Research Article

Phylogenetic Tracking of *Ica*-locus in MRSA from Different Sources at Ismailia City, Egypt

Ali Wahdan[1*], Mahmoud Ezzat[1], Fatma Youssef[2], Manal Munier[2], Elsayyad M. Ahmed[3], Marwa Abo Hashem[1]

[1]Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt; [2]Department of Clinical Pathology, Animal Health Research Institute, Ismailia, Egypt; [3]Department of Virology, Animal Health Research Institute (AHRI), Dokki, Giza, Egypt.

Abstract | Tracking of MRSA strains which encoded intracellular adhesion (*ica)* locus is a major threat for livestock and public health. As it indicates that the isolated strains have ability to biofilm formation leading to zoonotic risk and nosocomial infections. So, this study focused on tracking the *ica* locus in different hosts and other virulence factors with genetic analysis of recovered isolates. A total of 350 random samples were collected from animals and hospital acquired infected patients. The recovered isolates were identified biochemically by vitek2 compact, confirmed by PCR and tested for sensitivity against varieties of antimicrobials. Three multi-drug resistant isolates carried *ica* from human abscess (A9), sputum of dog (A12), and cow mastitic milk (A13) were sequenced with other genome sequences retrieved from the GenBank. Ten samples (9.17%) were *S. aureus* from human, while 3 samples (1.24%) were *S. aureus* and 2 samples (0.83%) were coagulase negative staphylococcus (CONS) from animal sources. The multiple sequences alignments (MSA) and phylogenetic tracking proved the genetic similarity among A9, A12 and A13 from (human, animal, food of animal origin respectively) reached to 94 %. In conclusion, although the low isolation rate of confirmed MRSA in Ismailia governorate, the three selected MRSA strains invigorate the possibility of transmission among different sources of food, animals, and human, which causes nosocomial infections.

Keywords | *Ica*-MRSA, PCR, Virulence factors, Resistant factors, Phylogenomics.

Received | August 11, 2022; **Accepted** | September 01, 2022; **Published** | September 15, 2022
***Correspondence** | Ali Wahdan, Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt; **Email:** dr_aly_w@yahoo.com
Citation | Wahdan A, Ezzat M, Youssef F, Munier M, Ahmed SM, Hashem MA (2022). Phylogenetic tracking of *Ica*-locus in MRSA from different sources at ismailia city, egypt.. Adv. Anim. Vet. Sci. 10(10): 2124-2132.
DOI | http://dx.doi.org/10.17582/journal.aavs/2022/10.10.2124.2132
ISSN (Online) | 2307-8316

INTRODUCTION

Staphylococci are common bacterial pathogens that may infect both animals and humans, resulting in a high number of hospital and community-acquired diseases each year. *Staphylococcal* infections have a significant economic and clinical impact due to a range of factors, including increased antibiotic resistance and a lack of effective immunizations (Asante et al., 2020).

Staphylococcus aureus is a prevalent human and animal infection that causes a variety of clinical symptoms (Tong et al., 2015); (Becker et al., 2017). *S. aureus* and other *Staphylococcal* species are considered public health pathogens due to their ability to rapidly acquire multiple resistance genes (Lakhundi and Zhang, 2018). Thus, methicillin-resistant *S. aureus* (MRSA) and coagulase-negative Staphylococci are among the most common causes of healthcare-associated infections (MR-CoNS) (Becker et al., 2020).

Staphylococcus aureus causes major difficulties in animals, including suppurated illness, mastitis, arthritis, and urinary

tract infection. In humans, *S. aureus* is a major cause of food poisoning, pneumonia, surgical wound infections, and nosocomial bacteremia. (John and Harvin, 2007). Meanwhile, CoNS, especially *S. epidermidis* and *S. haemolyticus*, have emerged as prevalent causative agents of nosocomial infections, particularly those linked with indwelling devices. Because they actively assist in horizontal resistance transmission, they offer a significant concern in the final stages of multidrug resistance (Becker et al., 2014).

Staphylococcus aureus generates a diverse set of extracellular proteins and polysaccharides, some of which are associated with virulence. Vaccines are not currently available, despite the fact that antibodies may neutralize *Staphylococcal* toxins and enzymes. Most antibiotics used to treat infections are resistant to some kinds of bacteria that infect hospitalized patients, with vancomycin being the only remaining medicine that has not acquired resistance (Foster, 1996) (Tong et al., 2015). Multidrug resistance strains can be discovered in persons, pets, foodstuff, other species, and the ecosystem (Sabat et al., 2013; Rossi et al., 2019).

Biofilm development allows germs to compete with human immunity while also protecting microbes from antimicrobial agents. This trait is associated with bacterial pathogenicity and several chronic bacterial diseases (Vancraeynest et al., 2004). Because they play a significant role in the production of polysaccharide intercellular adhesion, intercellular adhesion A and B (*icaA* and *icaD*) are responsible for *Staphylococci* biofilm growth (Fitzpatrick et al., 2005; Rohde et al., 2007).

Multiple of the genetic markers of resistance may have been exchanged among many *staphylococcal* species from varied environments and hosts, according to genomic studies (Xu et al., 2022). Due to local variations in control practices and specific characteristics of circulating clones, the overall geographic distribution of MRSA, this study focused on phylogenetic tracking the *Ica* locus in MRSA isolated from different sources with detection of some virulence and antibiotic resistance factors of the recovered isolates.

MATERIALS AND METHODS

Ethical Approval And Study Area

The investigation was carried out in accordance with Egyptian regulations on animal experimentation and veterinary medicine practice. The study is not an animal experiment, but rather the collection of samples in accordance with Egyptian Veterinary Practice. All human samples were collected with the permission of the Ismailia hospital. Veterinary samples were obtained from veterinary clinics, farms, and marketplaces throughout the governorates of Ismailia. Various areas in Egypt's Ismailia governorate were studied between 2020 and 2022.

Sampling

Different samples were collected from different sources in sterile manner as follow; 25 g from raw meat, processed milk products and poultry meat, 5ml raw milk and 43 lesion swabs from farms and veterinary clinics. Twenty five blood samples, 30 urine samples and 54 swabs from lesions of infected patients at Ismailia hospital. All swabs were transported in 10 ml peptone water (Power and Johnson, 2009). Urine and other samples were transported in sterile container for bacteriological examination. All sampled were prepared and processed according to instructions of (Eaton et al., 1995) (Quinn et al., 2015).

Isolation And Vitek2 Characterization Of The Isolates

According to (Quinn et al., 2011) samples were grown in peptone water for 24 hours at 37°C before being transferred to 5 % sheep blood agar and mannitol salt agar. After incubating all inoculation plates at 37°C for 24-48 hours, colonies were detected. *Staphylococcus* colonies were evaluated visually and biochemically by Vitek 2 compact utilizing Gram positive cards after the turbidity was adjusted to 0.53 to 0.63 according to (Rampacci et al., 2021).

Pcr Recognition Of Some Virulence And Resistance Genes

MRSA DNA was extracted from purified bacterial cells using the QIAamp DNA Mini Kit (Invitrogen, USA), and the methods were followed exactly (Ezzat et al., 2018). From the confirmed strains *nuc,ica, spa, mecA, blaZ, Coa* genes were detected, PCR was performed using specific sets of primers (Metabion, Germany), and the cycling conditions were performed as in (Table 1) according to (Ezzat et al., 2018). The final volume was 25 µl (12.5 µl Go Taq® Green Master Mix 2X, µl (20 pmol) for each forward and reverse primer, 5µl sample DNA, and PCR water up to 25 µl). The negative control was PCR water, and the positive control was graciously donated by A.H.R I, Dokki, Egypt). The proper bands were detected using 1% agarose gel electrophoresis at 100 Volt for 45 minutes (Applichem GmbH, Darmstadt, Germany) and photographed using a UV transilluminator. (Germany, Fermentas, Thermo Scientific), 100 bp ladder was utilized.

Alignment And Pylogenetic Comparison Of Ica Locus In Mrsa From Different Sources

Three isolates harbored to *mecA* and *ica* genes (human abscess (A9), sputum of dog (A12), and cow mastitic milk (A13) were aligned and sequenced. QIAquick PCR Product extraction kit was used to purify the PCR (Qiagen Inc. Valencia CA). Cycle sequencing kit Bigdye Terminator V3.1. Gene sequencing was conducted using an Applied

Table 1: Cycles conditions of PCR

Gene	Intial denaturation	denaturation	Annealing	Extension	Cycles	Final extension
mecA	94°C 5 min.	94°C 30 sec.	50°C 30 sec.	72°C 30 sec.	35	72°C 10 min.
coa	94°C 5 min.	94°C 30 sec.	55°C 40 sec.	72°C 45 sec.	35	72°C 10 min.
spa	94°C 5 min.	94°C 30 sec.	55°C 30 sec.	72°C 30 sec.	35	72°C 7 min.
blaZ	94°C 5 min.	94°C 30 sec.	50°C 40 sec.	72°C 50 sec.	35	72°C 10 min.
icaA	94°C 5 min.	94°C 30 sec.	49°C 1 min.	72°C 1 min.	35	72°C 10 min.
Nuc	94°C 5 min.	94°C 30 sec.	55°C 40 sec.	72°C 40 sec.	35	72°C 10 min.

Table 2: Prevalence of negative and positive coagulase *Staphylococcus* isolated from different sources:

Species	Sources of the sample	No. samples	Bacteriological finding			
			No. of coagulase positive *Staph*	%	No of coagulase negative *Staph*	%
Human	Lesion swabs (Wound, pus)	54	7	12.96	0	0
	Blood cultures	25	2	8	0	0
	Urine culture	30	1	3.33	0	0
	Total	109	10	9.17	0	0
Animal	raw meat	52	0	0	1 *S. xylosus*	1.92
	raw milk	51	1	1.96	1 *S. saprophyticus*	1.96
	milk product	50	0	0	0	0
	Poultry	45	0	0	0	0
	lesion swabs	43	2	4.6	0	0
	Total	241	3	1.24	2	0.83

Table 3: Results of virulence and antibiotic resistant genes

	Code	Source of samples	PCR results					
			nuc	*ica*	*spa*	*mecA*	*blaZ*	*coa*
Human origin	1	abscess	+	+	+	+	+	+
	2	abscess	+	+	+	+	_	_
	3	blood	+	_	+	+	+	_
	4	blood	_	_	+	+	_	_
	5	abscess	+	+	+	+	+	+
	6	abscess	+	_	+	_	_	+
	7	urine	+	_	+	+	+	+
	8	abscess	+	+	+	+	+	+
	9	abscess	+	+	+	+	+	+
	10	wound	+	+	+	+	+	+
Animal origin	11	abscess from cat	+	+	+	+	+	+
	12	Sputum from dog	+	+	+	+	+	+
	13	Mastitic milk	+	+	+	+	+	+
S.saprophyticus	14	Raw milk	-	-	-	-	-	-
S.xylosus (CONS	15	Raw meat	-	-	-	-	-	-

Total	12	9	13	12	10	10
% Percent	80%	60%	86.6%	80%	66.6%	66.6%

Biosystems 3130 genetic analyzer (HITACHI, Japan) and a Perkin-Elmer cat-number (4336817) (Perkin-Elmer, Foster City, CA) as (Wahdan et al., 2020). Using the Basic local alignment tool for nucleotide (BLASTN), the sequences were collected from the NCBI, and high similar sequences for the *ica* gene were chosen and downloaded in fatsa format (Tamura et al., 2013). Multiple sequence alignments (MSA) were utilized in conjunction with the UGEN software and the MUSCLE method to investigate the evolutionary relationship between our isolates and strains in the GenBank database (Okonechnikov et al., 2012). The phylogenetic tree was constructed in IQ-TREE (Nguyen et al., 2015) using the maximum likelihood method, Model Finder to choose the best-fitting model, and values from 1000 bootstrap replicates. The tree was scaled to show the amount of substitutions per site, with branch length indicating how many substitutions there are per site. The evolutionary tree was shown, manipulated, and annotated using the Interactive Tree of Life (iTOL) (Letunic and Bork, 2021).

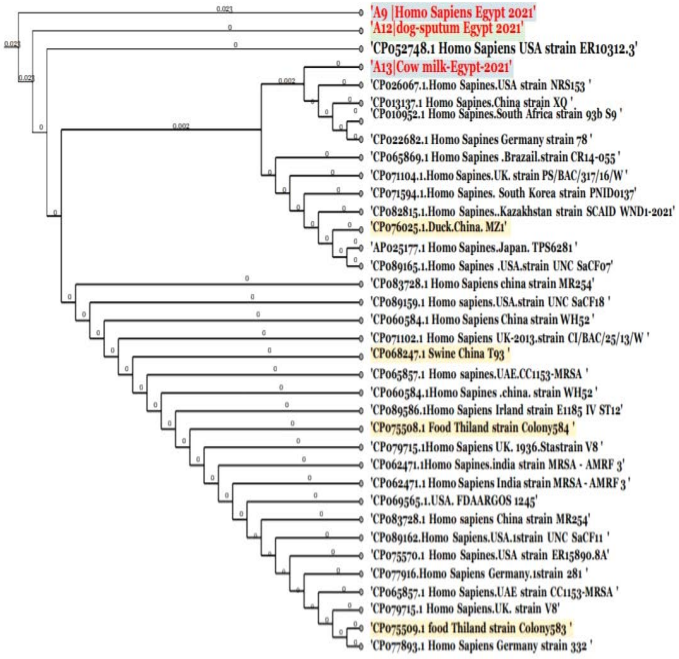

Figure 1: Phylogenetic analysis of *ica* locus sequence. (A9): human sample "abscess", (A12): dog sample "sputum", and (A13): cow mastitic milk. All other sequences are derived from the NCBI GenBank database.

RESULTS

Regarding to phenotypic characterization, large, round, convex, often with beta hemolysis on blood agar and yellow colonies with yellow precipitation on mannitol salt agar were selected and confirmed biochemically. Among 350 specimens from humans, animals, food of animal origin examined in this study, 15 contained *Staphylococcus* with percentage 4.3% from different sources as shown in (Table 2). Ten *S. aureus* (9.17%) was recovered from 109 human samples. Seven isolates from wounds and pus swab samples, 2 isolates from blood and one isolate from urine samples. Three *S. aureus* (1.24%) and 2 *staphylococcus* (CONS) (0.83%) were recovered from 241 animal samples. All recovered colonies were confirmed and some virulence genes (*nuc, ica, spa, mecA, blaZ, coa*) were marked by PCR as shown in (Table 3). Twelve isolates (80%) were *nuc* gene positive, 9 isolates (60%) were *ica* gene positive, 13 isolates (86.6%) were *spa* gene positive, 12 isolates (80%) were *mecA* gene positive, 10 isolates (66.6%) were positive for both *blaZ* and *coa* genes. CONS were negative for all tested genes.

Three isolates harbored to *mecA* and *ica* genes (human abscess (A9), sputum of dog (A12), and cow mastitic milk (A13) were aligned and sequenced and taken accession numbers (On994655, On994656, and On994657) at GenBank. Multiple sequences alignments and phylogenetic analysis of (Figure 1), proved that the sequence identity was 94% between 3 selected *S. aureus*. Our isolates are closely related to the isolates from different geographical locations, 96% identity with isolates from USA, India, Germany, Thiland, China, UK, Irland and 95% for 1 isolate from Kazakhstan, all clustered together. The phylogenetic tree of *ica* clustered the Egyptian three isolates with the other 33 *S. aureus* isolates. It proved that all have the same *ica* except for two nucleotides difference from C to G at position 144 in the isolates of Germany (CP022682.1 Homo Sapines) and Brazail (CP065869.1 Homo Sapines) other single nucleotide polymorphism (SNP) from C to T in isolates from Germany (CP022682.1 Homo Sapines), Brazail (CP065869.1 Homo Sapines), China (CP076025.1Duck) and South Korea (CP071594.1 Homo Sapines) at position 351 of our isolate's sequences.

In this study there was a genetic relatedness among A9, A12 and A13 from (human, animal, food of animal origin respectively) with 94 % genetic similarity which applied by sequencing on PCR product on *ica* gene. On the other hand, to investigate the evolutionary relationship between the sequenced gene from this isolates and strains available in the GenBank database, it revealed that there is genetic similarity between human isolate (A9) and swine isolate in china (CP068247.1) and food isolate in

Thiland (CP075508.1) with 96%. There is genetic similarity between animal isolate (A12) and human isolates in Germany (CP077916.1), USA(CP089159.1) and china(CP083728.1) with 96%. There is genetic similarity between food of animal origin isolate (A13) and human isolate in USA (CP026067.1) and duck (CP076025.1) isolate in china with 95%.

DISCUSSION

This study was designed to determine the prevalence of coagulase positive and coagulase negative *staphylococcus* isolates from different sources (human, animals, food of animal origin) from Ismailia governorate and their genetic relatedness to *S. aureus* isolates from human, animal, and food from animal origin.

Our findings were virtually identical to those of (El-Jakee et al., 2008) who used bacterial culture techniques to determine the presence of *S. aureus* in samples collected from unwell individuals and found 14 *S. aureus* strains with a 28% success rate, while the percentage of *S. aureus* isolates from septic wound was higher with a percentage of 45% (Kim and Hong, 2019) found that 161 of the 198 refugee health research participants volunteered to submit a nose swab. *S. aureus* was found in 9 (5.6%) of the 161 nasal swabs. MRSA was detected in four (2.5%) of the genotypes. These findings take into consideration research indicating that the most of hospital-acquired infections are caused by *S. aureus.* In this study, 2 *S. aureus* isolates were found in sputum from a dog with respiratory symptoms and an abscess from a cat, which corresponded with previous findings. (Lu and McEwan, 2007) who investigated the microbiota on healthy skin in 10 dogs and 10 cats. Surprisingly, they discovered *S. aureus* in nine (90%) of the dogs and four (40%) of the cats, but no *S. intermedius* isolates.

The occurrence of *Staphylococcus* in raw milk and raw meat may be linked to filthy worker hands, poor water quality used to wash utensils and carcasses, unsanitary transportation, improper storage, and contamination from mastitic cattle resuming to the milking practices. The current findings are consistent with the findings of (Normanno et al., 2007; De Boer et al., 2009; Ezzat et al., 2014). In this respect our results were less than the results of (Fa Mansour and Am Basha, 2009) and (Bhargava et al., 2011) who discovered that S. aureus was present in 20% and 20.5% of meat samples from different markets, respectively.

On the other hand, our results were nearly to (Goja et al., 2013) and (Ezzat et al., 2014) who retrieved *S. aureus* from raw beef meal in percentages of 5.55%, 12%, and 10%, respectively, and were less than the outcomes of (Kitai et al., 2005; Van Loo et al., 2007; Pu et al., 2009) that was 65%, 46% , 39.2% respectively. Therefore in this paper, the frequency of *Staphylococcus* recovered from unpasteurized milk is lower than the frequency of *Staphylococcus* recovered from pasteurized milk. (Fagundes et al., 2010) who confirmed that 7.3% of those likely *Staphylococcus* could transfer to consumers through milk from animals with mild mastitis. This result is noticeably lower than the findings of (Singh and Baxi, 1982) (Ezzat et al., 2014) They reported that the incidence of *Staphylococcus* in raw milk samples was (21%) and (18.5%). The findings of this study differ from those of previous studies undertaken by (Abou-Khadra et al., 2020) discovered that *S. aureus* was present in 20% of raw milk samples from Sharkia Governorate. The incidence of *Staphylococcus* isolates from frozen raw chickens was low, which was consistent with the findings of (De Boer et al., 2009), who reported that imported chicken goods such as frozen filets had a low frequency of Staphylococcus. These findings differed by 2.6% from those of (Ezzat et al., 2018) , who recovered S. aureus from fresh raw chicken.

The high rate of MRSA isolation in this study was considered as an extension of previous studies conducted by other investigator teams in Egypt (Ammar et al., 2016) and Bangladesh (Nusrat et al., 2015). In this study, some of virulence and resistance genes (*nuc, coa, spa, ica, mecA, blacZ*) were found using PCR technique, which is the most promising since it can detect just a few microbes in clinical samples yet is quick, inexpensive, and sensitive.

All negative coagulase *Staphylococcus* isolates showed no evidence of having virulence factors which was confirmed by PCR detection for virulence genes (*nuc, coa, spa, ica*). This finding is disagreed with (Xu et al., 2022) who supposed that some CoNS present these genes. In the same context (Quinn et al., 2015) Apart from a little quantity of toxins and exoenzymes, *Staphylococcus* species that were previously believed to be harmless members of the microbiota of people and animals, like the majority of CoNS, were shown to be deficient in virtually all of the virulence determinants outlined for *S. aureus*.

Furthermore, all CoNS showed no evidence of multidrug resistance, which was confirmed by PCR testing for resistance genes (*mecA, blacZ*), demonstrating that this kind of Staphylococcus strain has no clinical value; so, my findings are perfectly consistent with the literature (Becker et al., 2014) whic indicated that CoNS regarded both animal and human microflora as harmless invaders.

Our findings on virulence and resistance genes were substantially identical to those of another investigation by (Parth et al., 2016) who discovered that 32 of 53 isolates tested positive for the *coa* gene and had three unique prod-

uct sizes of 723, 812, and 1000 bp. Similar results were obtained by (Himabindu et al., 2009) who revealed that the size of PCR products generated in human samples following *S. aureus* amplification. (Schlegelova et al., 2003) illustrated that *coa* gene of *S. aureus* isolates from lactating cows and people was found to vary in size from 650 to 1050 bp. (Reinoso et al., 2008) furthermore, PCR amplification of the *S. aureus coa* gene discovered in both human and cow mild mastitis and food specimens generated seven different *coa* types from 45 *S. aureus* with amplicon sizes ranging from 400 to 1000bp.The same result was obtained by (Himabindu et al., 2009) who found that isolates that were coagulase negative by tube coagulase test were coagulase positive by gene PCR assay.

In this study the prevalence of *ica* gene among *S. aureus* isolates is 69.2% which is similar to (Foroutan et al., 2022) who claimed that a total of 30 *S. aureus* isolates were studied to assess the influence of biofilm forming capacity on antibiotic resistance patterns, with 77.4% developing strong biofilms. The frequency of encoding biofilm genes was as follows: *icaA* (9.4%), *icaD* (9.4%), and 75%. The *spa* gene was detected in all *S. aureus* isolates (100%), which is perfectly compatible with earlier findings (Eid et al., 2018) who detected the *spa* locus in all strains examined. Conventional PCR methods were capable of amplifying the protein A DNA (*spa*) bands (Parth et al., 2016) which is a component of the cell wall of *S. aureus* and is covalently attached to the peptidoglycan.

The current findings suggest a significant incidence of multidrug resistant *S. aureus* isolates comparable to those indicated by (Quinn et al., 2015) who discovered that in areas where antibiotics can be obtained without a prescription, the development and spread of resistance is accelerated. Similarly, in nations with no clear treatment guidelines, doctors regularly over-prescribe antibiotics and the general populace over-uses them. Also, (Food and Administration, 2014) observed that 62% of critical antibiotics for human health are marketed to food animal producers and utilized on farms, which is likely to be a significant contributor to the high incidence of multidrug resistant S. aureus isolates in animal feeds. These findings suggest that we are approaching a hazardous point when we will be unable to develop effective medications for these pathogens, (Organization, 2015) who indicated that opposition is at an all-time high throughout the world.

Every day, new resistance mechanisms emerge and spread around the world, endangering our ability to control common infectious diseases. In addition to, (Hellen et al., 2015) Drug-resistant infections have been linked to 58000 newborn sepsis fatalities in India. There is no monitoring system in Egypt that tells us how many people died as a result of the high frequency of antibiotic resistance. (Epidemiologists, 2015) confirmed that *Staphylococcus* infections in hospitals were virtually impossible to cure. (Cosgrove et al., 2005) methicillin resistance in *S. aureus* bacteremia has been linked to a significant increase in hospitalization duration and expenses. (Healy et al., 2004) tested Eight patients (47%) positive for MRSA, seven (88%) of eight patients developed septic shock, and vancomycin was used as the first line of therapy with *S. aureus* bacteremia. Despite this, three patients (38%) died and three acquired complications requiring long-term antibiotic treatment. (Control and Prevention, 2001) According to a compilation of information from January 1992 to June 2001, S. aureus septicemia was related with death rates ranging from 15% to 60%.

The prevalence of MRSA in this research was greater than in previous studies by (Ezzat et al., 2014) who discovered 96 patients with MRSA from 348 patients with *S. aureus* bacteremia, (El-Jakee et al., 2008) who demonstrated that *S. aureus* isolates from patients were sensitive to methicillin (Lee et al., 2001) who said that MRSA is a major problem in Korea, the rate of methicillin resistance among human *S. aureus* isolates in Korea is greater than 50%. In addition to, (Normanno et al., 2007; Van Loo et al., 2007; De Boer et al., 2009; Pu et al., 2009) they observed that MRSA was present in 3.75%, 2%, 11.9%, and 5% of raw beef samples, respectively. These findings contradicted the results previously reported by (Kitai et al., 2005) who found two distinct MRSA bacteria (0.5%) in 444 raw chicken meat items from Japanese retailers. In a Korean investigation that comprised 930 slaughterhouse and retail meat samples, MRSA was discovered in two chicken meat samples (0.2%) but not in any beef samples (Kwon et al., 2006). In an Italian study of 1634 food samples, six (0.4%) MRSA bacteria were found in bovine milk and cheese (Normanno et al., 2007). Many prior investigations have shown MRSA in 48.3% of mastitis cases by (Guimarães et al., 2017), 15.5% by (Wang et al., 2015), 11.6% by (Jamali et al., 2014) and 2.5% by (Moon et al., 2007). MRSA may also contaminate food and goods without altering milk according to (Parisi et al., 2016), MRSA is spread to people by mastitic milk or direct contact with dairy cows, presenting severe issues about food safety and public health.

In this study there was a genetic relatedness among A9, A12 and A13 from (human, animal, food of animal origin respectively) with 94 % genetic similarity which applied by sequencing on PCR product on *ica* gene. The evolutionary link between the sequenced genes from these isolates and strains accessible in the GenBank database indicated that there is 96 % genetic similarity between human isolate (A9) (On994655), swine isolate in China, and food isolate in Thailand. There is 96 % genetic similarity between

sputum from dog isolate (A12) (On994656) and human isolates from Germany, the United States, and China recorded by (Decline et al., 2020). Source of MRSA transmission is due to direct contact with humans or several animals, where dogs infected with MRSA act as a reservoir and then transmit to other animals or humans. There is genetic similarity between food of animal origin isolate (A13 mastitic milk from cow) (On994657) and human isolate in USA, duck isolate in china with 95%. This result agree with (Lee, 2003) which discovered that six of the animal isolates were similar to human isolates, pointing to a probable source of human illnesses caused by ingesting contaminated food products derived from these animals, (Organization, 2015) that mentioned 1 in 5 resistant infections in humans are caused by germs from food and animals, (Frieden, 2013) recorded 22 % of antibiotic-resistant illness in humans is in fact linked to food and (Van Loo et al., 2007) who claimed that MRSA from an animal reservoir has recently invaded the human population and is currently responsible for more than 20% of all MRSA in the Netherlands. In addition to (Weese, 2010) He stated that there is no doubt that MRSA may be present in foods and that it may play a role in human disease.

CONCLUSIONS AND RECOMMENDATION

The isolation rate of *Staphylococcus* from examined 350 humans, animals, food specimens was 4.3%. Thirteen isolate was confirmed as *S. aureus* and 2 isolates were CONS. PCR analysis revealed that *nuc, spa, ica, coa, mecA,* and *blaZ* genes were detected in coagulase positive *Staphylococcus* examined isolates. While, no virulence or antibiotic resistance genes were detected in CONS. The multiple sequences alignments (MSA) and phylogenetic tracking of 3 selected MRSA strains from (human (A9), animal (A12), food of animal origin (A13)) proved the genetic similarity reached to 94 %, which invigorate the possibility of transmission among different sources of food, animals, and human, which causes nosocomial infections.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

NOVELTY STAEMENT

The study focused on the phylogenetic tracking and possibility of transmission of MRSA strains isolated from human, animal, food of animal origin.

AUTHOR'S CONTRIBUTION

ME, FY, and MA H supervised, designed the study and took apart in writing the manuscript. MM collected the samples, identified the recovered strains and participated in writing of the manuscript. EMA analyzed the recorded data. AW participated in molecular detection of isolates and took apart in writing the manuscript.

REFERENCES

Abou-Khadra S., El-Shorbagy I., El-Azzouny M. (2020). Rapid detection of enterotoxigenic *Staphylococcus aureus* isolated from raw cow milk in Sharkia governorate, Egypt. Adv. Anim. Vet. Sci. 8: 11-17. https://doi.org/10.17582/journal.aavs/2020/8.s1.11.17

Ammar A., Attia A., El-Aziz A., Abd El Hamid M., El-Demerdash A. (2016). Class 1 integron and associated gene cassettes mediating multiple-drug resistance in some food borne pathogens. Int. Food Res. J. 23.

Asante J., Amoako D.G., Abia A.L., Somboro A.M., Govinden U., Bester L.A., Essack S.Y. (2020). Review of clinically and epidemiologically relevant coagulase-negative Staphylococci in Africa. Microbial Drug Resist. 26: 951-970. https://doi.org/10.1089/mdr.2019.0381

Becker K., Ballhausen B., Kahl B.C., Köck R. (2017). The clinical impact of livestock-associated methicillin-resistant *Staphylococcus aureus* of the clonal complex 398 for humans. Vet. Microbiol. 200: 33-38. https://doi.org/10.1016/j.vetmic.2015.11.013

Becker K., Both A., Weißelberg S., Heilmann C., Rohde H., (2020). Emergence of coagulase-negative staphylococci. Expert review of anti-infective therapy 18, 349-366. https://doi.org/10.1080/14787210.2020.1730813

Becker, K., Heilmann, C., Peters, G., 2014. Coagulase-negative staphylococci. Clin. Microbiol. Rev. 27: 870-926. https://doi.org/10.1128/CMR.00109-13

Bhargava K., Wang X., Donabedian S., Zervos M., da Rocha L., Zhang Y. (2011). Methicillin-resistant *Staphylococcus aureus* in retail meat, Detroit, Michigan, USA. Emerg. Infect. Dis. 17: 1135. https://doi.org/10.3201/eid1706.101905

Control C.f.D., Prevention (2001). National Nosocomial Infections Surveillance (NNIS) System report: data summary from January 1992-June 2001, issued August 2001. Am. J. Infect. Control. 29: 404-421. https://doi.org/10.1067/mic.2001.119952

Cosgrove S.E., Qi Y., Kaye K.S., Harbarth S., Karchmer A.W., Carmeli Y. (2005). The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. Infect. Cont. Hosp. Epidemiol. 26: 166-174. https://doi.org/10.1086/502522

De Boer E., Zwartkruis-Nahuis J., Wit B., Huijsdens X., De Neeling A., Bosch T., Van Oosterom R., Vila A., Heuvelink A. (2009). Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. Int. J. Food Microbiol. 134: 52-56. https://doi.org/10.1016/j.ijfoodmicro.2008.12.007

Decline V., Effendi M.H., Rahmaniar R.P., Yanestria S.M., Harijani N. (2020). Profile of antibiotic-resistant and presence of methicillin-resistant *Staphylococcus aureus* from nasal swab of dogs from several animal clinics in Surabaya,

Indonesia. Int. J. One Health. 6: 90-94. https://doi.org/10.14202/IJOH.2020.90-94

Eaton A.D., Clesceri L.S., Greenberg A., Franson M. (1995). APHA. AWWA, WEF, Standard methods for the examination of water and wastewater. 19th edn, Washington, DC, USA.

Eid H., Mohamed G., El Borolos I. (2018). Phenotypic and Genotypic Detection of Virulence Factors of *Staphylococcus Aureus* Isolated from Meat and Meat Products. Suez Canal Vet. Med. J. SCVMJ 23: 59-79. https://doi.org/10.21608/scvmj.2018.60277

El-Jakee J., Nagwa A.S., Bakry M., Zouelfakar S.A., Elgabry E., El-Said W. (2008). Characteristics of *Staphylococcus aureus* strains isolated from human and animal sources. Am Eurasian J. Agric. Environ. Sci. 4: 221-229.

Epidemiologists T (2015). Program and Abstracts Book (Atlanta).

Ezzat M., Shabana I., Riad E., Abbas S. (2014). Molecular characterization of *Staphylococcus aureus* isolated from meat, milk and their products. Suez Canal Vet. Med. J. SCVMJ. 19: 137-152. https://doi.org/10.21608/scvmj.2014.76373

Ezzat M., Wahdan A., Yousef F., Munier M. (2018). Genetic variation of *E. coli* strains isolated from poultry slaughterhouses at ismailia governorate, egypt. Adv. Anim. Vet. Sci. 6: 531-536. https://doi.org/10.17582/journal.aavs/2018/6.12.531.536

Fa Mansour A., Am Basha O. (2009). Hygienic status of imported frozen beef in alexandria markets. Assiut vet. Med. J. 55: 1-10. https://doi.org/10.21608/avmj.2009.174599

Fagundes H., Barchesi L., Nader Filho A., Ferreira L.M., Oliveira C.A.F. (2010). Occurrence of *Staphylococcus aureus* in raw milk produced in dairy farms in São Paulo state, Brazil. Brazilian J. Microbiol. 41: 376-380. https://doi.org/10.1590/S1517-83822010000200018

Fitzpatrick F., Humphreys H., O'gara J. (2005). The genetics of staphylococcal biofilm formation—will a greater understanding of pathogenesis lead to better management of device-related infection? Clin. Microbiol. Infect. 11: 967-973. https://doi.org/10.1111/j.1469-0691.2005.01274.x

Food U., Administration D (2014). FDA annual summary report on antimicrobials sold or distributed in 2012 for use in food-producing animals. (Rockville, MD: US Food and Drug Administration).

Foroutan S., Eslampour M.A., Emaneini M., Jabalameli F., Akbari G. (2022). Characterization of Biofilm Formation Ability, Virulence Factors and Antibiotic Resistance Pattern of *Staphylococcus aureus* Isolates from Subclinical Bovine Mastitis. Iranian J. Vet. Med. 16: 145.

Foster T. (1996). Staphylococcus. Med. Microbiol. 4th edition.

Frieden T. (2013). Antibiotic resistance threats in the United States. Centers Dis. Cont. Prev. 114.

Goja A., Ahmed T., Saeed S., Dirar H. (2013). Isolation and identification of Staphylococcus spp. in fresh beef. Pak. J. Nutrit. 12: 114. https://doi.org/10.3923/pjn.2013.114.120

Guimarães F., Manzi M., Joaquim S., Richini-Pereira V., Langoni H. (2017). Outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA)-associated mastitis in a closed dairy herd. J. Dairy Sci. 100: 726-730. https://doi.org/10.3168/jds.2016-11700

Healy C.M., Hulten K.G., Palazzi D.L., Campbell J.R., Baker C.J. (2004). Emergence of new strains of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. Clin. Infect. Dis. 39: 1460-1466. https://doi.org/10.1086/425321

Hellen G., Molly M.-P., Suraj P., Sumanth G., Jordan L., Devra B., Andrea W., Ramanan L. (2015). State of the World's Antibiotics. Center for Disease Dynamics, Economics & Policy: Washington, DC, 100.

Himabindu M., Muthamilselvan D.S., Bishi D.K., Verma R.S. (2009). Molecular analysis of coagulase gene polymorphism in clinical isolates of methicillin resistant *Staphylococcus aureus* by restriction fragment length polymorphism based genotyping. Am. J. Infect. Dis. 5: 163-169. https://doi.org/10.3844/ajidsp.2009.163.169

Jamali H., Radmehr B., Ismail S. (2014). Prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from bovine clinical mastitis. J. Dairy Sci. 97: 2226-2230. https://doi.org/10.3168/jds.2013-7509

John J.F., Harvin A.M. (2007). History and evolution of antibiotic resistance in coagulase-negative staphylococci: Susceptibility profiles of new anti-staphylococcal agents. Therapeut. Clin. Risk Manag. 3: 1143.

Kim H., Hong S. (2019). Role of the circadian clock in fine-tuning the process of leaf senescence in plants. Translat. Med. Aging. 3: 26-30. https://doi.org/10.1016/j.tma.2018.12.001

Kitai S., Shimizu A., Kawano J., Sato E., Nakano C., Kitagawa H., Fujio K., Matsumura K., Yasuda R., Inamoto T. (2005). Prevalence and characterization of *Staphylococcus aureus* and enterotoxigenic *Staphylococcus aureus* in retail raw chicken meat throughout Japan. J. Vet. Med. Sci. 67: 269-274. https://doi.org/10.1292/jvms.67.269

Kwon N.H., Park K.T., Jung W.K., Youn H.Y., Lee Y., Kim S.H., Bae W., Lim J.Y., Kim J.Y., Kim J.M. (2006). Characteristics of methicillin resistant *Staphylococcus aureus* isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. Vet. Microbiol. 117: 304-312. https://doi.org/10.1016/j.vetmic.2006.05.006

Lakhundi S., Zhang K. (2018). Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. Clin. Microbiol. Rev. 31: e00020-00018. https://doi.org/10.1128/CMR.00020-18

Lee H.J., Suh J.T., Kim Y.S., Lenz W., Bierbaum G., Schaal K.P. (2001). Typing and antimicrobial susceptibilities of methicillin resistant *Staphylococcus aureus* (MRSA) strains isolated in a hospital in Korea. J. Korean Med. Sci. 16: 381-385. https://doi.org/10.3346/jkms.2001.16.4.381

Lee J.H. (2003). Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl. Environ. Microbiol. 69: 6489-6494. https://doi.org/10.1128/AEM.69.11.6489-6494.2003

Letunic I., Bork P. (2021). Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. 49: W293-W296. https://doi.org/10.1093/nar/gkab301

Lu Y.F., McEwan N.A. (2007). Staphylococcal and micrococcal adherence to canine and feline corneocytes: quantification using a simple adhesion assay. Vet. Dermatol. 18: 29-35. https://doi.org/10.1111/j.1365-3164.2007.00567.x

Moon J.-S., Lee A.-R., Kang H.-M., Lee E.-S., Kim M.-N., Paik Y., Park Y.H., Joo Y.-S., Koo H. (2007). Phenotypic and genetic antibiogram of methicillin-resistant staphylococci isolated from bovine mastitis in Korea. J. Dairy Sci. 90: 1176-1185. https://doi.org/10.3168/jds.S0022-0302(07)71604-1

Nguyen L.-T., Schmidt H.A., Von Haeseler A., Minh B.Q. (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecul.

Biol. Evolut. 32: 268-274. https://doi.org/10.1093/molbev/msu300

Normanno G., Corrente M., La Salandra G., Dambrosio A., Quaglia N., Parisi A., Greco G., Bellacicco A., Virgilio S., Celano G. (2007). Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. Int. J. Food Microbiol. 117: 219-222. https://doi.org/10.1016/j.ijfoodmicro.2007.04.006

Nusrat J., Ifra T., Mrityunjoy A. (2015). Detection of methicillin-resistant *Staphylococcus aureus* within raw milk and cheese samples. Int. Food Res. J. 22: 2629.

Okonechnikov K., Golosova O., Fursov M., Team U. (2012). Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics. 28: 1166-1167. https://doi.org/10.1093/bioinformatics/bts091

Organization W.H. (2015). World health statistics 2015. World Health Organization.

Parisi A., Caruso M., Normanno G., Latorre L., Sottili R., Miccolupo A., Fraccalvieri R., Santagada G. (2016). Prevalence, antimicrobial susceptibility and molecular typing of methicillin-resistant *Staphylococcus aureus* (MRSA) in bulk tank milk from southern Italy. Food Microbiol. 58: 36-42. https://doi.org/10.1016/j.fm.2016.03.004

Parth F., Chauhan H., Bhagat A., Chandel B., Dadawala A., Kher H. (2016). Detection of virulence associated factors from *Staphylococcus aureus* isolated from bovine mastitis. Buffalo Bullet. 35: 687-696.

Power D.A., Johnson J.A. (2009). Difco™ & BBL™ manual. Manual of Microbiological Culture Media. 359-360.

Pu S., Han F., Ge B. (2009). Isolation and characterization of methicillin-resistant *Staphylococcus aureus* strains from Louisiana retail meats. Appl. Environ. Microbiol. 75: 265-267. https://doi.org/10.1128/AEM.01110-08

Quinn P.J., Markey B.K., Leonard F., FitzPatrick E., Fanning S. (2015). Concise Rev. Vet. Microbiol.

Quinn P.J., Markey B.K., Leonard F.C., Hartigan P., Fanning S., Fitzpatrick E. (2011). Veterinary microbiology and microbial disease. John Wiley & Sons.

Rampacci E., Trotta M., Fani C., Silvestri S., Stefanetti V., Brachelente C., Mencacci A., Passamonti F. (2021). Comparative performances of Vitek-2, disk diffusion, and broth microdilution for antimicrobial susceptibility testing of canine Staphylococcus pseudintermedius. J. Clin. Microbiol. 59: e00349-00321. https://doi.org/10.1128/JCM.00349-21

Reinoso E., El-Sayed A., Lämmler C., Bogni C., Zschöck M. (2008). Genotyping of *Staphylococcus aureus* isolated from humans, bovine subclinical mastitis and food samples in Argentina. Microbiolog. Res. 163: 314-322. https://doi.org/10.1016/j.micres.2006.05.013

Rohde H., Burandt E.C., Siemssen N., Frommelt L., Burdelski C., Wurster S., Scherpe S., Davies A.P., Harris L.G., Horstkotte M.A. (2007). Polysaccharide intercellular adhesin or protein factors in biofilm accumulation of Staphylococcus epidermidis and *Staphylococcus aureus* isolated from prosthetic hip and knee joint infections. Biomaterials. 28: 1711-1720. https://doi.org/10.1016/j.biomaterials.2006.11.046

Rossi B., Bonsaglia E., Castilho I., Dantas S., Salina A., Langoni H., Pantoja J., Budri P., Fitzgerald-Hughes D., Júnior A.F. (2019). Genotyping of long term persistent *Staphylococcus aureus* in bovine subclinical mastitis. Microbial Pathogen. 132: 45-50. https://doi.org/10.1016/j.micpath.2019.04.031

Sabat A.J., Köck R., Akkerboom V., Hendrix R., Skov R.L., Becker K., Friedrich A.W. (2013). Novel organization of the arginine catabolic mobile element and staphylococcal cassette chromosome mec composite island and its horizontal transfer between distinct *Staphylococcus aureus* genotypes. Antimicrobial Agents Chemother. 57: 5774-5777. https://doi.org/10.1128/AAC.01321-13

Schlegelova J., Dendis M., Benedık J., Babak V., Ryšánek D. (2003). *Staphylococcus aureus* isolates from dairy cows and humans on a farm differ in coagulase genotype. Vet. Microbiol. 92: 327-334. https://doi.org/10.1016/S0378-1135(02)00409-1

Singh K., Baxi K. (1982). Studies on the etiology, in vitro sensitivity and treatment of subclinical mastitis in milch animals. Indian Vet. J.

Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Molecul. Biol. Evol. 30: 2725-2729. https://doi.org/10.1093/molbev/mst197

Tong S.Y., Schaumburg F., Ellington M.J., Corander J., Pichon B., Leendertz F., Bentley S.D., Parkhill J., Holt D.C., Peters G. (2015). Novel staphylococcal species that form part of a *Staphylococcus aureus*-related complex: the non-pigmented Staphylococcus argenteus sp. nov. and the non-human primate-associated Staphylococcus schweitzeri sp. nov. Int. J. Systemat. Evolut. Microbiol. 65: 15. https://doi.org/10.1099/ijs.0.062752-0

Van Loo I.H., Diederen B.M., Savelkoul P.H., Woudenberg J.H., Roosendaal R., Van Belkum A., Lemmens-den Toom N., Verhulst C., Van Keulen P.H., Kluytmans J.A. (2007). Methicillin-resistant *Staphylococcus aureus* in meat products, the Netherlands. Emerg. Infect. Dis. 13: 1753. https://doi.org/10.3201/eid1311.070358

Vancraeynest D., Hermans K., Haesebrouck F. (2004). Genotypic and phenotypic screening of high and low virulence *Staphylococcus aureus* isolates from rabbits for biofilm formation and MSCRAMMs. Vet. Microbiol. 103: 241-247. https://doi.org/10.1016/j.vetmic.2004.09.002

Wahdan A., Riad E.M., Enany S. (2020). Genetic differentiation of Mycobacterium bovis and Mycobacterium tuberculosis isolated from cattle and human sources in, Egypt (Suez Canal area). Comparat. Immunol. Microbiol. Infect. Dis. 73: 101553. https://doi.org/10.1016/j.cimid.2020.101553

Wang D., Wang Z., Yan Z., Wu J., Ali T., Li J., Lv Y., Han B. (2015). Bovine mastitis *Staphylococcus aureus*: antibiotic susceptibility profile, resistance genes and molecular typing of methicillin-resistant and methicillin-sensitive strains in China. Infect. Genet. Evol. 31: 9-16. https://doi.org/10.1016/j.meegid.2014.12.039

Weese J.S. (2010). Methicillin-resistant *Staphylococcus aureus* in animals. ILAR journal 51, 233-244. https://doi.org/10.1093/ilar.51.3.233

Xu Z., Chen L., Chen X., Tang A., Huang D., Pan Q., Fang Z. (2022). Prevalence and Molecular Characterization of Methicillin-Resistant Staphylococci Recovered from Public Shared Bicycles in China. Int. J. Environ. Res. Pub. Health. 19: 4492. https://doi.org/10.3390/ijerph19084492