ORIGINAL ARTICLE

Veterinary Research Forum. 2023; 14 (5) 243 - 248
doi: 10.30466/vrf.2022.551346.3434
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Molecular detection of methicillin-resistant *Staphylococcus aureus* isolated from different foodstuffs in Egypt

Doha Elsayed Naeim*, Ibrahim Elsayed Eldesoukey, Amgad Ahmed Moawad, Ashraf Mohammed Ahmed

Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh, Egypt.

Article Info

Article history:

Received: 04 April 2022
Accepted: 27 June 2022
Available online: 15 May 2023

Keywords:

Egypt
Food
MRSA
Polymerase chain reaction

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is a major public health problem. Therefore, this study was aimed to estimate the prevalence of MRSA in various food products. A total number of 204 food samples including raw milk (n = 30), cheese (n = 60), chicken (n = 25), beef (n = 24) and fish (n = 65) were collected from August to November of 2021 within different localities in Kafr El-Sheikh governorate, the northern region of Egypt. All samples were assessed through a series of bacteriological and biochemical techniques to identify MRSA. Out of 204 samples, 52(25.49%) isolates were presumptively identified as MRSA on oxacillin resistance screening agar base media. Of these 52 isolates, 17(32.69%) were characterized as coagulase-positive. For the molecular confirmation of MRSA, all isolates were subjected to polymerase chain reaction assays to detect *mecA* and *mecC*. In addition, *mecA* was identified in all the isolates (100%), whereas, none was positive for *mecC*. Therefore, based on the detection of *mecA*, the overall occurrence rate of MRSA among the samples was 8.33%. The isolates were also subjected to antimicrobial susceptibility tests. Cefoxitin, cefuroxime, oxacillin and amoxicillin-clavulanic acid were completely resistant (100%) to the isolates, however, susceptible to vancomycin and ciprofloxacin. Raw milk had the highest prevalence of MRSA (13.30%), followed by chicken (12.00%), fish (9.20%), cheese (5.00%) and beef (4.20%). Due to the possibility of transmission of these strains to humans, the high prevalence of MRSA in various foodstuffs in Egypt poses a potential public health risk.

Introduction

Antimicrobial resistance is one of the most serious worldwide issue today. It leads to millions of fatalities, long-term impairments and higher medical expenditures. It also has a negative influence on livelihoods, poses a danger to food security and causes animal deaths.[1] Antimicrobial resistance is widely regarded as one of the most serious threat to humanity in the 21st century.[2] Epidemiological investigation has demonstrated a direct relationship between the extensive use of antimicrobials, whether in human or veterinary medicine, and the spread of resistant bacterial strains.[3] These resistant bacteria have the potential to infect humans, thereby, causing severe systemic infections. Most *Staphylococcus aureus* strains are penicillin- and derivative-resistant due to penicillinase production.[4] Also, certain strains, known as methicillin-resistant *S. aureus* (MRSA), are resistant to methicillin.[5] The MRSA strains induce resistance through a highly trans-missible mobile genetic element-staphylococcal cassette chromosome mec (SCCmec)-which harbors *mecA* and its analog *mecC* encode penicillin-binding protein 2a (PBP2a).[6] By interfering with the drugs ability to bind to cell wall proteins, both genes provide resistance to methicillin and other beta-lactam antibiotics.[7]

Recently, MRSA has emerged as a significant public healthcare concern and a potentially lethal infective agent due to its ineffective response to various antibiotic classes.[7] Moreover, it is regarded as the most prevalent pathogen in human nosocomial infections.[3] The MRSA is frequently found in animal-sourced foodstuffs intended for human consumption, especially meat.[8] Therefore, the presence of MRSA in animal-sourced foodstuffs such as meat, milk, dairy products and fish strongly suggests the potential transmission of these resistant strains to humans,[9] which adds to the evidence of its zoonotic potential.[10] Globally, many studies have identified MRSA in several animal-sourced foodstuffs including raw milk,[11]

***Correspondence:**

Doha Elsayed Naeim, BSc, MSc
Department of Bacteriology, Mycology, and Immunology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh, Egypt
E-mail: doha_naeim@vet.kfs.edu.eg

dairy products,[12] retail meat and meat products,[13] retail chicken,[14,15] and fish,[16] with varying prevalence rates. Currently, data on the presence of MRSA in food, especially in dairy products, meat products and fish are scarce. Therefore, this study conducted a molecular investigation of MRSA strains in some foodstuffs including raw milk, cheese, chicken, beef and fish in Egypt.

Materials and Methods

Sample collection. From August to November of 2021, a total number of 204 samples including 90 dairy products (30 milk, and 60 cheese samples), 49 meat products (25 chicken, and 24 beef samples), and 65 fish samples were collected from local markets within different localities in Kafr El-Sheikh governorate, the northern region of Egypt. Each sample was labeled, placed in an icebox and transferred immediately to bacteriology laboratory, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh, Egypt for bacteriological examination. Ethical approval was obtained from Research, Publication and Ethics Committee of the Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh, Egypt. The committee ensures compliance with all relevant Egyptian legislations.

Preparation of samples. Briefly, samples (25.00 g or mL) were placed in stomacher bags containing 225 mL of buffered peptone water. After homogenization of the samples at 320 rpm for 2 min and overnight incubation at 37.00 ˚C, 0.10 mL aliquots were inoculated into tubes containing 10.00 mL of tryptic soy broth (TSB; Oxoid, Basingstoke, UK) supplemented with 6.50% NaCl, as previously described by Tang *et al*.[17]

Isolation and identification of *S. aureus* and MRSA. The MRSA was isolated as previously described by Aklilu and Chia[22] with some modifications. Briefly, 0.10 mL of the inoculated TSB was plated on oxacillin resistance screening agar base plates (ORSAB; Oxoid). After incubation at 37.00 ˚C for 24 to 48 hr, representative intense blue-colored colonies on ORSAB (presumptive identified as MRSA) were further subjected to Gram staining for morphological confirmation and standard biochemical tests including catalase and coagulase tests for confirmation of *S. aureus*.

Antimicrobial susceptibility testing. The antibiotic sensitivity phenotypes of bacterial isolates were determined using the Kirby-Bauer disc diffusion assay on Mueller-Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) interpretive criteria.[18] The following antimicrobials were used: sulfamethoxazole/trimethoprim (SXT) 23.75/1.25 µg; cefoxitin (FOX) 30.00 µg; oxacillin (OXA) 1.00 µg; cefuroxime (CXM) 30.00 µg; amoxicillin (AMX) 10.00 µg; amoxicillin/clavulanic acid (AMC) 20.00/10.00 µg; vancomycin (VAN) 30.00 µg; ampicillin (AMP) 10.00 µg; ciprofloxacin (CIP) 5.00 µg; and cefazolin (CFZ) 30.00 µg. The discs were purchased from Oxoid and the results were recorded in accordance with CLSI guidelines.[18]

DNA extraction and molecular detection of *mecA* and *mecC*. Following the manufacturer's instructions, DNA was extracted from all isolates using the QIAamp DNA micro kit (Qiagen, Hilden, Germany) and bacterial coagulase-positive status was confirmed. Polymerase chain reaction (PCR) assays were used to detect *mecA* and *mecC* responsible for resistance to methicillin and other beta-lactam antibiotics in *S. aureus* strains. To detect *mecA*, PCR with primers (Fisher Scientific Company LLC., Pittsburgh, USA) *mecA*-F (5′ GTAGAAATGACTGAACGTCCGATAA ′3) and *mecA*-R (5′ CCAATTCCACATTGTTTCGGTCTA A ′3) was performed to amplify the 310 bp gene as previously described by McClure *et al*.[19] To detect *mecC*, *mecC*-F (5′ GCTCCTAATGCTAATGCA′3) and *mecC*-R (5′ TAAGCAATAA TGACTACC ′3) primers were used to amplify the 304 bp gene according to a previously described protocol by Cuny *et al*.[20] The negative control for *mecA* and *mecC* was deionized water. All PCR assays were performed in a total volume of 25.00 µL which included 12.50 µL of Emerald Amp GT PCR master mix (2X premix), 1.00 µL of 20.00 pmol of each forward and reverse primer, 5.00 µL of bacterial DNA template, and 5.50 µL nuclease-free water. The PCR assays were performed as uniplex reactions using a T3 Thermal cycler (Master cycler; Eppendorf, Hamburg, Germany) under the following cycling conditions: Initial denaturation at 94.00 ˚C for 5 min followed by 35 cycles of denaturation at 94.00 ˚C for 30 sec, annealing at 50.00 ˚C for 30 sec, extension at 72.00 ˚C for 30 sec and a final extension at 72.00 ˚C for 10 min. Finally, each amplified PCR product was electrophoresed on 1.50% agarose (Sigma-Aldrich, St. Louis, USA) prepared in 1X Tris Borate EDTA (TBE) buffer (New England BioLabs Inc., Ipswich, USA) and then stained with 0.50 µg mL^{-1} ethidium bromide (Sigma-Aldrich). The gel was then examined and photographed under a UV transilluminator (Fisher Scientific Company LLC).

Results

Prevalence of MRSA in foodstuffs. A total number of 204 samples of different foodstuffs of animal origin were screened for MRSA among which 52 (25.49%) isolates were presumptively identified on ORSAB media as MRSA. Of these 52 isolates, 17(32.69%) were identified as coagulase-positive *S. aureus* (Table 1).

PCR-based molecular detection of *mecA* and *mecC* by PCR. For the molecular detection of MRSA, all coagulase-positive *S. aureus* identified in this study were screened for the presence of both *mecA* and *mecC* using uniplex PCR assays. Interestingly, *mecA* (gene responsible of methicillin-resistance) was identified in all the isolates examined (100%; Fig. 1), whereas, *mecC* was not detected in any isolate. Consequently, based on the detection of *mecA*, the overall prevalence of MRSA in the examined samples was 8.33%. Raw milk had the highest prevalence

of MRSA (13.30%) followed by chicken (12.00%), fish (9.20%), cheese (5.00%), and beef (4.20%) (Table 1).

Table 1. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in foodstuffs of animal origin. Data are presented as number (%) of samples.

Sample	No.	ORSAB media	Coagulase test	*mecA*	*mecC*
Dairy products					
Milk	30	10 (33.33)	4(13.33)	4(13.33)	0(0.00)
Cheese	60	10(16.66)	3(5.00)	3(5.00)	0(0.00)
Meat products					
Beef	24	8(33.33)	1(4.16)	1(4.16)	0(0.00)
Chicken	25	8(32.00)	3(12.00)	3(12.00)	0(0.00)
Fish	65	21(32.30)	6(9.23)	6(9.23)	0(0.00)
Total	204	50(24.50)	17(8.33)	17(8.33)	0(0.00)

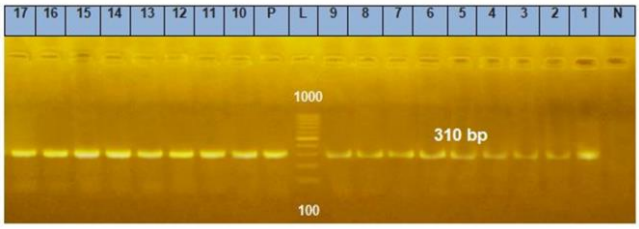

Fig. 1. Agarose gel electrophoresis patterns of the PCR product of amplicon (310 bp) of *mecA* (Lane L: 100 bp DNA ladder as a molecular-size DNA marker, Lane P: Positive control for *mecA*, Lane N: Negative control (deionized water) for *mecA*, Lanes (1-17): Analyzed samples of *S. aureus* isolates showing the positive 310 bp amplicon of *mecA*

Antimicrobial susceptibility testing of MRSA isolates. Antimicrobial susceptibility testing revealed variable rates of MRSA isolates resistance to the tested antimicrobial agents. Overall, absolute resistance (100%) was observed against cefoxitin, cefuroxime, oxacillin and amoxicillin/clavulanic acid, whereas, perceptible resistance was observed against ampicillin, amoxicillin, cefazolin and sulfamethoxazole/trimethoprim with 63.46%, 61.53%, 42.30% and 36.53% of the isolates being resistant, respectively. Conversely, lower resistance was observed against vancomycin and ciprofloxacin by 28.84% and 25.00%, respectively (Table 2). All isolates were found to be multidrug-resistant (MDR) indicating they were resistant to at least three different classes of antibiotics.

Discussion

MRSA is widely regarded as one of the most serious threat to healthcare facilities worldwide due to the emergence and dissemination of resistant isolates that are resistant to methicillin, other β-lactams, andalso several antibiotic classes.[21] Recently, several reports have documented the widespread of these strains outside the hospital environment in animals and the community.[22] In addition, MRSA is a major public health concern because of its ability to contaminate animal-origin foods and infect humans and animals.[23]

The detection of *mecA* or its analog *mecC* is the gold standard for identifying MRSA species.[24] Recently, it has been discovered that methicillin resistance in *S. aureus* is caused by factors other than *mecA*. A novel *mecA* homolog, *mecC*, was discovered in bovine and human isolates from the United Kingdom and Denmark.[25] Only 70.00% of the nucleotides in this gene is identical to those in *mecA* and only 63.00% of the amino acids is identical to those in the original PBP2a (encoded by *mecA*).[10] Interestingly, *mecA* was identified in all the examined isolates in this study, whereas, *mecC* was not detected in any of them which was in agreement with the findings of recent investigations.[26,27]

This study investigated the prevalence of MRSA in different foodstuffs, raw milk, cheese, chicken, beef and fish. Based on the PCR amplification results of *mecA* and *mecC*, only 17(8.33%) samples out of 204 tested were contaminated by MRSA. From beef, chicken, cheese, raw milk and fish samples, 1(4.20%), 3(12.00%), 3(5.00%), 4(13.30%) and 6(9.20%) isolates were obtained, respectively. Similar findings were reported in India by Mohanta and Mazumder[28] and in Italy by Basanisi *et al*.[29] with prevalence rates of 8.93% and 8.33%, respectively. Nevertheless, a higher prevalence of MRSA in foodstuffs was reported in Brazil by Costa *et al*. (28.10%),[8] in Sudan by Yahya Ahmed *et al*.[30] (17.00%), and in Algeria by Chaalal *et al*.[31] (16.90%), whereas, lower prevalence rates of 5.60% and 4.10% were recorded in China by Song *et al*.[32] and in Algeria by Titouche *et al*.,[33] respectively. These variations in the prevalence rate of MRSA could be attributed to many factors including sample type and area of sampling.

Table 2. Antimicrobial susceptibility testing of *S. aureus* isolates. Data are presented as number (%) of samples.

Antimicrobials	Milk (n = 16)	Cheese (n =9)	Chicken (n = 5)	Beef (n = 8)	Fish (n = 14)	Total (n = 52)
Cefoxitin	16 (100)	9 (100)	5 (100)	8 (100)	14 (100)	52 (100)
Cefuroxime	16 (100)	9 (100)	5 (100)	8 (100)	14 (100)	52 (100)
Amoxicillin/clavulanic acid	16 (100)	9 (100)	5 (100)	8 (100)	14 (100)	52 (100)
Oxacillin	16 (100)	9 (100)	5 (100)	8 (100)	14 (100)	52 (100)
Ampicillin	8 (50.00)	5 (55.55)	4 (80.00)	6 (75.00)	9 (64.29)	33 (63.46)
Amoxicillin	10 (62.50)	5 (55.55)	3 (60.00)	5 (62.50)	9 (64.29)	32 (61.53)
Cefazolin	6 (37.50)	3 (33.33)	4 (80.00)	3 (37.50)	5 (35.71)	22 (42.30)
Sulfamethoxazole/trimethoprim	5 (31.20)	2 (22.22)	1 (20.00)	4 (50.00)	7 (50.00)	19 (36.53)
Vancomycin	4 (25.00)	3 (33.33)	3 (60.00)	2 (25.00)	3 (21.42)	15 (28.84)
Ciprofloxacin	1 (6.25)	1 (11.11)	2 (40.00)	4 (50.00)	4 (28.57)	13 (25.00)

The MRSA is frequently found in meat and meat products and it plays a substantial role in human food poisoning infection.[34] In this study, four MRSA strains were identified out of the examined 49(8.16%) chicken and beef samples. Previous estimates of the worldwide prevalence of MRSA in meat have ranged from < 1.00% in the Netherlands as reported by de Boer *et al.*[35] to 11.90% in Korea as reported by Kwon *et al*.[36] In Egypt, Whole chicken carcasses had a higher prevalence rate (44.00%).[37] Meat contamination with MRSA could indicate improper handling and processing by individuals.[38] Dressed carcasses sanitary state is mainly determined by slaughterhouse hygiene and worker skill.[39]

Out of 30 raw milk samples, 4(13.30%) MRSA isolates were identified in this study. These results were consistent with the findings of a recent study in Brazil by Silva *et al.*[3] that identified MRSA in raw milk with a prevalence rate of 13.70%. Nevertheless, other studies in Egypt reported high 100% and 87.50% prevalence rates. [31,40] However, a lower prevalence rate was previously reported in China (2.74%) by Wang *et al*.[41] and in South Africa (1.20%) by Pekana and Green.[42]

Regarding the prevalence of MRSA in cheese, our findings revealed a rate of 5.00%. A higher prevalence rate of MRSA (40.00%) was reported in Egypt by Al-Ashmawy *et al*.[12] However, in a more recent investigation in Brazil, a prevalence rate (of MRSA) of 13.70% in cheese was reported by Silva *et al.*[3] Compared to other food products, the low prevalence of MRSA in cheese might be attributed partially to the acidity of the product which could limit survival of most bacteria including *S. aureus*.[12]

In the present study, the prevalence of MRSA in fish was 9.20% which was consistent with the findings obtained in Nigeria[43] (6.60%) and Malaysia[44] (8.75%). Although higher prevalence rates of 35.00% and 30.00% were reported in other studies in Brazil and Malaysia, respectively. [8,45]

Furthermore, all MRSA isolates in this study were examined for antimicrobial resistance against 10 antimicrobial agents belonging to different classes of antibiotics. There was absolute resistance to cefoxitin, cefuroxime, oxacillin, amoxicillin/clavulanic acid, ampicillin, amoxicillin and cefazolin. These observations supported the findings of earlier studies.[46,47] The observed resistance was probably due to a plasmid-encoded penicillinase β-lactamase produced by *S. aureus* with *bla*.[48] However, we detected significant susceptibility against vancomycin and ciprofloxacin. MRSA strains frequently develop MDR as reported by Shahid *et al*.[27] and Fri *et al*.[49] In the current study, MDR (resistance to at least three different antibiotic classes) was observed among MRSA isolates. This finding was consistent with those of previous studies.[46,50] In our investigation, we found that milk was the most important dietary source for antibiotic resistance in humans followed by cheese, fish, chicken and meat in the order. This variation in antibiotic resistance patterns could be due to the differences in the sources of isolates, geographical locations and the overall antibiotics used.

The MRSA was found in 8.33% of the samples tested. It was the most prevalent in raw milk (13.30%) followed by chicken (12.00%), fish (9.20%), cheese (5.00%) and beef (4.20%). These findings suggested that antibiotic-resistant *S. aureus* strains were not only nosocomial, but also prevalent in food. Our investigation was confined to the presence of *S. aureus* in various meals and the items examined were often consumed by locals in the area. As a consequence, we believed that resistance rates against tested antibiotics were found to be high among the isolates. Presence of multidrug-resistant *S. aureus* strains was a significant issue that should be highlighted in terms of public health and food safety.

Acknowledgments

The authors are thankful to The Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh, Egypt.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. WHO, UNEP. Antimicrobial resistance and the United Nations Sustainable Development Cooperation Framework. 2021.
2. Mahdi WKM, Hassuna NA, Esmail MA, et al. Molecular typing of methicillin resistant *Staphylococcus aureus* colonizing Egyptian healthcare workers and patients. Int J Curr Microbiol Appl Sci 2016; 5(6): 687-698.
3. Silva V, Capelo JL, Igrejas G, et al. Molecular epidemiology of Staphylococcus aureus lineages in wild animals in Europe: a review. Antibiotics (Basel) 2020; 9(3): 122. doi: 10.3390/antibiotics9030122.
4. Peacock SJ, Paterson GK. Mechanisms of methicillin resistance in Staphylococcus aureus. Annu Rev Biochem 2015; 84: 577-601.
5. Gajdács M. The continuing threat of methicillin-resistant *Staphylococcus aureus*. Antibiotics (Basel) 2019; 8(2): 52. doi: 10.3390/antibiotics8020052.
6. Paterson GK, Morgan FJ, Harrison EM, *et al.* Prevalence and properties of *mecC* methicillin-resistant *Staphylococcus aureus* (MRSA) in bovine bulk tank milk in Great Britain. J Antimicrob Chemother 2014; 69(3): 598-602.
7. Kamal RM, Bayoumi MA, Abd El Aal SFA. MRSA detection in raw milk, some dairy products and hands of dairy workers in Egypt, a mini-survey. Food Control 2013; 33(1): 49-53.

8. Costa WL, Ferreira Jdos S, Carvalho JS, et al. Methicillin-resistant Staphylococcus aureus in raw meats and prepared foods in public hospitals in salvador, Bahia, Brazil. J Food Sci 2015; 80(1): M147-M150.
9. Huber H, Ziegler D, Pflüger V, *et al.* Prevalence and characteristics of methicillin-resistant coagulase-negative staphylococci from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons. BMC Vet Res 2011; 7(1): 6. doi:10.1186/ 1746-6148-7-6.
10. Aires-de-Sousa M. Methicillin-resistant *Staphylococcus aureus* among animals: current overview. Clin Microbiol Infect 2017; 23(6): 373-380.
11. Ben Said M, Abbassi MS, Bianchini V, et al. Genetic characterization and antimicrobial resistance of *Staphylococcus aureus* isolated from bovine milk in Tunisia. Lett Appl Microbiol 2016; 63(6): 473-481.
12. Al-Ashmawy MA, Sallam KI, Abd-Elghany SM, et al. Prevalence, molecular characterization, and anti-microbial susceptibility of methicillin-resistant *Staphylococcus aureus* Isolated from milk and dairy products. Foodborne Pathog Dis 2016; 13(3): 156-162.
13. Chairat S, Gharsa H, Lozano C, et al. Characterization of *Staphylococcus aureus* from raw meat samples in Tunisia: Detection of clonal lineage ST398 from the African continent. Foodborne Pathog Dis 2015; 12(8): 686-692.
14. Abolghait SK, Fathi AG, Youssef FM, et al. Methicillin-resistant Staphylococcus aureus (MRSA) isolated from chicken meat and giblets often produces staphylo-coccal enterotoxin B (SEB) in non-refrigerated raw chicken livers. Int J Food Microbiol 2020; 328: 108669. doi: 10.1016/j.ijfoodmicro.2020.108669..
15. Okorie-Kanu OJ, Anyanwu MU, Ezenduka EV, et al. Molecular epidemiology, genetic diversity and antimicrobial resistance of *Staphylococcus aureus* isolated from chicken and pig carcasses, and carcass handlers. PLoS One 2020; 15(5): e0232913. doi: 10.1371/journal.pone.0232913.
16. Hammad AM, Watanabe W, Fujii T, et al. Occurrence and characteristics of methicillin-resistant and -susceptible *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci from Japanese retail ready-to-eat raw fish. Int J Food Microbiol 2012; 156(3): 286-289.
17. Tang Y, Qiao Z, Wang Z, et al. The prevalence of Staphylococcus aureus and the occurrence of MRSA CC398 in monkey feces in a zoo park in eastern China. Animals (Basel) 2021; 11(3): 732 doi: 10.3390/ ani11030732.
18. CLSI. Performance standards for antimicrobial susceptibility testing, 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute: 2020.
19. McClure JA, Conly JM, Lau V, et al. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. J Clin Microbiol 2006; 44(3): 1141-1144.
20. Cuny C, Layer F, Strommenger B, et al. Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a Novel mecA homologue in humans in Germany. PLoS One 2011; 6(9): e24360 doi: 10.1371/journal. pone.0024360.
21. Islam MA, Alam MM, Uddin MS, et al. Detection of methicillin-resistant *staphylococcus aureus* (MRSA) from animal and human origin in Bangladesh by poly-merase chain reaction. Bangl J Vet Med 2011; 9(2): 161-166.
22. Aklilu E, Chia HY. First mecC and mecA positive livestock-associated methicillin resistant *Staphylococcus aureus* (mecC MRSA/LA-MRSA) from dairy cattle in Malaysia. Microorganisms 2020, 8(2): 147. doi: 10.3390/microorganisms8020147.
23. Petinaki E, Spiliopoulou I. Methicillin-resistant *Staphylococcus aureus* among companion and food-chain animals: impact of human contacts. Clin Microbiol Infect 2012; 18(7): 626-634.
24. Aljahani AH, Alarjani KM, Hassan ZK, et al. Molecular detection of methicillin heat-resistant *Staphylococcus aureus* strains in pasteurized camel milk in Saudi Arabia. Biosci Rep 2020; 40(4): BSR20193470. doi: 10.1042/BSR20193470.
25. García-Álvarez L, Holden MT, Lindsay H, et al. Meticillin-resistant *Staphylococcus aureus* with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 2011; 11(8): 595-603.
26. Venugopal N, Mitra S, Tewari R, et al. Molecular detection and typing of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci isolated from cattle, animal handlers, and their environment from Karnataka, Southern Province of India. Vet world 2019; 12(11): 1760-1768.
27. Shahid AH, Nazir KHMNH, El Zowalaty ME, et al. Molecular detection of vancomycin and methicillin resistance in *Staphylococcus aureus* isolated from food processing environments. One Health 2021; 13: 100276. doi: 10.1016/j.onehlt.2021.100276.
28. Mohanta A, Mazumder PB. Detection of staphylococci in raw milk and milk products and evaluation of their antibiotic sensitivity: a report from Southern Assam, Indian. IOSR J Environ Sci Toxicol Food Technol 2015; 9(1): 17-22.
29. Basanisi MG, La Bella G, Nobili G, et al. Genotyping of methicillin-resistant Staphylococcus aureus (MRSA) isolated from milk and dairy products in South Italy. Food Microbiol 2017; 62: 141-146.
30. Yahya Ahmed MY, Abdalbagi Ali H, Mohammed Taher

Gorish B, et al. Molecular detection of Staphylococcal enterotoxins and mecA genes products in selected food samples collected from different areas in Khartoum State. Int J Microbiol 2021; 2021: 5520573. doi: 10.1155/2021/5520573.
31. Chaalal W, Chaalal N, Bourafa N, et al. Characterization of *Staphylococcus aureus* isolated from food products in Western Algeria. Foodborne Pathog Dis 2018; 15(6): 353-360.
32. Song M, Bai Y, Xu J, et al. Genetic diversity and virulence potential of *Staphylococcus aureus* isolates from raw and processed food commodities in Shanghai. Int J Food Microbiol 2015; 195: 1-8.
33. Titouche Y, Hakem A, Houali K, et al. Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) ST8 in raw milk and traditional dairy products in the Tizi Ouzou area of Algeria. J Dairy Sci 2019; 102(8): 6876-6884.
34. Datta S, Akter A, Shah IG, et al. Microbiological quality assessment of raw meat and meat products, and antibiotic susceptibility of isolated *Staphylococcus aureus*. Agric Food Anal Bacteriol 2012; 2(3): 187-194.
35. de Boer E, Zwartkruis-Nahuis JTM, Wit B, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. Int J Food Microbiol 2009; 134(1-2): 52-56.
36. Kwon NH, Park KT, Jung WK, et al. Characteristics of methicillin resistant *Staphylococcus aureus* isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. Vet Microbiol 2006; 117(2-4): 304-312.
37. Karmi M. Prevalence of methicillin-resistant *Staphylococcus aureus* in poultry meat in Qena, Egypt. Vet World 2013; 6(10): 711-715.
38. Hatakka M, Björkroth KJ, Asplund K, et al. Genotypes and enterotoxicity of *Staphylococcus aureus* isolated from the hands and nasal cavities of flight-catering employees. J Food Prot 2000; 63(11): 1487-1491.
39. Manna SK, Brahmane MP, Manna C, et al. Occurrence, virulence characteristics and antimicrobial resistance of Escherichia coli O157 in slaughtered cattle and diarrhoeic calves in West Bengal, India. Lett Appl Microbiol 2006; 43(4): 405-409.
40. Algammal AM, Hetta HF, Elkelish A, *et al.* Methicillin-resistant *Staphylococcus aureus* (MRSA): One health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. Infect Drug Resist 2020; 13: 3255-3265.
41. Wang D, Wang Z, Yan Z, et al. Bovine mastitis *Staphylococcus aureus*: antibiotic susceptibility profile, resistance genes and molecular typing of methicillin-resistant and methicillin-sensitive strains in China. Infect Genet Evol 2015; 31: 9-16.
42. Pekana A, Green E. Antimicrobial resistance profiles of *Staphylococcus aureus* isolated from meat carcasses and bovine milk in abattoirs and dairy farms of the Eastern Cape, South Africa. Int J Environ Res Public Health 2018; 15(10): 2223, doi: 10.3390/ ijerph15102223.
43. Grema HA, Geidam YA, Gadzama GB, et al. Methicillin resistant Staphyloccus aureus (MRSA): a review. Adv Anim Vet Sci 2015; 3(2): 79-98.
44. Atyah MA, Zamri-Saad M, Siti-Zahrah A. First report of methicillin-resistant *Staphylococcus aureus* from cage-cultured tilapia (*Oreochromis niloticus*). Vet Microbiol 2010; 144(3-4): 502-504.
45. Othman BR, Kuan CH, Mohammed AS, et al. Occurrence of methicillin-resistant *Staphylococcus aureus* in raw shellfish at retail markets in Malaysia and antibacterial efficacies of black seed (*Nigella sativa*) oil against MRSA. Food Control 2018; 90: 324-331.
46. El-Deeb W, Fayez M, Elmoslemany A, et al. Methicillin resistant *Staphylococcus aureus* among goat farms in Eastern province, Saudi Arabia: Prevalence and risk factors. Prev Vet Med 2018; 156: 84-90.
47. Abdel-Moein KA, Zaher HM. Occurrence of multidrug-resistant methicillin-resistant *Staphylococcus aureus* among healthy farm animals: a public health concern. Int J Vet Sci Med 2019; 7(1): 55-60.
48. Babic M, Hujer AM, Bonomo RA. What's new in antibiotic resistance? Focus on beta-lactamases. Drug Resist Updat 2006; 9(3): 142-156.
49. Fri J, Njom HA, Ateba CN, et al. Antibiotic resistance and virulence gene characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from healthy edible marine fish. Int J Microbiol 2020; 2020: 803903. doi: 10.1155/2020/9803903.
50. El-Adawy H, Ahmed M, Hotzel H, *et al*. Characterization of methicillin-resistant *Staphylococcus aureus* isolated from healthy turkeys and broilers using DNA microarrays. Front microbiol 2016; 7: 2019. doi: 10.3389/fmicb. 2016.02019.