Damanhour Journal of Veterinary Sciences 7 (1), (2021) 28-31

Damanhour Journal of Veterinary Sciences

Journal homepage: https://djvs.journals.ekb.eg/

E-ISSN 2636-3003 | ISSN 2636-3011

Prevalence, antibiogram, and expression of enterotoxin-coding genes of Staphylococcus aureus in bovine raw meat, liver, milk, and kariesh cheese

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A B S T R A C T

The objectives of the present study were firstly to investigate the prevalence of Staphylococcus aureus (S. aureus) in bovine meat, liver, raw milk, and kariesh cheese retailed in Egypt. Secondly, the antimicrobial resistance profiling of the recovered S. aureus isolates was examined. Thirdly, detection of the coding genes of S. aureus-enterotoxins (SE) including SEA, SEB, SEC, and SED was screened using PCR. The obtained results of the present study showed that S. aureus was isolated from retailed kariesh cheese, raw milk, raw liver, and raw meat at 80%, 70%, 65%, and 50%, respectively. Kariesh cheese had significantly the highest total S. aureus count (3.55 ± 0.19 log 10 cfu/g), followed by raw liver (3.08 ± 0.13 log 10 cfu/g), raw milk (3.04 ± 0.17 log 10 cfu/mL), and raw meat (2.39 ± 0.08 log 10 cfu/g), respectively. Additionally, 80%, 55%, 50%, and 25% of the examined kariesh cheese, raw liver, raw milk, and raw meat, respectively exceeded Egyptian limits of S. aureus in meat and dairies. Besides, S. aureus isolates showed clear multidrug resistance profiling. PCR testing of selected S. aureus isolates for harboring Staphylococcal enterotoxin-coding genes revealed that none of the tested genes were detected in isolates recovered from raw meat. However, some isolates recovered from raw milk, kariesh cheese, and raw liver harbored SEA, SEC, and SED. Therefore, strict hygienic measures should be adopted during handling, processing, and serving of such meat and dairies.

Keywords: Bovine meat; liver; S. aureus; kariesh cheese; milk, antimicrobial resistance

1. Introduction

Bovine meat, liver, milk, and kariesh cheese are considered as primary sources of animal derived protein rich in essential amino acids, vitamins, minerals, and provide humans with an adequate part of their needs from energy (Morshdy et al., 2019).

Microbial contamination of meat and dairies is controlled by the hygienic practices followed during milking, slaughtering, skinning, evisceration, and further processing. The sources of the microbial contamination of the meat and dairies involve the animal itself (as hair, skin, excreta, etc.), the operator (hands, hair, clothes, etc.), the use of contaminated raw materials, washing water, collecting containers, and equipment (Aberle et al., 2001; Darwish et al., 2018). Therefore, there is a large need for continuous monitoring of the microbial quality of the retailed meat and dairy products in Egypt.

Staphylococcus aureus (S. aureus) is a significant cause of foodborne diseases, particularly, foodborne intoxications worldwide (Darwish et al., 2022). It causes about 241,000 illnesses per year in the United State alone. Symptoms of human intoxication by S. aureus-enterotoxins are characterized by their rapid onset (1-6 hours post ingestion of contaminated foods), vomiting, nausea, abdominal cramps, and diarrhea (Hennekinne et al., 2012). S. aureus and staphylococcal enterotoxins (SEs) were isolated and detected in raw meat and meat products in Zaria, Nigeria (Ndahi et al., 2014), in raw meat in Iowa, USA (Thapaliya et al., 2017), in meat products (Morshdy et al., 2019), and raw chicken meat and livers in Egypt (Abolghait et al., 2020). In addition, raw milk, and dairy products such as cheese were reported to harbor S. aureus as reported in Egypt (Al-Ashmawy et al., 2016; Hegab et al., 2020), in artisan dairies in Italy (Johler et al., 2018), and in Banat region in Romania (Morar et al., 2021).

Antimicrobial resistance was developed foodborne pathogens through the abuse and the uncontrolled usage of antimicrobials during livestock production (Darwish et al., 2013). However, the role of the bovine meat, liver, raw milk, and kariesh cheese as potential sources of multidrug resistant S. aureus., in Egypt has received less attention.

This study was taken to investigate the prevalence rates of S. aureus in the retailed bovine meat liver, raw milk, and kariesh cheese in Egypt. Furthermore, screening of the antimicrobial resistance of the recovered S. aureus was done using the disk diffusion assay. In addition, detection of the coding genes of S. aureus-enterotoxins including SEA, SEB, SEC, and SED was done using PCR.

2. Material and Methods

2.1.Collection of samples:

Eighty random samples including 20 each of cattle meat (round, 100 g), liver (100 g), raw milk (200 ml), and kariesh cheese (100 g) were collected directly and randomly from butchery shops, farmers, and retail stores at different sanitation levels at Zagazig, and Mansoura cities, Egypt. The samples were cooled and transferred without delay to the laboratory for bacteriological examination.

2.2.Sample preparation:

The collected samples were prepared for bacteriological examination according to APHA (2001). In brief, 10 grams from each collected sample were mixed with 90 mL of 1% sterile peptone water (Oxoid CM9, UK), then blended for 3 min at 3000 rpm, then the resultant mixture was allowed to stand for 15 min at room temperature.

2.3.Isolation and identification of S. aureus:

The isolation and identification procedures of S. aureus were done according to APHA (2001). In brief, 0.1 mL of each prepared homogenate was cultured over Baird Parker agar (Oxoid, UK) plate supplemented with egg yolk emulsion using surface spreading technique by the use of a sterile glass spreader. Plates were kept on inverted positions and incubated at 37°C for 48 h. S. aureus colonies appear as black, shiny, circular, smooth, and convex with narrow white margin and surrounded by a clear zone extending into the opaque medium. For further biochemical examination, five suspected S. aureus colonies were purified on nutrient agar slopes. S. aureus colonies were subjected to morphological, biochemical, and serological identification. On biochemical examination, S. aureus isolates were positive for catalase, coagulase, hemolysis, and showed yellow colonies surrounded by halo zones in the mannitol test.

2.4.Antimicrobial susceptibility of the recovered S. aureus isolates:

Antimicrobial sensitivity of eight recovered isolates of S. aureus was tested using the disk diffusion method. Antimicrobial discs were bought from Oxoid Limited, Hampshire, UK. Nutrient agar plates acted as a culture medium during antimicrobial sensitivity testing of S. aureus. The guidelines of the Clinical and Laboratory Standards Institute (CLSI) guidelines (Wayne, 2013) were applied. In addition, the Multiple Antibiotic Resistance (MAR) index for each tested S. aureus isolate was

determined according to the formula stipulated by Singh et al. (2010) as follow:

MAR index= No. of resistance / Total No. of tested antibiotics

The used antimicrobial sensitivity discs (Oxoid Limited, Basingstoke, Hampshire, UK) were ampicillin (10 µg; AM), cephalothin (30 µg; CET), chloramphenicol (30 µg; C), ciprofloxacin (5 µg; CIP), enrofloxacin (5 µg; ENR), erythromycin (15 µg) (E), gentamicin (10 µg) (GEN), kanamycin (30 µg) (K), nalidixic acid (30 µg) (NA), neomycin (30 µg) (N), oxacillin (1 µg) (OX), oxytetracycline (30 µg) (OXY), penicillin (10 IU) (P), and trimethoprim/sulfamethoxazole (25 µg) (SXT).

2.5.Molecular identification of Staphylococcal enterotoxins:

Bacterial DNA was extracted from the cultured and identified S. aureus isolates using Genomic DNA extraction kit according to the instructions of the manufacturer (Alliance Global, Dubai, UAE). Primer pairs for S. aureus enterotoxin genes including SEA, SEB, SEC, and SED were purchased from Metabion International, Gmbh, Germany, and displayed in Table 1.

PCR amplification reactions were performed according to Darwish et al. (2018) on a Thermal Cycler (Master cycler, Eppendorf, Germany) using a uniplex PCR approach. The PCR cycling conditions started with an initial denaturation at 95°C for 1 min, followed by 35 cycles each is consisting of denaturation at 95°C for 15 sec, annealing at 50°C for 30 sec, , and an extension at 72°C for 1 min. A final extension step at 72°C for 7 min was followed and ended by holding at 4°C. Amplified PCR products were run on 1.5% agarose gel electrophoresis (AppliChem, GmbH, Germany) in 1x Tris Borate EDTA buffer stained with ethidium bromide then visualized on a UV transilluminator. DNA Ladder (100 bp, Qiagen, GmbH) was used a DNA marker.

2.6.Statistical analysis:

S. aureus counts were transferred into base-10 logarithms of cfu/g. Data were analyzed using one-way ANOVA procedure of SPSS v.23 (SPSS Inc., Chicago, Illinois,

The USA). Tukey's multiple comparison tests were used to detect significant differences among S. aureus counts in the examined samples. Data were expressed as means ± SD, with a p value of 0.05 is considered significant.

3. Results and Discussion

S. aureus is a major foodborne pathogen that is responsible for many nosocomial infections worldwide and many cases of foodborne intoxications via production of heat-stable enterotoxins such as SEA, SEB, SEC, and SED (Darwish et al., 2022). Herein, S. aureus was isolated from retailed raw milk, kariesh cheese, raw liver, and raw meat at variable rates. Kariesh cheese had the highest prevalence rate of S. aureus at 80%, followed by raw milk at 70%, raw liver at 65%, and raw meat at 50%, respectively (Fig. 1). Likely, kariesh cheese had significantly ($p < 0.05$) the highest total S. aureus count (3.55 ± 0.19 log 10 cfu/g), followed by raw liver (3.08 ± 0.13 log 10 cfu/g), raw milk (3.04 ± 0.17 log 10 cfu/mL), and raw meat (2.39 ± 0.08 log 10 cfu/g), respectively (Fig. 2). Comparing the recorded S. aureus counts in the present study with the established maximum permissible limits set (2 log 10 cfu/g for raw milk, meat, and liver, while cheese must be free from S. aureus and its toxins) by Egypt Organization for Standardization (EOS, 2005) revealed that 50%, 80%, 55%, and 25% of the examined raw milk, kariesh cheese, raw liver, and raw meat exceeded that limit, respectively (Fig. 3).

In agreement with the obtained results of the present study Osman et al. (2015) isolated S. aureus from raw meat retailed in Cairo, Egypt. In addition, Zeinhom et al. (2015) recorded an average count of S. aureus at 4.04 ± 3.28 log cfu/mL in milk, and 4.24 ± 3.71 log cfu/mL in feta cheese in samples collected from Beni-Suef, Egypt with lower S. aureus prevalence rates at12% for each of milk and feta cheese. Al-Ashmawy et al. (2016) isolated S. aureus at 75%, 65%, 40%, 50%, and 35% in raw milk, Damietta cheese, kariesh cheese, ice cream, and yogurt samples, respectively with mean counts of 3.49, 3.71, 2.93, 3.40, and 3.23 log 10 cfu/g in these dairy products, respectively. They added that all positive samples exceeded EOS limits. Hassan et al. (2018) detected S. aureus at 36%, 52%, 64%, and 12% in minced meat, beef burger, kofta, and luncheon retailed in Gharbia Governorate, Egypt, respectively. They added that 16 samples (64%) of minced meat, 22 samples (88%) of beef burger, 25 samples (100%) of kofta and 11 samples (44%) of luncheon exceeded the established Egyptian MPL of S. aureus. Besides, Naas et al. (2019) isolated S. aureus at 23% from meat, and meat products collected from Libyan retail markets. Higher prevalence rate was recorded in cheese retailed in Romania as S. aureus was isolated from 138 out of 169 tested samples with a prevalence rate of 81.6% (Morar et al., 2021).

Staphylococci can be found on the skin, hair, and nails of food handlers (Darwish et al., 2022; Zeinhom et al., 2015). Additionally, washing water used in the cleaning of the animal carcasses is also considered as an additional source of S. aureus (Darwish et al., 2018). Such sources might explain the recorded isolation of S. aureus from retailed milk, liver, and meat in the present study. Kariesh cheese had the highest contamination rate which could be explained as kariesh cheese was collected from rural areas in Egypt where minimum level of hygiene is adopted. Kariesh cheese is one of the most famous soft cheeses in Egypt, which is manufactured locally by farmers under less hygienic conditions, and without heat treatment of milk, and retailed at markets open to air (Elafify et al., 2022).

This variation in the isolation rates of S. aureus among different studies could be attributed to the differences in the hygienic practices adopted during milking and processing of milk, fecal contamination, infected udder or uncleaned utensils and equipment or originated from the milkers' hands, cross contamination during slaughtering, evisceration (Karns et al., 2005).

The recovered S. aureus isolates were subjected to antimicrobial sensitivity testing. Interestingly, all tested isolates (100%) showed multidrug resistance profiling with at least resistance to three tested antimicrobial classes. The recovered S. aureus isolates showed resistance to the tested antimicrobials in the following order: 87.5% to erythromycin, gentamicin, kanamycin, nalidixic acid, neomycin, and oxacillin > 75% to oxytetracycline > 62.5% to cephalothin > 37.5% to ampicillin, ciprofloxacin, and enrofloxacin > 25% to penicillin, and trimethoprim/sulfamethoxazole > 12.5% to chloramphenicol, respectively (Fig. 4). The calculated MAR index for the recovered S. aureus isolates in the current study ranged between 0.214 to 0.929 with an average of 0.598 (Table 2). Similarly, S. aureus isolates recovered from raw milk, and feta cheese retailed in Beni-Suef, Egypt showed marked antimicrobial resistance to ciprofloxacin, oxacillin, tetracycline, and gentamycin (Zeinhom et al., 2015). Furthermore, Morar et al. (2021) reported that S. aureus isolated from artisanal cheese in Romania showed a multidrug resistance profiling as the isolates were resistant to enrofloxacin (86.2%), neomycin (63.6%), kanamycin (41.4%), tetracycline (38.8%), ciprofloxacin (30%), erythromycin (22.4%,), oxacillin (16.3%), ampicillin (5.5%), and gentamicin (4.1%). The uncontrolled usage of antimicrobials in dairy farms and during intensive livestock production without a proper veterinary supervision led to development of multidrug resistance among foodborne pathogens (Alsayeqh et al., 2021).

PCR testing of eight randomly selected S. aureus isolates for harboring Staphylococcal enterotoxin-coding genes revealed that none of the tested genes were detected in S. aureus isolates recovered from raw meat. SEB-coding gene was also not detected in any tested S. aureus isolate. One S. aureus isolate recovered from raw milk harbored SEA only. While one S. aureus isolate recovered from kariesh cheese harbored SEA, SEC, and SED. Likely, one S. aureus isolate recovered from raw liver harbored SEA, and SEC only (Fig. 5). Detection of enterotoxins in the identified S. aureus isolates in the present study agrees with Shawish and Al-Humam (2016) who detected at least one of the S. aureus enterotoxins (SEA, SEB, SEC, and SED) in the examined beef products sold in Egypt and Saudi Arabia. Furthermore, Al-Ashmawy et al. (2016) detected SEA, SEB, and SEC in all recovered S. aureus isolates from raw milk, Damietta cheese, kariesh cheese, ice cream, and yogurt samples collected from Mansoura city, Egypt. In addition, Hassan et al. (2018) detected SEA, SEB, SEC, and SED in S. aureus isolated from beef mince, beef burger, kofta, and luncheon collected from Gharbia Governorate, Egypt. However, Zeinhom et al. (2015) detected only SEB from S. aureus isolates recovered from raw milk and feta cheese collected from Beni-Suef, Egypt.

S. aureus is responsible for many cases of food poisoning outbreaks worldwide. For instances, Center for Disease prevention and Control (CDC) reported a S. aureus-caused food poisoning outbreak in a military unit, US, 2012 (CDC, 2013). Furthermore, European Food Safety Association reported that 293 food poisoning outbreaks were linked to S. aureus in Europe during 2011 (EFSA, 2011). These results suggest that raw milk, kariesh cheese, raw liver are potential sources for S. aureus enterotoxins.

4. Conclusion

The obtained results of the present study revealed contamination of the retailed raw milk, kariesh cheese, raw liver, and raw meat with S. aureus at variable rates that exceeded the established Egyptian limits on several occasions. In addition, the recovered S. aureus isolates showed marked resistance to antimicrobials with remarkable multidrug resistance. Besides, the genes coding S. aureus enterotoxins were detected in the examined raw milk, kariesh cheese and raw liver. Therefore, adoption of strict hygiene should be followed with efficient heat treatment or preservation of the retailed meat and dairies.

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Table 1: Oligonucleotide primer sequences used in the study

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
SEA (F)	5′ TTGGAAACGGTTAAAACGAA′3	120	Rall et al. (2008)
SEA (R)	5′ GAACCTTCCCATCAAAAACA ′3		
SEB (F)	5′ TCGCATCAAACTGACAAACG ′3	478	
SEB (R)	5′ GCGGTACTCTATAAGTGCC ′3		
SEC (F)	5′ GACATAAAAGCTAGGAATTT ′3	257	
SEC (R)	5′ AAATCGGATTAACATTATCC ′3		
SED (F)	5′ CTAGTTTGGTAATATCTCCT ′3	317	
SED (R)	5′ TAATGCTATATCTTATAGGG ′3		

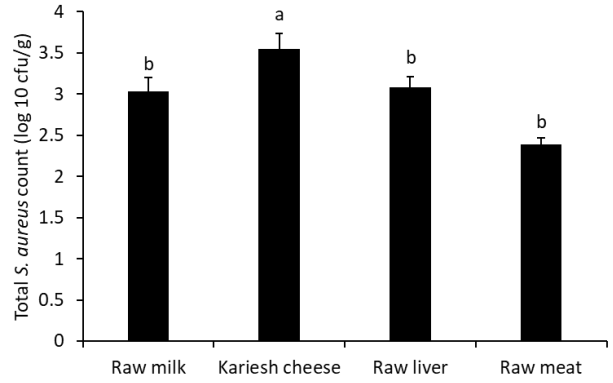

Fig. 2: Total S. aureus count (log 10 cfu/g) in the examined raw milk, kariesh cheese, raw liver, and raw meat (n = 20/each). Columns carrying different letter (a, b, c) are significantly different at $p < 0.05$.

Isolate	Resistance profile	MAR value
S. aureus 1	AM, CET, C, CIP, E, GEN, K, NA, N, OX, OXY, P, SXT	0.929
S. aureus 2	AM, CET, CIP, ENR, E, GEN, K, NA, N, OX, OXY	0.786
S. aureus 3	CET, CIP, ENR, E, GEN, K, NA, N, OX, OXY	0.714
S. aureus 4	CET, GEN, K, NA, N, OX, OXY, P, SXT	0.643
S. aureus 5	ENR, E, GEN, K, NA, N, OX, OXY	0.571
S. aureus 6	E, GEN, K, NA, N, OX, OXY	0.5
S. aureus 7	E, GEN, K, NA, N, OX	0.429
S. aureus 8	AM, CET, E	0.214
	Average	0.598

AM: ampicillin, CET: cephalothin, C: chloramphenicol, CIP: ciprofloxacin, ENR: enrofloxacin, E: erythromycin, G: gentamicin, K: kanamycin, NA: nalidixic acid, N: neomycin, OX: oxacillin, OXY: oxytetracycline, P: penicillin, and SXT: trimethoprim/sulfamethoxazole.

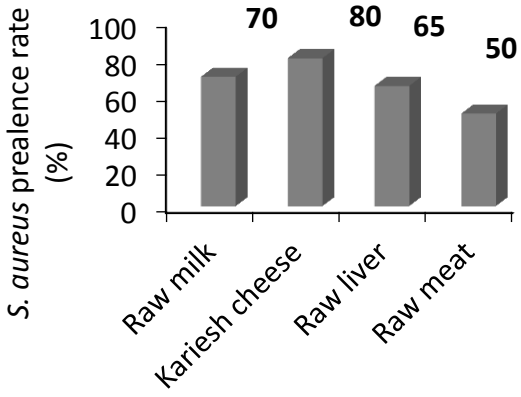

Fig. 1: Prevalence rate (%) of S. aureus in the examined raw milk, kariesh cheese, raw liver, and raw meat (n = 20/each)

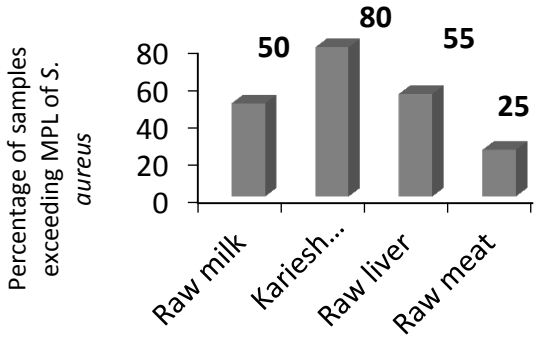

Fig. 3: Percentages of samples exceeding maximum permissible limits of S. aureus (2 log 10 cfu/g for raw milk, meat, and liver, while cheese must be free from S. aureus and its toxins) in the examined raw milk, kariesh cheese, raw liver, and raw meat (n = 20/each)

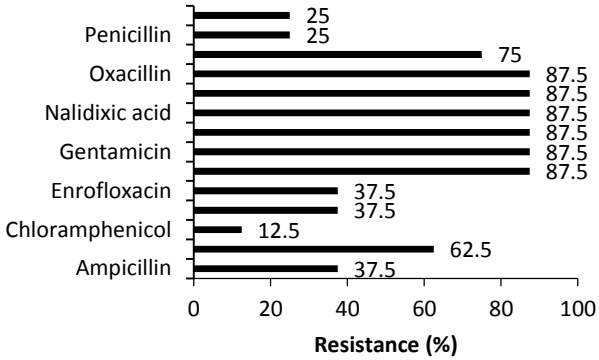

Fig. 4: Antimicrobial resistance rates (%) of the recovered S. aureus isolates from raw meat, liver, milk, and kariesh cheese

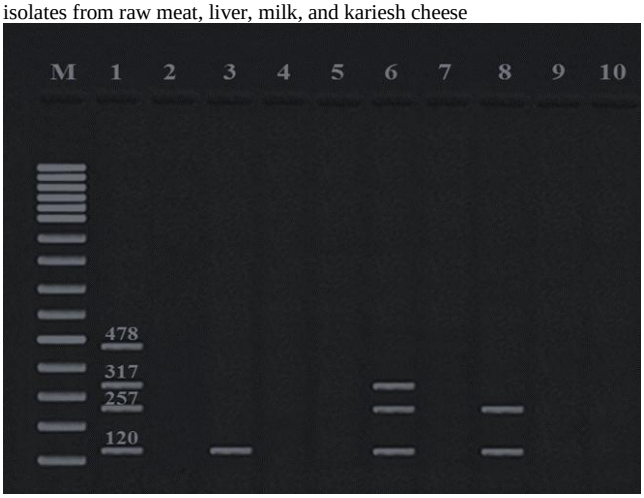

Fig. 5: DNA expression of S. aureus enterotoxin-coding genes by using multiplex PCR. Agarose gel electrophoresis of multiplex PCR of SEA (120 bp), SEB (478 bp), SEC (257 bp), and SED (317 bp) enterotoxin genes. Lane M: 100 bp DNA ladder as a molecular size DNA marker. Lane 1: Control positive for SEA, SEB, SEC, and SED genes. Lane 2: Control negative. Lanes 3, 4: S. aureus colonies recovered from raw milk. Lanes 5, 6: S. aureus colonies recovered from kariesh cheese. Lanes 7, 8: S. aureus colonies recovered from raw liver. Lanes 9, 10: S. aureus colonies recovered from raw meat