ASSESSMENT OF MICROBIAL LOAD IN RAW MEAT SAMPLES FROM OPEN MARKETS OF PRODDATUR, Y.S.R. KADAPA DISTRICT, ANDHRA PRADESH

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ABSTRACT

The present investigation was aimed at assessing the microbial load in raw meat samples procured from open markets of Proddatur city, YSR Kadapa District, Andhra Pradesh. Thirty chevon and thirty chicken samples were collected separately, from different local open markets of the city and were subjected for Total Aerobic Plate count, E. *coli* count, *Staphylococcus aureus* count, detection of Salmonella and Yeast and Mould counts, following standard microbiological procedures. The range of Total Aerobic Plate count of chevon samples varied from 5.97 – 7.67 $\log_{10}$CFU/cm^2, whereas the E. *coli* counts varied from 1.86 to 2.28$\log_{10}$CFU/cm^2, the Staph. *aureus* counts ranged from 2.39 – 4.73 $\log_{10}$CFU/cm^{2}and Yeast and Mould counts fluctuated from 0 to 5.92 $\log_{10}$CFU/cm^2. Salmonella was detected in 10% of chevon samples. Chicken samples had a mean + S.E. Total Aerobic Plate count of 7.03 ± 1.65 $\log_{10}$CFU/cm^2, E. coli counts of 2.98 ± 0.42 $\log_{10}$CFU/cm^2, Staph. *aureus* counts of 3.58 ± 0.16$\log_{10}$CFU/cm^{2}and Yeast and Mould counts of 2.71 ± 0.22 $\log_{10}$CFU/cm^2 .20 % of chicken samples were Positive for Salmonella detection. Meat consumers should be cautious about the meat handlers while purchasing the meat and also should be well aware about the proper measures like cleaning and cooking the meat to overcome microbial contamination.

Key Words : Microbial load, Total Plate count, E. *coli* count, *Staph.aureus* count, Salmonellae, Yeast and Mould counts.

INTRODUCTION

Meat is an excellent source of protein in human diet, also rich in fat, vitamins and minerals, low in carbohydrate content, and is delicious, appetizing and easily digestible food item. Thewhole nutritional requisite can be met easily and efficiently if reasonable amount of meat is included in the diet. However, Meat, which is rich in protein is a good medium for growth of microbes, and with sufficient water activity, is highly susceptible to microbial contaminations, which supports the growth

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of both spoilage and pathogenic bacteria and can cause its spoilage and food borne infections in human, ensuing economic and health losses (Komba*et al.,* 2012).

Muscles of healthy animals are essentially sterile, do not contain microorganisms, however, meat tissues get contaminated during the various stages of pre and post slaughter (Warriss, 2000; Alvarez *et al.,* 2009;Adzitey, 2011;Adzitey and Huda, 2012;Adzitey and Huda, 2011) and transportation (Ercolini*et al.,* 2006).A great diversity of microbes inhabits fresh meat generally. However, only certain types may become dominant depending on pH, composition, texture, storage temperature, and the modus of transportation of raw meat (Ercolini*et al.,* 2006; Li *et al.,* 2006; Adu-Gyamfi*et al.,* 2012). Raw meat harbors many important pathogenic microbes i.e. Salmonella spp., Campylobacter jejuni/coli, Yersinia enterocolitica, E. coli, S. aureus and, to some extent, Listeria monocytogenes. Improper handling and control of these pathogens, may lead to occurrence of food borne ill-nesses, making the meat a risk for human health ((Nørrung*et al.,* 2009). Meat provides an ideal condition for the proliferation of different spoilage bacteria (Mayr*et al.,* 2003) thus making meat highly perishable.

Contaminated raw or undercooked poultry and red meats are particularly important in transmitting food-borne pathogens (Zhao *et al.,* 2001). Retail meat harbors all the bacteria that are already present in meat as inherent contamination through infection and also that are introduced during handling, improper dressing, cleaning, insanitary condition and retailing. Meat can also be contaminated at several points throughout the processing procedures. Higher microbial load in retail cuts could be a consequence of increase in exposed surface area, more readily available water, nutrient and greater oxygen penetration which leads to spoilage of meat. Meat borne zoonotic diseases such as Salmonellosis, Campylobacteriosis, E.coli enteritis and food poisoning by Clostridium, Staphylococcus, etc. are the major problems encountered by the consumers eating contaminated meat. Foodborne infections, which still remain one of the major problems of public health worldwide, have been linked to the consumption of meat. Meat infected with microorganisms is the cause of many food-borne diseases (WHO,1997).

Slaughtering of livestock continue to escalate as a result of the enhanced demand for meat and its products (Warris, 2010). Meat has been and continues to be an important constituent of our daily repasts, since it provides us with proteins and serves as source of energy (Stufflebeam, 1983).

The sanitary conditions of abattoirs and its surrounding environment are chief facets contributing to bacterial contamination of meat (Gill *et al.,* 2000). Contaminations can be compounded during transportation, storage and handling of meat at the butcher shops (Adzitey, 2011; Adzitey*et al.,* 2011). In developing countries the abattoir environment, its sanitary level, transportation and storage conditions not only contaminate but also enhance the growth of different types of spoilage (Adzitey*et al.,* 2011; Adzitey*et al.,* 2014)as well as pathogenic bacteria in meat. Mukhopadhyay*et al.,*(2009) reported

that, increased total aerobic counts on meat may be due to the impact of hot and humid climate areas. To control the food-borne illnesses and to keep the microbial load of raw meat under check, the food safety requirements should be followed stringently in accordance with HACCP (Hazard analysis critical control point).

Consumers in developed countries acquired an escalated consciousness for microbiologically safe food.There is a need to produce better quality and disease free meat, especially in most developing countries. It is an illustrious fact that meat coming in contact with microbial populationduring production, processing, transportation and distribution, presents a challenging hazard to meat industry,which poses problems of infection, spoilage and intoxications (Ramasastry*et al.,* 1999; Dhanze*et al.,* 2012). To increase meat quality, assurance in accordance with microbial load assessment is deemed necessary.

Hence the present study has been carried out with an objective to "Assess the Microbial Load in Raw Meat Samples from Open Markets of Proddatur, Y.S.R. Kadapa District, Andhra Pradesh", to create awareness among the consumers regarding the safety levels of meat they consume.

MATERIALS AND METHODS

Sample collection and Processing :Goat and chicken meat samples were collected from thirty different open market meat outlets in Proddatur town, Y.S.R. Kadapa district, Andhra Pradesh, India. Sampling was carried out by swabbing the muscular surface of fore and hind quarter of each goat carcass as well as from total chicken carcass surface, after flaying and washing. An area marked within a sterile frame of 10 cm X 10 cm on each site of the carcass was identified and a sterile cotton swab was rotated on the surface of carcass for 30 seconds and swabs were transferred to a screw-capped test tube containing 10 ml of sterile maintenance medium (0.85% NaCl and 0.1% peptone) (Bell, 1997). The tubes were transported to lab at 4^{O}C and processed for further analysis within four hours.

Serial decimal dilution was then carried out with one (1) ml of each samples in nine (9) ml of 0.1% peptone water (pH 7.0, sterilized at 121^{O}C for 15 mins.) to obtain the neat(dilution of 10^{-1}).Tenfold serial dilutions were prepared as $10^{-3,}$ $10^{-4,}$ $10^{-5,}$ 10^{-6}, 10^{-7},by transferring 1 ml of aliquot from diluted tube to dilution blank and inoculated onto petri dishes with appropriate media.

Total Plate Count : Total Aerobic plate count was carried out on total plate count agar as described by (Obeng*et al*., 2013). The medium was autoclaved and maintained at 46^{O} C. Samples were serially diluted decimally and an aliquot of 1 ml of each of serial dilution was transferred to the petri dishes (4 inch diameter) and molten agar (15-20 ml) was poured on it. Plates were gently swirled to uniformly mix the sample and incubated at 37^{O} C for 24 hours, before the colonies were counted and reported as CFU/cm^2, using a digital colony counter (Deep Vision Colony Counter, Model : 362).After 24hrs of incubation, plates with countable colonies (30-300cfu) wereidentified, and TPC was determined from appropriate plates as CFU/cm^2.

Enumeration of *Escherichia coli:* *Escherichia coli* were enumerated on Eosin Methylene Blue agar (Himedia Laboratories, Mumbai) by plating an appropriate dilution on plates followed by aerobic incubation at 37^{O} C for 24hrs. After incubation E. coli were counted as colonies with distinct metallic sheen (Bhandare*et al.,* 2007).

Enumeration of *Staphylococcus aureus* : Baird Parker agar (Himedia Laboratories, Mumbai), a selective medium for the isolation and counting of coagulase positive staphylococci was used for the enumeration of *Staphylococcus aureus* as described by (Bhandare*et al.,* 2007). Enumeration of *S.aureus* was done by spreading an appropriate dilution of sample on agar plates followed by aerobic incubation at 37^{O} C for 48hrs. Further confirmation of S.aureus was carried out by grams staining and catalase testing.

Isolation and identification of *Salmonella*: Presence of salmonella in meat sample was established by pre-enrichment of meat sample in lactose broth followed by enrichment in tetra-thionate broth and final detection on Bismuth Sulphite agar as recommended by WHO procedures.

Enumeration of Yeastand Mould Count: Yeast and Mould counts load was estimated by incubating the appropriate dilution of the sample on Potato Dextrose agar(Himedia Laboratories, Mumbai), followed by incubation at 37^{O}C for 5 days.

Statistical Analysis: Microbial counts (CFU/cm^2) were represented as $\log_{10}$ CFU/cm^2 and means were calculated. Microbial counts were compared by ANOVA using SPSS software 19.0.

RESULTS AND DISCUSSION

Total Plate Counts : In the present study, the mean Total Plate Count of chevon samples collected from local market of Proddatur were $6.79 \pm 1.02\log_{10}$ CFU/cm^2, with a range of 5.97 – 7.67. The higher prevalence of microorganisms may possibly be ascribed to unhygienic and inapt handling of meat during slaughtering, dressing and evisceration of goat by the local butchers (Mukhopadhyay*et al*., 1998). In general,Meat is transported to the markets in unhygienic meat vans, motor cycles, and often on bicycles. It is also a familiar practice to see people carrying carcasses on their bare shoulders (Obeng*et al*., 2013). As per the observations ofBhandare*et al*.,(2007), contamination of meat with microorganisms has been a consequence of the unhygienic practices of meat processing in developing countries. Introduction of saliva on the meat, from meat sellers who were busily conversing, coughing, and sneezing also might result in contamination. Mukhopadhyay*et al*., (2009) opined that, hot and humid climate areas contribute to escalating total aerobic counts on meat; and that also could be a contributing factor for the high total aerobic counts of the meat in this study.

The observations in the present study were in consonance with the findings of Keshab Prasad Sharma and Chattopadhyay (2015), who observed that the raw mutton samples sold in the open markets of Kolkata had a General Viable Count,ranging from 2×10^6 CFU/gm to 1.1×10^7 CFU/gm in

different (Thigh, Neck, Groin) meat parts of mutton.Singh *et al*., (2014)recorded a SPC of 6.96 ± 0.78 (log_{10}CFU/gm) in the raw chevon procured from local markets of Agra. Similar findings were reported by Ahmad*et al*., (2013), who recorded Aerobic Plate Count of 6.92±2.16 log_{10} CFU/cm^2 in mutton samples and 6.62± 1.12 log_{10} CFU/cm^2 in chevon samples collected from various abattoirs of Lahore city. Analogous interpretations were also reported by Bhandare*et al.*, (2007) in Sheep/Goat carcasses; Haque*et al.*, (2008)in goat meat and Lambey*et al.* (2010) in chevon.

The mean Total Plate Count of microbes in chicken samples collected from local market of Proddatur were 7.03 ± 1.65log_{10} CFU/cm^2, with a range of 6.48 – 7.69.The higher counts might be due to the contamination from butcher's practices. Anachinaba*et al*., (2015) analyzed that butchers,who handle meat did not pay appropriateinterestto their personal hygiene and carried the meat with unclean hands and clothing. Meats are sold in the open markets sometimes in sieves (or) without sieves. Meats were put on tables which are not well cleaned before and after the day's work and also in the open, exposing the meat to houseflies. Scanty sanitation was observed in the immediate environment where meats are sold. Adziteye*t al*. (2014) observed similar unhygienic practices in meat handling. Food can be infected with microorganisms as a consequence of "coughing" and "sneezing" from those who handle and process these foods (Okonko*et al*. 2008, Koffi-Nevry*et al*. (2011)also declared that, "careless sneezing and coughing among butchers can cause contamination of the products". The afore-mentioned practices add to the high microbial load.

The findings in the present study corroborate with the results of Ahmad *et al*., (2013), who found out that the Aerobic Plate Counts of Chicken meat samples collected from differentretail outlets of Lahore city were 7.22± 2.11 log_{10} CFU/cm^2, while Singh *et al*., (2014) observed that the SPC of poultry meat samples obtained from local markets of Agra were, 6.75±0.04 (log_{10}CFU/gm). Keshab Prasad Sharma and Chattopadhyay (2015), recorded that the General Viable Count of poultry varied from 2.4 X 10^6 CFU/gmto 4.1 X 10^7 CFU/ gm in different (Thigh, Neck, Groin) meat parts of poultry gathered from the open markets of Kolkata. The present findings are also comparable to the findings of Alvarez-Astorga*et al.*, (2002) fromretail chicken by-products in Spain, Hassan *et al*.,(2010) from retail meat shops in Karachi, Pakistan and Obeng*et al*., (2013) in the Northern Region of Ghana.

***E. coli*Counts :**The mean *E. coli* Count in chevon samples collected from local market of Proddatur were 2.02 ± 0.48log_{10} CFU/cm^2, with a range of 1.86 – 2.28.*E. coli*existence in several outlets is a sign of fecal contamination of the meat, which may be owed to unhygienic handling of meat starting, from slaughtering, butchering equipments, handling, transportation, and processing (Warris, 2010).

The findings in the present study were congruent with the findings ofAhmad *et al*., (2013), who found out that the *E.*

coli Counts of mutton samples collected from various retail outlets of Lahore city were 2.78± 1.10$\log_{10}$ CFU/cm^{2}and of the same counts in chevon samples were 1.94±1.12$\log_{10}$ CFU/cm^2. Similar findings were reported by Bhandare*et al*., (2007) and Doyle (2007).

The mean *E. coli*Count in Chicken meat samples collected from local market of Proddatur were 2.98 ± 0.42$\log_{10}$ CFU/cm^2, with a range of 2.76 – 3.28. The usual practice of washing the carcass with the same water in which intestines and offal had been sluiced, was considered as one of the principal reasons for enhanced microbial counts of the carcasses (Mukhopadhyay*et al*., 2009).

The findings in the present study were harmonious with the findings of Ahmad *et al*., (2013), who found out that the *E. coli* Counts of chicken meat samples collected from differentretail outlets of Lahore city were 2.74± 1.13 $\log_{10}$ CFU/cm^2. Correspondingly parallel findings were reported by of Alvarez-Astorga*et al.*, (2002) fromretail chicken by-products in Spain and Adu-Gyamfi*et al*., (2012) in chicken sold in Accra, Ghana.

Stap.aureus* Counts :**Postgate (2000) reported that staphylococcus spp. is an ingredient of the normal flora on the skin of humans and animals, which can be passed on from person to meat and meat products through unhygienic practices. The mean ***Stap.aureusCount in chevon samples collected from local market of Proddatur were 3.47 ± 0.21$\log_{10}$CFU/cm^2, with a range of 2.39 – 4.73.Animals are slaughtered in abattoirs and sometimes in backyards without observing strict hygienic practices. Suchmeat isfurther contaminated by butchers, producing unhygienic meat (Norrung. *et al*.,(2009).

The findings in the present study were in agreement with the findings of Singh *et al*., (2014), who reported that the Staphylococcus Counts raw chevon meat procured from local markets of Agra were 3.84 ±0.12 $\log_{10}$ CFU/gm. Whereas, Ahmad *et al*., (2013), recorded *Staph. aureus*Count of 2.96±1.66 $\log_{10}$ CFU/cm^2 in mutton samples and 3.07± 1.45 $\log_{10}$ CFU/cm^2 in chevon samples collected from various retail outlets of Lahore city. Similar findings were reported by Haque.*et al.*, (2008)in goat meat and of Tassew*et al*., (2010)in minced meat.

Chicken samples collected from local market of Proddatur, had a mean ***Stap.aureus***count of3.58 ± 0.16 $\log_{10}$ CFU/cm^{2}with a range of 2.46 – 4.92. Anabsoluteunawareness on the part of the meat handlers/butchers in hygienic treatment of carcasses during slaughter and retailing processes might be the corefactor for turning out meat with elevated microbial load (Mukhopadhyay*et al*., 2009).

The results obtained from the present study correlate well with the findings of Ahmad *et al*., (2013), and the author statedthat, the mean ***Stap.aureus***Counts of chicken meat samples collected from different retail outlets of Lahore city were 3.80±1.34 $\log_{10}$ CFU/cm^2.Besides, Singh *et al*., (2014) observed that the Staphylococcus Counts of poultry meat samples obtained from local markets of Agra were, 3.35± 0.10 ($\log_{10}$CFU/gm). Collateral findings

were also reported by VoidarouC.D.*et al.,* (2011)in poultry meat.

Salmonella detection : Meat and poultry carcasses and their parts are recurrently contaminated with pathogens, which get in contact with the carcasses from the intestinal tract or from fecalmaterial on feet and feathers. Cross contaminations is a specific problem and have been frequently published to control pathogens in the chain right through from hatcheries to the home made preparations. Animals with certain illness may lead to higher possibility of mistakes, such as gastrointestinal ruptures, in the processing plant, which would lead to increased microbial contamination and cross-contamination (Singer *et al.,*2007).

In the present investigation, Salmonella contamination has been recognized in 10% of the chevon samples (3 samples) collected from local market of Proddatur. The high prevalence of Salmonella can be attributed to the usage of contaminated water for washing of carcasses.Ahmad *et al*., (2013), assessed the microbial load of chevon samples collected from different retail outlets of Lahore city, and reported that 10 % of the samples procured from goat abattoirs and 10 % of the samples obtained from goat retail outlets were positive for Salmonellae. However, Keshab Prasad Sharma and Chattopadhyay (2015) detected a prevalence of Salmonella in 2 % of the raw mutton samples sold in the open markets of Kolkata.

Salmonella is one of the recurrently isolated bacteria from the abattoir environment and gastrointestinal tract of all farm and wild animals, particularlyin poultry (Norrung*et al.* 2009, EFSA, 2007). The chicken samples collected from local market of Proddaturrevealed pervasiveness of Salmonella up to 20 % (5 samples). Ahmad *et al*., (2013), observed that 25 % of chicken samples collected from different retail outlets of Lahore city were positive for Salmonellae.Maharjan*et al*., (2006)also noticed Salmonella Species in raw chicken samples of a Local Market in Kathmandu, Nepal.

Yeast and Mould Counts : The mean Yeast and Mould Count in chevon samples collected from local market of Proddatur were $2.94 \pm 0.15 \log_{10}$ CFU/ cm^2, with a range of 0 – 5.92. The present study inferences were covenant with the interpretations ofMukhopadhyay*et al*., (1998), who testified that the Yeast and MouldCount of mutton samples collected from different retail shops of Namakkal city, Tamil Nadu,were $\log_{10}$ 2.9 CFU/gm, and also informed a Count of $\log_{10}$6.90 CFU/gmin chevon samples collected from different retail outlets of Pondicherry(2009). Similar conclusions were reported by Duffy *et al.*, (2001) in lamb carcasses processed in United States.

The mean Yeast and MouldCount of chicken samples collected from local market of Proddatur were $2.71 \pm 0.22 \log_{10}$ CFU/cm^2.The findings in the present study concurs with the outcomes of Santosh Kumar *et al*., (2014), that the Yeast and Mould Counts of market slaughtered chicken were 2.91 ± 0.22 $\log_{10}$ CFU/gm. Similar findings were reported by Anand*et al.*, (1989) in dressed chicken.

CONCLUSION

A microbial check must be carried out periodically and meat hygiene practices are essential for public health point of view with objectives to safeguard the diseased free meat to the consumers, to arrest the spread of disease among meat consumers, to trace the source of disease by proper examination of animals prior and after the slaughter of meat, to check the spread of diseases which have a definite life cycle, to encourage the honest butchers and retailers, to promote the trade of quality meat and meat products across the country and eliminating the risk of rejections of foreign consignments of meat.

The present study reveals the poor quality of meat sold in the open markets of Proddatur, by increase in the microbial load, which might be attributed to unhygienic production, processing, transportation and storage measures. Raw meat should be properly handled with suitable hygienic measures to safeguard the consumer health. Consumers also should be aware of the microbial quality of meat available from the market and simple measures like cleaning and proper cooking of meat should be followed before consuming.

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Table 1 : Mean and Range of Total Plate, *E.coli, Staph. aureus*, Salmonella and Yeast &Mould counts of chevon and chicken samplescollected from open market meat outlets in Proddatur town, Y.S.R. Kadapa district, Andhra Pradesh, India

S. No.	Sample		Total Plate Counts (log 10 cfu /cm^2)	*E. coli* Counts (log 10 cfu /cm^2)	*Stap. aureus* Counts (log 10 cfu /cm^2)	Detection of Salmonella	Yeast &Mould count (log 10 cfu /cm^2)
1.	**Chevon**	Mean ± S.E.	$6.79^a \pm 1.02$	$2.02^a \pm 0.48$	$3.47^a \pm 0.21$	10 % Positive	$2.94^a \pm 0.15$
		Range	5.97 – 7.67	1.86 – 2.28	2.39 – 4.73		0 – 5.92
2.	**Chicken**	Mean ± S.E.	$7.03^b \pm 1.65$	$2.98^b \pm 0.42$	$3.58^a \pm 0.16$	20 % Positive	$2.71^a \pm 0.22$
		Range	6.48 – 7.69	2.76 – 3.28	2.46 – 4.92		0 – 6.02

n = 30