Molecular detection of Staphylococcal enterotoxins and mecA genes products in selected food samples collected from different areas in Khartoum state.

Mohammed Yahya  (mohammedyhaya707@gmail.com) 
Sudan University of Science and Technology

Hashim Abdalbagi Ali 
Sudan University for Science and Technology

Babbiker Mohammed Taher Gorish 
Omdurman Islamic University

Sara Omer Ali 
Sudan University of Science and Technology

Eman Saif Aldein Abdalrhim 
Sudan University of Science and Technology

Mawada Hamza Mergani 
Sudan University of Science and Technology

Asmaa Abass Abd Elgadir 
Sudan University of Science and Technology

Somaya Khalid Mohammed 
Sudan University of Science and Technology

Salma Omer Ahmed 
Sudan University of Science and Technology

Naglaa Alsaeid Musa 
Sudan University of Science and Technology

Alaa Saeed Ahmed 
Sudan University of Science and Technology

Wafaa Mohammed Abdalla 
Sudan University of Science and Technology

Yousif Fadlallah Hamedelnil 
Sudan University of Science and Technology

Ahmed Ibrahim Hashim 
Sudan University of Science and Technology

Hisham N. Altayeb 
King Abdulaziz University
Research article

**Keywords:** Food samples, S. aureus, Sensitivity test, enterotoxin gene, mecA gene, PCR

**DOI:** https://doi.org/10.21203/rs.3.rs-59354/v3

**License:** ☕️ 📧 This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background: Staphylococcal food poisoning is an intoxication that results from the consumption of improperly prepared or stored foods containing sufficient amounts of one or more preformed S. aureus enterotoxins. Nowadays many researchers worldwide noted an emergence of resistant strains Staphylococci particularly for the antibiotic methicillin. Therefore, this study was aimed to determine the existence of Staphylococcus aureus and its enterotoxins, mecA genes in selected food samples.

Results: A total of 400 selected food samples were collected from different areas in Khartoum state. The selected foods included cheese, meat products, fish and raw milk. One hundred sample from each type of food were cultivated and the resultant growth yielded 137 (34.25 %) S. aureus, 126 (31.5%) bacteria other than S. aureus and 137 (34.25%) yielded no growth. Eighty-four of the137 S. aureus isolates were randomly selected and tested for the presence of mecA and enterotoxin genes. Oxacillin sensitivity test showed that 15(11%) of 137 S. aureus isolates were Oxacillin resistant. The PCR assay showed that the mecA gene was detected in 15 of 84 (17%) S. aureus isolates. Simultaneously, only 2 (2.385%) out of 84 S. aureus isolates showed an enterotoxin B gene product.

Conclusion: There was a relatively moderate prevalence of methicillin-resistant staphylococcus aureus with very low frequency of enterotoxin B gene in different kinds of selected food samples that collected from Khartoum state. These findings elucidate the increased risk on public in Khartoum being affected by Staphylococcal food poisoning upon consumption of dairy or meat products prepared in unhygienic conditions that could lead to intoxication by Staphylococcus aureus enterotoxins.

Background

Food-borne diseases (FBD) remain one of the greatest concerns in public health and food safety, they are caused by many pathogens that contaminate food and food products (1). Many food sources may serve as a substrate for many microorganisms which are transmitted during harvesting, storage or food processing and handling by multiple environmental sources such as water, soil, insects, or even by the handlers (2).

Staphylococcal Food Poisoning (SFP) is an intoxication that results from the consumption of improperly prepared or stored foods containing sufficient amounts of one or more preformed enterotoxins (3, 4). A wide variety of foods support the growth of Staphylococcus aureus and are ideal for enterotoxin production including milk, meat, meat products, dairy products, and ready-to-eat food (5, 7).

Although Staphylococcus aureus may produce a large variety of enterotoxins, 95% of food poisoning outbreaks are caused by classical enterotoxins: A, B, C, D and E (6). Since these toxic proteins are capable of tolerating high temperatures up to 100°C for several minutes, improperly cooked food contaminated with bacteria or its preformed toxins in sufficient amounts could lead to Staphylococcal food poisoning within a few hours characterized by symptoms including nausea, vomiting and diarrhea (8). Some strains of Staphylococcus aureus have the ability to resist heat and drying; hence, it can easily contaminate
foods. This contamination might come from food handlers or from the environment, where the bacteria multiply and release toxins in uncooked or inadequately cooked foods, especially if the foods are unrefrigerated (9). The consumption of foods of animal origin contaminated with MRSA or MRSA preformed enterotoxins could lead to serious threats to the well-being of humans due to uncountable clinical implications (10).

Non-hygienic handling practices, working conditions, and improper storage and refrigeration; can all increase the opportunity for food contamination. So, it is important to follow the standard practices in food handling such as hand washing, proper cooking, proper storage and others to reduce or prevent food contamination (11, 14).

There is paucity of information in Sudan regarding the role *Staphylococcus aureus* in food poisoning and the presence of enterotoxins in *mecA* genes in common consumed foods in Khartoum State. Hence, this study was conducted to determine the prevalence of enterotoxins and *mecA* genes in foods commonly consumed in Khartoum State, Sudan.

**Result**

**Prevalence of *S. aureus* isolates in food samples**

The Presence of *S. aureus* was observed in 137 (34.25%) of the 400 food samples collected from different areas in Khartoum state. However, 126 (31.5%) of the 400 samples were identified as bacteria other than *S. aureus* and 137 (34.25%) samples did not yield any growth (Table 1). All isolated *S. aureus* confirmed by detection of the 16S rRNA housekeeping gene product which correspond to 756 bp band size (Figure 1).

**Detection of *mecA* and enterotoxins genes among the *S. aureus* isolates**

The 84 different *S. aureus* isolates were randomly selected from a total of 137 *S. aureus* isolates. The selected isolates were further examined for the presence *mecA* and enterotoxin genes using specific primer in a conventional PCR assay. The *mecA* gene was detected in 15 (17%) *S. aureus* isolates (in samples number 6, 13, 55, 63 and 81 of meat and samples number 2, 11, 47, 72 for cheese and in samples number 19, 28, 34 and 49 of milk and in samples number 9 and 38 of fish) (Table 2), (Figure 1, 2).

However, only 2 (2.385%) of 84 *S. aureus* isolates were showed an enterotoxin B gene product (both isolates were from cheese samples and the samples ID were 16 and 31), while the rest of 82 isolates were negative. All isolates were negative for other enterotoxins gene (other than *seb* gene) (Table 2), (Additional figure 1).

**Meat isolates antimicrobial susceptibility characteristics**

Presence of *S. aureus* was observed in 30 (30%) of the 100 Milk samples (Table 3) of which 11 (36.7%) isolates were detected in raw beef and 19 (63.3%) were identified in restaurants meat. However, 20 (20%) samples showed a growth of bacteria other than *S. aureus* and 50 (50%) samples showed no growth.
tested meat *S. aureus* isolates, were susceptible to ciprofloxacin. The resistant rates of meat *S. aureus* isolates to gentamycin was 4 (13.3%) and it was higher than that identified to the antibiotics oxacillin and vancomycin with percentage of 2 (6.6%) and 5 (16.7%) respectively (Table 4). The PCR assay for enterotoxin gene products showed that none of the meat *S. aureus* isolate produce enterotoxins genes products (Additional figure 1).

**Cheese isolates antimicrobial susceptibility characteristics**

The examination of 100 cheese samples collected from different area in Khartoum state revealed that the occurrence of *S. aureus* Isolate was 20 (20%). While bacteria others than *S. aureus* were represented 4%. However, none of the rest 76(76%) showed any growth on the agar plate surface (Table 3). We found that all isolates were susceptible to both gentamicin, ciprofloxacin antibiotics. However, vancomycin showed growth-inhibition zones with 17 (85%) isolates out of 20 positive samples. Only one (5%) cheese *S. aureus* isolate was resistant to oxacillin (Table 4). Enterotoxin gene B (*seb*) was detected only in 2(10%) of cheese isolates (samples ID were 16 and 31). While none of other types of enterotoxin genes products were detected among these isolates (Additional figure 1).

**Antimicrobial Profile for fish *S. aureus* isolates**

In this study a total of 100 fish samples (50 salted fish 50 raw fish) were analyzed for the presence of bacterial pathogens. The study revealed 24/100 (24%) of the fish samples had *S. aureus* contamination (Table 3). Antibiotic susceptibility of *S. aureus* was tested using the agar disc diffusion method. The results have shown that all fish originated *S. aureus* isolates were susceptible to both ciprofloxacin and gentamicin antibiotics. In contrast, the resistance rate was only 8% to both vancomycin and oxacillin antibiotics (Table 4). No enterotoxin gene product was detected during the gel electrophoresis procedure which applied after successful cycles of conventional PCR (Additional figure 1).

**Antimicrobial Profile for milk *S. aureus* isolates**

Out of 100 milk samples collected from different areas in Khartoum state 63(63%) were identified as *S. aureus*, 26(26%) were identified as bacteria others than *S. aureus* and 11(11%) showed no growth (Table 3). The antimicrobial susceptibility test was done to all *S. aureus* isolates and the result showed that the highest susceptibility rate was recorded to ciprofloxacin and gentamicin with a percentage of 98.4% (62/63 isolates) and 87.3% (55/63 isolates) respectively, followed by oxacillin with a percentage of 84% (53/63). However, the least potent antibiotic was vancomycin with a percentage of 65% (41/63) (Table 4). The enterotoxin genes results reveal that no *S. aureus* isolate produces such genes (Additional figure 1).

**Table 1. Distribution of bacteria isolated from selected food samples purchased from retailers in Khartoum State**
Isolate | Number | Percentage
--- | --- | ---
*Staphylococcus aureus* | 137 | 34.25%
Others bacteria | 126 | 31.5%
No growth | 137 | 34.25%
Total | 400 | 100%

Table 2. Distribution of *mecA* and *enterotoxin* genes among *Staphylococcus aureus* isolates

| Type of gene detected | Positive | Negative | Total |
|-----------------------|----------|----------|-------|
mecA gene              | 15 (17.9%) | 69 (82.1%) | 84 (100%) |
Enterotoxin B gene     | 2 (2.38%)  | 82 (97.62%) | 84 (100%) |
Other enterotoxin genes| 0 (0%)    | 84 (100%)  | 84 (100%) |

Table 3. Distribution of *Staphylococcus aureus* isolates according to the type of food samples
Table 4. Show Antimicrobial sensitivity pattern of *Staphylococcus aureus* that isolated from different food material samples

| Sample | S. aureus Isolates | Other Bacteria Isolates | No growth | Total |
|--------|--------------------|-------------------------|-----------|-------|
| Meat   | 30 (30%)           | 20 (20%)                | 50 (50%)  | 100 (100%) |
| Cheese | 20 (20%)           | 4 (4%)                  | 76 (76%)  | 100 (100%) |
| Fish   | 24 (24%)           | 76 (76%)                | 0 (0%)    | 100 (100%) |
| Milk   | 63 (63%)           | 26 (26%)                | 11 (11%)  | 100 (100%) |
| Total  | 137                | 126                     | 137       | 400    |

Table 4. Show Antimicrobial sensitivity pattern of *Staphylococcus aureus* that isolated from different food material samples

| Isolates | Pattern  | Antibiotics | Gentamycin (10mg) | Ciprofloxacin (5mg) | Oxacillin (5mg) | Vancomycin (30mg) |
|----------|----------|-------------|-------------------|---------------------|-----------------|-------------------|
| Meat     | Sensitive| 26 (86.7%)  | 30 (100%)         | 28 (93.4%)          | 25 (83.3%)      |
|          | Resistant| 4 (13.3%)   | 0 (0%)            | 2 (6.6%)            | 5 (16.7%)       |
| Cheese   | Sensitive| 20 (100%)   | 20 (100%)         | 19 (95%)            | 17 (85%)        |
|          | Resistant| 0 (0%)      | 0 (0%)            | 1 (5%)              | 3 (15%)         |
| Fish     | Sensitive| 24 (100%)   | 100 (100%)        | 22 (92%)            | 22 (92%)        |
|          | Resistant| 0 (0%)      | 0 (0%)            | 2 (8%)              | 2 (8%)          |
| Milk     | Sensitive| 55 (87.3%)  | 62 (98.4%)        | 53 (84%)            | 41 (65%)        |
|          | Resistant| 8 (12.7%)   | 1 (1.6%)          | 10 (16%)            | 22 (35%)        |
| Total    |          | 137         | 137               | 137                 | 137             |

Discussion
In this study, the prevalence of \textit{S. aureus} and MRSA and enterotoxin gene products were investigated in various food samples collected from Khartoum state markets (400 samples of milk, cheese, fish, meat). Identification of the bacteria isolated from the selected foods through conventional methods yielded a total of 137 (34.3\%) \textit{S. aureus} isolates. Similar reports on foods contaminated with \textit{S. aureus} from Italy and India revealed much lower percentage yielding 17.1\% (15) and 12.01\% (16) respectively. The previous studies conducted to detect \textit{S. aureus} in various foods revealed that the contamination levels with \textit{S. aureus} reported were lower than those obtained in this study. On the other hand, a study in Greece reported 47.8\% of north-central and north-eastern Greece foods; which is much higher contamination level compared to those reported in this study (3). Those great discrepancies between our finding and other studies results may be due to variation in foods, habits, cooking behaviors, food keeping hygiene in addition to environmental factors like the weather temperature and moist which significantly affect bacterial growth in food materials.

In this study resistance gene (\textit{mecA}) of \textit{S. aureus} that responsible for resistance to $\beta$-lactam antimicrobials was detected by using PCR and we find 15 (17\%) of \textit{S. aureus} isolates are positive for the \textit{mecA} gene while 69 (83\%) were negative. Comparing our finding to previous studies results we realize that most previous studies results showed a significantly higher prevalence than our finding for example in a study done by Khayri in Makkah city he found that about 44.4\% of his \textit{S. aureus} isolates were positive for \textit{mecA} gene (4). Similarly, Papadopoulos and his colleagues found that 81.3\% of their \textit{S. aureus} isolates were positive for the \textit{mecA} gene (3). This variation could be due to the difference in the antibiotic protocol applied by the doctors for their patients in different countries or due to the extensive usage of methicillin antibiotics their communities or by doctors during treatment prescription \textit{in these} countries and eventually lead to a high prevalence rate of MRSA. On the contrary, the results reported in this study are higher than those reported by Novak \textit{et al} (5) where the \textit{mecA} gene prevalence was only 11.4\%. Low rates of \textit{mecA} gene were also reported in Egypt, Brazil and China where the prevalence of \textit{mecA} gene was 5.1\%, 9\% and 7.9\%, respectively (6-8). The variation between the results may be due to variation of samples sources and the use of different molecular techniques in different countries for the detection of \textit{mecA} gene product.

In this study, one hundred raw meat samples were obtained from different supermarkets and restaurants in Khartoum state. These samples are examined for the presence of \textit{S. aureus}. Thirty (30\%) samples were found to be contaminated with \textit{S. aureus}. These findings highlight the high potential risk for consumers of meat and dairy products especially in the absence of strict hygienic and preventive measures to avoid \textit{Staphylococcus aureus} enterotoxins (SEs) production in foods. In other comparative studies, similar results were reported from USA where the prevalence of \textit{S. aureus} in meat samples was 29.0\% (23). Much lower results were reported in South Africa, who reported that \textit{S. aureus} was 26.5\% (24). Also, our result is lower than that obtained by Das \textit{et al.} in India who reported that out of 65 samples \textit{S. aureus} incidence
was in 46.1% (25). In our study, none of the meat *S. aureus isolates* were resistant to ciprofloxacin and 13.3% were resistant to gentamicin. In disagreement with our result Das *et al.* in India found that 16.66% of *S. aureus* meat isolates were resistant to ciprofloxacin (25) and Pu S *et al.*, in Louisiana found that 13.0% were resistant to ciprofloxacin. Also, in contrast to our findings. Pu S *et al.* Louisiana found that 3.0% were resistant to gentamicin (26). Vancomycin-resistant *Staphylococcus aureus* (VRSA) is a type of antibiotic-resistant *S. aureus* which have developed a resistance and can no longer be treated with vancomycin. This study showed that 16.6% of the meat isolates were resistant to vancomycin, which suggests that the contamination may be coming from VRSA carrier's food handlers and processors, however Das *et al.* found that 3.33% of the isolates were resistant to vancomycin (VRSA) which is low compared with our findings (25). Methicillin-resistant *S. aureus* (MRSA) strains have acquired a gene that makes them resistant to nearly all beta-Lactam antibiotics, animal-adapted MRSA strains also exist although it's in small percentage but it's of a clinical importance and may cause serious problems to immunocompromised individuals as well as healthy ones (carriers). In this study 6.6% of meat isolates were resistant to oxacillin, this finding was high compared to Inge *et al.* results whom found that 2.5% of *S. aureus* meat isolates were resistant to oxacillin (27), and low compared to Das *et al.* results whom found that 23.3% of *S. aureus* isolates were resistant to oxacillin (25).

Investigations through PCR technique on enterotoxin genes in this study showed the absence of enterotoxin genes in meat *S. aureus* isolates. A similar study in Denmark demonstrated the presence of enterotoxin genes in only 0.2% of the isolates (28). On the other hand, report from China demonstrated prevalence of 46.0% enterotoxin gene (29) and Bergdoll, found that the percentage of enterotoxigenic strains of *S. aureus* is estimated to be around 25% (30). Moreover, most of *S. aureus* food isolates are not SEs producers, thus considerable research effort is still required for a better understanding of the interactions between *S. aureus* and the food matrix and of the mechanism of SEs production in foods stuffs (31). The data obtained in this study probably underestimated the enterotoxigenic properties of the analyzed strains, since the possible presence of newly described SEs was not considered and the sample size was too small to represent *S. aureus* contaminated meat effectively. However, there is always the possibility of mutation at the level of the corresponding gene, leading to the absence of its detection. Therefore, a positive PCR shows the presence of the enterotoxin genes but a negative PCR does not point to the absence of the corresponding operon because there are many types of enterotoxin genes and we determine only one type (31).

In this study, one hundred white cheese samples collected from different retailers in Khartoum State to detect the rate of *S. aureus* contamination in cheese sample in this study is higher than that reported in Iran and Japan where the reported contamination rates were 16% and 13.3% respectively (32,33), and lower than that reported in Turkey which was 37.5% (34). However, the results in this study disagree with a previous report in Khartoum State where no *Staphylococcus aureus* were found on white cheese (35).
This may be due to variation in sample sources where the cheese was manufactured, the retailers where cheese samples were purchased from, the sampling area, season and environment, all these factors might affect the rate of contamination with microorganisms. The rate of sensitivity of cheese S. aureus isolates to gentamicin and ciprofloxacin was 100%; these findings opposes those reported in the U.S.A, where 75% of S. aureus isolates were resistant to gentamicin (36) and in Iraq where 25% of S. aureus isolates were resistant to ciprofloxacin (37). The resistance of cheese S. aureus to Oxacillin in this study was (5%) which was lower than that reported in Iraq which was 20% (38). The sensitivity to vancomycin was 85% which is lower than that reported in Switzerland which was 100% (39, 40).

The molecular detection of Staphylococcus aureus enterotoxins (A, B, C, D and E) genes among cheese isolates resulted in the detection of seb gene in 10% of the 20 isolates, that was lower than the results obtained by Salheen in Sudan who reported 20% of seb gene was detected in cheese samples (41). The variation between results could be due to several factors such as sample source, geographical origin, the sensitivity of identification methods and sample size can affect the outcomes.

In this study 108 of fish samples were examined. The contamination rate of fish samples in this study is very low (22%) when compared with a previous report in Khartoum State 72% (42), Egypt 93% (43), India 100% (44) and relatively lower than that reported in Spain (27%) (45). Antimicrobial susceptibility results for fish S. aureus isolates showed 100% sensitivity to ciprofloxacin, which was in agreements with reports in Egypt (43), Turkey (46) and very close to a study in also nearly similar to the Portugal 98% (47). However, lower results in India was 48.5% (48) and in Egypt was 57% (49). gentamycin also showed an efficacy rate of 100% among all S. aureus isolates, which is similar to reports from Egypt (49), Turkey (46), and that of Vázquez-sánchez et al., in Spain (45), and slightly similar to results reported in Egypt 97% (43) and in Portugal, 92% (47). In this study, S. aureus isolated from fish samples showed a sensitivity rate of 92% to vancomycin, which is similar to those, reported in Egypt 91% (43) and Portugal 90% (47) respectively. Another study in Egypt reported higher result 100% (49), while other studies in Turkey reported much lower sensitivity to vancomycin, which was 83% and 78% respectively (46,50). The oxacillin showed 92% potency against fish S. aureus isolates which was higher than that reported in Portugal 62% (47), while it was relatively lower than those reported in Spain and Turkey where both reported 100% (45, 46). The variation between these results could be due to contribution of several factors such as source of samples, geographical origin, the sensitivity of identification methods and sample size can affect the outcomes.

The molecular detection of the enterotoxins genes among fish S. aureus isolates gave no band for all genes meaning that none of the all isolates possess such gene in their genetic material this finding was in agreement with those reported in Turkey and USA (46, 51), where no enterotoxins B, C and E genes were found in their food S. aureus isolates. However, a study in Tanzania, reported that enterotoxin B and C genes were detected in 0.3% with the absence of enterotoxin A gene (52), while a study from Turkey reported enterotoxins A and D genes in 10.5% of their samples (46). Variation among researcher studies
results and our findings could be due to several factors such as samples source, geographical origin, the sensitivity of identification methods and sample size can affect the outcomes.

In this study, *S. aureus* was isolated in 63% of raw milk samples, which is relatively close to that reported in Brazil 68% (53) and Malaysia 60% (54). However, this finding was lower than those reported in Turkey 75% (55) and Egypt 82% (56). The prevalence of this study is higher than those reported in two different studies in Iraq with prevalence of 53.33%, 43.5%, respectively (57, 58). Moreover, the prevalence of *S. aureus* in this study is too high compared to reports from Sudan and Egypt where the levels were 30% and 24.8% respectively (59,60). *S. aureus* isolated from milk samples in this study were highly sensitive to ciprofloxacin 98.4%, which is close to a report from a study in Bangladesh 93.3% (61). However, relatively lower susceptibility levels were reported in India and Bangladesh where the rate was 80% and 83.3% respectively (62, 63). The sensitivity to gentamycin in this study 87% is lower than that reported in Ethiopia 90% (64, 63), 100% (65) and higher compared to another report in Sudan which was 47.6% (59). While slightly similar to results obtained by Beyene, in Ethiopia, and Thaker *et al.*, in India whom reported that 90% of *S. aureus* isolates were sensitive to gentamicin (64, 63). Reports from other researchers were indicated a higher level of susceptibility rate to gentamicin for instance Abraha *et al.*, in Ethiopia reported 100% of milk *S. aureus* isolate are susceptible to gentamycin (65). In this study, vancomycin susceptibility test was determined for all milk originated *S. aureus* and the result showed that 65% of the *S. aureus* isolates were susceptible to vancomycin, this finding was completely closed to findings reported by Idbeis, in Basra in Iraq, AL –Marsomy, and Bendahou *et al.*, in North Morocco who mentioned that *S. aureus* isolated from raw milk and milk product showed sensitivity to vancomycin of 100% (66,67,68). On the contrary studies in Ethiopia and Iraq reported 100% resistance to Vancomycin (65, 69). The sensitivity to Oxacillin 84% in this study is higher than that reported in India 70% (63) and lower than that reported in Bangladesh 100% (62). All milk *S. aureus* isolates tested for the presence of enterotoxins genes yielded negative results. Similar findings were reported in Hungary (70). The variations between results reported in this study and other reports could be attributed to several factors such as samples source, geographical origin, the sensitivity of identification methods and sample size can affect the outcomes.

**Methods**

**Collection of selected food samples**

A total of 400 samples were collected from different areas in Khartoum state (Khartoum, Omdurman, East Nile and Khartoum North), during 2018. The type of foods included Cheese, Meat products, Fish and Raw milk. Each sample was aseptically collected, fifteen grams of cheese samples were collected from different retailers by using sterile container, meat samples were collected randomly from supermarkets and restaurants using disposable blades, a small piece of raw meat had been spiltted and transferred to the lab in sterile containers, small pieces fish inner tissues were collected by sterile blade and placed in
sterile plain containers and milk samples were collected in sterile containers and stored in a refrigerator at 4°C in microbiology laboratory until examined.

**Isolation and identification of coagulase positive Staphylococcus isolates**

Meat, fish and cheese samples were enriched in peptone water. The raw milk and the enriched peptone water samples were swabbed and inoculated in Blood agar medium, Mannitol salt agar medium and MacConkey's agar medium and incubated aerobically at 37°C for 24-48 hrs. The presence of *Staphylococcus aureus* was confirmed based on colony morphology; Gram's reaction and other biochemical tests including catalase test, coagulase test and DNase test.

**Antimicrobial susceptibility testing of coagulase positive Staphylococci**

The antimicrobial susceptibility test was done by disk diffusion method using Mueller-Hinton agar plates (oxoid) according to (12). Where 4 antimicrobial agents belonging to different classes were selected including ciprofloxacin (5 μg), gentamicin (10 μg), oxacillin (5 μg) and vancomycin (30 μg). The *S. aureus* ATCC 52923 Control strain was used.

**DNA Extraction**

DNA was extracted by simple boiling method, in which the extracted product was done from overnight isolates on Nutrient Agar. A loop full of bacterial colony was picked from an isolate and suspended in 300μl of sterile distilled water and 10μl of proteinases K was added and incubated at 60°C for 60 minutes. Then incubated at 100°C in a water bath for 15 minutes, and then the suspension was centrifuged at high speed (10000 rpm for 10 min). The supernatant containing the genomic DNA was transferred into a fresh sterile Eppendorf tube and stored at -20°C until to be used for PCR (13).

**PCR Detection of 16s rRNA gene**

All samples were confirmed as *S. aureus* by using specific housekeeping gene primer (16s), showed in Table 5. Negative samples were excluded. The DNA amplifications were performed from a volume of 25 μL of a mixture containing 2 μL Maxime PCR Premix, 0.5 μL of each primer, 2 μL of template DNA and 20 μL of double distilled water. The amplification conditions were included three steps: initial denaturation at 94°C for 5 min; 35 successful cycles of denaturation at 94°C for 45sec, annealing at 50°C for 45sec, and extension at 72°C for 45 sec; and the final extension at 72°C for 7 min (14).

**PCR Detection of Staphylococcal Enterotoxins Genes**

Multiplex PCR, amplification was done using (CLASSIC K960, UK) thermo cycle. PCR amplification of *Staphylococcal* enterotoxins (SE) genes, namely (*sea*, *seb*, *sec*, *sed* and *see*) was performed using Maxime PCR Premix kit (iNtRON, Korea) and specific primers listed in Table 5. The PCR assay was carried out in a total volume of 25 μL of mixture containing 2 μL Maxime PCR Premix, 0.5 μL of each of the toxin
gene-specific primers (5 μL), 2 μL of template DNA and 16 μL of double distilled water. The amplification conditions included three steps: initial denaturation at 94°C for 5 min; 35 successful cycles of denaturation at 94°C for 45sec, annealing at 50°C for 45sec, and extension at 72°C for 45 sec; and the final extension at 72°C for 7 min.

PCR Detection of *mecA* Gene

Primers were used for the detection of *mecA* gene, showed in table 5. DNA amplification was done using Maxime PCR Premix kit (iNtRON, Korea). The PCR assay was carried out in a total volume of 20 μL of mixture containing 2 μL Maxime PCR Premix, 0.5 μL of each of the gene-specific primers (5 μL), 2 μL of template DNA and 13 μL of double distilled water. The amplification conditions included three steps: initial denaturation at 94°C for 5 min; 35 successful cycles of denaturation at 94°C for 45sec, annealing at 52°C for 45sec, and extension at 72°C for 45 sec; and the final extension at 72°C for 7 min.

Table 5. Primers used for detection of *S. aureus* housekeeping gene, enterotoxins and *mecA* genes.

| Primer        | Sequence 5' – 3'          | Product size (bp) |
|---------------|---------------------------|-------------------|
| **Housekeeping gene primers** |                           |                   |
| Staph 756-F   | AACTCTGTTATTTAGGAAGAACA   | -                 |
| Staph 750-R   | CCACCTTCCTCCGGTTTGTCACC  | 756               |
| **Enterotoxins genes primers** |                       |                   |
| SA-Ua- F      | TGTATGTATGGAGGTGTAAC      | -                 |
| SA-A- R       | ATTAACCGAAGGTCTCTGT       | 270               |
| SA-B-R        | ATAGTGACGAGTTAGGTA        | 165               |
| ENT-C-R       | AAGTGACGAGTTAGGTA         | 102               |
| SA-D-R        | TTTCCGGAAAATCACCTTAA      | 303               |
| SA-E-R        | GCCAAAGCTGTCTGAG          | 213               |
| **mecA gene primers** |                       |                   |
| MecA1 – F     | AACTCTGTTATTTAGGAAGAACA  | -                 |
| MecA1 – R     | CCACCTTCCTCCGGTTTGTCACC  | 310               |

Ua: Universal, f: Forward, r: Reverse
Quality control

All samples were aseptically collected and analyzed, positive control which was a well-known enterotoxin and mecA genes producing *Staphylococcus aureus* and negative control which was sterile distilled water were included during PCR running.

List Of Abbreviations

FBD: Food borne diseases

*S. aureus*: *Staphylococcus aureus*

PCR: polymerase chain reaction

MRSA: Methicillin-Resistant *Staphylococcus aureus*

SEs: *Staphylococcus aureus* enterotoxins

VRSA: Vancomycin-Resistant *Staphylococcus aureus*

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interest.

Funding

The authors received no specific funding for this work.

Authors’ contributions
MY, HA, BG, SO, ES, MH, performed main experiments, AA, SK, SO, NA, AS collected samples and information. MY, HA, WM, HN, YF, AI designed the experiments and wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors gratefully acknowledge for his great effort Microbiology lab staff in Sudan university of science and technology. We also express our thanks and appreciation to all salles man in all markets whom provide us the samples to achieve this research.

References

1- Abd Elsalam, EE. Rapid Method for Detection of Staphylococcus aureus Enterotoxins in Food. B.V.Sc. Cairo University. 2008.
2- Ubiebi CO. Isolation and Identification of Bacterial Isolates from Poultry and Fish Feeds sold In Abraka, Delta State, Nigeria. Journal of Industrial Technology, 2017; 2(1): 14- 20.
3- Niveditha P, Shylaja R, Radhika M, Murali S, Harshvardhan B. A novel mPCR for the detection of prominent toxins in MRSA strains of S. aureus recovered from diverse sources. Int J Res Biol Sci. 2014, 2 (4): 1-3.
4- Schelin J, Wallin-Carlquist N, Cohn MT, Lindqvist R, Barker GC, Rådström P. The formation of Staphylococcus aureus enterotoxin in food environments and advances in risk assessment. Virulence. 2011; 2(6):580-592. doi:10.4161/viru.2.6.18122
5- Aydin A, Sudagidan M, Muratoglu K. Prevalence of staphylococcal enterotoxins, toxin genes and genetic-relatedness of foodborne Staphylococcus aureus strains isolated in the Marmara Region of Turkey. Int J Food Microbiol. 2011;148(2):99-106. doi: 10.1016/j.ijfoodmicro.2011.05.007.
6- Letertre C, Perelle S, Dilasser F, Fach P. Identification of a new putative enterotoxin SEU encoded by the egc cluster of Staphylococcus aureus. J Appl Microbiol. 2003;95(1):38-43. doi:10.1046/j.1365-2672.2003. 01957.x
7- Techer C, Salmain M, Tranquet O, Echasserieau Valérie, Le Moigne V, Hennekinne J, Gautier M, Val Florence. Detection and quantification of staphylococcal enterotoxin A in foods with specific and sensitive polyclonal antibodies, Food Control. 2013; https://doi.org/10.1016/j.foodcont.2012.11.021.
8- Carroll K C, Butel, J, Morse S. Jawetz Melnick & Adelberg Medical Microbiology 27th edition. New York. Mc Graw Hill Professional, 2012; p203-208.
9- Black JG. Microbiology Principles and Explorations”.7thed. United States of America: John Wiley and Sons, 2008; Pp 684-685
10- Monecke S, Coombs G, Shore AC, et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant Staphylococcus aureus. PLoS One. 2011; doi: 10.1371/journal.pone.0017936
11- Ezzeldeen NA, Mansour HA, Ahmed AA. Phenotypic and Molecular Identification of Staphylococcus aureus Isolated from some Egyptian Salted Fish”. World Applied Science Journal. 2015; 15 (12):1703-1712.
12- Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility
13- Compain F, Babosan A, Brisse S, et al. Multiplex PCR for detection of seven virulence factors and K1/K2 capsular serotypes of Klebsiella pneumoniae. J Clin Microbiol. 2014;52(12):4377-4380. doi:10.1128/JCM.02316-14.

14- Mahmoud L H. Molecular detection of Staphylococcus aureus toxin genes isolated from different clinical samples in Khartoum state. PhD thesis. Sudan University of Science and Technology.

15- Traversa A, Gariano GR, Gallina S, Bianchi DM, Orusa R, Domenis L, Cavallerio P, Fossati L, Serra R, Decastelli L. Methicillin resistance in Staphylococcus aureus strains isolated from food and wild animal carcasses in Italy. Food Microbiol. 2015; 52:154-8. doi: 10.1016/j.fm.2015.07.012.

16- Sivakumar M, Dubal ZB, Kumar A. Virulent methicillin resistant Staphylococcus aureus (MRSA) in street vended foods. J Food Sci Technol. 2019; 56, 1116–1126 https://doi.org/10.1007/s13197-019-03572-5.

17- Papadopoulos P, Papadopoulos T, Angelidis AS, Kotzamanidis C, Zdragas A, Papa A, Filioussis G, Sergelidis D. Prevalence antimicrobial susceptibility and characterization of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus isolated from dairy industries in north-central and north-eastern Greece. Int J Food Microbiol. 2019; 16; 291:35-41. doi: 10.1016/j.ijfoodmicro.2018.11.007.

18- Khayri A, Al-Ouqaili M, Hassan A. Molecular detection of MEC A and FEM A genes in methicillin-resistant Staphylococcus aureus in human milk. Memórias do Instituto Oswaldo Cruz, 2009; 95(1), 29-33.

20- Rania M K, Mohamed A B, Salah FA. 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, https://doi.org/10.1016/j.foodcont.2013.02.017

21- Rizek CF, Matté MH, Dropa M, Mamizuka EM, de Almeida LM, Lincopan N, Matté GR, Germano PM. Identification of Staphylococcus aureus carrying the mecA gene in ready-to-eat food products sold in Brazil. Foodborne Pathog Dis. 2011; 8(4):561-3. doi: 10.1089/fpd.2010.0706.

22- Wang W, Baloch Z, Jiang T, Zhang C, Peng Z, Li F, Fanning S, Ma A, Xu J. Enterotoxigenicity and Antimicrobial Resistance of Staphylococcus aureus Isolated from Retail Food in China. Front Microbiol. 2017; 21; 8:2256. doi: 10.3389/fmicb.2017.02256.

23- Kelman A, Soong YA, Dupuy N, Shafer D, Richbourg W, Johnson K, Brown T, Kestler E, Li Y, Zheng J, McDermott P, Meng J. Antimicrobial susceptibility of Staphylococcus aureus from retail ground meats. J Food Prot. 2011; 74(10):1625-9. doi: 10.4315/0362-028X.JFP-10-571

24- Ramatla T, Nhlabiseng M, Kutswa G, Moeti T, Oriel MM, Thekisoe, Michelo S. “Identification of Rodent Species That Infest Poultry Houses in Mafikeng, North West Province, South Africa.” International Journal of Zoology, 2019.

25- Das, Piyali and Pranab Behari Mazumder. “Prevalence of Staphylococcus in raw meat samples in Southern Assam, India.” (2016); 9(1), 23–29.

26- Pu S, Wang F, Ge B. Characterization of toxin genes and antimicrobial susceptibility of Staphylococcus aureus isolates from Louisiana retail meats. Foodborne Pathog Dis. 2011; 8(2):299-306. doi: 10.1089/fpd.2010.0679.
27- van Loo IH, Dieder BM, Savelkoul PH, Woudenbergh JH, Roosendaal R, van Belkum A, Lemmens-den Toom N, Verhulst C, van Keulen PH, Kluymans JA. Methicillin-resistant Staphylococcus aureus in meat products, the Netherlands. Emerg Infect Dis. 2007; 13(11):1753-5. doi: 10.3201/eid1311.070358.
28- Larsen HD, Huda A, Eriksen NH, Jensen NE. Differences between Danish bovine and human Staphylococcus aureus isolates in possession of superantigens. Vet Microbiol. 2000 Sep 25;76(2):153-62. doi: 10.1016/s0378-1135(00)00232-7.

29- Li S, Wang P, Zhao J, Zhou L, Zhang P, Fu C, Meng J, Wang X. Characterization of Toxin Genes and Antimicrobial Susceptibility of Staphylococcus aureus from Retail Raw Chicken Meat. J Food Prot. 2018; 81(4):528-533. doi: 10.4315/0362-028X.JFP-17-309.
30- Bergdoll MS. Staphylococcus aureus. In: Food borne Bacterial Pathogens, Doyle, M. (Ed.). CRC Press, New York, USA, ISBN: 9780824778668, 1989; PP: 463-523.

31- Le Loir Y, Baron F, Gautier M. Staphylococcus aureus and food poisoning. Genet Mol Res. 2003; 31;2(1):63-76.

32- Shanehbandi D, Baradaran B, Sadigh-Eteghad S, Zarreddar H. Occurrence of Methicillin Resistant and Enterotoxigenic Staphylococcus aureus in Traditional Cheeses in the North West of Iran. ISRN Microbiol. 2014; doi: 10.1155/2014/129580.

33- Katsuda K, Hata E, Kobayashi H, et al. Molecular typing of Staphylococcus aureus isolated from bovine mastitic milk on the basis of toxin genes and coagulase gene polymorphisms. Veterinary Microbiology. 2005; 105(3-4):301-305. DOI: 10.1016/j.vetmic.2004.12.004.

34- Gucekoglu A, Kevenk TO, Uyanik T, Cadirci O, Terzi G, Alisarli M. Detection of enterotoxigenic Staphylococcus aureus in raw milk and dairy products by multiplex PCR. Journal of Food Science. 2012; 77(11): 1750-3841.

35- Mohamed TA, Kalid AE, Saaciki AM. Isolation of Staphylococcus aureus from semi preserved udaneen food. Research Journal of Microbiology, 2014; 9(5); 246-250.
36- Seguin JC, Walker RD, Caron JP, Kloos WE, George CG, Hollis RJ, Jones RN, Pfaller MA. Methicillin-resistant Staphylococcus aureus outbreak in a veterinary teaching hospital: potential human-to-animal transmission. J Clin Microbiol. 1999; 37(5):1459-63. doi: 10.1128/JCM.37.5.1459-1463.
37- Jaber, NN. Isolation and biotyping of Staphylococcus aureus from white cheese in Basrah local markets. Journal of Veterinary Research, 2011; 10(2).
38- Alshammary AH, and Galfoori MH. Detection of methicillin or multidrug resistant Staphylococcus aureus (MRSA) in locally produced raw milk and soft cheese in Baihadad markets Iraq. Journal of Veterinary medicine, 2013; 37(2); 226-231.

39- Gentilini E, Denamiel G, Llorente P, Godaly S, Rebuelto M, DeGregorio O. Antimicrobial susceptibility of Staphylococcus aureus isolated from bovine mastitis in Argentina. J Dairy Sci. 2000; 83(6):1224-7. doi: 10.3168/jds. S0022-0302(00)74988-5.

40- Gentilini E, Denamiel G, Llorente P, Godaly S, Rebuelto M, DeGregorio O. Antimicrobial susceptibility of Staphylococcus aureus isolated from bovine mastitis in Argentina. J Dairy Sci. 2000; 83(6):1224-7. doi: 10.3168/jds. S0022-0302(00)74988-5.

41- Salheen EM. Molecular detection of staphylococcus aureus enterotoxin B in milk and milk product
using PCR. M.Sc. Sudan University of Science and Technology. 2016.
42. Mohammed TA, Khalid A.E, Saadabi, AM. PCR Detection of Staphylococcal Enterotoxin A and B genes in Staphylococcus aureus isolates from salted fermented fish. Microbiology journal, 2014; 4:51-56.
43. Ezzeldeen NA, Mansour HA, Ahmed,AA. Phenotypic and Molecular Identification of Staphylococcus aureus Isolated from some Egyptian Salted Fish”. World Applied Science Journal, 2011; 15 (12):1703-1712.
44. Geetha S, Govinda RV, Mudula KN, Ram RN, Ramesh BK. Some aspects of biochemical and microbial analysis of sundry fish Trichurus Linnaeus, 1758, from the east coast of Visakhapatnam”. I.J.A.B.R., 2014; 4(4): 462-465.
45- Vázquez-Sánchez D, López-Cabo M, Saá-ibusquiza P, Rodríguez-Herrera JJ. Incidence and characterization of Staphylococcus aureus in fishery products marketed in Galicia (Northwest Spain). Int J Food Microbiol. 2012;157(2):286-96. doi: 10.1016/j.ijfoodmicro.2012.05.021.
46. Arslan S, Özdemir F. Molecular characterization and detection of enterotoxins, methicillin resistance genes and antimicrobial resistance of Staphylococcus aureus from fish and ground beef. Polish Journal of Veterinary Sciences. 2017; 20(1):85-94. DOI: 10.1515/pjvs-2017-0012.
47. Pereira V, Lopes C, Castro A, Silva J, Gibbs P, Teixeira P. Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of Staphylococcus aureus isolates from various foods in Portugal. Food Microbiol. 2009; 26(3):278-82. doi: 10.1016/j.fm.2008.12.008.
48. Parmar BC, Pal M., Dhami AJ. In vitro antimicrobial drug susceptibility pattern of Staphylococcus aureus strain”. Indian journal of field Veterinarians, 2006; 2(1): 46-48.
49. Afifi SI, Al-Newery HA. Bacteriological studies on some virulence factors of Staphylococcus aureus isolated from chicken and Nile tilapia”. J.Chem. Environ,Healt, 2017; 3(1): 20-36.
50. Guven K, Mutlu MB, Gulbandilar A, Cakir P. Occurrence and characterization of Staphylococcus aureus isolated from meat and dairy products consumed in Turkey”. J Food Safety, 2010; 30:196-212.
51. Pu S, Wang F, Ge B. Characterization of toxin genes and antimicrobial susceptibility of Staphylococcus aureus isolates from Louisiana retail meats”. Foodborne Pathog D, 2013; 8:299-306.
52. Ali H H. Isolation and identification of Staphylococcus bacteria from fish of fresh water and its antibiotics sensitivity in Mosul city”. Basrah J Vet Res, 2014; 13(1):33–42.
53. de Oliveira L P, Barros L S S, Silva, V C, Cirqueira M G. Study of Staphylococcus aureus in raw and pasteurized milk consumed in the Reconcavo area of the State of Bahia. Food Processing & Technology. 2011; 2(6): 128.
54- Chye FY, Abdullah A, Ayob MK. Bacteriological quality and safety of raw milk in Malaysia. Food Microbiology. 2004; 21(5):535-541. DOI: 10.1016/j.fm.2003.11.007.
55- Ekici K, Bozkurt H, Isleyici, O. Isolation of some Pathogens from raw milk of different milch animals. Pakistan Journal of Nutrition. 2014; 3 (3): 161-162.
56- Agban N M, Ahmad S A. Detection and Identification of Staphylococcus Aureus Enterotoxins in Some Milk Products and their Handlers. Egyptian Journal of Medical Microbiology. 2013; 22 (2): 101-108.
57- Al-khafaji, H M. Detection of Enterotoxins Genes in Staphylococci Isolated from Milk and Cheese. Ph D. Thesis, College of Science-University of Baghdad. 2013.
58- Mustafa J Y. Isolation of some bacterial causative agent of bovine mastitis, with extraction and
purification of Staphylococcus aureus B lactamase. M Sc. Thesis, College of Veterinary Medicine, University of Basrah. 2007.

59- Sanaa OY, Nazik A, Ibtisam Z. Incidence of Some Potential Pathogens in Raw Milk in Khartoum North (Sudan) and Their Susceptibility to Antimicrobial Agents. Journal of Animal and Veterinary Advances, 2005; 4: 341-344.

60- Abdel-hameed K G, El-Malt LM. Public health hazard of the Staphylococcus aureus isolated from raw milk and ice cream in Qena governorate. Journal of Veterinary Medicine. 2009; 55 (121):191-200.

61- Islam MA, Kabir M L, Rahman MT. Molecular detection and characterization of staphylococcus aureus isolated from raw milk sold in different markets of bangladesh. Journal of Veterinary Medicine. 2016; 14 (2): 277-282.

62- Jahan M, Rahman M., Parvej S, Chowdhury Md., Haque E, Talukder AK. and Ahmed S. Isolation and characterization of Staphylococcus aureus from raw cow milk in Bangladesh. Journal of advanced veterinary and animal research. 2016; 2(1): 49-55.

63- Thaker HC, Manoj NB, Jitendra BN. Isolation and identification of Staphylococcus aureus from milk and milk products and their drug resistance patterns in Anand, Gujarat. Veterinary World. 2013; 10-13.

64- Beyene G F. Antimicrobial Susceptibility of Staphylococcus aureus in Cow Milk, Afar Ethiopia. International Journal of Modern Chemistry and Applied Science. 2016; 3 (1): 280- 283.

65- Abraha H, Hadish G, Aligaz B, Eyas G, Workelule K. Antimicrobial resistance profile of Staphylococcus aureus isolated from raw cow milk and fresh fruit juice in Mekelle, Tigray, Ethiopia. Journal of Veterinary Medicine and Animal Health, 2018 10(4), 106-113.

66- Idbeis HI. Detection of enterotoxin genes (sea-see) of Staphylococcus aureus isolated from raw milk by multiplex PCR and study of their pathogenicity. M. Sc. Thesis. College of Veterinary Medicine-University of Basrah (Microbiology). 2008.

67- AL - Marsomy H M. Isolation and diagnostic some bacterial causes of mastitis in cows and role of lactobacillus secretion on inhibition growth of Staphylococcus aureus. M.Sc., Thesis, College of Veterinary Medicine, University of Baghdad. 2008.

68- Bendahou A, Lebbadi M, Ennanei L, Essadqui FZ, Abid M. Characterization of Staphylococcus species isolated from raw milk and milk products (lben and jben) in North Morocco. J Infect Dev Ctries. 2008; 2(3):218-25. doi: 10.3855/jidc.266.

69- AL - Saady A A. Extraction and characterization of surface adherence protein from methicillin resistance Staphylococcus aureus and study of the pathogenic effects. Ph.D., Thesis, College of science, University of Baghdad. 2007.

70- Peles F, Wagner M, Varga L, Hein I, Rieck P, Gutser K, Keresztúri P, Kardos G, Turcsányi I, Béri B, Szabó A. Characterization of Staphylococcus aureus strains isolated from bovine milk in Hungary', International Journal of Food Microbiology, 2007; 118 (2)186-193. https://doi.org/10.1016/j.ijfoodmicro.2007.07.010

Figures
Figure 1

PCR amplification of 16S rRNA gene of S. aureus on 2% agarose gel electrophoresis. Lane 1 DNA ladder: MW 100-1500 bp fragments. Lanes 5, 6 and 7 shows a typical band size of (756 bp) corresponding to 16S rRNA of positive control isolates (isolates IDs 13, 55, and 63 respectively). Lanes 2, 3, 4 and 8 are negative samples.

Figure 2
PCR amplification of mecA gene of S. aureus on 2% agarose gel electrophoresis. Lane 1 DNA ladder: MW 100-1500 bp fragments. Lanes 2, 4, 5, and 6 are typical band size of (310bp) corresponding to mecA gene products of S. aureus isolated from samples number 13, 55, 63, and 81. Lanes 3, 7, 8, and 9 are negative sample.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- AdditionalFigure1.png