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 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 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 diarrhea (8). Because of the resistance of *Staphylococcus aureus* to the heat and drying, foods can easily become

contaminated from food handlers or from the environment, it multiply and release 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 common consumed foods in Khartoum state.

Result

A total of 400 samples (milk 100, cheese 100, fish100, and meat 100) were collected from different areas in Khartoum state. Out of 400 samples cultivated 263 were 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 where yielded no growth (Table 1). Of 137 *S. aureus* isolates 84 were randomly selected and examined for the presence mecA and enterotoxin genes products. The result show that mecA gene was detected in 15 (17%) isolates (of which the distribution of mecA according to the types of samples were 17% in milk, 15% in fish, 20% in meat and 20% in cheese) (Table 2), (Fig. 2 and additional Fig. 1). While only 2 (2.385%) out of 84 *S. aureus* isolates were show an enterotoxin B gene product (both isolates were from cheese samples) (Table 2), (additional Fig. 1).

Our result show that out of hundred raw meat samples examined 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. All isolates, 30 (100%) were sensitive to ciprofloxacin. While 26(86.7%) were sensitive to gentamycin and 4 (13.3%) were resistant. Additionally, the sensitivity test results also revealed that 28(93.4%) out of the 30 *S. aureus isolates* were sensitive to oxacillin and 25 (83,3%) were sensitive to vancomycin (Table 4). All 30 *S. aureus* isolates investigated for the presence of enterotoxin genes by multiplex PCR; no enterotoxins genes were detected (additional Fig. 1).

In this research we find that out of hundred cheese samples examined 20 (20%) isolates were identified as *Staphylococcus aureus*, 4(4%) were identified as bacteria others than *Staphylococcus aureus* while 76(76%) were show no growth (Table 3). The antimicrobial sensitivity pattern of was examined in All of twenty cheese *Staphylococcus aureus* isolates. All isolates were sensitive to Gentamicin, Ciprofloxacin. 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 only 2(10%) isolates (additional Fig. 1).

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 rest 84 (76%) were other bacteria (Table 3). All isolated *S. aureus* showed complete sensitivity to ciprofloxacin and gentamicin (100%). While Vancomycin and oxacillin were showed efficacy rate of 92% against S. aureus

isolates and therefore the resistance rate was 8% both mentioned antibiotic (Table 4). All 24 isolated S. aureus were confirmed by detection of the presence of 16Shousekeeping gene and Enterotoxin genes A, B, C, D and E were also investigated in the confirmed *S. aureus* but there was no enterotoxin gene detected (additional Fig. 1).

In this research a total of 100 milk samples were collected from different areas in Khartoum state. 63(63%) of isolates were identified as *S. aureus*, 26(26%) were identified as others than *S. aureus* and 11(11%) were show no growth (Table 3). The antimicrobial susceptibility test of isolated *S. aureus* was showed high sensitivity rate to Ciprofloxacin 98.4%, 87.3% to Gentamicin, 84% to Oxacillin and 65% to Vancomycin (Table 4). The results for enterotoxins genes reveal that there is no *S. aureus* isolate produce such gene (additional Fig. 1).

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

Isolate	Number	Percentage
S. aureus	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
mecA 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	*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

Antibiotics		Gentamycin(10 mg)	Ciprofloxacin(5 mg)	Oxacillin(5 mg)	Vancomycin(30 mg)
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 theire 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 is dramatically disagreed with our result. On the other hand, study 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 exciding 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 factor 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 significant 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 different 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 infection which could cause by *S. aureus in* these countries and eventually lead to high prevalence rate of MRSA.

In contrary, in study conducted by Novak *et al* in 2000 the mecA gene prevalence was lower than our finding 11.4% (5). Other studies done 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 source of samples 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 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* was 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 suggest 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 the PCR results showed 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 food 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 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 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 was disagreed with Mohamed *et al.*, who reported 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 was disagreed with Seguin *et al.*, in 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 result reported by Alshammary 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 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 source of sampling, geographical origin, 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 result reported by Vázquez-sánchez *et al.*, in Spain (27%) (45).

Antimicrobial susceptibility results for fish S.areus isolates showed that 100% sensitivity for ciprofloxacin, which was in agreements with that of Ezzeldeen *et al.*, in Egypt (43) and Arsland and Ozdemer 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) Arsland and Ozdemer 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 asinsitivity 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 Arsland and Ozdemer, 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 Arsland and Ozdemer, 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 sampling, geographical origin, 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 Arsland and Ozdemer, 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 Arsland and Ozdemer in Turkey whom 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 percentage of 30%, 24.8% respectively (59, 60). The variation of these results among researcher could be due to several factors such as source of sampling, geographical origin, 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 whom reported a sensitivity rate of 93.3% (61). While lower level of ciprofloxacin potency was detected by Jahan *et al*., in Bangladesh and Thaker *et al*., in India whom 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 whom 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 was indicated 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 Basrha (Iraqi), AL –Marsomy, and Bendahou *et al*., in North Morocco whom 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 isolated *S. aureus* 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 source of sampling, 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 whom recorded that the isolated *S. aureus* from dairy milk farms were showed no enterotoxins (70). The variation of these results among researcher could be due to several factors such as source of sampling, 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, 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 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 others 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 (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 6o°C for 60 minutes. Then incubated at 100°C in a water bath for 15 minutes, and then 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 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	CCACCTTCCTCCGGTTTGTCACC	756
Enterotoxins genes primers		
SA-Ua- F	TGTATGTATGGAGGTGTAAC	-
SA-A- R	ATTAACCGAAGGTTCTGT	270
SA-B-R	ATAGTGACGAGTTAGGTA	165
ENT-C-R	AAGTACATTTTGTAAGTTCC	102
SA-D-R	TTCGGGAAAATCACCCTTAA	303
SA-E-R	GCCAAAGCTGTCTGAG	213
mecA gene primers		
MecA1 – F	AACTCTGTTATTAGGGAAGAACA	-
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.

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

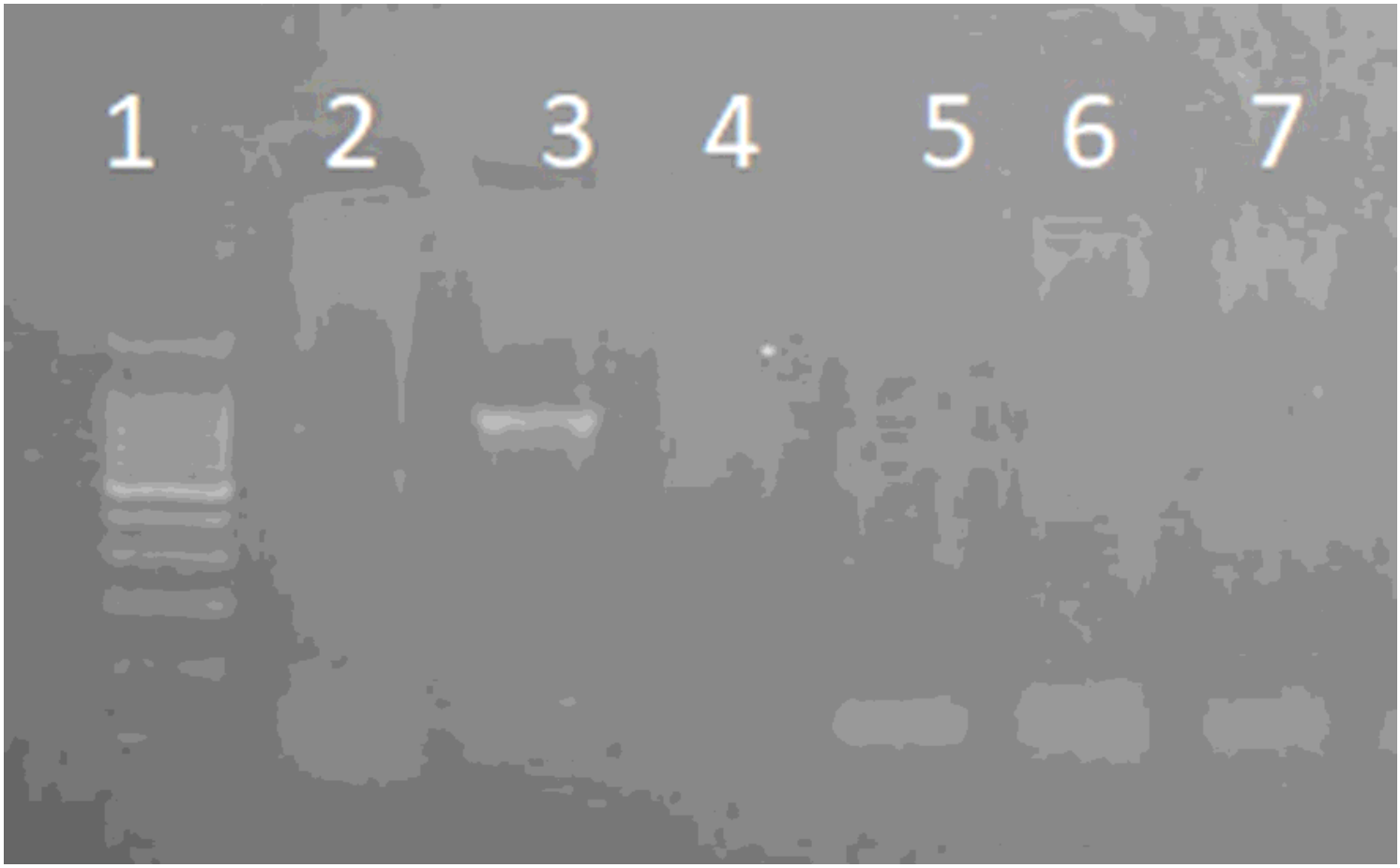

Figure 1

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.

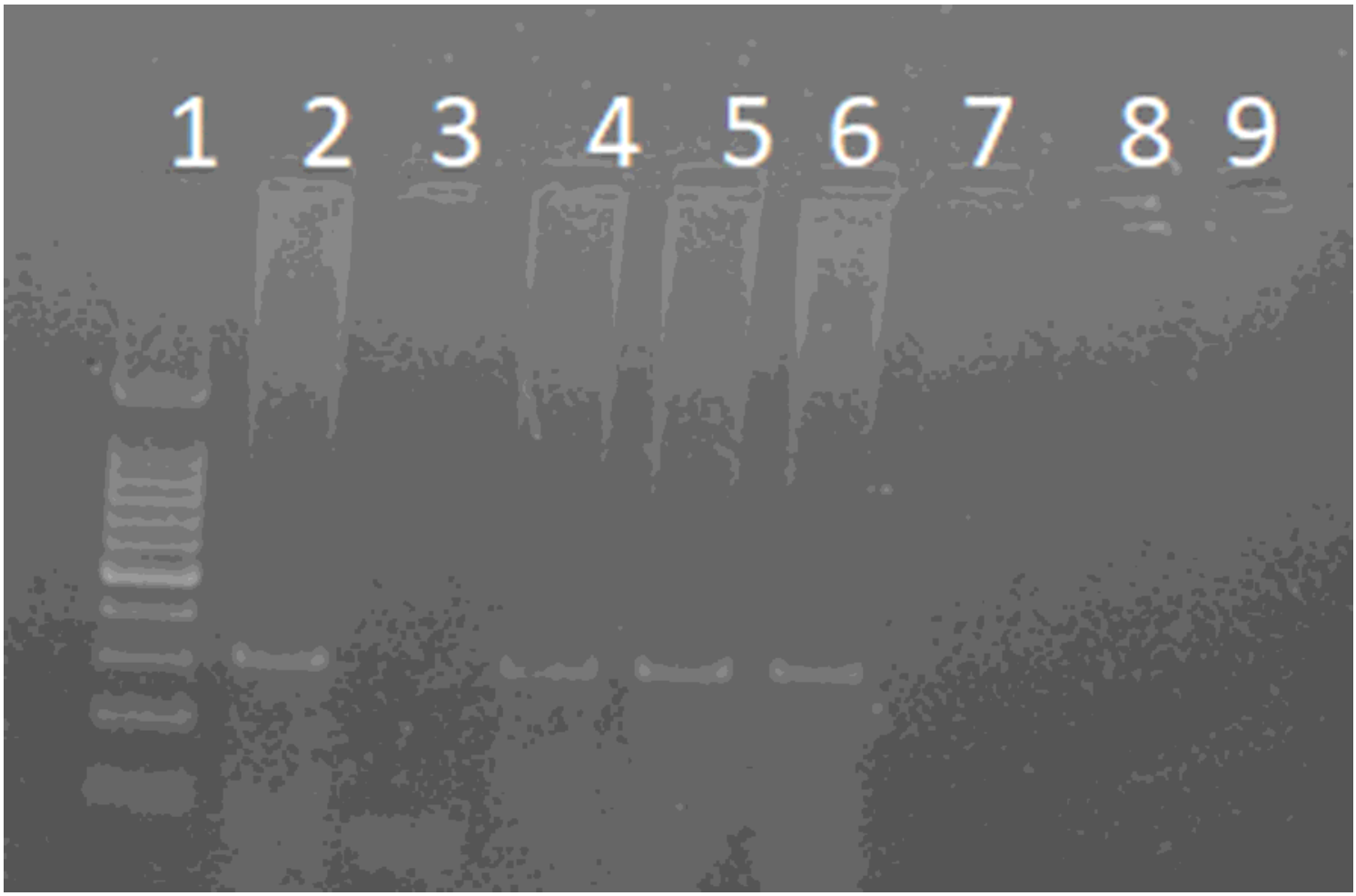

Figure 2

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.