

Original Research Article

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Isolation, Identification and Detection of *Staphylococcus aureus* in Raw Chicken and Frozen Chicken Meat Products in Ludhiana, India by Standard Isolation Techniques and PCR Assay

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ABSTRACT

Staphylococcus aureus is one of the major food contaminant with life threatening potential in both humans and animals. This pathogen is one of the indicator organism to monitor hygienic condition(s) during slaughter and processing of the meat samples. The present study was aimed at finding the prevalence of *Staphylococcus aureus* in raw and frozen chicken meat products purchased from different retail outlets and local butcher shops across the Ludhiana city. In the present study a total of 100 raw chicken meat samples were collected (80 fresh raw samples and 20 frozen chicken meat products). During this study, 100 chicken meat samples were inoculated in mannitol salt agar for the selective isolation of *S. aureus* and were later characterized by a combination of microscopic and bio-molecular tests. Results of the study revealed that, 31 samples were containing *S. aureus*, which is 31% of total samples. The isolation techniques and PCR assay were found to be more specific, reliable and expeditive methods to corroborate the presence of *Staphylococcus aureus* in raw and frozen meat products.

Keywords

Staphylococcus aureus, Retail meat, Frozen meat products and PCR

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Introduction

The genus *Staphylococcus* is the most important genus present in the family Micrococcaceae having in its ambit thirty-two species. The members of this group are Gram-

positive, spherical in shape, non-spore forming, non-motile with limited capsule formation (Harris *et al.*, 2002). These bacteria grow well on most routine laboratory media at 37°C. Colonies of the most *Staphylococci* spp. that grow on solid media are circular,

smooth, opaque, raised, with white to pigments of different colors. Staphylococci are known to be facultative anaerobes, usually oxidase negative and catalase-positive. Coagulase production by staphylococci organism cause hemolysis of blood, but the pattern of hemolysis depends on both the source of the blood and the staphylococcal strain (Moraveji *et al.*, 2014). The biochemical characters of different species of staphylococci have been well documented. Staphylococci are known to be ubiquitous in nature and are usually isolated from the outer body surfaces of mammals and birds besides also from blood, genitourinary tract, intestines, upper respiratory tract and other organs of the body. Staphylococci are the most common bacteria found in the environment where poultry are hatched, reared, and processed. They are also isolated from the skin and nares, feet and beak of healthy chickens. *Staphylococcus aureus* is one of the major foodborne pathogens in fresh and ready-to-eat products and recognized for causing various infections around the world. There are many foodborne diseases associated with *Staphylococcus* spp. where food handlers who have staphylococcal lesions of the skin, especially of the nasopharyngeal region and the hands, or who are carriers. Most of the contamination of chicken meat due to *S. aureus* was found due to cross-contamination, inadequate heat treatment of the foodstuff and improper storage resulted into outbreaks of food poisoning.

Materials and Methods

Collection and processing of samples

A total of 100 samples of poultry meat (80 raw chicken meat and 20 frozen meat) samples were collected from different retail shops in vicinity of Ludhiana. About 100 grams of meat samples were collected in dry, clean and sterile polythene bags and transported to the laboratory for

microbiological analysis within one hour or refrigerated at 4°C till further analysis was carried out.

These samples were then processed no later than 48 hours after purchase. These samples were then swabbed with sterile cotton swabs and inoculated onto the Brain Heart Infusion broth (BHI) and then incubated overnight at 37°C. On the next day, the swabs were streaked onto the different media plates like Brain Heart Infusion Agar (BHI), Mannitol Salt Agar (MSA) for isolation of *Staphylococcus* spp.

Identification of bacterial isolates

The bacterial colonies were isolated after incubation. These colonies were subjected to Gram's staining for identification and requisite biochemical tests were carried out to further confirm the presence of the pathogen. The final confirmation of the organism was done by using molecular technique like PCR assay.

Biochemical characterization

S. aureus suspected colonies were subjected to various biochemical tests like the Catalase test and *Staphylococcus aureus* identification kit (HIMEDIA) for Voges Proskauer, Alkaline phosphatase, ONPG, Urease, Arginine Utilisation and various carbohydrate utilization tests including Mannitol, Sucrose, Lactose, Arabinose, Raffinose, Trehalose, and Maltose utilization tests.

Molecular characterization

The DNA was extracted from suspected colonies and tissues using Himedia DNA extraction kits. The extracted DNA was subjected to PCR for the detection of bacterial DNA in the samples using published primers and probes.

Polymerase Chain Reaction (PCR)

The DNA extracted was subjected to polymerase chain reaction using specific primers for *Staphylococcus aureus*. The 25 µl reaction mixture for PCR was prepared that consisted of 13 µl Mastermix (Promega), 1 µl each of 20 pmol/µl Forward primer and Reverse primer, 5 µl of DNA template and 5 µl of Nuclease free water. PCR was performed on C1000 touch thermocycler (Bio-Rad, USA) with the following conditions; an initial denaturation at 95°C for 5 minutes and later 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 1 minute for *S. aureus* and extension at 72°C for 1 minute. The final extension followed at 72°C for 10 minutes. The PCR products were run on 1.5% agarose along with 100 bp DNA molecular weight marker (New England Biolabs, USA) at 5V/cm and visualized using a gel documentation system (AlphaImager, Alpha Innotech, USA).

Results and Discussion

A total of 100 meat samples (80 fresh raw samples and 20 frozen chicken meat products) were examined for the presence of *S. aureus*. The *S. aureus* was isolated from a total of 31 samples in fresh raw meat (28) and frozen chicken meat products (03) with a prevalence of 31% (Table no.1) which were Catalase positive and later confirmed by PCR detection at 118 bp targeting *nuc*- gene sequence.

The samples following the standard protocol were streaked on Mannitol Salt Agar (MSA) (Chapman, 1945) for selective culture of *S. aureus* and yellow colonies with yellow zones in the media were obtained (Fig. 1). Gram's staining performed on suspected colonies showed the presence of Gram positive cocci organisms (Fig.2) discrete or in groups typical of *S. aureus* (Murdoch and Greenlees, 2004). The isolation results for *S. aureus* 31 out of

100 samples i.e. 31% are in congruence to the observations of Banga (2018), Gonclaves-Tenorio *et al.*, (2018), Saliha *et al.*, (2018), Shylaja *et al.*, (2018), Wei-Wei *et al.*, (2018), Reddy and Pusapukdepod (2019) and Zelalem *et al.*, (2019) as 52%, 39.9%, 42%, 21%, 12.5%, 37.7% and 21%, respectively from poultry meat and/or meat products.

Gundogan *et al.*, (2005) undertook study on one hundred and fifty samples of raw chicken parts (giblets, carcass) for the presence of *Staphylococcus aureus* and found 80 samples i.e. 53.3% prevalence of *S. aureus*. Saikia and Joshi (2010) demonstrated the presence of microbial contaminants in retail chicken meat samples in North East India. They isolated different pathogenic microorganism viz. *Escherichia coli* (98%), *Staphylococcus aureus* (20%), *Yersinia enterocolitica* (23%), *Salmonella typhi* (20%) from chicken raw meat samples collected from the local meat markets. Ruban *et al.*, (2012) reported prevalence of common food borne pathogens like *Salmonella spp.*, *Staphylococcus* and *E. coli* in chicken meat obtained from markets in Bangalore under different processing conditions. The colonies picked from Mannitol Salt Agar (MSA) were subjected to Catalase test, which showed positive reactivity (Foster, 1996). Furthermore, biochemical test kit (Himedia) was used in the study for confirming the presence of *S. aureus* (Fig. 3) with the help of 12 tests for identification of *S. aureus* namely MR test, Voges Proskauer, Citrate utilization, Indole, Glucuronidase, Nitrate reduction, ONPG, Lysine utilization tests and 4 different carbohydrates utilization tests. The results from kit confirmed the presence of *S. aureus*. All the samples were confirmed primarily with the help of its growth characteristics on selective media and then with the help of biochemical and immunological testing kits. Das and Mazumdar (2016) found the prevalence of staphylococcus from raw meat

samples in Southern Assam. They collected 65 raw meat samples (chicken and goat) from various regions in and around Southern Assam and reported 17 samples (48.57%) from chicken were positive for *Staphylococci* spp. based on screening of staphylococcal isolates was done on the basis of morphology,

Gram's stain, catalase, coagulase and mannitol fermentation tests according to standard protocols. In another study from retail outlets of Chennai, India the prevalence of different biotypes of *S. aureus* i.e. Clonal Complex 398 in chicken meat was carried out.

Table.1 Comparison of the detection of *Staphylococcus aureus* in meat samples using various techniques

Techniques	Total fresh meat samples (80)		Frozen Samples (20)	
	<i>Staphylococcus aureus</i>	%	<i>Staphylococcus aureus</i>	%
Isolation	28	35	03	15
PCR	28	35	03	15
Total	28	35	03	15
Overall Total	(28+03)= 31 (31%)			

Fig.1 Growth of *S. aureus* on Mannitol Salt Agar



Fig.2 Microscopic view, note the typical grapes like clusters of *S. aureus* from culture. Gram's stain x 100

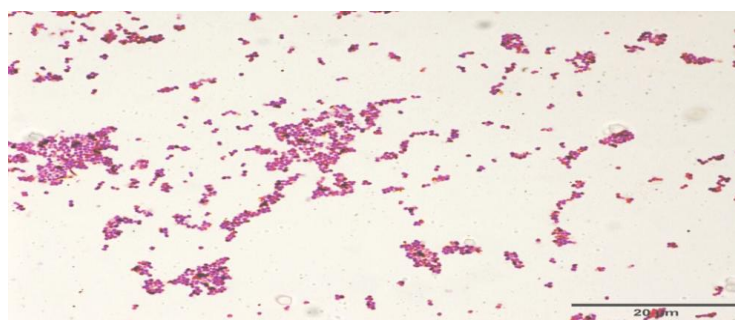


Fig.3 Biochemical test for *S. aureus* using kit by HiMedia showing positive reaction for *S. aureus*

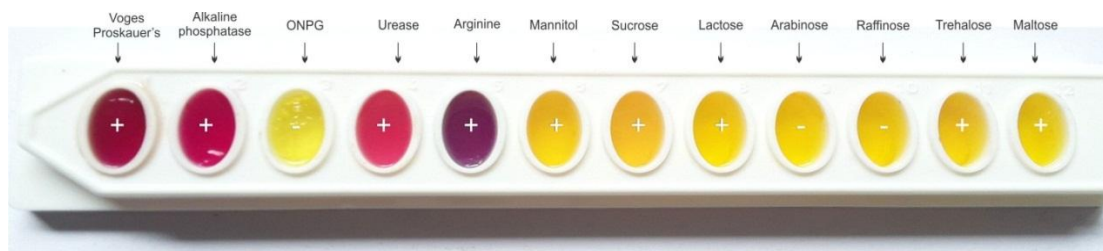
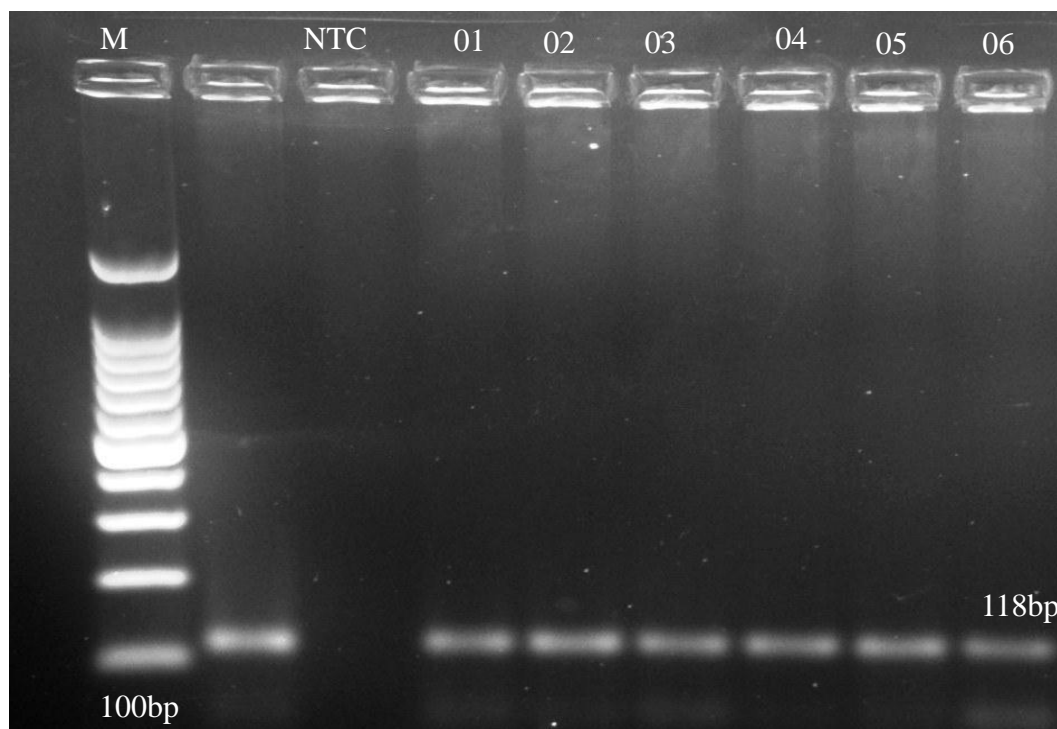


Fig.4 Molecular identification of *S. aureus* at 118 bp targeting *nuc* gene. M=100bp DNA ladder; L1to L6= test samples showing distinct bands at 118bp; PTC= Positive template control; NTC= Negative template control



In the present study, standard PCR assay (Fig. 4) was employed to confirm the presence of *Staphylococcus aureus* by targeting *nuc* gene which amplified at target size of 118bp using published primers (Manga and Vyletelova 2013). *Staphylococcus aureus* was isolated from 28 fresh meat sample out of 80 (35%) and 03 out of 20 (15%) samples from the frozen meat with an overall prevalence of 29 (29%) from the fresh and frozen meat samples. Other scientists have also used PCR

for confirmation of *S. aureus*. Also in another study, Rusenova and Rusenov (2017) have used PCR for confirmation of isolated *Staphylococcus aureus* by amplification of DNA. Ruban *et al.*, (2018) used PCR to confirm prevalence of *S. aureus* from 40 chicken isolates collected from retail outlets of Chennai, India, further suggesting its zoonotic potential. Shylaja *et al.*, (2018) studied the incidence of *Staphylococcus aureus* in different meat and meat products

samples. Out of 30 samples of chicken 17 (56.66%), chicken nuggets 18(60.00%), sausages 16 (53.33%), and burgers samples 17 (56.66%), respectively were positive for *Staphylococcus aureus* by cultural method, whereas PCR assay revealed the incidence of *Staphylococcus aureus* to be 18 (60.00%), 19 (63.33%), 17 (56.66%) and 18 (60.00%), respectively. The prevalence of *Staphylococcus aureus* by cultural method and PCR assay was determined in different meat and meat products samples. In a study in China it was observed that there were 12.5% of foodborne bacterial outbreaks caused by *S. aureus*, which showed the third most frequently occurring pathogen(Wei-Wei *et al.*, 2018).

A total of 1105 samples collected from chicken meat processing plant and retail shops had 333 (30.13%) prevalence of *S. aureus*. *Staphylococcus aureus* isolated from fecal (28.88%), skin swabs (40%), intestinal mucosa (34.81%), and 45.18% environmental samples collected from processing plant had an overall prevalence of 37.77% (Reddy and Pusapukdepod 2019).

In conclusions, the present study revealed high proportion of *Staphylococcus* species (31%) in raw chicken meat and frozen chicken meat products. Thirty one percent (31%) isolation rate of *Staphylococcus* species from retail shops signals the existence of poor hygienic practices and consequently, its public health implication. Routine isolation, supplemented with molecular technique like PCR helped in better comprehension of meat pathogens.

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