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Prevalence and Virulence Characterization of *Staphylococcus aureus* Isolates from Chicken Meat and Ready to Eat Chicken Products to Assess Hygiene and Consumer Safety

Vijaya Singh Thakur[1], Bhavana Gupta[1], R.V. Singh[1], Anju Nayak[2], Sanjay Shukla[2] **10.18805/ajdfr.DR-1905**

ABSTRACT

Background: Meat and meat products are good source of proteins and essential amino acids for health. These products are contaminated with various pathogens like *S. aureus, Salmonella* spp, *E coli* and *Listeria* spp., *S. aureus* is one of most important food borne pathogen associated with food products. The present study was carried out to estimate prevalence of *S. aureus* and their molecular characterization in raw chicken meat and ready-to-eat chicken products in Jabalpur city.

Methods: The samples were collected from different retails outlets and processed as per standard microbiological procedures for isolation of *S. aureus* and subjected further for molecular characterization and processed to assess the prevalence of *S. aureus*. A total of 100 raw chicken meat and 70 ready to eat chicken meat products (chicken samosa, momos, pattis, tikka, barbeque *etc*.) have been collected.

Result: The overall prevalence of *S. aureus* was observed 38.82% with 38.00% in raw chicken meat and 40.00% in ready-to-eat chicken (RTE) products. The molecular study revealed that all the *S. aureus* isolates were positive for 16s ribosomal RNA (*rRNA*). Out of 66 isolates, 27 (71.05%) from raw chicken meat and 12 (42.85%) from ready to eat chicken meat products isolates were found to be positive for *sea* gene where as 3 (7.8%) isolates from raw chicken meat and 6 (21.42%) from ready-to-eat chicken products were found positive for *nuc* gene respectively.

Key words: Consumer safety, Molecular characterization, Raw chicken meat, Ready-to-eat (RTE) chicken products, *S. aureus*.

INTRODUCTION

Meat and meat products are one of the important sources of high quality nutrients with high biological value proteins and essential amino acids. World population is growing at a rate of 1.0% per year in developed countries and 2.5% per year in the developing countries. India stands at 5th rank (6.3 million tons) and accounts for 3% of the total world meat production (220 million tones) including poultry meat (Islam *et al.,* 2016) and the per capita meat consumption is running at 5.0 to 5.5 kilograms (11 to 12 pounds) per year (FAO, 2011). As per the Ministry of Fisheries and Animal Husbandry of India, poultry is contributing nearly 47.86% of total meat production and per capita consumption of poultry meat is estimated at around 3.1 kg.

Meat consumption pattern in majority of the countries are culture dependent and in India, meat consumption pattern is controlled by customs, tradition and religious taboos. Of the various meats consumed in India, poultry meat occupies the major share among various sections because of its versatility, relatively low cost; no social and religious taboo associated with its consumption and is considered to be lean with low fat content (Bai *et al.,* 2022).

Staphylococcal food-borne disease (SFD) is a gastrointestinal illness with rapid onset caused by consuming foods contaminated with enterotoxins produced by the bacterium (Bousbia *et al*., 2018). Approximately 0.5 ng/mL concentration of staphylococcal enterotoxin (SEs) in contaminated food may cause a large outbreak. Staphylococcal enterotoxin A (*sea*) is one of the most important cause of gastroenteritis and more than 50% of food poisoning (FP) is caused by *S. aureus.* It is a Gram-positive, non-spore forming organism which can grow at optimum temperature of 30-37°C, pH 7-7.5 and NaCl concentration upto 15-20%. It is a commensal and opportunistic pathogen that can cause wide spectrum of

[1]Department of Veterinary Public Health and Epidemiology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India.

[2]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India.

Corresponding Author: Bhavana Gupta, Department of Veterinary Public Health and Epidemiology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India. Email: dr.bvgvetgupta@rediffmail.com

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infection from superficial skin infection to severe and potentially fatal invasive diseases. It is found on the skin and nose (about 25%) of healthy people and animals without causing illness but has ability to produce toxins which leads to food poisoning in human being and mastitis in animals (CDC, 2016). Although pathogen produces various toxins but the food poisoning prevalence is mainly due to 22 different enterotoxins (Hennekinne *et al.,* 2012). Most of the *S. aureus* food poisoning occurrences are caused by A, B, C, D and E enterotoxins (Montville and Mathews, 2008 and FDA, 2012). More than 90% *S. aureus* strains producing enterotoxin are also producing coagulase and thermostable nuclease (Jay *et al*., 2005).

Now a days, the demand for chicken meat and processed chicken meat are at rise due to consumer's preference for poultry meat over other animal proteins as it is ubiquitously accepted meat because of different religious and social taboos in eating of pork, water buffalo and beef meat and due to its relatively low cost. Meat may be excellent source for various type microorganism *viz*. virus, bacteria, parasites, toxins etc. In meat, the common bacteria leads to infections are *Salmonella, Campylobacter*, *S. aureus, C. perfringens*, pathogenic *E. coli etc*., so consumption of raw or improperly cooked or contaminated meat may results in various types of food borne infectious diseases (Kadariya *et al.,* 2014). Pathogens may gain entry into meat due to unhygienic production, transportation or processing and some time, post processing contamination from various sources such as water, raw ingredients, environment and food handlers.

MATERIALS AND METHODS

The present research work was performed out during the November 2017- June 2018 in the Department of Veterinary Public Heath, College of Veterinary Science and A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P.), India.

Table 1: Distribution of samples raw chicken meat and ready to eat chicken (RTE) products collected from different sources.

Sample of chicken		No. of samples	Total samples
Raw chicken meat		100	100
Ready to eat (RTE) chicken products		70	70
Chicken samosa		10	10
Chicken pattis		10	10
Chicken momos	Fried	15	30
	Steamed	15	
Chicken barbeque		10	10
Chicken tikka		10	10
Total			170

Collection of samples

A total of 170 samples (100 raw chicken and 70 RTE chicken products consisting of chicken samosa, chicken pattis, chicken momos, chicken barbeque and chicken tikka) (Table 1) were collected from different retail outlets in Jabalpur city. The samples were collected in properly sterilized polythene bags and transferred to laboratory in chilled condition for bacteriological examination on ice and stored at 4°C till further processing. All samples were processed for isolation of *S. aureus* within 24 hrs of arrival in the laboratory.

Enrichment and selective media

One ml of diluted sample was inoculated in 5 ml *Staphylococcal* enrichment broth having 5.5% salt and mixed thoroughly and incubated overnight at 37°C. The enrichment inoculum (0.1 ml) was streaked on Baird Parker (egg yolk tellurite) agar plates and incubated at 37°C for 18-24 hrs. Colonies showing shiny, jet-black (halo around colony) were picked up and considered as presumptive *S. aureus*.

Morphological and biochemical identification of *S. aureus* isolates

The presumptive isolates of *S. aureus* were microscopically and biochemically characterized on the basis of colony morphology, Gram's staining, coagulase , catalase, oxidase test, indole, MR, VP and thermonuclease tests according to method described by Cruickshank *et al.* (1975) and Agarwal *et al.* (2003).

Polymerase chain reaction for detection of 16S*rRNA*, *nuc* and *sea* genes of *S. aureus*

The template DNA was prepared by boiling and snap chilling method to detect 16S *rRNA*, *nuc* and *sea* genes of *S. aureus*. PCR amplification for the individual genes was setup in 25 µl of reactions. The PCR protocol was initially standardized by varying the annealing temperature (50-60°C) and cyclic conditions. The standardized amplification reaction started with initial denaturation at 94°C for 5 min, followed by 30 cycles each having denaturation at 94°C for 1 min, annealing at 50°C for 16S *rRNA* gene, 55°C for *nuc* gene for 30 sec and *sea* gene for 57°C for 2 min and extension at 72°C for 1 min, with final extension for 10 min at 72°C. Amplified products were analyzed by agarose gel (1.0%) electrophoresis and primers used to detect genes of *S. aureus* are listed in Table 2 (Fig 1-4).

Table 2: Details of primers used for PCR reaction.

Target gene	Oligonucleotide sequence (5′-3′)	Product size (bp)	Annealing temperature	Reference
16S*rRNA*	F: GTA GGT GGC AAG CGT TAT CC	228	50°C	Abeer *et al.,* (2010)
	R: CGC ACA TCA GCG TCA G			
nuc	F: GCG ATT GAT GGT GAT ACG GTT	270	55°C	Brakstad *et al.,* (1992)
	R: AGC CAA GCC TTG ACG AAC TAA AGC			
Sea	F: GGT TAT CAA TGT GCG GGT GG	102	57°C	Mehrotra *et al.,* (2000)
	R: CGG CAC TTT TTT CTC TTC GG			

RESULTS AND DISCUSSION

Out of 170 samples of raw chicken meat and its products examined, 66 samples were found positive for *S. aureus* as depicted in Table 3, Fig 1. The occurrence of *S. aureus* in raw chicken meat was 38% and in case of chicken meat products (RTE), the occurrence was 40.00% in chicken samosa (70%), chicken momos (50%), chicken pattis (30%), chicken tikka (20%) and chicken barbeque (10%).

Virulence characterization of *S. aureus* isolates by PCR

Presumptive isolates were selected on the basis of colony characteristics and biochemical testing and subjected for virulence characterization by PCR (Table 4).

The main source of *S. aureus* in food is skin and the nose of healthy people, animals and birds as about 25% healthy individuals possess the organism without showing any illness (CDC, 2016).

Similar prevalence studies conducted by various workers have also reported *S. aureus* to be associated with variety of meat and meat products including chicken meat having varying percentage. Saikia and Joshi (2010), (Hanson *et al*., 2011; Zargar *et al*., 2014) have found comparatively less prevalence in chicken meat 20%, 17.8% and 15.7%, respectively whereas Kargirwar, 2004 and Abdulrahman *et al*. (2015) reported higher prevalence percentage than our study results, although Das and Mazumder (2016) found quite similar prevalence with present study at 48.57%. In case of ready-to-eat chicken meat products, Abd-El-Malek (2017) had reported 16.3% prevalence while Shafizi *et al.* (2016) observed 53.00% prevalence. Oguttu *et al.* (2014) conducted

Table 3: Prevalence of *S. aureus* isolates from raw chicken meat and ready to eat chicken (RTE) products.

Samples	Sample type		No. of samples	No. of samples positive for *S. aureus*	Prevalence of *S. aureus* (%)
Raw chicken meat (38%)	Raw chicken meat		100	38	38%
Ready to eat chicken products (RTE) (40%)	Chicken samosa		10	7	70%
	Chicken momos	Steamed	15	9	60%
		Fried	15	6	40%
	Chicken pattis		10	3	30%
	Chicken tikka		10	2	20%
	Chicken barbeque		10	1	10%
	Prevalence		170	66	38.82%

Table 4: Virulence characterization of *S. aureus* in raw chicken meat and ready to eat chicken (RTE) products.

Name of gene	Sources of Isolates (N=66)						Total
	Raw chicken meat (n=38)	Ready to eat chicken products (RTE) (n=28)					
		Chicken samosa	Chicken pattis	Chicken momos	Chicken barbeque	Chicken tikka	
16S *rRNA*	38	7	3	15	1	2	66
nuc	0	2	0	3	0	0	05
sea	24	2	0	8	0	1	35
nuc and *sea* both	3	0	0	1	0	0	04

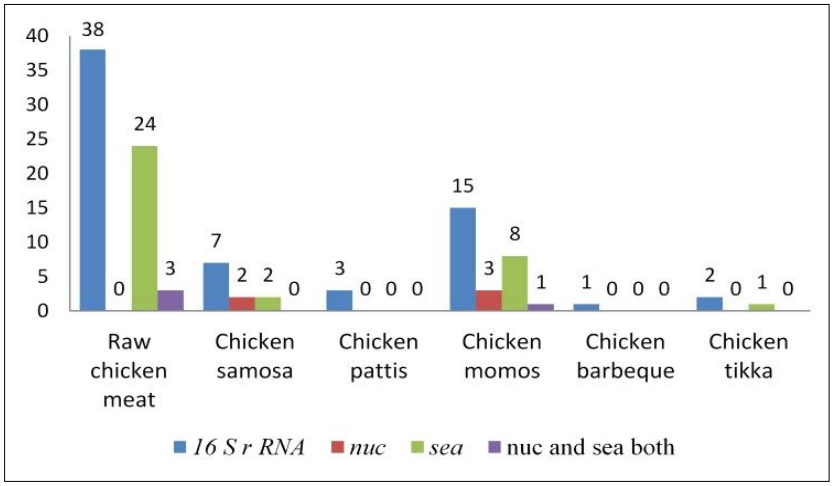


Fig 1: Distribution of *16 S rRNA* , *nuc* and *sea* genes in raw chicken meat and ready to eat chicken (RTE) products.

study in Tshwane South Africa and reported results in accordance to our study with 44.00% prevalence in ready-to-eat (RTE) chicken. Difference prevalence rate were reported by various workers might be due to variation in hygienic standards maintained at different steps in food chain starting from farm to table or due to variation in isolation protocol. The higher prevalence in ready-to-eat chicken meat products than raw chicken meat is indicative of post cooking contamination during handling or inappropriate cooking procedure.

Colony characteristics, Gram staining and different biochemical tests like oxidase, catalase used for presumptive identification of *S. aureus* were found quite effective in this study and enrichment media SB broth and selective media Baird Parker agar were used quite effectively. All isolates were further subjected to detect staphylococcal enterotoxin genes A (*sea*) and 59.09% isolates were found to be positive for *sea* with high percent was observed in raw chicken meat (71.05%) than ready to eat chicken meat products (42.85%). In present study 6.06% (04) isolates found to possess both *nuc* and *sea* gene.

Madahi *et al*. (2014) reported in their study conducted in Iran, 33.33% of isolates fromchicken nuggets producing *sea*. Similarly Gupta *et al*. (2014) have also reported 42.22% prevalence of *sea* gene with 31.11% was from raw fish

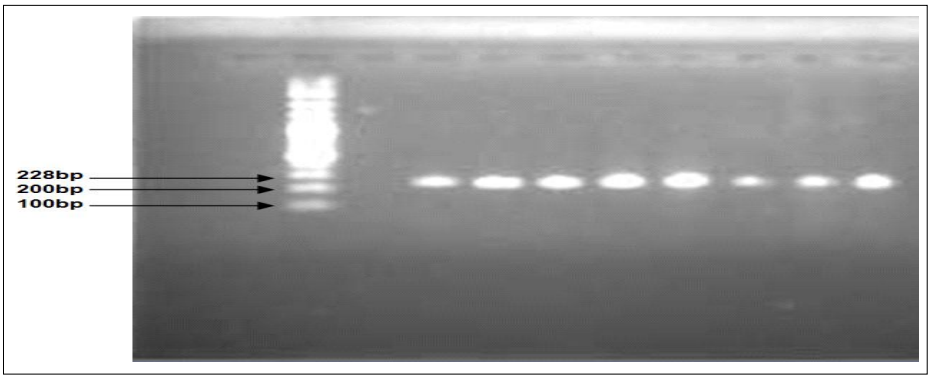


Fig 2: Agarose gel electrophoresis showing amplified PCR product of *16S rRNA* of *S. aureus* isolates from raw chicken and its products (228 bp; L:2-9).

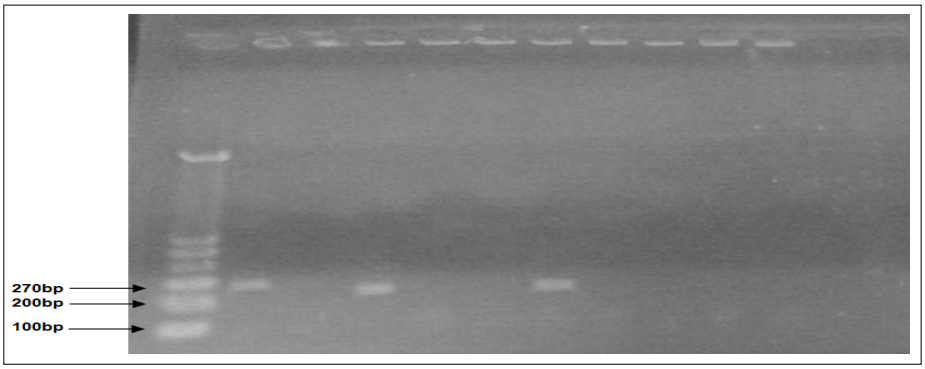


Fig 3: Agarose gel showing amplified PCR product of *nuc* of *S. aureus* isolates from raw chicken and its products (270 bp; L:1, 3 and 6).

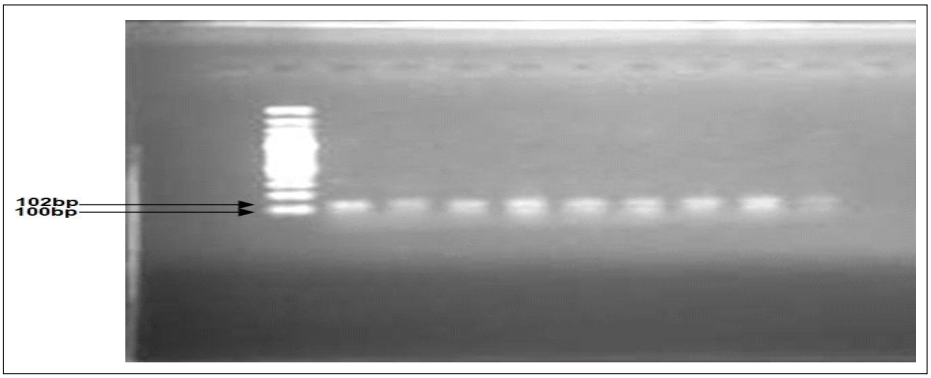


Fig 4: Agarose gel showing amplified PCR product of *sea* of *S. aureus* isolates from raw chicken its products (102 bp; L:1-9).

samples and 11.11% from RTE fish productsas found with present study.

CONCLUSION

Presence of *nuc* and *sea* positive strain in raw chicken as well as ready to eat chicken meat products (RTE) indicates unhygienic handling of meat and meat products and contamination of food items at the time of processing or post processing. As enterotoxins are heat stable and even though organism may be destroyed by various methods of cooking and processing, The presence of *sea* gene might be food intoxication due to already produced toxin in raw or in ready to eat products. The study revealed that the chicken meat and meat products in the study area were contaminated with *S. aureus* which indicates poor hygienic practices in slaughter house and cold chain failure at retail outlets.

Conflict of interest

All authors declare that they have no conflict of interest.

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