four antibiotics.			

As shown in Table (2), it was observed that the prevalence of MRSA colonization in farm workers was

36.8% that was nearly similar to that of Elemo et al., (2017) (39.6%) while it was lower than that of Sarkar et al., (2014) (70%). On contrary, it was higher than that obtained by Mekuria et al., (2013) (13.2%). Studies had confirmed that the persistence of MRSA carriage in farmers was always associated with duration of animal contact and it was noticed that livestock-associated methicillin resistant *S. aureus* (LA-MRSA) prevalence dropped during a low exposure period (Graveland et al., 2011). In the current study, most of farm workers were living in farms or had their own animals so they had a contact with one or more species of animals throughout the day. This observation may be considered as strong evidence for the relation between animal exposure and the persistence of MRSA carriage. Also, it could explain the higher prevalence in farm worker than HCWs.

As shown in Table (3), it was observed that the prevalence of MRSA in females (32%) was higher than males (30.6%) that could be attributed the fact that females constituted the most of HCWs. This result was in harmony with Sadek et al., (2013) who found higher incidence of MRSA in females (60.5%) than in males (39.5%) and Hefzy et al., (2016) who found that the prevalence of MRSA was higher in females (67.3%) than in males (32.7%).

The age wise positivity of MRSA infection was recorded in Table (4). It was observed that the highest prevalence was found in age group (20-40 years) (38.5%) then the age group (>40 years) (23.2%) and finally the age group (less than 20 years). This result may be traced back to the fact that the age group (20-40 years) represented the active age of occupation where direct contact with infected patients and animals was likely to occur.

The presented data in Table (5) showed higher prevalence in patients with autoimmune diseases (60%) followed by the group of apparently healthy individuals (43.4%) and lastly patients with chronic diseases (17.2%). The increased prevalence in patients with autoimmune diseases may throw the light on the effect of immune status on colonization of MRSA where immunodeficiency may increase the ability of MRSA colonization or even infection (Grimbacher et al., 2016).

The antibiotic resistance of MRSA positive human isolates was tabulated in Table (6). It was observed that most isolates showed higher resistance for cefoxitin (92.5%) that may be attributed to using a specific media for MRSA isolation which contained oxacillin supplementation that was the same generation of cefoxitin beta-lactam antibiotic. Similar result was obtained by Sadek et al., (2013). Also, it was noticed that tetracycline resistance was 42.5% that could be explained by due to the frequent using of tetracycline in both human and animal medication programs. In addition, linezolid resistance was observed in 25% of isolates that could be considered as increased percentage because linezolid resistance was very rare and it required a specific *cfr* gene mutation by bacteria to adapt it and none of the examined humans informed that they took linezolid. This mutation started in coagulase negative *Staphylococci* that was found and harbored in animals specially livestock as bovine and many of examined humans informed that they were in contact with bovine or most of patients who come in contact with HCWs were farmers so it could transmitted in between human by contact and environmental contamination. This finding was confirmed by that of Garcı´ et al., (2010) who recorded linezolid resistant *S. aureus* (LRSA) outbreak in ICU in Spain 2010 where 12 cases with no history of linezolid intake become colonized from a patient who harbored it at first after linezolid medication intake for 5 days. LRSA was extremely uncommon where only 8 cases from the United States, cases from Germany and 1 case each from the United Kingdom, Brazil and Colombia have been reported. Moreover, vancomycin resistance was observed in 12.5% of human isolates. Vancomycin resistant *S. aureus* (VRSA) strains were rarely found these days so our finding could be considered as high prevalence that could be attributed to unwise use of vancomycin in hospitals and by individuals. Usually vancomycin was used as first-line antibiotics for treating serious MRSA infections. The first VRSA was discovered in a patient in United States (Lindgren, 2017).

As shown in Table (7), 28 milk samples collected from two different dairy cattle farms were examined for detection of MRSA and only 6 samples were identified as MRSA (21.4%). Nearly similar results were recorded in Hong Kong (21.3%), Turkey (17.5%) and India (29%) (Spohr et al., 2011) while it was higher than recorded in Korea (3.2%), Malaysia (1.4%) and Japan (0.9%) (Li et al., 2017). These different frequencies may be due to different animal populations studied or the implemented methodologies. Also, swabs from 26 cups of milking machine were examined for MRSA and 22 were identified as MRSA contaminated (84.6%) that was considered very high percentage and could transmit MRSA to milk and handlers by contact. There were few studies taking

milking machine in concern; Spanu et al., (2016) found that all milking machines were positive for MRSA in a sheep farm in Italy. In addition, 62 cattle nasal swabs were tested and MRSA was identified in 33 (53.2%). 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%).

In Table (10) the relationship between occurrence of MRSA and mastitis in both milk samples and cattle nasal swabs was studied. It was found that MRSA was detected in milk samples collected from mastitic and healthy cows with the percentage of 66.67 and 33.3%, respectively. This results was higher than recorded by Zutic et al., (2012) (5.9%), Rai and Tiwari, (2016) (7.4%) and Guimarães et al., (2017) (12.2%) in infected cows.

The antibiotic resistance of MRSA isolates obtained from cattle sources was tabulated in Table (9). It was observed that most isolates showed higher resistance for cefoxitin (78.7%) in examined MRSA isolates followed by tetracycline (42.6%), vancomycin (24%) then linezolid (16%). Two samples were found to be resistant for the four antibiotics. Osman et al., (2015) noticed very low resistance in Vancomycin (8.5%), tetracycline (25.5%) and Elemo et al., (2017) from Ethiopia observed resistance as following ; vancomycin (3%), cefoxitin (60.8%), tetracycline (54.9%). Linezolid resistance has not previously detected in coagulase positive *S. aureus* isolated from bovine and it was only isolated from coagulase negative *Staphylococci*. *Staphylococci* can exhibit linezolid resistance by acquisition of *cfr* gene, which was originally identified in a bovine *Staphylococcus sciuri* isolated in 2000 and was subsequently detected in a clinical MRSA isolate in 2005. So far, cfr-carrying staphylococci have spread worldwide, even causing several outbreaks (Cai et al., 2015). So the detection of LRSA from *S. aureus* of bovine origin may be originated from human source or it may harbor the same gene as in coagulase negative *staphylococci* but both possibilities could not confirmed because multi locus sequencing typing (MLST) was not available during the study.

On conclusion, detection of MRSA in both cattle and human samples constituted a public hazard as well as the development of antibiotic resistance. Also, there was a relationship between animal exposure and the persistence of MRSA carriage that explained the higher prevalence in farm worker than HCWs. In addition, the increased prevalence in patients with autoimmune diseases may throw the light on the effect of immune status on colonization of MRSA. Moreover, the recorded higher tetracycline resistance may be due to the frequent using of tetracycline in both human and animal medication programs.

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