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Prevalence of *Coagulase-Positive Staphylococci* (CPS) in Chicken Meat Sold in Chennai Metropolis and its Suburbs

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A B S T R A C T

A study was carried out to determine the prevalence of *Coagulase-Positive Staphylococci* (CPS) in chicken processing tools such as defeathering machine, wooden log and also chicken meat sold at butchers' shop in five different locations in and around Chennai city. A total of 150 samples were assessed and each fifty represented the processing tools and chicken meat. Mean CPS count in defeathering machine, wooden log used for fabrication and chicken meat were 3.16, 2.97 and 3.78 log cfu/sq.in., or g of sample, respectively. One way analysis of variance to assess the effect of location on CPS count revealed that mean CPS count in defeathering machine and chicken meat samples did not statistically differed between locations whereas mean CPS count in wooden log differed significantly ($p<0.05$) between locations. Correlation studies revealed the existence of highly significant ($p\leq0.01$) correlation between CPS count in chicken meat and defeathering machine and also with that of wooden log. Predictive modeling studies in chicken meat at two different static temperatures to envisage the growth kinetics of CPS during transport and at consumers' kitchen revealed that the level of 10^6cfu/g, required to elaborate enterotoxins, would be reached when the meat is left at 29.8°C for approximately 10 hrs and 30 minutes. The same level would be reached when the meat is kept at 7.5°C for approximately 22 days. The investigation highlighted that these organisms are very common and constitute a risk for consumers' health. Further, it became evident that the hygiene practices are not being followed at the butchers' shops. Results of predictive modeling studies showed that there is absolutely less or no risk of enterotoxin production in raw chicken meat while consider existing consumer practices.

Introduction

Chennai is the sixth most populous city in India wherein 70% of the population is non-vegetarian and consumes predominantly chicken meat. With respect to poultry processing, 'wet market' system is largely being followed in which the broiler chickens, are processed in butchers' shop adopting ritual slaughter methods and sold to the consumer as hot meat. This often lacks basic facilities, meat inspection and personnel hygiene of the butchers' involved etc., and thus adds up to the microbial load of chicken meat. Bhaisare *et al.,* (2014) reported that poultry meats are often found contaminated

with potentially pathogenic microorganisms. Several studies have also indicated the prevalence of drug resistant strains of *S. aureus* in foods, environment (Tambekar *et al.,* 2011; Ruban *et al.,* 2012; Agarwal *et al.,* 2012), chicken meat and its products (Boer *et al.,* 2009; Pesavento *et al.,* 2007). Shale *et al.,* 2005 and Kadariya *et al.,* 2014 have reported that *Staphylococcus aureus* has been consistently shown to be one of the most important micro-organisms responsible for food poisoning outbreaks worldwide. Further, in a pilot study to investigate food poisoning cases in Hyderabad, Sudershan *et al.,* (2014) pointed that *Staphylococcus aureus* was the etiological agent in most cases of food poisoning. Nema *et al.,* 2007 also reported an outbreak of staphylococcal food poisoning in a social gathering after the consumption of a snack called "Bhalla" made up of potato balls fried in vegetable oil. Keeping above points in view, the present study was proposed to assess the prevalence of coagulase positive *Staphylococci* in chicken meat sold in Chennai Metropolis and its suburbs.

Materials and Methods

Microbial analysis

A total of 150 samples, each fifty representing, defeathering machine, wooden log and chicken meat from butchers' shops in five different locations in and around Chennai city, were aseptically collected and transported to Food and Industrial Microbiology Laboratory, College of Food and Dairy Technology at 4°C in insulated and refrigerated container and enumerated for Coagulase Positive *Staphylococci* using pour plate technique described by APHA, 1984.

Predictive modeling

A predictive modeling study in chicken meat has been carried out using Combase growth model to envisage the growth kinetics of CPS at two different static temperatures (29.8°C and 7.5°C). *S. aureus* was considered as model micro-organism. Staphylococcal counts should reach approximately 10^6 cfu/g to produce enterotoxin (Necidová *et al.,* 2009 and, Pelisser *et al.,* 2009). Hence, in the present study, the time required to attain this level in chicken meat at 29.8°C and 7.5°C were also predicted.

Statistical analysis

The Analysis of Variance and Pearson correlation were carried out using SPSS statistical software.

Results and Discussion

The mean±SE values of CPS count in defeathering machine, wooden log and chicken meat sold at butchers' shop in five different locations in and around Chennai are presented in Table 1. The Mean CPS count in defeathering machines at different locations ranged between 2.88 and 3.62 $\log_{10}$cfu/sq.in., and the counts did not significantly varied between locations studied. Mean CPS count in wooden log from different locations ranged between 2.85 and 3.22 $\log_{10}$cfu/sq.in., and the counts significantly ($p \leq 0.05$) varied between locations. Mean CPS count in wooden logs at locations II,III and IV were significantly higher ($p \leq 0.05$) however did not significantly differ among them. Similarly, Mean CPS count in locations V was significantly ($p \leq 0.05$) lower however did not significantly differ from that of locations I and III. Mean CPS count in chicken meat obtained from different locations ranged between 3.70 and 3.96 $\log_{10}$cfu/g and the counts did not significantly varied between locations studied. The results of the correlation studies (Table 2) revealed that there is highly significant ($p \leq 0.01$) correlation between CPS counts in chicken meat, defeathering machine and wooden log.

Table.1 Mean ± SE coagulase positive staphylococcal count in chicken processing tools and chicken meat

Location	Time of collection (min. after slaughter)	Temp. of the meat (°C)	Defeathering machine ($\log_{10}$cfu/sq.in)	Wooden log ($\log_{10}$cfu/sq.in)	Chicken meat ($\log_{10}$cfu/sq.in)
I	192	29.4 ± 0.83^{ab}	3.15 ± 0.37	2.85 ± 0.10^{ab}	3.77 ± 0.10
II	150	31.9 ± 0.73^{b}	3.62 ± 0.14	3.22 ± 0.11^{c}	3.96 ± 0.10
III	204	29.9 ± 0.78^{ab}	3.12 ± 0.35	2.96 ± 0.13^{abc}	3.76 ± 0.07
IV	234	29.1 ± 1.04^{a}	2.88 ± 0.49	3.11 ± 0.12^{bc}	3.73 ± 0.12
V	222	28.8 ± 0.86^{a}	3.05 ± 0.37	2.74 ± 0.07^{a}	3.70 ± 0.08
Overall Mean± SE	**200.4**	**29.8±0.40**	**3.16±0.16**	**2.97±0.05**	**3.78 ±0.04**

Table.2 Correlations coefficient between CPS counts in chicken meat, wooden log and defeathering machine

		CPS - chicken meat	CPS - wooden log	CPS- defeathering machine
CPS - chicken meat	Pearson Correlation	1	**.525****	**.688****
	Sig. (2-tailed)		.000	.000
	N	50	50	50
**. Correlation is significant at the 0.01 level (2-tailed).				
*. Correlation is significant at the 0.05 level (2-tailed).				

Table.3 Effect of temperature and pH on maximum growth rate, doubling time and time to attain CPS count of 10^{6}cfu/g of chicken meat at Consumer kitchen

Stage in supply chain	Temp (°C)	pH of meat	Initial CPS count (log cfu/g)	Max. growth rate (log cfu/g)	Doubling Time (hrs)	Time to attain 10^{6}cfu/g of chicken meat (hrs)	Lag phase duration (hrs)	Observation time (hrs)
At the consumer kitchen	29.8	7.1	3.78	0.39	0.773	**10.57**	0.71	17.41
At the consumer kitchen	7.5	7.1	3.78	0.008	35.964	**515.16**	34.96	546.3

Predictive modeling studies in chicken meat at two different static temperatures viz., 29.8°C and 7.5°C envisaged that the level of 10^6cfu/g, required to elaborate enterotoxins, would be reached when the meat is left at 29.8°C for approximately 10 hrs and 30 minutes. The same level would be reached when the meat is kept at 7.5°C for approximately 22 days. The duration of lag phase was extremely longer when the meat was expected to be stored at latter temperature compared to former. Conversely, the growth rate and doubling time were extremely higher and shorter, respectively at 29.8°C than at 7.5°C (Table 3).

Evaluation of meat for specific bacterial population provides information about the process, personal and environmental hygiene adopted during different unit operations of meat processing and thereby confers an opportunity to improve the processing conditions in order to ensure safe supply of meat to the intended consumers. The genus *Staphylococcus* is present in skin and nasal flora of humans and various animals. To date, seven species of coagulase-positive staphylococci (CPS) have been identified (Devriese *et al.,* 2005; Freney *et al.,* 1999). This present investigation studied the presence of CPS in chicken meat marketed in Chennai city.

The CPS was detected in almost all chicken samples examined. Badhe *et al.,* (2013) also exactly observed similar findings while screening market chicken samples in Bangalore city, India and reported the hundred per cent prevalence of *Staphylococcus aureus,* in chicken meat samples obtained from outlets with minimum facilities compared to the meat samples obtained from outlets with better facilities and hygiene. Further, the results of present study are in accordance with Normanno *et al.,* (2005) who observed the higher incidence of

CPS in foodstuffs marketed in Italy and also reported that the meat products analysed showed the highest prevalence ranging from 17.1% for the ripened meat product samples to 48.1% of the ''other meat product'' samples, i.e. foods prepared with fresh meat, roast beef, dishes prepared with ground meat, meat skewers, rolls, etc. However, our findings are in contrast to the results observed by Arul kumar and Saravanan (2011) who reported that out of 210 meat samples collected, 6.67% were positive for *S. aureus* and the colony count was $1.03 \pm 0.08 \log_{10}$ cfu/g. The lower count obtained in the latter study might be attributed to the lesser (12 h) incubation period after plating at 37°C. Kitai *et al.,* (2005) also observed higher prevalence (65.8%) of *S. aureus* in 444 samples of raw chicken meat screened. In the present study, while considering the CPS counts in defeathering machine and wooden log that the CPS count in chicken meat should have been higher that the results obtained. This comparatively less count might be due to the act of skin removal in later stage of chicken processing or lower transfer rate of CPS from processing tools.

Similar to chicken meat, high prevalence of CPS was also observed in wooden log and defeathering machine used for chicken processing. Geornaras *et al.,* (1995) also reported that in a poultry processing plant, transport cages, "rubber fingers", defeathering curtains, shackles and conveyor belts repeatedly showed aerobic plate counts in excess of $5.0 \log_{10}$ CFU/25cm^2. Further, *Listeria* spp., presumptive *Salmonella* and *Staphylococcus aureus* were isolated from 27.6, 51.7 and 24.1% of all product samples, respectively, and *Listeria* and *Staphylococcus aureus* were also isolated from selected equipment surfaces. Arnold and Silver, 2000 also stated that during processing of poultry meat products, broiler carcasses come in contact with many solid surfaces. Bacteria

from the carcasses can attach to wet equipment surfaces, form biofilms, and provide a source of cross-contamination for subsequent carcasses. Gundogan and Ataol (2013) also stated that Staphylococci may attach to the contact surfaces in both milk and meat processing industries, form biofilms and survive on them. Their attachment to food contact surfaces in food processing plants and subsequent biofilm formation pose a risk of contamination in milk and meat products. While assessing microbial status of chicken portions and portioning equipment, Holder *et al.,* (2007) also observed that the mean *S. aureus* count on equipment, hands and utensils ranged between 1.37 and 3.53 $\log_{10}$ cfu per swab. In the present study, the high prevalence of CPS in processing tools revealed that these opportunistic pathogens are very common in environment and it is very likely that the contamination of chicken meat might be occurred during processing and handling. The variation in the CPS count of wooden log at different locations might be due to the act of scrapping the surface of the former with the knife at periodical interval.

The results of correlation studies showed that these processing tools could act as a source of contamination of chicken meat. FAO (1991) also emphasized that failure to sterilize all knives and equipment regularly will result in carcass contamination.

Results of predictive modeling showed that the extremely longer duration of lag phase at 7.5°C and comparatively higher growth rate and shorter doubling time at 29.8°C would be attributed to the shorter time requirement to attain the level of 10^6cfu/g at 29.8°C. ICMSF, 1996 also specified that the optimum growth temperature for *S. aureus* is between 35 and 40°C with growth limits at about 7 and 48°C. Further, the commission stated that at 10°C there is a long lag time (>20h) and when growth commences it is very slow. Similar results were also observed in the present study. Staphylococcal enterotoxins are generally produced under a more limited range of conditions compared with growth but are similarly affected by factors affecting growth (ICMSF, 1996). While consider these facts and existing consumer practices there is absolutely less or no risk of staphylococcal enterotoxin production in raw chicken meat.

Effective cleaning and sanitation of defeathering machine as well as replacement of wooden log with circular band saw will possibly improve the microbial quality of chicken meat.

Further, butchers' should be trained for hygienic slaughter and dressing of poultry as well as cleaning and sanitation of premises.

As the study reveals that the incidence of CPS is high in chicken meat, norms can be set for CPS as a process hygiene criteria in addition to *Salmonella* as far as developing countries are concerned.

Acknowledgement

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