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Studies on the incidence of *Staphylococcus aureus* and its enterotoxins in different meat and meat products

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Abstract
Staphylococcus aureus is an important food borne gastrointestinal agent and regarded as an emerging pathogen, due to its ability to grow in many foods. Present study was envisaged on the incidence of *Staphylococcus. aureus* in different meat and meat products samples. Out of 240, samples (30 each) of chicken samples, beef samples, mutton samples, pork samples, chicken nuggets, sausages and burgers samples, 21(70.00%), 17(56.66%), 19(63.33%), 15(50.00%), 19(63.33%), 18(60.00%), 16(53.33%) and 17(56.66%) respectively were positive by cultural method, whereas PCR assay revealed 22(73.33%), 18(60.00%), 20(66.66%), 16(53.33%), 20(66.66%), 19(63.33%), 17(56.66%) and 18(60.00%) respectively. Enterotoxin targeting *sea*, *seb* and *tsst-1* genes was carried among the PCR positives which 15(68.18%), 4(18.18%), 2(9.09%) and 1(4.55%) were positive for SEA, SEB, SEA+SEB and TSST-1 in chicken samples respectively, 13(72.22%), 3(16.67%), 1(5.56%) and 1(5.56%) were positive for SEA, SEB, SEA+SEB and TSST-1in beef samples respectively, 14(70.00%), 3(15.00%), 2(10.00%) and 1(5.00%) were positive for SEA, SEB, SEA+SEB and TSST-1 in mutton respectively, .12(75.00%), 2(12.5%), 1(6.25%) were positive for SEA, SEB, SEA+SEB in pork respectively, 14(70.00%), 3(15.00%), 2(10.00%) and 1(5.00%) were positive for SEA, SEB, SEA+SEB and TSST-1in chicken nuggets respectively, 15(78.95%), 5(26.32%), 3(15.79%) and 2(10.53%) were positive for SEA, SEB, SEA+SEB and TSST-1in sausages respectively, 14(82.35%), 3(17.65%), 2(11.76%) and 1(5.88%) were positive for SEA, SEB, SEA+SEB and TSST-1 in burger respectively, 13(72.22%), 3(16.67%), 1(5.56%) and 1(5.56%) were positive for SEA, SEB, SEA+SEB and TSST-1 respectively.

Keywords: *Staphylococcus aureus*, meat and meat products, nuc gene, *Staphylococcal toxins*

Introduction
Staphylococci microorganisms are gram-positive, spherical cells, non-motile, non-spore forming, aerobic and facultative anaerobic, about 1 µm in diameter arranged in irregular clusters. Single cocci, pairs, tetrads and chains are also seen in liquid cultures and clinical material. The *Staphylococci* were first described by the Scottish surgeon, Sir Alexander Ogston as the cause of a number of pyogenic (pus forming) infections in humans. *Staphylococci* are typical of other gram-positive bacteria in having a requirement for certain organic compounds in their nutrition. Amino acids are required as nitrogen sources and thiamine and nicotinic acid are required among B vitamins. When grown anaerobically they appear to require uracil. In one minimal medium for aerobic growth and enterotoxin production, monosodium glutamate (MSG) and soy sauce served as C, N and energy source (Hennekine *et al.*, 2012) [12]

Anterior nares are the natural niche of *S. aureus* in humans and the microbe is frequently present on the skin, skin glands, axillae, inguinal and perineal areas also in (moist habitats) mucous membranes of many individuals (Jay *et al.*, 2005) [16].

S. aureus is among the leading causes of food-borne diseases (FBDs) worldwide for two reasons. Firstly *S. aureus* is often present in food contaminated by humans. As estimated 30-50% of human population carries *S. aureus*, mainly in the nasopharynx or on the hands where the organisms can persist without causing any damage. Coughing, sneezing or food handling combined with poor hygiene may cause contamination, especially after heat treatment. When it comes to raw foods, contamination from animal origins is more frequent (i.e. mastitis). The second reason is that *S. aureus* is capable of growing and producing toxins in a wide variety of foods (Ortega *et al.*, 2010) [29]

S. aureus is a pathogen capable of producing various toxins, named pyrogenic toxic superantigens, including the toxic-shock syndrome toxin (e.g. TSST-1) and staphylococcal enterotoxins (SEs). Staphylococcal Enterotoxins A and B (SEA and SEB) were the first

described SEs. For a long time, only five SEs designated SEA to SEE were reported in the literature and because they all were discovered when some of the major food poisoning outbreaks occurred, all SEs were described as an emetic substances, some being stronger than others. Many authors have reported the production of one or several enterotoxins by other *Staphylococcus* species such as *S. cohnii*, *S. xylosus*, *S. haemolyticus* and *S. epidermidis* (Ortega *et al.*, 2010) [29].

Keeping in view of the public health significance of *S. aureus*, this study was designed to isolate and identify *S. aureus* from different meat and meat products and molecular characterization of the isolates to find out the *S. aureus* and its toxins by using specific primers.

Materials and Methods

Isolation and Identification

All the samples were collected aseptically in sterile polythene bags and transferred on ice to the laboratory at the earliest possible for the further analysis.

About 10 g of each sample (chicken, mutton, beef, pork, turkey meat, chicken nuggets, sausages and burger) was homogenized in mortar and pistil and enriched into 90 ml TSB (trypatone soy broth) broth in individual sterile polythene bags and incubated at 37 ^{0}C for 24hrs. The enriched inoculum from broth was streaked onto Mannitol salt agar plates and incubated at 37 ^{0}C for 24hrs and yellow colour colonies were noticed. The presumptive colonies of *S. aureus* were picked up and confirmed by standard biochemical tests.

Molecular characterization

The genomic DNA isolation was isolated carried out by phenol: chloroform: iso amyl alcohol method from the bacterial strain *S. aureus* standardize PCR assay for detection. All the enriched samples were subjected to PCR analysis for the presence of *S. aureus* using primers specific to *nuc* gene. These positive samples were further examined for the presence of *S. aurues*, heat stable enterotoxin using primers specific to *sea*, *seb* and *tsst-1* (Table: 1)

Table 1: Details of primers used in this study

Target gene	Primer	Primer sequence	Amplification product (bp)	Reference
Nuc gene	nuc F	GCGATTGATGGTGATACGGTT	279	Jung *et. al.* (2015)
	nuc R	AGCCAAGCCTTGACGAACTAAAGC		
Sea gene	SEA 1	TTGGAAACGGTTAAAACGAA	120	Madahi *et. al.* (2014) [21]
	SEA 2	GAACCTTCCCATCAAAAACA		
Seb gene	SEB 1	TCGCATCAAACTGACAAACG	478	Rall *et.al.* (2008)
	SEB 2	GCAGGTACTCTATAAGTGCC		
Tst gene	TSST 1	ATGGCAGCATCAGCTTGATA	350	Momtaz *et. al*. (2010)
	TSST 2	TTTCCAATAACCACCCGTTT		

For molecular characterization of the isolates the genomic DNA extraction was carried out by boiling and snap chilling method. In this method about 1000 µl of the 24h inoculums from the selective enrichment was centrifuged at 6000rpm for 5 min and the pellet was resuspended in 200 µl of molecular grade water and boiled for 15 min in a boiling water bath. The micro centrifuge tubes were transferred immediately onto ice, later it was centrifuged at 13000rpm for 5min and the supernatant was used as template for PCR assay.

For PCR technique, five µl of supernatant used as template. PCR was done in 25 µl reaction mixture (table 2) containing 5 µl of template, 0.2 µl of dNTP's, 2.5 µl of forward primers, 2.5 µl of reverse primers, 2.5 µl of buffer with MgCl2, 0.3 µl of 10x Taq polymerase and 12.0 µl of nuclease free water. Amplification was done in thermal cycler following standardized conditions. The amplified DNA fragments were resolved by agarose gelelectrophoresis stained with ethidium bromide (0.5 µg/ml) and detected the bands in the Gel doc instrument.

Table 2: Components of reaction mixture

S. No	Name of the reagent	Quantity (µl)
1	10X Taq polymerase buffer	2.5
2	dNTP mix	0.2
3	Primer F	2.5
4	Primer R	2.5
5	Taq DNA polymerase	0.3
6	Purified DNA/ Bacterial lysate	5
7	Nuclease free water	12

Results and Discussion

The results of the samples by cultural and PCR assay (*nuc*, *sea*, *seb* and *tsst-1* genes) are presented in table: 3 & 4.

Out of 30 chicken samples, 21(70.0%) were positive for *S. aureus* by cultural method and 22(73.33%) by PCR method. Higher incidence of 83.0% and 81.18% was reported by Suleiman *et al.* (2013) [33] and Momtaz *et al.* (2013) [25] respectively by the cultural method than the present study (70.0%). Low incidence of 30.0% was reported Gundogan *et al.* (2005) [10], Schlegelova *et al.* (2004), Alvarez *et al.* (2002) [1] and 25.0% and 17.8% incidence was reported by Febler *et al* (2011) [7] and Hanson *et al.* (2011) [11] respectively. Higher incidence (73.33%) in the present study by PCR was more than the incidence of 67.6%, 65.8%, 55.5% and 53.3% reported by Buyukcangaz *et al.* (2013) [6], Kitai *et al.* (2005) [19], Gencay *et al.* (2010) [9] and Gundogan *et al.* (2005) [10] respectively. Low incidence of 42.1%, 41.0%, 25.0% and 22.7% in chicken was reported by Lubna *et al.* (2015) [20], Waters *et al.* (2011) [35], Bhargav *et al.* (2011) [4] and Momtaz *et al.* (2013) [25] respectively by PCR assay. Out of 22 positives samples, 15(68.18%) were positive for SEA toxin. Lower incidence (61.8%, 17.2%, 2% and 0%) than the present study was reported by Methange *et al.* (2015) [23], Zargar *et al.* (2014) [36], Lubna *et. al.* (2015) [20] and Symth *et al.* (2005) [32] respectively. An incidence of 18.18% for SEB toxin was observed in the present study, which was lower incidence (2%) than the present study was reported by Lubna *et al.* (2015) [20] and Symth *et al.* (2005) [32] and (0%) was reported by Wang *et al.* (2002). 9.09% incidence was observed for SEA+SEB toxins in the present study. For TSST-1 toxin in the present study the incidence was 4.55%, which was higher (8.9%) than the present study was reported by Lubna *et al.* (2015) [20] and lower incidence (present study was reported by Symth *et al.* (2005) [32]

Table 3: Culture and PCR results of different natural samples for *S. aureus* in meat and meat products

S. No	Sample	No of samples	Culture method		PCR assay		% of culture method compared to PCR
			No	%	No	%	
1	Chicken meat	30.0	21.0	70.00	22.0	73.33	95.45
2	Mutton	30.0	19.0	63.33	20.0	66.66	95.00
3	Beef	30.0	17.0	56.66	18.0	60.00	94.44
4	Pork	30.0	15.0	50.00	16.0	53.33	93.73
5	Turkey meat	30.0	19.0	63.33	20.0	66.66	95.00
6	Chicken nuggets	30.0	18.0	60.00	19.0	63.33	94.73
7	Sausages	30.0	16.0	53.33	17.0	56.66	94.12
8	Burger	30.0	17.0	56.66	18.0	60.00	94.44
Total		240.0	142.0	59.17	150.0	62.50	94.67

Table 4: PCR results of different natural samples for toxins in meat and meat products

S. No	Sample	No of samples	Positive by PCR (Nuc gene)		Positive for SEA		Positive for SEB		Positive for SEA+SEB		Positive for TSST	
			No	%	No	%	No	%	No	%	No	%
1	Chicken meat	30.0	22.0	73.33	15.0	68.18	4.0	18.18	2.0	9.09	1.0	4.55
2	Mutton	30.0	20.0	66.66	14.0	70.00	3.0	15.00	2.0	10.00	1.0	5.00
3	Beef	30.0	18.0	60.00	13.0	72.22	3.0	16.67	1.0	5.56	1.0	5.56
4	Pork	30.0	16.0	53.33	12.0	75.00	2.0	12.50	1.0	6.25	0.0	0.00
5	Turkey meat	30.0	20.0	66.66	14.0	70.00	3.0	15.00	2.0	10.00	1.0	5.00
6	Chicken nuggets	30.0	19.0	63.33	15.0	78.95	5.0	26.32	3.0	15.79	2.0	10.53
7	Sausages	30.0	17.0	56.66	14.0	82.35	3.0	17.65	2.0	11.76	1.0	5.88
8	Burger	30.0	18.0	60.00	13.0	72.22	3.0	16.67	1.0	5.56	1.0	5.56
Total		240.0	150.0	62.5	110.0	73.33	26.0	17.33	14.0	9.33	8.0	5.33

Out of 30 beef samples, 17(56.66%) were positive for *S. aureus* by cultural method and 18(60.0%) by PCR assay. Higher incidence than the present study i.e. 76.3%, 69 Jackson.2% and 65.6% was reported by Rahimi *et al.* (2013) [21, 31], *et al*. (2013) [14] and Lubna *et al.* (2015) [20] respectively by the cultural method. Low incidence of 42.0%, 33.3%, 28.0%, 20.5%, 14.8%, 10.0% and 6.9% in beef samples than the present study (56.66%) by the cultural method was reported by Jay *et al.* (1962) [15], Van loo *et al.* (2007), Kelman *et al.* (2011) [17], Bhargava *et al.* (2011) [4], Zargar *et al.* (2014) [36], Hee *et al.* (2008) [13] and Hanson *et al.* (2011) [11] respectively. An incidence of 65.6% which is almost similar to the incidence of present study (60.0%) by the PCR assay in beef samples was reported by Hee *et al.* (2008) [17] by the PCR assay. Out of 18 positives for *S. aureus* by PCR in beef samples, 13(72.22%) were positive for SEA toxin by PCR. Lower incidence (62.5%. 28.5% and 0%) than the present study was reported by Rahimi *et al*. (2013) [21, 31], Zargar *et al.* (2014) [36] and Wang *et. al.* (2002) respectively. An incidence of 16.67% for SEB toxin was observed in the present study which was higher than the incidence (0%) reported by Wang *et al*. (2002). 5.56% incidence was observed for SEA+SEB toxins in the present study. An incidence of 5.56%, for TSST-1 toxin was observed in the present study, which was higher than the incidence (19.2%) reported by Symth *et al*. (2005) [32]

Out of 30 mutton samples, 19(63.33%) were positive for *S. aureus* by cultural method and 20(66.67%) by PCR assay. Lower incidence of than the present study 41.0%, 18.0%, 15%. 15%. 7.62% and 6.20% by the cultural method than the present study (63.33%) was reported by Rahimi *et al.* (2013) [21, 31], Normanno *et al.* (2007) [28], Zargar *et al.* (2014) [36], Anu *et al*. (2015), Nashwa *et al.* (2015) [27] and Boer *et al.* (2009) [5] respectively. The incidence reported by Gundogan *et al.* (2005) [10] was 66.7%, which is similar to the present study by the PCR assay. Out of 20 positive samples, 14(70.00%) were positive for SEA toxin. Higher incidence (100%) than the present study was reported by Khalifa *et al.* (2015) [18, 27]. Lower incidence (1.25% and 1.2%) was reported by Rahimi *et al.* (2013) [21, 31] and Rasoul Mashouf *et al.* (2015), whereas zero incidence was reported by Zargar *et al.* (2014) [36] and Symth *et al*. (2005) [32]. An incidence of 15.00% for SEB toxin was observed in the present study, which was higher (4.3%) than the incidence reported by Khalifa *et al.* (2015) [18, 27] and zero percent incidence was reported by Symth *et al.* (2005) [32], Mashouf *et al*. (2015) and Morshdy *et al.* (2013) [26]. 10.0% incidence was observed for SEA+SEB toxins in the present study whereas zero percent incidence was reported by Morshdy *et al*. (2013) [26]. For TSST-1 toxin in the present study the incidence was 5.00%, whereas no incidence was reported by Symth *et al.* (2005) [32]

Out of 30 pork samples, 15(50.0%) were positive for *S. aureus* by cultural method and 16(53.33%) by PCR assay. The incidence in the present study (50.0%) by cultural method was similar to the incidence reported by Jackson *et al.* (2013) [14]. Lower incidence i.e. 49.3%, 45.3%, 43.3%, 42.0%, 27.0%, 25.9%, 18.2%, 12.0% and 7.0% was reported by Buyukcakgaz *et al.* (2013) [6], Van loo *et al.* (2007), Lubna *et al.* (2015) [20], Waters *et al.* (2011) [35], Jay *et al.* (1962) [15], Atanassova *et al.* (2001) [3], Hanson *et al.* (2011) [11], Kelman *et al.* (2011) [17] and Hee *et al.* (2008) [17] respectively. Higher incidence of 67.56% was reported by Velasco *et al.* (2014) and slightly lower incidence (51.1%) is reported by Antanassova *et al.* (2001) [3] by the PCR assay than the present study (53.33%). Out of 16 positives samples, 12(75.00%) and 2(12.5%) were positive for SEA and SEB toxins respectively. 6.25% incidence was observed for SEA+SEB toxins in the present study, which was higher than the incidence (1.4%) reported by Mirzai *et al.* (2012) [24]. For TSST-1 toxin in the present study the incidence was zero (0), which was lower than the incidence (1.4%) reported by Mirzai *et al.* (2012) [24]

Out of 30 turkey meat samples, 19(63.33%) were positive for *S. aureus* by cultural method and 20(66.67%) by PCR assay. Lower incidence (56.0%, 19.4% and 16.6%) than the present study was reported by Kelman *et al.* (2011) [17], Hanson *et al.*

(2011) [11] and Zargar *et al.* (2014) [36] respectively by the cultural method. Higher incidence of 77.0% was reported by Waters *et al.* (2011) [35] by the PCR assay than the present study (66.66%). Lower incidence of 64.2%, 56.0%, 54.54%, 22.0% and 19.4% by the PCR was reported by Lubna *et al.* (2015) [20], Kelman *et al.* (2011) [17], Gencay *et al.* (2010) [9], Febler *et al.* (2011) [7] and Hanson *et al.* (2011) [11] respectively. Out of 20 positives 14(70.00%) were positive for SEA toxin. No incidence of SEA was reported by Lubna *et al.* (2015) [20]. An incidence of 15.00% for SEB toxin was observed in the present study, which was higher than the incidence (3.1%) reported by Fooladi *et al.* (2010) [8] and Jakee *et al.* (2013) [14], whereas zero percent incidence was reported by Lubna *et al.* (2015) [20]. 10.0% incidence was observed for SEA+SEB toxins in the present study. For TSST-1 toxin in the present study the incidence was 5.0%, whereas zero percent incidence reported by Lubna *et al.* (2015) [20]

Out of 30 chicken nuggets, 18(60.0%) were positive for *S. aureus* by cultural method and 19(63.33%) by PCR method. Low incidence of 6.42% than the present study (60.0%) was reported by Madahi *et al.* (2014) [21] by the cultural method. The incidence in the present study (63.33%) was almost similar to the incidence of 60.7% reported by Morshdy *et al.* (2013) [26] by the PCR assay. Out of 19 positives 15(78.95%) were positive for SEA toxin. Lower incidence (33.33%) than the present study was reported by Madahi *et al.* (2014) [21]. An incidence of 26.32% for SEB toxin was observed in the present study, which was higher than the incidence (4.16%) reported by Madahi *et al.* (2014) [21]. 15.78% An incidence of 15.79% and 10.53% was observed for SEA+SEB and TSST-1 toxins respectively in the present study

Out of 30 sausages samples, 16(53.33%) were positive for *S. aureus* by cultural method and 17(56.66%) by PCR assay. Lower incidence of 43.68% than the present study was reported by Pumtag *et al.* (2006) [30], whereas 39.3% incidence was reported by Alae El Deein *et al.* (2013) by the cultural method. An incidence of 6.66% was reported by Marwa *et al.* (2013) [22] by the PCR assay, which was less than the incidence in the present study (56.66%). Out of 17 positives 14 (82.35%) were positive for SEA toxin. Higher incidence (88.9%) than the present study was reported by Ala El Deein *et al.* (2013), whereas lower incidence (60.7%) was reported by Morshdy *et al.* (2013) [26]. An incidence of 17.65% for SEB toxin was observed in the present study, which was higher than the findings (11.1%) reported by Ala El Deein *et al.* (2013). 11.76% incidence was observed for SEA+SEB toxins in the present study, whereas zero incidence was observed by Morshdy *et al.* (2013) [26]. 5.88% incidence for TSST-1 toxin was observed in the present study

Out of 30 burgers samples, 17(56.6%) were positive for *S. aureus* by cultural method and 18(60.0%) by PCR assay. An incidence of 50.0% reported by Febler *et al.* (2011) [7] by cultural method was almost similar to the incidence in the present study (56.6%). Higher incidence (60.7%) than the present study was reported by Ala El DeeinS *et al.* (2013). Low incidence of 24.0% and 4.0% by cultural method than the present study was reported by Sharhtaz *et al.* (2012) and Ala EI said *et al.* (2005) respectively. Low incidence of 21.4% and 13.33% by the PCR assay was reported by Morshdy *et al.* (2013) [26] and Marwa *et al.* (2013) [22] by the PCR assay than in the present study (60.0%).Out of 18(60.00%) were positives 13(72.22%) were positive for SEA toxin. Higher incidence (75.0%) than the present study was reported by Ala El Deein *et al.* (2013), whereas lower incidence (21.4%) was reported by Morshdy *et al.* (2013) [26]. An incidence of 16.67% for SEB toxin was observed in the present study, which was higher than the (12.5%) reported by Ala El Deein *et al.* (2013). 5.56% incidence was observed for both SEA+SEB and TSST-1 toxins respectively in the present study

Conclusion

The incidence of *Staphylococcus aureus* in chicken meat and chicken nuggets samples is quite high and alarming and so necessary precautionary methods should be followed while handling and consuming chicken products.

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