

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

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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. Now days many researchers worldwide noted an emerging of resistant strains *Staphylococci* especially for the antibiotic Methicillin. Therefore, this study was aimed to determine the existence of *Staphylococcus aureus* and its enterotoxins, *mecA* genes in food samples.

Results: A total of 400 samples were collected from different areas in Khartoum state. The type of foods included Cheese, Meat products, Fish and Raw milk, 100 samples for each. out of 400 samples cultivated 137 (34.25 %) isolates were identified as *S. aureus*, 126 (31.5%) were identified as bacteria other than *S. aureus* and 137 (34.25%) were yield no growth. Of 137 *S. aureus* isolates, 84 were randomly selected and examined for the presence *mecA* and enterotoxin genes products. Oxacillin sensitivity test showed that 15(11%) of 137 *S.aureus* isolates were Oxacillin resistant. The PCR assay showed that *mecA* gene was detected in 15 of 84 (17%) *S. aureus* isolates. While only 2 (2.385%) out of 84 *S. aureus* isolates were show 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 food samples which collected from Khartoum state. 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 *Staphylococcus aureus* enterotoxins production in foods.

Background

Food-borne diseases (FBD) remain one of the greatest concerns in public health and food safety, they are caused by a large variety of 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, raw milk, meat, meat products, dairy products, and ready-to-eat food (5, 7).

Staphylococcus aureus may produce a large variety of enterotoxins but 95% of food poisoning outbreaks are caused by classical enterotoxins: A, B, C, D and E (6). These toxic proteins withstand exposure to 100°C for several minutes, when ingested as preformed toxins in contaminated food, microgram amounts of toxin within a few hours can induce the symptoms of *staphylococcal* food poisoning: nausea, vomiting and diarrhoea (8). Because of the resistance of *Staphylococcus aureus* to the heat and drying, foods can easily become contaminated from food handlers or environment, it multiplies and releases a toxin in uncooked or inadequately cooked foods, especially if the foods are unrefrigerated (9).

The occurrence of *Staphylococcus aureus* and MRSA in foods of animal origin and potential risk of transmission to humans through foods if consumed without maintaining adequate hygienic standards pose a

serious threat to the well-being of humans due to uncountable clinical implications (10).

Non-hygienic handling practices, working conditions, and improper storage and refrigeration; all these can 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). In our country, only few information is available about the occurrence of virulence genes among the *Staphylococci* isolated from foods, so this study conducted to spot in enterotoxins and *mecA* genes in commonly consumed foods in Khartoum state.

Result

A total of 400 samples (milk 100, cheese 100, fish 100, and meat 100) were collected from different areas in Khartoum state. Of the 400 samples that were cultivated, 263 showed a significant growth of which 137 isolates (34.25%) were identified as *S. aureus*, and 126 isolates (31.5%) were identified as bacteria other than *S. aureus*. However, 137 (34.25%) samples did not yield any growth (Table 1). Of 137 *S. aureus* isolates, 84 isolates were randomly selected and examined for the presence *mecA* and enterotoxin genes products. The result indicated that *mecA* gene was detected in 15 (17%) isolates (of which the distribution of *mecA* according to the types of samples was 17% in milk, 15% in fish, 20% in meat and 20% in cheese). However, the rest 69 (83%) isolates were negative for *mecA* gene when examined by PCR and the products visualized by 2% gel electrophoresis versus DNA ladder of MW 100-1500 bp fragments (Table 2), (Figure 1,2) (Additional figure 1). While only 2 (2.385%) of 84 *S. aureus* isolates were showed an enterotoxin B gene product (both isolates were from cheese samples), while the rest 82 isolates were negative. All isolates were negative for other enterotoxins gene product (other than *seb* gene) (Table 2), (Additional figure 2).

Our result show that among one hundred of examined raw meat samples, 30 (30%) were identified as *S. aureus* (Table 3) of which 11 (36.7%) isolated from raw beef and 19 (63.3%) from restaurants meat. However, 20 (20%) samples showed a growth of bacteria other than *S. aureus* and 50 (50%) samples showed no growth. All 30 (100%) meat *staphylococcal* isolates, were sensitive to ciprofloxacin. While 26 (86.7%) were sensitive to gentamicin and 4 (13.3%) were resistant. In addition, the sensitivity test results revealed that the efficacy rates of oxacillin and vancomycin versus 30 meat *S. aureus* isolates were 28 (93.4%) and 25 (83.3%) respectively while the resistant rates for the same mentioned antibiotics were 2 (6.6%) and 5 (16.7%) respectively (Table 4). All 30 *S. aureus* isolates investigated for the presence of enterotoxin genes by multiplex PCR; and the result demonstrate that there was no *S. aureus* isolate produce enterotoxins genes products (Additional figure 2)

In this research we find that out of hundred examined cheese samples, 20 (20%) isolates were identified as *Staphylococcus aureus*, while only 4 isolates (4%) were identified as bacteria others than *Staphylococcus aureus*. However, 76 (76%) did not show any growth on the agar plate surface (Table 3). The pattern of antimicrobial susceptibility was examined among the twenty cheese *Staphylococcus aureus* isolates. All isolates were sensitive to Gentamicin, Ciprofloxacin 20 (100%). Instead, 17 (85%) isolates were sensitive to Vancomycin and 3 (15%) were resistant. However, 19 (95%) were sensitive to oxacillin and 1 (5%) was resistant (Table 4). All 20 cheese *Staphylococcus aureus* isolates were investigated for the presence of enterotoxin genes by multiplex PCR, and *seb* was detected in 2 (10%) isolates only, while no other enterotoxin genes products were detected among these isolates (Additional figure 2).

In our research we examine a total of 100 fish samples of which 50 were salted fish samples while the rest were collected from raw fish. The result find that *Staphylococcus aureus* was isolated in 24 (24%) fish samples while the remaining 84 (76%) were other bacteria (Table 3). All fish originated *S. aureus* isolates showed a sensitivity rates of (100 %) when tested against both ciprofloxacin and gentamicin antibiotics. While Vancomycin and oxacillin were showed efficacy rate of 92% against fish *S. aureus* isolates and therefore the resistance rate was 8% for both mentioned antibiotic (Table 4). All 24 isolated *S. aureus* were confirmed by detection of the presence of 16S housekeeping gene and also examined for the presence of enterotoxin genes A, B, C, D and E. the gel electrophoresis showed confirmatory band size of 16 s *S. aureus* gene. No enterotoxin gene product detected during the gel electrophoresis procedure which applied after successful cycles of conventional PCR (Additional figure 2).

In this research, a total of 100 milk samples were collected from different areas in Khartoum state. 63(63%) of the isolates 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 of *S. aureus* showed a high sensitivity rate to Ciprofloxacin and Gentamicin with percentage of 98.4% (62/63 isolates) and 87.3% (55/63 isolates) respectively, meaning that the resistant rates for the same antibiotics were 1.6% and 12.7 % respectively. However 84% (53/63) of *S. aureus* isolates were sensitive to Oxacillin and only 16% (10/63) were resistant. In contrary only 65% (41/63) *S. aureus* isolates were sensitive to Vancomycin and 35% (22/63) of milk *S. aureus* isolates were resistant to the same antibiotic (Table 4). The results for enterotoxins genes reveal that there is no *S. aureus* isolate produce such gene (Additional figure 2).

Table 1. Shows Frequencies and percentage of isolates among all food samples that cultivated in this study

Isolate	Number	Percentage
<i>S. aureus</i>	137	34.25%
Others	126	31.5%
No growth	137	34.25%
Total	400	100%

Table 2. Frequencies of *mecA* gene and enterotoxin gene among all *S. aureus* Isolates

Type of gene detected	Positive	Negative	Total
<i>mecA</i> gene	15 (17.9%)	69 (82.1%)	84 (100%)
Enterotoxin B gene	2 (2.38%)	82 (97,62%)	84 (100%)
Other enterotoxin gene	0 (0%)	84 (100%)	84(100%)

Table 3. Percentage of *S. aureus* isolates according to the type of food samples

Sample	<i>S. aureus</i> 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%)
		76 (76%)	0 (0%)	
Fish	24 (24%)			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

Antibiotics		Gentamycin(10mg)	Ciprofloxacin(5mg)	Oxacillin(5mg)	Vancomycin(30mg)
Meat Isolates	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 Isolates	Sensitive	20 (100%)	20 (100%)	19 (95%)	17 (85%)
	Resistant	0 (0%)	0 (0%)	1 (5%)	3 (15%)
Fish Isolates	Sensitive	24 (100%)	100(100%)	22 (92%)	22 (92%)
	Resistant	0 (0%)	0 (0%)	2 (8%)	2 (8%)
Milk isolates	Sensitive	55 (87.3%)	62 (98.4%)	53 (84%)	41 (65%)
	Resistant	8 (12.7%)	1 (1.6%)	10 (16%)	22 (35%)
Total of isolates		137	137	137	137

Discussion

In this study, the prevalence of *S. aureus* and MRSA and enterotoxin gene products were investigated in various food samples collected from markets in Khartoum state (400 samples of milk, cheese, fish, meat). A total of 137 (34.25 %) *S. aureus* isolated after cultivation and performing required biochemical tests. The previous studies conducted to detect *S. aureus* in various foods revealed that the contamination levels with *S. aureus* have been observed to be lower than that obtained by our study group. For instance, study in Italy conducted by Traversa and his colleagues found that only 17.1% of different kind of their foods are contaminated by *S. aureus* (15). Similarly, another study conducted by Sivakumar and his colleagues in India and their results revealed that only 12.01% of Indian food contaminated by *S. aureus* (16) and the later finding dramatically disagreed with our result. On the other hand, a study was done in Greece by Papadopoulos and *et al.*, found that 47.8% of north-central and north-eastern Greece foods are contaminated by *S. aureus* (3) this finding is greatly exciting our results. 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 affect greatly the bacterial growth in food materials.

Resistance gene (*mecA*) in *S. aureus* witch responsible for resistance to β -lactam antimicrobials was detected by using PCR among only 84 randomly selected *S.aureus* Isolates, and we find that 17% of *S. aureus* positive for *mecA* gene while 69 (83%) were negative. Most previous *mecA* gene detection studies results showed a significantly higher prevalence than our finding as in study done by Khayri in Makkah city he found that about 44.4 % of his *S. aureus* isolates were positive for *mecA* gene (4). Similarly, Papadopoulos and his colleagues found that 81.3% of their isolates were positive for the *mecA* gene (3). This variation could be due to the difference in the antibiotic protocol that applied by the doctors for their patients or due to the extensive usage of methicillin antibiotic in their communities or by doctors to treat the infection which could cause by *S. aureus* in these countries and eventually lead to a high prevalence rate of MRSA.

In contrary, in a study conducted by Novak *et al* in 2000, the *mecA* gene prevalence was lower than our finding 11.4 % (5). Other studies have done by Kamal *et al* in Egypt, Rizek in Brazil and wang *et al* in China found that the prevalence of *mecA* gene was 5.1%, 9% and 7.9% respectively (6-8). The variation in the results may be due to variation of samples sources and usage different molecular techniques in different countries for detection of *mecA* gene product.

In this study, one hundred raw meat samples were obtained from different supermarkets and restaurants in Khartoum state and examined for the presence of *S. aureus*. Thirty (30%) samples were found to be contaminated with *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 *Staphylococcus aureus* enterotoxins (SEs) production in foods. In other comparative studies, similar results were presented by Kelman *et al.* (23) in Washington who reported that the prevalence of *S. aureus* in meat samples was 29.0%. However, in the present study results are higher than that obtained by Ramatla *et al.* in South Africa who reported that *S. aureus* was in 26.5% (24), and lower than those obtained by Das *et al.* in India who reported that out of 65 samples *S. aureus* incidence was in 46.1% (25). The variation in the prevalence may be due to the variation in community hygiene in certain countries and variation in the environment especially the moisture and temperature which affect the growth of *S. aureus*.

In our study, none of the meat *S. aureus* isolates were resistant to ciprofloxacin and 13.3% were resistant to gentamicin. 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.* in 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 were 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).

In this study, our PCR studies results showed that there were no enterotoxin genes in meat *S. aureus* Isolates, Larsen *et al* in Denmark nearly found a similar results which showed that only 0.2% of isolates were enterotoxin gene possessing *S. aureus* (28), meanwhile in contrast to our findings Lis *et al.*, in China found that the prevalence of enterotoxin gene possessing *S. aureus* was 46.0% (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 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 the absence of the corresponding operon (31).

In this study, one hundred sample white cheese were collected from different retailers in Khartoum State to detect the presence of *staphylococcus aureus*. In which 20 (20%) isolates were detected; that was higher than the results obtained by Shanehbanali *et al.*, in Iran and Katsuda *et al.*, in Japan, whom isolated *Staphylococcus aureus* from white cheese with the percentage of 16% and 13.3% respectively (32,33), and lower than the results obtained by Gucukoglu *et al.*, in Turkey who reported *Staphylococcus aureus* 37.5% in white cheese (34). This study disagreed with Mohamed *et al.*, who reported the absence of *Staphylococcus aureus* in white cheese in Khartoum (35).

All of the twenty cheese *S. aureus* isolates were sensitive to gentamicin and ciprofloxacin (100%) this finding has disagreed with Seguin *et al.*, in the U.S.A, who estimated 75% resistant to gentamicin (36) and Jaber *et al.*, who measured 25% resistant to ciprofloxacin in Iraq (37). However, among cheese *S. aureus* isolates only one sample (5%) was resistant to oxacillin, which lower than the result reported by Alshammery and Galfoori in Iraq which showed higher resistance to oxacillin observed in 6 isolates (20%) (38). Additionally, 17 (85%) out of 20 cheese *S. aureus* isolates were sensitive to vancomycin these result lower than the result obtained by Valsangiacomo *et al.*, in Switzerland who reported 100% sensitivity to vancomycin (39,40)

The molecular detection of *Staphylococcus aureus* enterotoxins (A, B, C, D and E); 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 (41). The variation of these results among researcher 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 out 108 fish samples, 22% of samples were contaminated with *S. aureus* which lower than result demonstrated by Mohammed *et al.*, in Khartoum state with percent of 72% (42), and Ezzeldeen *et al.*, in Egypt 93% (43), and also lower than that of Geetha *et al.*, in the East Coast of Visakhapatnam 100% (44) from dried salted fish, in contrast, it was higher than the result reported by Vázquez-sánchez *et al.*, in Spain (27%) (45).

Antimicrobial susceptibility results for fish *S. aureus* isolates showed that 100% sensitivity for ciprofloxacin, which was in agreements with that of Ezzeldeen *et al.*, in Egypt (43) and Arslan and Ozdemir in Turkey (46) and also nearly similar to the finding of Pereira *et al.*, in Portugal 98% (47). However, lower results obtained by Parmer *et al.*, in India 48.5% (48) and Afifi and A-Newery in Egypt 57% (49). Moreover, gentamicin sensitivity was 100% among *S. aureus* that isolated from fish samples which agreed with results found by Afifi and A-Newery in Egypt (49) Arslan and Ozdemir in Turke (46) and that of Vázquez-sánchez *et al.*, in Spain (45) and mild similar with results reported by Ezzeldeen *et al.*, in Egypt 97% (43) and Pereira *et al.*, in Portugal, 92% (47). In our study, the fish isolated *S. aureus* show a sensitivity rate of 92% to vancomycin which matched with the results of Ezzeldeen *et al.*, in Egypt 91% (43), and Pereira *et al.*, in Portugal 90% (47). However, our result nearly lower than that obtained by Afifi and A-Newery in Egypt, 100% (49) while Arslan and Ozdemir, in Turkey, and Guven *et al.*, in Turkey, concluded lower percentage of 83% and 78% respectively (46,50). The oxacillin has shown 92% potency against fish *S. aureus* isolates that was relatively similar to result obtained by Arslan and Ozdemir, in

Turkey 100% (46), and Vázquez-sánchez *et al.*, in Spain 100% (45) Whereas Pereira *et al.*, in Portugal found results lower than that of ours 62% (47). The variation of these results among researcher could be due to several factors such as source of samples, geographical origin, the sensitivity of identification methods and sample size can affect the outcomes.

The molecular analysis of the enterotoxins genes among fish isolated *S. aureus* gave no results for all genes which in agreement with result obtained by Arslan and Ozdemir, in Turkey and Pu *et al.*, in Louisiana (46,51), whom found no enterotoxins B, C and E genes. The current study was slightly lower than that obtained by Ali in Tanzania who detected enterotoxin B and C genes in 0.3% of his study samples, with the absence of enterotoxin A gene in all samples (52), while our result is significantly lower than that of Arslan and Ozdemir in Turkey who reported enterotoxins A and D genes in 10.5% of their samples (46). The variation of these results among researcher could be due to several factors such as source of sampling, geographical origin.

In this study *S. aureus* was isolated in 63% raw milk samples, which closes on with results obtained by de Oliveira *et al.*, in Brazil 68% (53) and Chye *et al.*, in Malaysia 60% (54). However, our finding was lower than results reported by Ekici *et al.*, in Turkey 75% (55) and Agban and Ahmad in Egypt 82% (56). On another hand the present study was higher than AL- Kafaje, in Baghdad, and Mustafa in Basrah, whom recorded that *S. aureus* was isolated from clinical and subclinical mastitis in cows in percentages of 53.33%, 43.5% respectively (57,58). however, our result is dramatically higher than levels of contaminations which were reported by Yagoub *et al.* in Sudan and Abdel- Hameed and El-Malt in Egypt whom isolated *S. aureus* from raw milk with the percentage of 30%, 24.8% respectively (59,60). The variation of these results among researcher could be due to several factors such as the source of samples, geographical origin, the sensitivity of identification methods and sample size can affect the outcomes.

In relation to antimicrobial susceptibility testing, this result showed high sensitivity rate for milk *S. aureus* isolates to ciprofloxacin (98.4%) which was agreed with Islam *et al.*, findings in Bangladesh who reported a sensitivity rate of 93.3% (61). While the lower level of ciprofloxacin potency was detected by Jahan *et al.*, in Bangladesh and Thaker *et al.*, in India who recorded an 83.3% and 80.0% sensitivity rate for *S. aureus* raw milk isolates (62,63). Gentamicin sensitivity test showed 87% potency that was disagreed with that obtained by Yagoub *et al.*, in Sudan who recorded that 47.6% sensitivity (59). While slightly similar to that obtained by Beyene, in Ethiopia, and Thaker *et al.*, in India that both showed 90% of isolated *S. aureus* were sensitive to gentamicin (64, 63). Reports from other researchers were indicated a higher level of sensitivity to gentamicin as 100% reported by Abraha *et al.*, in Ethiopia (65). Vancomycin sensitivity showed 65%. Which incompatible with results reported by Idbeis, in Basra (Iraqi), 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 100% (66,67,68). In contrast Abraha *et al.*, in Ethiopia and Alsaady, in Baghdad reported that the resistant rate of isolated *S. aureus* from raw milk is 100% to vancomycin (65,69). The *S.aureus isolates* were sensitive to oxacillin (84%), which slightly higher than result achieved by Thaker *et al.*, in India 70% (63) and Jahan *et al.*, in Bangladesh showed complete sensitivity (100%) to oxacillin that was conversed to this result (62). The variation of these results among researcher could be due to several factors such as samples source, geographical origin, sensitivity of identification methods and sample size can affect the outcomes.

In this study, no enterotoxigenic milk *Staphylococci* were detected that was similar to that obtained Peles *et al.*, in Hungary who recorded that the isolated *S. aureus* from dairy milk farms were showed no enterotoxins (70).

The variation of these results among researcher findings could be due to several factors such as samples source, geographical origin, sensitivity of identification methods and sample size can affect the outcomes.

Methods

Sample collection and isolation of *S. aureus*

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 splitted and transferred to the lab in sterile containers, small pieces from 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. 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

The antimicrobial susceptibility test was done by disk diffusion method using Mueller-Hinton agar plates (oxid) 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 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 included three steps: initial denaturation at 94°C for 5 min; 35 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 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 *mecA* Gene

Primers were used for 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 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	AACTCTGTTATTAGGGAAGAACA	-
Staph 750-R	CCACCTTCCTCCGGTTTGTCCACC	756
Enterotoxins genes primers		
SA-Ua- F	TGTATGTATGGAGGTGTAAC	-
SA-A- R	ATTAACCGAAGGTTCTGT	270
SA-B-R	ATAGTGACGAGTTAGGTA	165
ENT-C-R	AAGTACATTTTGTAAAGTTCC	102
SA-D-R	TTCGGGAAAATCACCTTAA	303
SA-E-R	GCCAAAGCTGTCTGAG	213
<i>mecA</i> gene primers		
MecA1 – F	AACTCTGTTATTAGGGAAGAACA	-
MecA1 –R	CCACCTTCCTCCGGTTTGTCCACC	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.

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.

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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.

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Figures

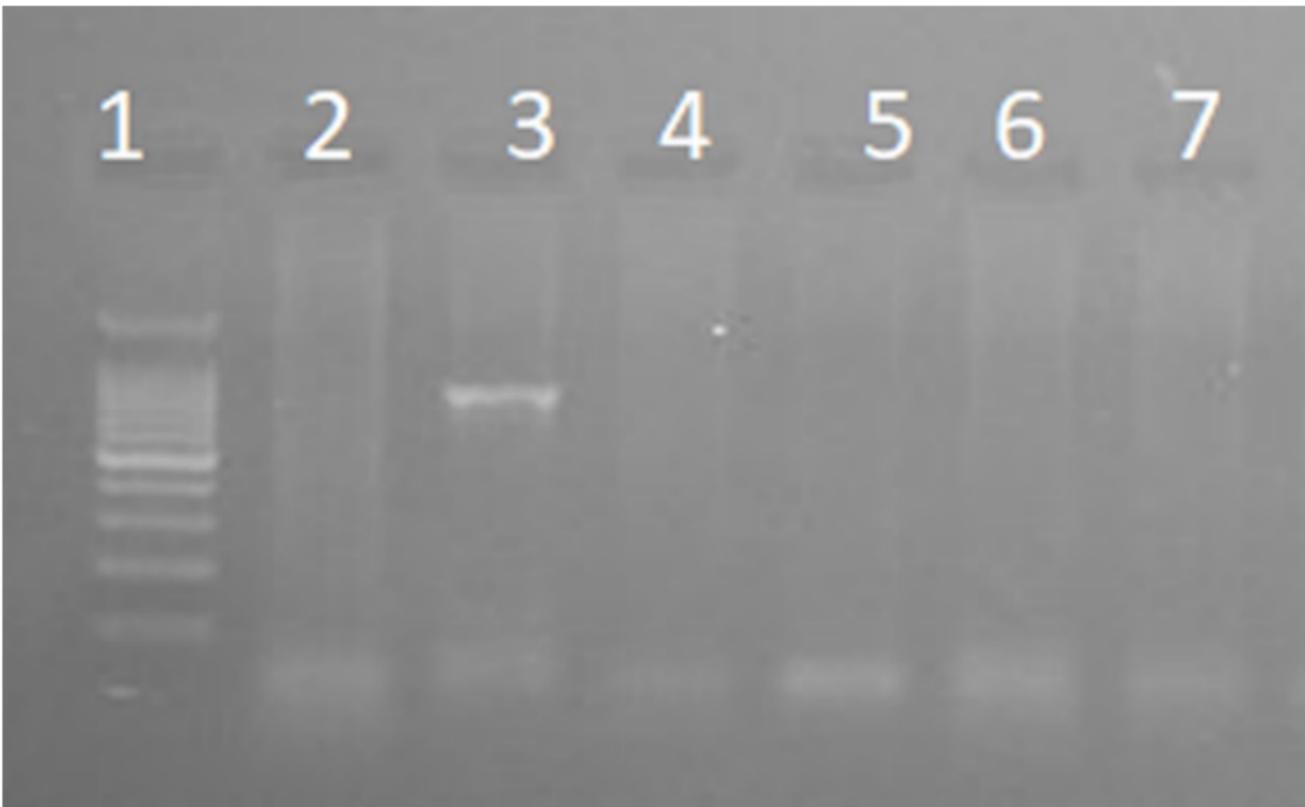


Figure 1

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

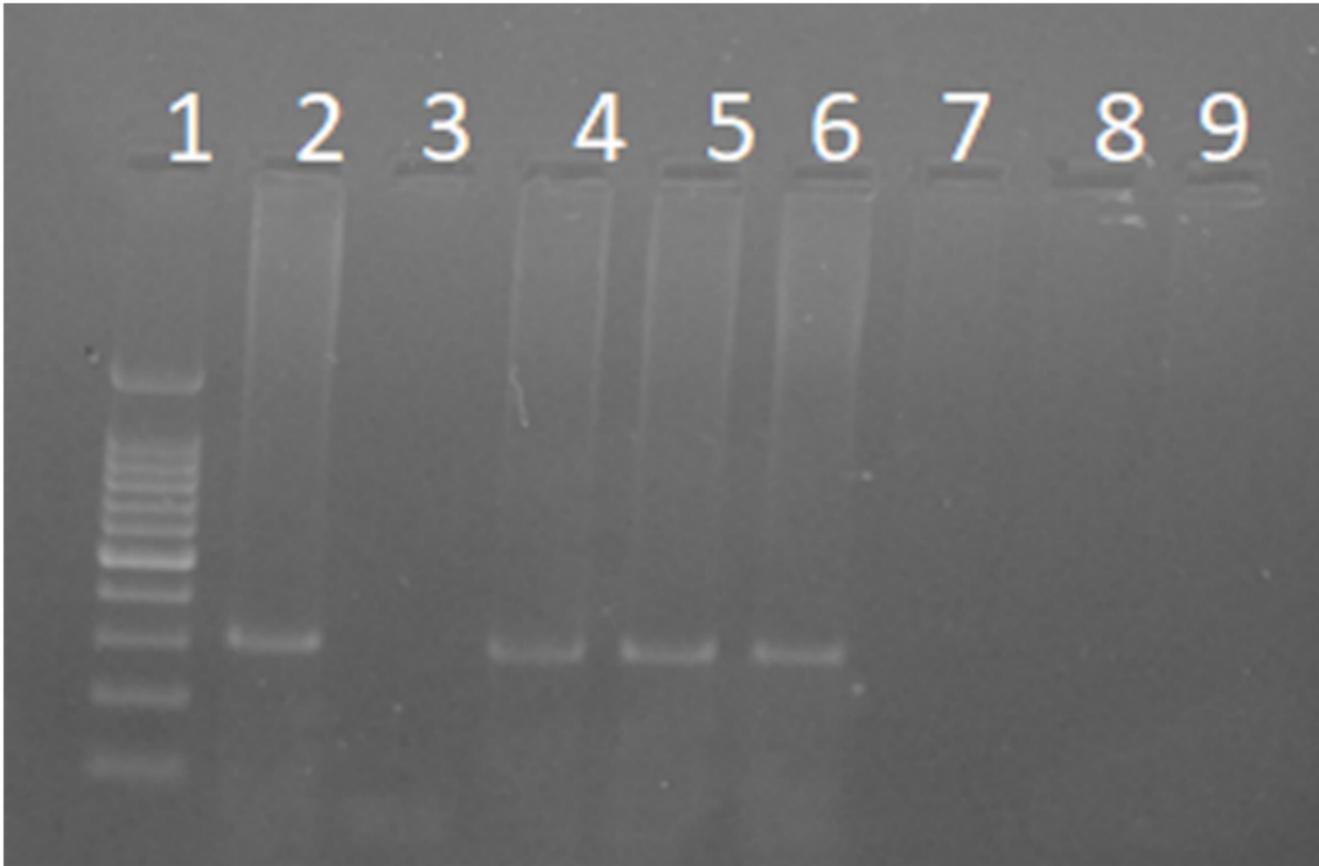


Figure 2

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. Lanes 3, 7, 8, and 9 are negative sample.

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