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Prevalence and potential risk factors associated with methicillin resistant *Staphylococcus aureus* infection in Dairy farms

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ABSTRACT

Objective: A cross sectional study was conducted between June 2017 and August 2018 in around Dakahlia governorate to identify Methicillin resistant *Staphylococcus aureus* (MRSA) and to determine risk factors associated with the occurrence of mastitis in dairy cows.

Design: Cross sectional study

Animals and samples: 130 dairy cattle (744 samples) and 181 environmental samples (36 bedding, 37 bulk tank milk BTM, 27 feed troughs, 27 water troughs, 27 milk linear and 27 worker's nasal swabs), with a total of 925 samples were studied.

Procedures: Three hundred and seventy-two quarters were examined to detect clinical and subclinical mastitis by physical examinations and California mastitis test. In addition, bacteriological isolation and identification of *Staphylococcus aureus* and MRSA were carried on the above milk samples of above mentioned quarters, teat swabs and environmental samples.

Results: The overall prevalence of *Staphylococcus aureus* and MRSA was 59.4 and 22.4, respectively. The MRSA were more prevalent in mastitic quarter ($X_2 = 31.146$, P-value = 0.000), Holstein Friesian breed (P-value = 0.021), Old aged dairy cattle (>8 years) (P-value = 0.000), Multiparous cows (P-value = 0.000), large herd size (≥ 2000) (P-value = 0.000).

Conclusion and clinical relevance: The present result indicates that MRSA is a major cause of mastitis in dairy farms at northern Egypt, where one of the key elements for controlling its spreading depends mainly on determining its potential risk factors responsible for its existence.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA), Risk factors, Antibiotic resistance, Dairy farms

1. INTRODUCTION

Mastitis is considered the most influencing disease in dairy sector, as it leads to massive losses in milk, culling and even death of infected cattle [1]. *Staphylococcus aureus* is one of the most crucial contagious mastitis pathogens in the dairy cattle [2]. *S. aureus* infection is estimated to be present in up to 90% of dairy farms with 35% of the economic loss in the dairy industry [3].

Antibiotics are utilized for both therapeutic and sub-therapeutic purposes in veterinary practice. Sub-therapeutically, antimicrobials are used to enhance feed efficiency and promote growth of animals [4]. Currently, There is increased public and scientific concern regarding extensive use of antimicrobials for therapeutic or sub-therapeutic purposes, due to the emergence and dissemination of multiple antibiotic resistant zoonotic bacterial pathogens [5]. Data from the USDA National Animal Health Monitoring System indicated that the main percent of farms that treated cows with an antibiotic were for treatment of mastitis by rate of 85.4% [6].

High-level resistance of *Staphylococcus aureus* to β -lactams is acquired by a *mecA* gene coding, a modified penicillin binding protein (PBP2a) was first observed in the early 1960's

[7]. For effective treatment and control of bovine mastitis, it is crucial to study the antimicrobial resistance mechanism and epidemiology of *Staphylococcus aureus* infections [8].

MRSA is frequently transmitted via environmental contamination; reaching the environment through animal manure and then it could be carried by air. There is an actual hazard of MRSA spreading from animal manure contaminating water courses and agriculture crops [9]. The age progression and high delivery rate (>7 calves) induce 13 times greater risk of IMI than lower delivery calving rate (3 calves), which could be related to calving influences on teat muscle relaxation and enlargement making udder much easier accessible to infection [10]. Due to the contagious character of MRSA that shed in milk during milking which becomes a source of infection to other healthy cows. Consequently, large herd size farms characterized by high risk of exposure to infection due to increased stocking density [11]. Interestingly, cross breeds are more susceptible to be infected by *Staphylococcus aureus* mastitis than local breeds, due to physiological and anatomical character difference of mammary gland and quantity of milk produced by cow. Besides, high milk production is related to

genetic selection, lead to increased genetic sensitivity to mastitis [12]. Therefore, Knowledge of MRSA is necessary to make decisions regarding antibiotic treatment and prerequisite for establishing control strategies in the area. This study aimed to investigate the prevalence of Methicillin-resistant *Staphylococcus aureus* and to assess the potential risk factors associated such infection in dairy farms at northern Egypt.

2. MATERIALS AND METHODS

2.1. Study area and design

The study was conducted at Dakahlia governorate which located northeast of Cairo 31.0832° latitude, 31.4913° longitude. Dakahlia has a mild climate that tends to be warm in winter with some rain that increases on the coasts, and is hot in summer; where the average annual temperatures range from 14 - 28 degrees Celsius. Its area is approximately 3,500 km² and it has a total human population of approximately 6,000,000. Total cattle population in Egypt is (4.950.000) [13]. The study started from June 2017 till August 2018.

Cross-sectional study was adopted; sampling collection was carried out by using stratified sampling technique. Four dairy farms with different levels of biosecurity measures (moderate to low) were selected for evaluating the epidemiological status of MRSA. To identify the potential risk factors for the prevalence of MRSA, questionnaires were used to gather information depending on observation, interviewing the dairy farmers and handlers and the field assessment of MRSA status. Questions about animals and farm level factors as well as inquire educational needs of dairy farmers, a animal level factors as general herd feature, herd size, breed, age, lactation phase, cow identification, individual milk production and whole milk yield were recorded. The farm level factors as housing type, biosecurity practices, milking system, barn floor bedding, dry cow therapy routines, mastitis handling record, treatment procedures of clinical mastitis cases, therapy records, the use and regularity of microbiological cultures tests, milking sequences, the use and the frequency of antimicrobial susceptibility tests were also recorded [14]. Three breeds of dairy cattle included in this study (Holstein-Friesian, Simmental and local breeds). A total of 925 samples were collected from 4 selected dairy farms were chosen conveniently relied on dairy cow availability.

2.2. California mastitis test (CMT)

A stream of milk from each quarter of the udder was placed in each of four shallow cups in the CMT paddle and the same amount of the reagent was added. A gentle circular motion was done in a horizontal plane. Positive samples displayed gel formation within a few seconds. The result was scored based on the gel formation and considered as negative if there was no gel formation and recorded as zero, or positive if there was gel

formation scoring from trace (T) to +3. If in any case one quarter was positive by the CMT then the cattle was considered positive [15].

2.3. Sample collection

A total of 925 samples were randomly collected from dairy cattle, 372 samples for each of individual quarter's milk and 372 teat skin swabs. Samples of dairy cattle were collected from 4 different dairy farms. Each sample was labelled with the following information; breed, age, lactation stage, parity, udder status and time of collection. In addition, a total of 181 different environmental samples were collected including BTM (37), milking linear (27), feed troughs (27), water troughs (27), bedding (36) and workers' nasal swabs (27).

2.4. Culturing and identification

For isolation and identification of *S. aureus*, a loopful of enriched samples was streaked onto selective media for *Staphylococcus aureus* (Baird-Parker agar, Oxoid, England). The typical colonies of *Staphylococcus aureus* are black, shiny, convex (2-4 mm size) with entire margins and clear zones, with an opaque precipitation zone after 48 hours. Pure isolated single colonies were picked up and sub-cultured on trypton soya broth (TSB) at 37°C/24 hours. Then, stored in buffered glycerol at -20 °C for further identification procedures [16]. The phenotypic identification of isolated *Staphylococcus aureus* was relied on colony morphology, gram staining and biochemical tests; positive catalase test and positive tube coagulase rabbit plasma test at 4 and 24 hrs. (Positive result is indicated by gelling of the plasma).

2.5. Cefoxitin disk diffusion test

Methicillin-resistance in *Staphylococcus aureus* was identified by cefoxitin screening. Disc diffusion test is employed by incubating *Staphylococcus aureus* on tryptone soya broth (TSB) impregnated with Cefoxitin (30µg) discs. MRSA is identified by assessing zone of inhibitions. The *Staphylococcus aureus* categorized as methicillin-resistant, if showed inhibition diameter zone ≤ 21mm. Cefoxitin induces mecA gene of MRSA and its results have been found in concordance to PCR. Thus, Cefoxitin disc diffusion test can be alternative to PCR for the detection of MRSA in resource constraint settings [17].

2.6. Statistical analysis

Collected data was coded and entered to MS Excel spreadsheet and checked for accuracy. After validation, it was transferred and processed using computer software SPSS version 23 for analysis. Pearson's chi-square tests were used when appropriate to analyze the proportions of categorical data. 95% Confidence Interval was computed and the results were considered significant at P < 0.05.

3. Results

3.1. Biosecurity status of the examined dairy farms throughout the study period.

The assumed score of biosecurity was examined in the four examined dairy farms based on the adopted biosecurity measures.

Table 1. The assumed scores of biosecurity level at the examined farms.

Measures	Farm I	Farm II	Farm III	Farm IV
1. Proper administration of antibiotics (Under Veterinary Supervision)	1	0	0	0
2. Milking (Occurred under hygienic procedure)	1	0	1	0
3. Restrict Access of visitors and vehicles.	1	0	1	0
4. Personnel hygiene (wearing coverall and boots)	0	0	0	0
5. Farm biosecurity level (acceptable biosecurity level)	1	0	1	0
6. Routine screening of milk (CMT)	1	0	0	0
7. In vitro testing of antibiotic before using it	0	0	0	0
8. wearing gloves during milking	1	0	0	0
9. Fore-milking teat cleaning and disinfection	1	1	1	0
10. Prior-milking teat cleaning and disinfection	1	1	1	0
11. Record keeping about antibiotic treatment	1	0	0	0
12. Having plane for treating sick animals with antibiotic	1	1	1	0
13. After administration of antibiotic, are the course of treatment completed	1	1	1	0
14. Treated cow marked as treated	1	1	1	0
15. Treated cow physically separated from other milking cows	1	0	0	0
16. Are treated cow Milked last	1	1	1	0
17. Are treated cow milked with separate milking unit	0	0	0	0
18. Are cow routinely Screened after antibiotic treatment by antibiotic residue detect kit	0	0	0	0
19. Milk Sampled for culture in case of mastitis	0	0	0	0
20. Bedding routinely changed	1	1	1	0
Assumed level	1	0	1	0

Moderate biosecurity=1; Low biosecurity=0

Table 1 reveals that both farms I and III had a moderate biosecurity level (1); however, farms II and IV had the lowest level (0).

Table 2. Scoring system for biosecurity level in the examined farms.

	Variable	Description and level
1	Proper administration of antibiotics	Under veterinary supervision=1; without supervision =0
2	Milking	Occurred under hygienic procedure=1; milking procedure not hygienic=0
3	Restrict Access of visitors and vehicles	Yes=1; No=0
4	Personnel hygiene	wearing coverall and boots=1; not wearing coverall and boots=0
5	Farm biosecurity level	acceptable biosecurity level=1; poor biosecurity level=0
6	Routine screening of milk	Regular California Mastitis testing (CMT)=1; not apply CMT=0
7	In vitro testing of antibiotic before using it	Yes=1; No=0
8	wearing gloves during milking	Yes=1; No=0
9	Fore-milking teat cleaning and disinfection	Yes=1; No=0
10	Prior-milking teat cleaning and disinfection	Yes=1; No=0
11	Record keeping about antibiotic treatment	Yes=1; No=0
12	Having plane for treating sick animals with antibiotic	Yes=1; No=0
13	After administration of antibiotic, are the course of treatment completed	Yes=1; No=0
14	Treated cow marked as treated	Yes=1; No=0
15	Treated cow physically separated from other milking cows	Yes=1; No=0
16	Are treated cow Milked last	Yes=1; No=0
17	Are treated cow milked with separate milking unit	Yes=1; No=0
18	Are cow routinely Screened after antibiotic treatment by antibiotic residue detect kit	Yes=1; No=0
19	Milk Sampled for culture in case of mastitis	Yes=1; No=0
20	Bedding routinely changed	Yes=1; No=0

3.2. Prevalence of MRSA

The results of MRSA prevalence in the examined dairy farms in relation to sample source are shown in **table 3**, there is a significant difference at P value = 0.000 and $\chi^2=58.515$ for MRSA isolation rates from different sources. A total of 744 animal samples were examined during the study period, out of

372 collected individual quarter milk samples, MRSA was isolated from 111 (29.8%). Whereas, it was recovered at a rate of 41% from positive *Staphylococcus aureus* isolates. On the same time, out of 372 collected teat swab samples before and after iodine teat dipping, 26 (7 %) isolates were identified as MRSA. It was noticed that all 26 positive detected MRSA from teat swabs were collected prior to dipping of teat by iodine.

Table 3. The prevalence of Methicillin-Resistance *Staphylococcus aureus* in relation to sample source (animal samples, workers and farm environment) in the examined dairy cattle farms at Dakahlia during (2017-2018).

Sample (No.)	Distribution of <i>S. aureus</i> (n=549)		Distribution of MRSA (n=207)		
	No	%	No.	<i>S. aureus</i> (%)	Total d (%)
Animal (n=744)					
Individual milk (n=372)	271	72.8	111	41	29.8
Teat skin swabs (n=372)	159	42.7	26	16.6	7
Environmental samples (n=181)					
Bedding (n=36)	21	58.3	14	66.7	38.9
BTM (n=37)	24	64.9	17	70.8	45.9
Feed (n=27)	18	66.7	10	55.6	37
Water (n=27)	18	66.7	11	61.1	40.7
Milking linear (n=27)	19	70.3	12	63.2	44.3
Workers nasal swab (n=27)	19	70.4	6	31.6	22.2

As regards to the environmental samples, a total of 181 samples including; 36 bedding, 27 feed, 27 milking linear, 37 BTM, 27 water and 27 nasal swabs were collected from the four examined farms as shown in **table 3**. Among the positive *Staphylococcus aureus*, MRSA was more prevalent in BTM followed by bedding, milking linear then water troughs (70.8%, 66.7%, 63.2% and 61.1%, respectively), while it was less prevalent in feed troughs and nasal swabs (55.6% and 31.6%, respectively). Meanwhile, the positive MRSA among collected environmental samples was 70 (38.7 %), while it was (58.8 %) out of 119 positive *Staphylococcus aureus*. The high percentage of positive MRSA samples among total collected environmental samples was from BTM followed by milking linear, water troughs then bedding (45.9%, 44.3%, 40.7 and 38.9%

respectively), while the lowest percentage was obtained from feed troughs and nasal swabs (37% and 22.2%, respectively).

Table 4. Animal-level risk factors associated with the prevalence of MRSA causing mastitis.

Risk factor	Categories	MRSA			
		Collect sample	No.	%	P value
Age	Young (<5 years)	32	7	21.9	
	Adults (5-8 years)	71	44	62	
	Old (>8 years)	27	25	92.6	0.000
Parity	Primiparous	20	3	15	
	Multiparous	110	73	66.3	0.000
Lactation stage	Early (<4 month)	72	43	60	
	Late (>4 month)	58	33	56.8	0.754
Breed	Holstein Friesian	90	59	65.6	
	Simmental	10	6	60	
	Local breed (Baladi)	30	11	36.7	0.021
Herd size	Large (>=2000)	57	45	78.9	
	Medium (1000-2000)	40	19	47.5	
	Small (200-1000)	33	12	36	0.000

3.3. Prevalence of MRSA in relation to clinical findings and results of CMT

According to cow level prevalence of MRSA in relation to CMT observation of tested individual milk samples, MRSA was isolated mostly from mastitic quarters followed by sub-clinically mastitic then healthy quarters as illustrated in Fig. (1), there is a significant difference at P-value=0.000 and $\chi^2=31.146$ for MRSA isolation rate from normal, mastitic and subclinical milk. Screening performed with CMT paddle resulting in categorizing tested quarters to following; 125 (33.6 %) healthy quarters, 114 (30.6 %) sub-clinically infected quarters and 133 (35.8 %) clinically infected quarters (**Figure 1**).

The distribution of MRSA in relation to seasonal variations was showed in **Figure 2**, it was notable that there is no significant effect of season upon MRSA prevalence at $\chi^2=4.015$. MRSA was more prevalent in summer (42.4%), followed by spring, winter and autumn at rates of 37%, 36% and 33%, respectively.

The potential risk factors at animal level associated with mastitis caused by MRSA are mainly focusing on breed, age, parity, lactation stage and herd size as illustrated in **table 4**. Low

prevalence of mastitis was recorded in local breeds at a rate of 36.7 % (11/30), while it was significantly higher in Holstein-Friesian breed at a rate of 65.6 % (59/60) and Simmental breed by 60% (6/10) at P-value=0.021 and $\chi^2=7.743$. Also, the association between mastitis and age of cattle was significant at P-value=0.000 and $\chi^2=30.951$. Elder age was more susceptible to mastitis than young age, at which mastitis distribution was 21.9% (7/32) within young age (less than 5 years) group, 62 % (44\71) among adult age (between 5 to 8 years) cows and 92.6 % (25\27) within old aged (more than 8 years) cows. The incidence of mastitis has significant association with parity at P-value=0.000 and $\chi^2=18.385$. Evidently, the occurrence of mastitis was greater in multiparous cows (66.3%) than primiparous cows (15%). In contrast, the association between mastitis and stage of lactation was insignificant statistically, where 60 % (43\72) was mastitic during early lactation phase, however 56.8 % (33\58) infected by mastitis during late lactation phase, incidence of mastitis slightly higher during early lactation than late lactation stage. In addition, there is a significant association between herd size and occurrence of mastitis at P-value=0.000 and $\chi^2=18.466$. The highest prevalence of MRSA observed in large size herds followed by medium and small herds at rates of 78.9%, 47.5% and 36%, respectively.

Table 5. Risk factors associated with the prevalence of MRSA causing mastitis depending on farm-biosecurity level.

Risk factor	Category	Proportion of farms (%)	Distribution of MRSA (%)
Restrict access of visitors and vehicles	Yes	2/4	20.1 %
	No	2/4	24.6 %
Record keeping of previous antibiotic treatment	Yes	2/4	20.1 %
	No	2/4	24.6 %
Veterinary supervision	Yes	¼	20 %
	No	2/4	23 %
Hygienic milking procedure	Yes	2/4	20.1
	No	2/4	24.6
Routine bacteriological culturing of animal and environmental samples and performing of antibiotic sensitivity test for selection of proper antibiotic	Yes	¼	20 %
	No	¾	23 %

Our results investigated that farms which suffering from lack of biosecurity measures exhibited higher prevalence of MRSA, on the contrary, farms that adopt such measures showed lower prevalence of MRSA form collected samples. Farms that lack of keeping records of previous antibiotic treatment and also those do not have veterinary supervision inside farm showed higher MRSA prevalence rates of 24.6% and 23%, respectively. On the contrary, farms adopted aforementioned elements of biosecurity measures, have lower rates 20.1% and 20% (Table 5).

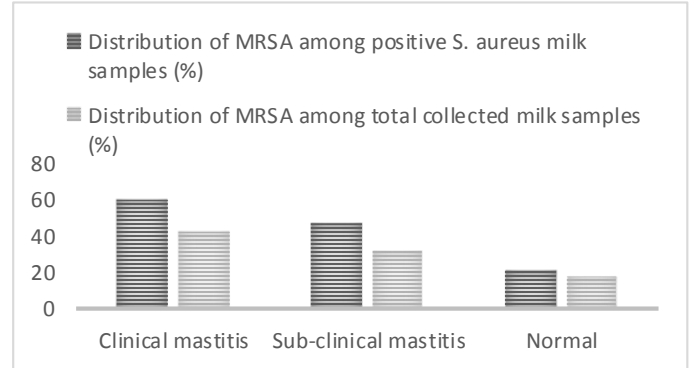


Figure 1. Prevalence of MRSA according to CMT screening which reveal milk to 3 categories; normal, clinical mastitis and sub-clinical mastitis (P-value = 0.000).

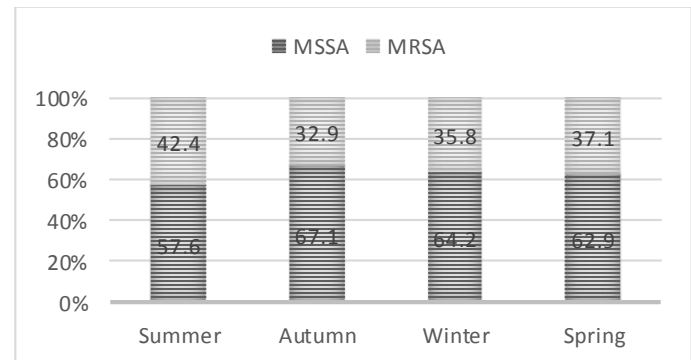


Figure 2. Prevalence of MSSA & MRSA in relation to season in the examined dairy farms during 2017-2018.

4. Discussion

The primary aim of animal hygiene and adopted biosecurity plans is preventing disease before its incidence, as it is well-known that an ounce of prevention is worth a pound of cure. Nowadays, MRSA has become reputable as a common problem in dairy farms with increasing frequency of their antimicrobial resistances. The study was conducted on large and middle-sized dairy farms in and around Dakahlia governorate to determine the prevalence of mastitis and assess the major risk factors associated with MRSA.

The appropriate levels of biosecurity in the examined farms can be justified on the basis of increased risk of MRSA infection, irrespective of pathogenicity and in relation to applied biosecurity level. On this basis, farms I and III with the moderate level of biosecurity assumed to provide more than 50 % protection of disease occurrence with occasionally adopted biosecurity measures as lacking of personnel hygiene (wearing coverall and gloves during milking), absence of prior in-vitro antibiotic testing, milking healthy and infected cows with the same unit, routine screening of antibiotic residues after treatment by antibiotics and absence of milk samples culturing in case of mastitis to determine causative agent. However, farms possess level (0) of biosecurity corresponds to absence of most biosecurity measures had a higher risk of MRSA occurrence. The most detrimental factor of mastitis detection was the culturing of milk sample in case of mastitis and determination of the causative agent, then applying of antibiotic sensitivity test to choose the most effective antibiotic, in addition to the presence of veterinary supervision that regulate administration of antibiotics. It has been elucidated that farms with a higher biosecurity score, giving concern to identify mastitis causative agents and selecting proper antibiotic to inhibit it under strict veterinary supervision had higher prognosis and less MRSA prevalence. Our findings are in accordance with those found by [12, 18-20].

The variations in MRSA prevalence from variable sources may be due to the differences in animal-husbandry systems, breeds, antimicrobial policies and regulations which may contribute to the variety of prevalence estimates. Our data agreed with those reported by [21, 22].

For effective inhibition of intra-mammary infection (IMI), it is important to know the prevalence and distribution of its causative pathogens and the risk factors associated with the mastitis. The overall percentage of *Staphylococcus aureus* isolated from collected individual raw milk samples was 72.8 %; our results are nearly similar to those previously obtained by [1, 21, 23] in Bangladesh, Brazil and South Africa. On the contrary, *Staphylococcus aureus* had lower prevalence rates; 41%, 11% and 5.5% from raw milk samples in studies conducted by [22, 24, 25], respectively.

The overall percentages of *Staphylococcus aureus* and MRSA from total collected animal and environmental samples were 59.4% and 22.4%, respectively. Similarly, to these findings, [26] recovered *Staphylococcus aureus* at the rate of 58% from both types of samples, but found lower MRSA prevalence rate of 3%.

The prevalence of MRSA from the total collected individual milk samples was 29.8%. Our findings go in harmony with those of [8, 21, 27, 28] in Pakistan, Jordan, South Africa and Germany. While, higher results were recorded by [29] who isolated MRSA from 60% of the examined individual milk samples. whereas, lower values of 3.7 % were found by [30]. When we examined

the prevalence of MRSA among positive *Staphylococcus aureus*, we investigated high rates at 41%. These findings are significantly higher than those recorded by [31-35] who identified MRSA among *Staphylococcus aureus* isolates at the rates of 4%, 0%, 0.6% ,16.7% and 7.4%, respectively. Nearly similar results were also investigated by [36] in India and [37] who revealed MRSA with percentages of 29% and 31%, respectively. Our mentioned data also supported by [38] results, who reported that MRSA is isolated at the rate of 52% from recovered *Staphylococcus aureus* in Egypt. On the other hand, higher prevalence rates of 60% and 64.1% were recorded by [12, 25] in Ethiopia.

In addition, *Staphylococcus aureus* was isolated from 58% of bedding samples nearly agreed with [39] who reported that approximately half of bedding samples harbor *Staphylococcus aureus*. However, the overall percentage of MRSA isolated from bedding in the examined dairy farms was 38.9%. Our findings go in harmony with [12] results, who attributed this level to poor hygienic muddy bedding which favor propagation and transmission of mastitis microbes.

Concerning to the presence of MRSA in the dairy farm environment, it was 38.7% from total collected environmental samples. These findings were markedly higher than records of [40] who reported lower prevalence level (25%) of MRSA. On the contrary, higher value of 46.7% was recorded by [41]. Among environmental samples, BTM was the main source of MRSA at the rate of 44%. These results were closely similar to [29] findings, who isolated MRSA from 45% of tested BTM. But, we recorded higher levels than [42] who demonstrated 6.1% MRSA prevalence rate from environmental samples. Also, MRSA prevalence rate among different types of environmental samples, bedding harbored 38.9% positive MRSA. Lower results were reported by [41] who isolated MRSA at rate 19%. Nearly similar to our findings of *Staphylococcus aureus* rates in teat swab samples (42.7%), [39] was isolated *Staphylococcus aureus* from teat swabs at the rate of (44%). Furthermore, with reference to the occurrence of *Staphylococcus aureus* and MRSA in milking linear, our findings are in alignment with [39] who reported that *Staphylococcus aureus* milking linear isolates represent 62% of the total collected milking linear samples. Farm workers represent possible source for MRSA transmission through dairy farm. Our findings demonstrated MRSA in (22.2%) from total collected nasal swabs, similarly to the findings of [43] who recorded (23%) positive MRSA from nasal swab samples.

The incidence of *Staphylococcus aureus* and MRSA in relation to the CMT was investigated, where within the total screened quarters, MRSA found more prevalent in mastitic milk at a rate of 41.4% followed by sub-clinically infected quarters 31.6%, then healthy quarters at a rate of 17.6%. Meanwhile, MRSA distribution among total positive *Staphylococcus aureus* from screened quarters, was highly prevalent from mastitic

quarters at the rate of 59.8% followed by sub-clinically mastitic quarters at the rate of 46.2% with the lowest prevalence rate from healthy quarters at rate of (21.2%). Our findings go in line with [12, 18] who indicated that pathogen was mostly associated with clinical mastitis. Whereas, the current data was higher than those recorded by [44] who reported that MRSA present in subclinical mastitic milk at percent of 5.9%. On the other hand, [43, 45, 46] revealed lower prevalence rates of MRSA from positive *Staphylococcus aureus* in comparing with our findings from mastitic milk as following; 12%, 17.2% and 4.4%, respectively. In contrast to our findings, [47, 48] reported that MRSA was more prevalent in sub-clinical mastitic milk than clinically mastitic milk. Regarding to the prevalence of *Staphylococcus aureus* within mastitic milk, our findings are in accordance with [39], but it was higher when compared to [27] findings, who found that the prevalence of *Staphylococcus aureus* was (28%), among them (28.57%) MRSA was detected.

MRSA incidence in relation to the seasonal changes was studied, where it was more prevalent in summer (42.4%), followed by spring, winter and autumn at rates of 37%, 36% and 33%, respectively. The seasonal variations could be attributed to the effect of climatic variations; temperature, humidity and rainfall rate in between seasons, besides higher udder hygiene was adopted during winter, where the lactation process mostly take place during this season. These results are similar to those found by [49, 50] who stated that Season possess little significant in MRSA incidence.

Risk factors for the occurrence of mastitis were investigated in the examined farms, where according to our data low prevalence of mastitis was recorded in local breeds, versus Holstein-Friesian breed at rate and Simmental breed. This variation could be attributed to the differences in anatomical and physiological features of mammary gland, in addition to genetic differences between breeds. The association between mastitis and age of cattle was remarkable. Elder age was more susceptible to mastitis than young age. The incidence of mastitis has significant association with parity. However, the association between mastitis and stage of lactation was less significant. In addition, there is a significant association between herd size and incidence of mastitis caused by MRSA. To elaborate, it was observed that large herd size characterized by increased stocking density and so higher risk of exposure to infections. The current study revealed that cows' breed, parity, age and herd size were the most detrimental risk factors associated with mastitis onset. Our findings agreed with those reported by [11, 12, 51-55]. Even though, findings of [18] conveyed no significant association between MRSA incidence and cows' breeds. Differences in the prevalence rates of mastitis from various findings could be due to variation in farm management practices, breeds of targeted cows, rate of production and variations in the study methods and materials employed by the investigators. Based on the observations made throughout the collection of samples, inadequate

hygiene and deprived farm management practices contributed to the high incidence rates of MRSA mastitis. Where, MRSA is a contagious pathogen which can spread from animal to animal or personnel by contact with cows during unhygienic milking practice. The increased prevalence of *Staphylococcus aureus* in older animals in this study can be attributed to increased susceptibility of pathogenic organisms in udder relaxed sphincter muscles of teats.

Moreover, the risk factors associated with the prevalence of MRSA causing mastitis depending on farm-biosecurity level was elaborated in this study. The studied biosecurity measures affecting MRSA prevalence were; restrict access of visitors and vehicles; record keeping of previous antibiotic treatment; veterinary supervision; hygienic milking procedure; routine bacteriological culturing of animal and environmental samples; and performing of antibiotic sensitivity test for selection of proper antibiotic. Our results investigated that farms which suffering from lack of biosecurity measures exhibited higher prevalence of MRSA, on the contrary, farms that adopt such measures showed lower prevalence of MRSA form collected samples. Farms that lack of keeping records of previous antibiotic treatment and also those do not have veterinary supervision inside farm showed higher prevalence rates. On the contrary, farms adopt both types of biosecurity measures, have lower rates. These results are similar to findings of [56-58], where authors confirmed that lack of pre and post dipping measures, udder towels used frequently on more than one cow, and not routinely use the gloves are the main factors associated with the high incidence of MRSA within the dairy farms.

Conclusion

For the development of effective MRSA prevention strategies, it is necessary to know which factors increase the risk for MRSA transmission into and within dairy herds. The risk factors for of MRSA into dairy herds are elder ages, multiparous cattle, and foreign breeds.

Research Ethics Committee permission

The current research work was permitted to be executed according to Standards of Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contribution

All authors had contributed equally.

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