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Genotyping of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolated From Bovine Mastitis

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) has a grown impact in veterinary medicine in the last two decades and triggered severe complicated multi-drug resistance mastitis in dairy herds. Therefore, we isolated *Staphylococcus aureus* from bovine mastitis in three different governorates in Egypt, during summer and winter seasons, assessed the sensitivity and resistance profile of the isolates to different antibiotics used in the field. Also, identified *mec*A and *coa* genes in MRSA isolates and utilized PCR RFLP for genotyping of the isolates. Our results revealed an isolation rate of 53.3% (64/120) of *S. aureus*, with significant difference between the governorates under investigation. Isolates demonstrated high rates of multidrug resistance (MDR), with MAR index ranged from 0.57 to 1. The highest MAR index was observed in 8 (12.5%) isolates recovered in the winter season. The *mec*A gene was identified in (59%) of the isolates which positively harbored *coa* gene amplicon with three different product sizes (670bp, 430bp, and 580bp) varied according to the location of sample collection. Restriction of *coa* gene by *Alu*I resulted in 3 different RFLP patterns of MRSA with pattern 1 was the most common and strongly related to MRSA isolates from Kafr-Elsheikh. In conclusion: our results identified a high rate of MDR MRSA causing bovine mastitis in Egypt due to three different genotypes based on RFLP PCR of the *coa* gene. Data analysis revealed genotypic relations among MRSA isolates in the same governorate without seasonal or species association.

Keywords: Bovine mastitis, Genotyping, RFLP PCR, MRSA.

Introduction

Mastitis is one of the most prevalent health issues that have an important impact on the dairy industry in Egypt. Mastitis poseses a significant threat to animal health, lowers milk production, leads to milk losses, animal replacement, culling, and a decline in the productivity [1, 2].

Climate change is one of the most critical issues facing our globe at present. These climate-associated changes comprise, rises in the average seasonal temperatures, increased winter or rainy season precipitation, and other changes [3, 4].

Climate changes may alter microbial pathogenicity and host susceptibility, leading to changes in disease incidence and severity [5]. The incidence of clinical mastitis may increase with the rise in the temperature-humidity index (THI) [6]. Previous studies have reported that hot weather, particularly above 24°C, has been connected to higher milk somatic cell count (SCC), more microorganisms, lower dry matter intake, and low immunity, resulting in a negative energy balance and dairy cattle are thus more vulnerable to illnesses [7, 8].

Staphylococcus aureus (*S. aureus*) is a facultative anaerobic gram-positive bacteria that affecting humans and animals. It is supposed to be the most widespread and frequent cause of all types of bovine mastitis [9]. *S. aureus* has extended multidrug resistance, causing it to pass through the immune system of the host more easily [10]. Methicillin- resistant *S. aureus* (MRSA) is every strain of *S. aureus* that has advanced resistance to beta-lactam antibiotics naturally or acquired (through horizontal gene transfer), also it may have a multiple drug resistance to beta-lactam antibiotics. This is a broad-spectrum antibiotics group

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includes penicillin derivatives such as methicillin, oxacillin, and cephalosporins [11].

Reducing MRSA isolation and exposure is commonly authorized to public health action [12]. Kaba, Kuhlmann [13] revealed a relationship between warmer temperatures and antibiotic resistance progress [14].

The coagulase enzyme is one of the main pathogenic factors in *S. aureus*, this enzyme is released and causes clotting of the host's plasma due to the conversion of fibrinogen to fibrin. *S. aureus* may be protected from phagocytosis action by the formation of fibrin. Researchers suggested that the production of coagulase enzyme is a crucial indicator for *S. aureus* genotyping [15].

Different methods for genotyping can be used to genetically type *S. aureus*. Several molecular techniques have been established and used in epidemiological research for the detection and association of *S. aureus* isolates [16]. Restriction Fragment Length Polymorphism (RFLP) utilizes restriction enzymes to examine specific patterns in DNA fragments to genetically differentiate between diverse organisms [17]. For epidemiological investigations of bovine mastitis, identification based on PCR-RFLP of the *coa* gene has been regarded as an available and precise typing method [18]. PCR-RFLP is a quick, repeatable, easy, and effective way to type *S. aureus* that has been isolated from different sources. With the aid of this typing process, genetic relationships between isolates from various origins can be established [19]. Molecular genotyping of *S. aureus* isolates linked to bovine mastitis may aid in the creation of more potent disease-control strategies. The coagulase gene is considered an accurately defined test to detect *S. aureus* in biological materials. Because of the varied sequences (81 bp tandem repeats) at its 3' end, the *coa* gene, which encodes the coagulase protein, is highly polymorphic and enables the differentiation of *S. aureus* species including MRSA [20].

MRSA genotyping can help to understand the epidemiology and transmission of MRSA, as well as to guide infection control and treatment strategies. Thus the current study aimed to isolate and identify MRSA from mastitis milk in dairy farms during the summer and winter seasons in different localities in Egypt. The antibiotic resistance profile of the isolates and genotyping by coagulase gene RFLP were also investigated.

Material and Methods

Collection of samples

The study received approval from the Agricultural Research Center Institutional Animal Care and Use Committee (ARC-IACUC) under approval number ARC-AHRI-68-24. Animal procedures were conducted following the ARRIVE guidelines. A total of 120 milk samples from mastitis cases were collected from El-Garbia, Kafr El Sheikh, and Monufia Governorates (40, each). From each governorate, 20 samples, each, were collected during Summer and Winter from cows and buffaloes (10, each). All samples were collected separately on sterile plastic syringes and transported immediately in an ice box to the laboratory for bacteriological examination.

Isolation and identification of S. aureus

From each milk sample, 10 ml were collected and centrifuged at 3,000 r.p.m and then the supernatant was discarded including the creamy layer. For enrichment, the sediment was inoculated into nutrient broth and incubated at 37°C for 24 h. A loopfull from the enriched sample was spread onto the surface of Baird Parker agar (Merck, Germany, VM 807406) as a selective medium for isolation. The isolation of *S. aureus* was performed according to ISO (ISO-6888-1 2021/Amd 1:2023) using Baired-Parker agar plates incubated under aerobic conditions for 48±2 hours at 37°C. The suspected colonies were subjected to biochemical examination (catalase, oxidase, and coagulase tests) as well as hemolysis testing on blood agar plates [21, 22].

Antimicrobial susceptibility testing and phenotypic detection of MRSA strains

Antimicrobial susceptibility test was performed for all *S. aureus* isolates using the Kirby - Bauer disk diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS), and the zones of inhibition were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

The used antimicrobial agents included β-lactams (amoxicillin/clavulanic; AMC 30 µg, and cefotaxim; CTX 30 µg), tetracycline (oxytetracycline; TET 30 µg), aminoglycoside (gentamycin; CN 10 µg), sulfonamide (sulfamethoxazole/trimethoprim; SXT 25 µg) and quinolones (ciprofloxacin; CIP 5 µg). Cefoxitin disc (30 µg) was used as a surrogate for the detection of MRSA in all the examined isolates according to CLSI performance *M100* [23]. An isolate was classified as MRSA strain when the inhibition zone diameter for cefoxitin was ≤ 21 mm [24]. Each isolate was examined in triplicate and the results of inhibition zone diameters for each antibiotic were interpreted according to the criteria recommended by [25]. According to Khan, Irfan [26], the resistance of an isolate to at least one antibiotic in three or more antibiotic classes is known as multidrug resistance

(MDR). Moreover, the proportion of the number of antibiotics to which *S. aureus* isolates revealed resistance, to the total number of antibiotics were tested: is defined and considered as the multiple antibiotic resistance (MAR) index [27].

Molecular detection of mecA gene in S. aureus isolates using conventional PCR

Bacterial DNA from suspected MRSA isolates (phenotypically exhibited resistance to cefoxitin) was extracted using QIAamp DNA Mini kit (Qiagen GmbH, Hilden, Germany, Catalogue no. 51304) according to manufacturer's instructions. The isolates were confirmed as MRSA by the amplification of *mec*A gene using specific primers with the sequences 5'-GTA GAA ATG ACT GAA CGT CCG ATA A-3') and 5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3'. ([28].

Genotyping of MRSA based on coagulase gene (coa) RFLP

Confirmed MRSA isolates were subjected to amplification of *coa* gene using the primers (5'-ATAGAGATGCTGGTACAGG -3') and (5'-GCTTCCGATTGTTCGATGC-3')[29]. The amplified PCR products of *coa* gene were restricted using *Alu*I endonuclease. Approximately 15 µl of coagulase PCR products were digested with 4U of restriction endonuclease *Alu*I after incubation at 37 °C for 1 h. After that, 10 µl of the digested PCR products of *coa* gene were analyzed by electrophoresis on 2 % agarose gel. The PCR-RFLP fingerprinting data were transformed into a binary code based on the presence or absence of each band, and the discriminatory power of the reaction was measured using the Simpson's index of diversity (*D*). A *D* value of more than 0.9 indicated good differentiation [30, 31].

Statistical analysis

The data were analyzed using R software version 4.3.1. The Chi-square test and Multiple Correspondence Analysis (MCA) were used for investigation and visualization of the relationships between categorical variables. $p < 0.05$ considered significant.

Results

Prevalence of S.aureus

Staphylococcus aureus was isolated from 53.3% (64/120) of the examined milk samples during winter and summer (Table 1). The isolation rate was 61% (37/60) and 45% (27/60) during winter and summer season, respectively, the rate during winter was higher than the rate of summer, this difference was statistically insignificant (*p*-value = 0.099). With regard to the locality, the highest rate of isolation was from Monufia with a percentage of 72.5% (29/40) followed by El-Gharbia (45.5%;18/40) then Kafr-Elsheikh (42.5%;17/40) with significant difference between the three governorates (*p*-value = 0.011).

Antimicrobial susceptibility testing and phenotypic detection of MRSA

The antibiotic resistance profiles of the 64 *S. aureus* isolates demonstrated high rates of resistance to amoxicillin/clavulanic and cefoxitin (100%, each), followed by cefotaxim (93.7%), gentamycin (79.6%), oxytetracycline (62.5%) and sulfamethoxazole/trimethoprim (51.5%). The lower resistance rate was observed with ciprofloxacin (34.3%). Regarding MDR, there are 11 different patterns recorded between the examined isolates. All *S. aureus* strains exhibited resistance to four antibiotics at least, with a high MAR index ranging from 0.57 to 1.00 with an average of 0.7. The highest MAR index was observed in 8 (12.5%) *S. aureus* isolates recovered in the winter season, the results are shown in Table (2).

Molecular detection of mecA gene in S. aureus isolates

A total of 22 *S. aureus* isolates which were determined phenotypically as MRSA using cefoxitin disk diffusion method were subjected to molecular confirmation by the amplification of *mec*A gene. Out of the examined 22 isolates, 13 (59%) harbored the *mec*A gene and were confirmed as MRSA (Figure 1). The higher isolation rate of MRSA strains was in the winter season (7/13) with a percentage of 63% and these isolates were from Monofia and Kafre El-sheikh Governorates, while only one isolate was from Gharbia governorate and also it was detected in the winter season.

Detection of coa gene in isolated MRSA strains

Conventional PCR results for the examined 13 MRSA isolates showed that all harbored *coa* gene amplicon with three different product sizes of 430 bp, 580 bp, and 670 bp (Figure 2). The product amplicon of 670bp was the most frequently present in all the examined isolates with a percentage of 61.5% (8/13), and was detected in dairy farms of Gharbia and Kafr El Sheikh Governorate, followed by the 580bp amplicon which was detected in 3 isolates (23.07%) in both cows and buffaloes in Monufia Governorate. Moreover, the product amplicon of 430bp was detected in two MRSA isolates (15.38%), which was recovered from both cows and buffaloes in Monufia and Kafr El Sheikh Governorate.

Genotyping of MRSA isolates by coa gene PCR-RFLP

Coagulase gene (*coa*) product amplicons were subjected to digestion and restriction with the *Alu*I enzyme using PCR-RFLP technique for genotyping of MRSA isolates. The agarose gel analysis of *Alu*I

RFLP patterns of *coa* gene revealed that each *coa* gene amplicon product produces one pattern, resulting in three genotypes for *coa* gene of MRSA that were distinct (Table 3 and Fig. 3). The first pattern of *coa* gene amplicon (670bp), it was restricted to 3 fragments with different lengths (320bp, 240bp and 210bp). The second pattern of RFLP for *coa* gene amplicon of 430bp length which was restricted to 3 bands of (240bp, 110bp, and 80bp). The third pattern, *coa* gene of 580bp length was restricted to three bands of different lengths (80bp, 170bp, and 330bp), and it was detected in 3 isolates (Table 3 and Fig. 3).

Multiple correspondence analysis (MCA) showed variability between MRSA genotypes based on the geographic area of isolation (Figure 4). The RFLP Pattern 1 appears to be closely associated with the Kafr El Sheikh governorate. The discriminatory power of RFLP PCR was calculated using the Simpson's index of diversity (*D*) and the data showed arelativly low discrimination power (*D*=0.58).

Discussion

S. aureus is an important etiological agent in the development and spread of mastitis causing serious economic losses associated with reduced milk production and poor animal health [32]. In the present study, the prevalence of *S .aureus* was 53.3%, this was nearly similar to the results reported by Lalita, Verma [33] who identified *S. aureus* from 50% of clinical mastitis milk samples. A lower isolation rate of 27.7% was reported by Liu, Li [34] and 29% was reported by Zhang, Li [35]. In addition, 2% to 50% and even higher prevalence of *S. aureus* mammary gland infection was also observed in another study [36]. Differences in the prevalence of the pathogen could be influenced by parity, type of sample, season, and locality [37]. Therefore, bacteriological examination at the herd level must be taken regularly to monitor udder health.

In our study, an isolation rate of 61% during winter was higher than the rate during summer (45%), although nonsignificant difference. Lower temperatures and higher humidity in winter may support the survival and transmission of *S. aureus* in the environment and on the cow's skin [38, 39]. A similar result was reported by Rychshanova, Mendybayeva [40] who found that *S. aureus* isolates were most often isolated in the winter months (60.9 %). In addition, Matallah, Bouayad [41] reported that crowded indoor conditions during winter affect the occurrence of the mastitis pathogen. In contrary, Etter, Naidoo [42] noted that in the warm months the number of cows with mastitis increases, which is caused by high humidity and poor hygiene of pens and bedding.

Intensive use of antimicrobial drugs increases the resistance to antimicrobials commonly used and increases the incidence of multi-drug resistant strains [43]. The resistance of *S. aureus* to antimicrobial agents is a global problem. A drug sensitivity test is required not only for effective therapy but also for monitoring the spread of resistant strains. In the current study, *S. aureus* isolates demonstrated high rates of resistance to amoxicillin/clavulinic and cefoxitin (100%, each), followed by cefotaxim (94.7%), gentamycin (73.6%), oxytetracycline (63.1%) and sulfamethoxazole/trimethoprim(51.5%). A lower resistance rate was detected with ciprofloxacin (31.5%). In accordance, many studies reported high levels of resistance rates of *S. aureus* against different groups of antimicrobials [41, 44]. Saeed, Mat Yazid [45] attributed the high resistance of *S. aureus* isolates to the regular usage of these antimicrobials for treatment of mastitis in Egypt. Moreover, the uncontrolled use of antibacterial agents in developing countries could be a reason for the increase of *S. aureus* strains resistant to all types of β-lactam antibiotics that are frequently used for empirical treatment of mastitis [46].

In the current study, eleven different antibiotic resistance patterns were detected, which proved the high rates of excessive multidrug resistance present in the study area. Most of the resistant strains were isolated during the winter months . Our results are consistent with data obtained by other researchers from the USA and Norway [40, 47, 48]. Several factors may support and increase the exposure and susceptibility of cattle to MRSA in the winter season such as lower immunity of cows and higher stress levels in winter due to changes in nutrition, housing, and management [49]. In Egypt, calving season mostly occurs in winter; this in turn lower the immunity of cows and make them more susceptible to MRSA and other pathogens [50]. Therefore, it is important to implement good hygiene and biosecurity practices, as well as regular monitoring and treatment of mastitis cases, to prevent the spread of multidrug resistant MRSA in bovine mastitis especially during winter and calving season.

The *mec*A gene is a genetic element that conserves resistance to methicillin and other beta-lactam antibiotics in *Staphylococci* [51]. It is important to monitor the prevalence, antimicrobial susceptibility, and molecular characteristics of MRSA strains involved in bovine mastitis, and to implement appropriate control measures to prevent their dissemination. In the current study, the *mec*A gene was identified in 59% (13/22) of the screened phenotypically positive MRSA isolates. A similar result of *mec*A prevalence was reported in Iran with a percentage of 54.54% (36/60) in the screened *S. aureus* strains [52]. Another study reported 52.2% (12/23) of MRSA from dairy mastitis in Iraq [53]. In India, a study found that 47.6% of *S. aureus* isolates from mastitis-affected cows were *mec*A-positive [54]. Another study in China reported that 49 of 103

(47%) *S. aureus* isolates from mastitic cows were *mec*A-positive [55]. A low incidence of the *mec*A gene was detected in Egypt (28.2%) [56], while a very low prevalence (1.78%) was detected in Brazil, in *S. aureus* isolates from mastitis cow milk [57]. These differences in the prevalence of MRSA may be due to different geographic locations or different sources of infection.

In the current study, 100% of *S. aureus* isolates showed phenotypic methicillin resistance while only 59% were positive for the *mec*A gene. This is consistent with Xu, Shah [58] results who stated that the variation between phenotypic methicillin resistance and the presence of the *mec*A gene in *S. aureus* isolates can be attributed to several factors. For instance; some *S. aureus* strains may exhibit methicillin resistance through other mechanisms such as modifications to other penicillin-binding proteins (PBPs), changes in cell wall composition or genetic diversity. Moreover, some isolates may have lower *mec*A gene expression which resulted in phenotypic resistance without gene detection [59].

Genetic approaches in addition to phenotypic tests precisely monitor and categorize MRSA strains. They also recommend more research on the pathogenicity, epidemiology, and transmission of these strains, as well as the improvement of effective measures for the prevention and control of infection.

The coagulase (*coa*) gene is a main virulence determenant for *S. aureus* strains [60]. Moreover, Motta, Coelho [61] and Zapotoczna, McCarthy [62] mentioned that the coagulase gene plays a crucial role in *Staphylococcus* virulence, and they found a high incidence of *coa* gene in *S. aureus* isolated from bovine.

According to the current study, *coa* gene was charactrized in all the examined MRSA isolates collected from the three Governorates in Egypt, and the three *coa* amplified bands (430bp, 580bp, and 670bp) were detected. The product amplicon of 670bp length was the most prevalent. Similarly, in Egypt, Gharib, Attia [63] reported 3 *coa* gene bands in *S. aureus* isolates from human and animal samples. However, TALEBI, Ahmadi [64], examined 26 isolates of *S. aureus* and found four products of *coa* gene. Coagulase gene amplicons of about 600 bp and 800 bp were present in *S. aureus* isolates from mastitis milk sources (15 isolates and 4 isolates, respectively) in Military dairy farm, Jammu, India [65]. In addition, the product amplicon of 670bp length was recorded in *S. aureus* strains isolated from dairy products and bovine mastitis in Iran [66]. While, MOUSTAFA, HAMMAD [67] reported that the majority of *S. aureus* isolates of mastitis milk samples collected from Monofyia Governorate carried one to four *coa* gene product amplicons ranging from 300 bp to 1000 bp. Furthermore, Karahan, Şahin [68] found that all *S. aureus* isolates collected from meat and surface samples of different animal species had *coa* genes with five different molecular lengths ranging from 500 to 1400 bp. Several studies revealed that the variation in the size and quantity of *coa* gene bands may be caused by the existence of several allelic *coa* genes in MRSA, which help one strain to produce multiple amplicons [60].

Climatic and temperature changes have a variety of effects on microbial biodiversity [69]. The most frequently cited mechanism is that rising temperatures enhance metabolism, which in turn accelerates ecological and evolutionary processes including speciation, mutation, and interactions, increasing population doubling times [70, 71]. Temperature variations have been shown to have an impact on the diversity of microorganisms at several levels: for example, abundance, phenology, distribution, and geographic range [72].

Widespread applications of RFLP have been reported for genotyping, for instance; DNA fingerprinting, gene mapping, and the diagnosis of genetic diseases [73]. According to a recent Iranian study by Gharibi, Ghadimipour [66], RFLP can be utilized to examine the diversity of *S. aureus* coagulase gene in food products. Typing is done using primers corresponding to a conserved area within the *coa* gene, and this *coa* gene polymorphism is used as an epidemiological marker [74]. Since the number of repetitive sequences in the *coa* gene varies, so can the length of the PCR products produced by various strains. Mutations in the restriction sites or the insertion or deletion of DNA sequences inside the amplified fragments are blamed for the variations in the patterns obtained from different isolates within the same subspecies [75].

In the present study, 13 MRSA isolates were examined with RFLP PCR technique based on *coa* gene digestion with *Alu*I enzyme to perceive if the temperature and climatic change affected MRSA genotypes. The result revealed three different genotypes of MRSA. Therefore, the genetic variation reported between the examined isolates was (3/13) 23.07%. The main prevalent genotype was the *coa* gene with 670bp length and it was identified in 8 out of 13 isolates. The 580bp *coa* amplicon was identidied in three isolated, while the *coa* product amplicon of 430bp was detected in two isolates.

Katsuda, Hata [76] reported that *S. aureus* isolated from mastitis milk was investigated by RFLP PCR and found that coagulase genotyping showed 15 patterns. Other investigators identified varied lengths of *coa* PCR products from 500 to 1400 bp [77]. Furthermore, Bhati, Nathawat [78] compared *S. aureus* isolates from native breed and crossbred cattle in India based on the RFLP technique, and they discovered that *S. aureus* isolated from the native breed of cattle enclosed 8 types of

coa gene, but that isolated from crossbred cattle only had 3 types of *coa*. They also noted that isolates from these two breeds did not exhibit any difference in the RFLP patterns derived from the comparable amplicons. Javid, Taku [65] recorded 2 *coa* gene RFLP patterns with 595 bp *coa* genotype being predominant in *S. aureus* isolated from mastitis milk samples demonstrating multiple sources of infection. Khazaie and Ahmadi [79] recorded that *coa* gene with the sizes of 490 bp, 680 bp, and 730 bp, was produced in the partial 3′ end area amplification of the *coa* gene between the MRSA isolates from bovine subclinical mastitis, and 3 distinct RFLP patterns were apparent. Furthermore, Elkady, Al-Askar [80] proved *coa* gene polymorphism when they characterized the examined MRSA isolates into 20 patterns with the RFLP technique, and the *coa* product length ranged from 243 to 972 bp and gave 3 to 7 restriction fragments.

In the current study, MRSA strains showed different patterns of coa gene RFLP which indicates genetic variation between MRSA isolates. Similar results were reported by Can, Elmalı [81] who found a genetic relationship among *S. aureus* strains isolated from raw cow's milk in Turkey, and Castañeda-Vázquez, Padilla-Ramírez [82] in the Jalisco government, Méxic.

Furthermore, the determination of MRSA isolates with the same RFLP pattern in mastitis infection in the different dairy farms indicates that certain genotypes have been spread in this area, and could be prevalent [83].

The current investigation recorded that the genotypes of MRSA varied according to the location (governorate) of sample collection. Moreover, the pathogenicity of *S. aureus* mastitis in Egypt could be related to coagulase gene of 670 bp length.

The discriminatory power of coagulase gene amplification and RFLP can be beneficial in the epidemiological investigation, to resistor and screen hospital- and community-acquired *S. aureus* infections **[**84**].** In the current study RFLP PCR showed arelativly low discrimination power (*D*=0.58), which may be due to the DNA regions under analysis had low variability or presence of many fragments with similar sizes which make RFLP difficult to distinguished. This result agreed with Huang, Chu [85]who stated that RFLP has low discrimination power in typing of *Yersinia pestis* isolated from the United States

Conclusion

Multidrug resistant MRSA are causing bovine mastitis in Egypt, these isolates belong to three different genotypes based on coagulase gene RFLP PCR with the pattern 1 of 670bp length the most common. More epidemiological studies are recommended to aid in the controle and treatment of MRSA infection in dairy cattle.

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Not applicable.

Author contributions

F.A.E. and A.T.T. design the study. F.A.E., A.T.T. and M.M.G.E performed sampling, bacterial isolation, antimicrobial sensitivity test and genotyping. F.A.E. and H.A.A. perform data analysis and wrote the manuscript. All authors read and agreed to the published version of the manuscript.

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Data availability

All data used have been included in the manuscript.

Conflict of interest

Declare they have no financial interests.

Ethical of approval

The study received approval from the Agricultural Research Center Institutional Animal Care and Use Committee (ARC-IACUC) under approval number ARC-AHRI-68-24. Animal procedures were conducted following the ARRIVE guidelines.

TABLE 1. Prevalence of *Staphylococcus aureus* isolates in milk samples collected from cows and buffalos during winter and summer at the three Governorates under investigation

Locality	Winter		Summer		Total
	Cow N=10	Buffalo N=10	Cow N=10	Buffalo N=10	
El-Gharbia	4	6	3	5	18/40 (45.5%)
Kafr-Elsheikh	4	5	5	3	17/40 (42.5%)
Monufia	9	9	4	7	29/40 (72.5%)
Total	37/60 (61%)		27/60 (45%)		64/120 (53.3%)

TABLE 2. Antibiotic resistance patterns of *Staphylococcus aureus* isolates in milk samples collected from cows and buffalos during winter and summer

Pattern	Antimicrobial resistance pattern	No of *S.aureus* isolates (%)	Winter		Summer		MAR index
			N	%	N	%	
1	AMC – FAX- CTX- CIP – CN-TE-SXT	8 (12.5%)	8	100	0	0%	1
2	AMC – FAX- CTX- CN-TE-SXT	8 (12.5%)	5	62.5	3	37.5	0.85
3	AMC – FAX- CTX- CN-TE	14(21.8%)	8	57.1	6	42.8	0.71
4	AMC – FAX- CTX- CIP – CN	6 (9.3%)	2	33.3	4	66.6	0.71
5	AMC – FAX- CTX- CIP – TE	4 (6.2%)	1	25	3	75	0.71
6	AMC – FAX- CTX- CN-SXT	6(9.3%)	3	50	3	50	0.71
7	AMC – FAX- CTX- TE-SXT	5 (7.8%)	2	40	3	60	0.71
8	AMC – FAX- CTX- CIP-SXT	3(4.6%)	0	0	3	100	0.71
9	AMC – FAX- CTX- CN	6(9.3%)	4	66.6	2	33.3	0.57
10	AMC – FAX- CIP – TE	1(1.5%)	0	0	1	100	0.57
11	AMC – FAX- SXT – CN	3(4.6%)	2	66.6	1	33.3	0.57
	Total	64	35	54.6	29	45.3	

AMC; amoxicillin/clavulanic, FAX; cefoxitin,CTX; cefotaxime, CN; gentamycin, CIP; ciprofloxacin, TE; oxytetracycline, SXT; sulfamethoxazole/trimethoprim, MAR: multiple antibiotic resistance index, N: Number.

TABLE 3. Coagulase genotypes, source, geographic region, and pattern of RFLP in examined MRSA isolates.

Code no. of samples	Animal species	Site of isolation	Coagulase product	Season of isolation	RFLP fragments	RFLP pattern
4	cow	Gharbia	670	winter	320, 240, 110	pattern 1
5	Buffalo	Monufia	430	summer	240, 110, 80	Pattern 2
6	cow	Monufia	670	summer	320, 240, 110	pattern 1
8	cow	Monufia	670	summer	320, 240, 110	pattern 1
13	Buffalo	Monufia	670	summer	320, 240, 110	pattern 1
14	Buffalo	Kafr El Sheikh	670	winter	320, 240, 110	pattern 1
15	cow	Kafr El Sheikh	670	winter	320, 240, 110	pattern 1
16	cow	Kafr El Sheikh	670	summer	320, 240, 110	pattern 1
17	Buffalo	Kafr El Sheikh	670	summer	320, 240, 110	pattern 1
18	cow	Kafr El Sheikh	580	summer	80, 170, 330	Pattern 3
19	cow	Monufia	580	winter	80, 170, 330	Pattern 3
20	Buffalo	Monufia	580	winter	80, 170, 330	Pattern 3
21	cow	Monufia	430	winter	240, 110, 80	Pattern 2

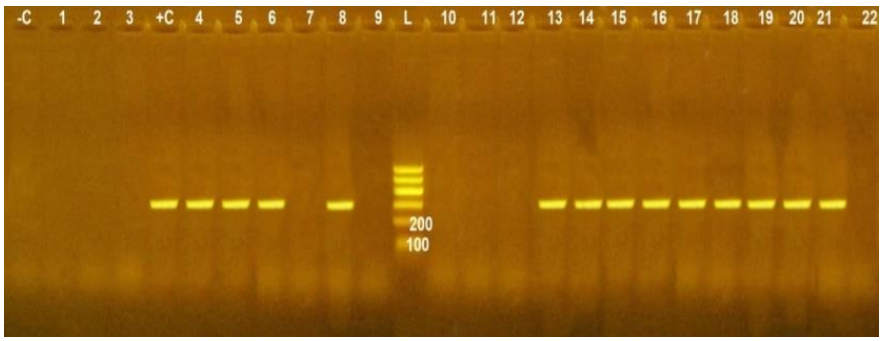

Fig. 1. Gel electrophoresis of *mec*A gene in *S. aureus* isolates on 1.5 % agarose gel. Lane L: ladder. 1 and 4: 6, 8,13: 21 positive amplification for *mec*A gene at 310 bp.

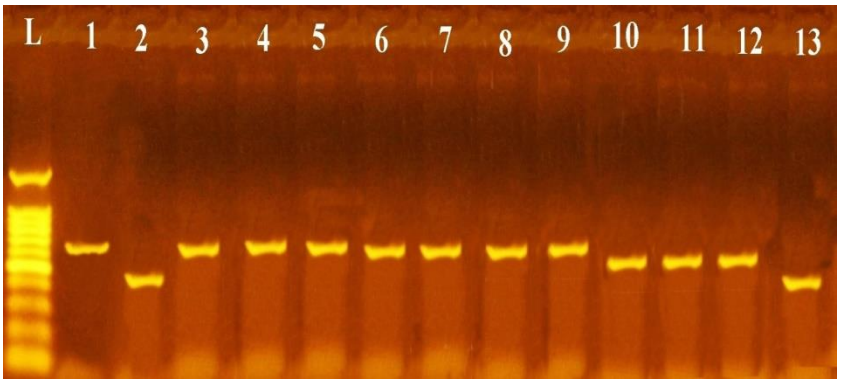

Fig. 2. Gel electrophoresis of *coa* gene in MRSA isolates on 1.5 % agarose gel. Lane L: ladder. 1 and 3: 9 for *coa* gene amplified product at 670 bp; lanes 2 and 13 for *coa* gene amplified product at 430 bp; lanes 10,11, and 12 for *coa* gene amplified product at 580 bp

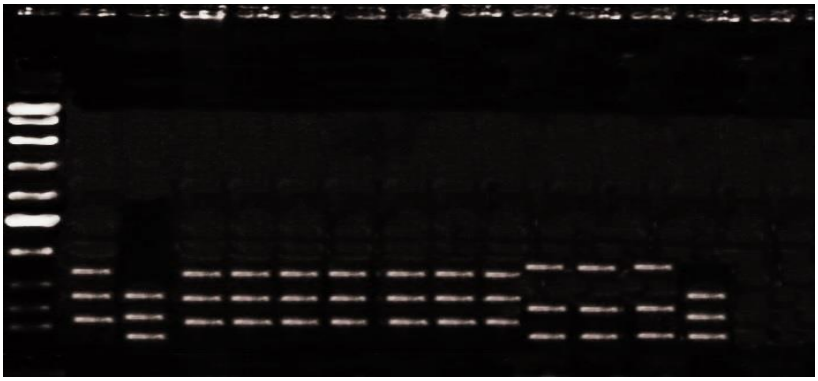

Fig. 3. Electrophoresis of *coa* RFLP after digestion by *AluI* enzyme on 1.5 % agarose gel. Lane L: ladder. 1, 3, and 4: 9 for pattern 1 of *coa* gene amplified product at 670 bp giving three bands(320, 240 and 110); lanes 2 and 13 for pattern 2 of *coa* gene amplified product at 430 bp giving three bands (240, 110 and 80); lane 10,11, and 12 for pattern 3 of *coa* gene amplified product at 580 bp giving 80, 170 and 330 bp band.

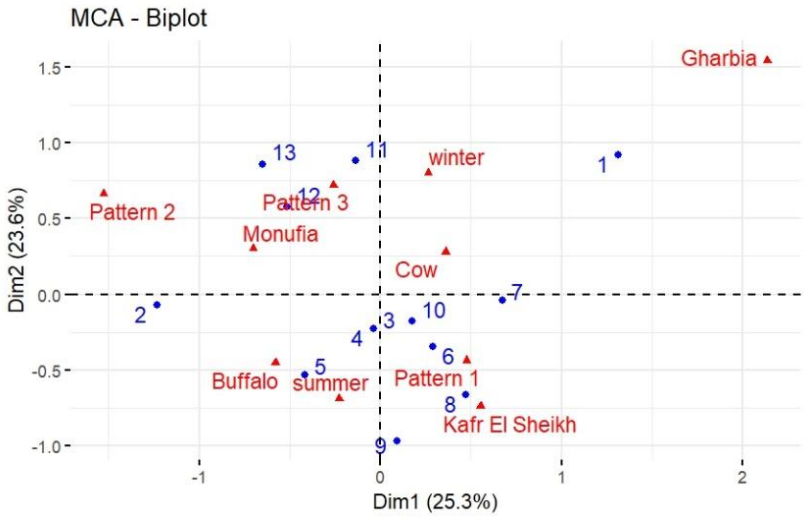

Fig. 4. Multiple correspondence analysis (MCA) plot showed that dimension 1and 2 representing 25.3% and 23.6% respectively from the total variability between the tested isolates based on the RFLP pattern. It also showed that Pattern 1 was more related to Kafr El sheik and they were closed to dimension 1. Blue points: tested isolates, red labels: variables.

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التنوع الجيني للاستافيلوكوكس اوريوس المقاومه للمسيسيللين المعزوله من التهاب الضرع في الماشيه

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[2] قسم الامراض المشتركة ، كلية الطب البيطري، الزقازيق، جامعة الزقازيق، مصر.

الملخص

المكورات العنقودية الذهبية المقاومة للميثيسيلين (MRSA) لها تأثير متزايد في الطب البيطري في العقود الماضية . وتسببت في التهاب الضرع المقاوم للأدوية المتعددة المعقدة و الشديدة في قطعان الألبان. هدفت هذه الدراسة إلى عزل المكورات العنقودية الذهبية المسببه لالتهاب الضرع البقري في ثلاث محافظات مختلفة في مصر ، خلال فصلي الصيف والشتاء. بالإضافة إلى ذلك ، قمنا بتقييم حساسية ومقاومة العزلات للمضادات الحيوية المختلفة المستخدمة في الحقل. حددنا أيضا جينات mecA و coa في عزلات MRSA واستخدمنا PCR RFLP للتنميط الجيني للعزلات. كشفت نتائجنا عن معدل عزل بنسبة 53.3٪ (120/64) من S المكورات العنقودية الذهبية ، مع اختلاف كبير بين المحافظات قيد الاختبار . أظهرت العزلات معدلات عالية من مقاومة الأدوية المتعددة (MDR) ، مع 11 نمطا مختلفا لمقاومة النمط الظاهري وتراوح مؤشر MAR في العزلات من 0.57 إلى 1. وقد لوحظ أعلى مؤشر MAR في 8 عزلات (12.5٪) تم استردادها في فصل الشتاء. تم التعرف علي جين mecA في (59٪) من العزلات التي تحتوي بشكل إيجابي على جين coa مع ثلاثة أحجام مختلفة للمنتج (670 bp و 430 bp و580 bp) تختلف وفقا لمكان عزل العينات.وعند استخدام انزيم القطع AluI لجين coa نتج 3 أنماط مختلفة من RFLP ل MRSA حيث كان النمط 1 كان الأكثر شيوعا ويرتبط ارتباطا وثيقا بعزلات MRSA من كفر الشيخ. في الختام: حددت نتائجنا معدلا مرتفعا من MRSA المقاوم للأدوية المتعددة التي تسبب التهاب الضرع البقري في مصر بسبب ثلاثة أنماط وراثية مختلفة تعتمد على RFLP PCR لجين COA. وقد كشف تحليل البيانات عن وجود علاقات وراثية بين عزلات MRSA في نفس المحافظة دون ارتباط موسمي أو نوعي.

الكلمات الدالة: التنوع الجيني، الاستافيلوكوكس اوريوس ،المقاومه للمسيسيللين، التهاب الضرع في الماشيه، البصمة الوراثيه RFLP