The Pharma Innovation Journal 2022; SP-11(1): 356-359

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(1): 356-359

www.thepharmajournal.com
Received: 13-11-2021
Accepted: 15-12-2021

Vadlamudi Gowthami
B.V.Sc &A.H, Department of Veterinary Public Health and Epidemiology, P.V. Narsimha Rao Telangana Veterinary University, Telangana, India

Sangam Sai Geetha
B.V.Sc &A.H, Department of Veterinary Public Health and Epidemiology, P.V. Narsimha Rao Telangana Veterinary University, Telangana, India

A Vijaya Kumar
Assistant Professor & Head, Department of Veterinary Public Health and Epidemiology, P.V. Narsimha Rao Telangana Veterinary University, Telangana, India

N Krishnaiah
Professor (Retd.), Department of Veterinary Public Health and Epidemiology, P.V. Narsimha Rao Telangana Veterinary University, Telangana, India

Corresponding Author
Vadlamudi Gowthami
B.V.Sc &A.H, Department of Veterinary Public Health and Epidemiology, P.V. Narsimha Rao Telangana Veterinary University, Telangana, India

Assessment of microbial load in raw chicken at retail outlets in and around Hyderabad, India

Vadlamudi Gowthami, Sangam Sai Geetha, A Vijaya Kumar and N Krishnaiah

Abstract
The present study aims to assess the contamination of microbes in the marketed raw chicken collected from retail outlets available in Hyderabad, India with a special emphasis on foodborne pathogens such as *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus.* A total of 75 samples containing leg, wing and breast were collected under sterile conditions from the retail outlets and transported to the laboratory on ice. The chicken meat samples were homogenized in a sterile glass homogenizer and enumeration of bacteria was done through standard cultural techniques and the three major foodborne pathogens were isolated and identified using conventional microbiological methods.
Mean values of Total viable count (TVC) in the leg was assessed to be 7.36 $\log_{10}$ CFU/g or cm^2 while that of wing is 3.26 $\log_{10}$ CFU/g and in the breast, it is found out to be 5.62 $\log_{10}$ CFU/g. Out of 25 samples of leg, ranges of TVC, *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* are 4.03 to 10.41, 2.95 to 9.75, 5.09 to 12.98, 1.96 to 10.67 $\log_{10}$ CFU/g respectively as mentioned in table 1. Among the 25 samples of wing, the ranges of TVC, *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* are 0.94 to 7.43, 1.96 to 9.76, 2.15 to 9.54 and 0.47 to 4.86 $\log_{10}$ CFU/g respectively as mentioned in table 2. In case of breast samples, ranges of Total viable count (TVC), *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* are 2.73 to 8.62, 0.76 to 5.01, 4.96 to 12.87 and 0.65 to 5.64 $\log_{10}$ CFU/g respectively as mentioned in table 3.
Higher bacterial load and the presence of intestinal microbes such as *E. coli*, *Salmonella* spp., in the present study indicates that the chicken is contaminated and the consumers are at higher risk of getting exposed to foodborne ailments when taken the raw or undercooked chicken.

Keywords: bacteriological quality, *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, Hyderabad

Introduction
Ensuring safe food supply has always been one of the biggest challenges faced by the producers, buyers and the public health officials in both developed and developing countries. It is well registered that contamination of meat with pathogens constitutes a major public health concern (Cohen *et al*., 2007) [1, 27]. Several epidemiological studies have implicated meat as major channel associated with ailments caused by food borne pathogens. The contaminated chicken can cause a wide variety of food hazards. *Salmonella* and *Staphylococcus aureus* are on the top of the list in terms of food poisoning and infections. Similarly, *E.coli* is also a major cause of food poisoning. Coliform bacteria, especially fecal coliforms are good microbial indicators of the possible presence of disease-causing bacteria and indicate the hygiene quality of the food. The high prevalence of diarrheal diseases in many developing countries suggests major problems with food safety.
Chicken is one of the most consumed high protein foods in India. Raw chicken is more susceptible to contamination and also frequently implicated in the spread of food borne illnesses. The microbiological quality of chicken meat depends on the slaughter process, sanitation during processing and packaging, maintenance of adequate cold chain storage from processing and then finally selling it to the consumers. Contamination of poultry carcasses and parts with *Salmonella* organisms is well documented and data are available for many parts of the world (Simmons *et al.*, 2003) [2]. A recent study by the Translational Genomics Research Institute showed that nearly half (47%) of the meat and poultry in US grocery stores was contaminated with *S. aureus*, with more than half (52%) of those bacteria being resistant to antibiotics. The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent years (Van *et al.*, 2007) [3, 28].

Public health research in countries like United States of America focusing on food qualities illustrated that retail outlets or stores in low socio-economic status populations were shown to be consistently exposed to food that is of lower microbial quality due to higher food safety violations. This further causes illness that would cost billions of dollars. The importance of different food borne diseases varies between countries depending on consumption of food, food processing, preparation, handling, storage techniques employed and population sensitivity (ICMSF, 2002) [4]. To control the food-borne illnesses and to restrain the microbial load of raw meat, the food safety requirements should be followed strictly in accordance with HACCP (Hazard analysis critical control point), but in developing countries like India, the sanitary level, transportation and storage conditions enhance the growth of different types of pathogenic bacteria in meat along with its contamination.

The present study was done to determine the prevalence and concentration of bacteria in chicken with special emphasis on *Salmonella spp., Staphylococcus aureus, Escherichia coli* in addition to total viable counts available in the market as the raw chicken available in retail outlets in Hyderabad, India is with questionable hygiene and due to overcrowding and inadequate sanitary conditions, food-borne infections are on rise in the city

Materials and Methods

Sample Collection

Around seventy five (75) samples of chicken meat were collected from retailers out of which 25 samples were of chicken breasts, 25 samples were of chicken wings and the remaining 25 were of chicken legs. Meat samples were collected in the units of 500gms each in polythene covers. 25 g of each sample were incised using a sterile knife and put in sterile plastic bags and then transferred to the laboratory in ice bags. Later, the samples were evaluated for the presence of various foodborne bacteria by conventional cultural methods and then estimation of total plate count.

Isolation Procedure

Total viable count: Total viable count is carried out using Pour Plate technique. In Pour Plate technique, successive dilutions of the inoculum (serially diluting the original specimen of old broth culture) is added to the sterile petri plates containing the melted and cooled(40-45 ^{o}C) agar medium and thoroughly mixed by gently swirling the plates and then left aside to solidify. Once the media solidifies, the plates are then incubated at 37 ^{o}C for 24-48 h in an inverted position. After incubation, the presence of individual colonies growing throughout the medium was determined from the respective plates.

Isolation and identification of *Escherichia coli*

E.coli were enumerated on Eosin Methylene Blue (EMB) agar using the Streak Plate Technique. In this technique, 1gm of sample is mixed in 9 ml of PBS and incubated for 24 hrs. Then using a loop (sterilized), a loopful of inoculum is withdrawn and is streaked over the agar surface present in the petri plate in a way that it thins out the bacteria. The streaked plates were incubated at 37 ^{o}C for 24-48hrs in an inverted position and the *E. coli* colonies were counted with distinct metallic sheen (Bhandare *el al*., 2007) [6]. It is further confirmed by performing biochemical tests.

Isolation and identification of *Salmonella*

The loopful of inoculum was streaked onto the *Salmonella*-Shigella (SS) agar and then incubated at 37 ^{o}C for 48hrs. The presumable *Salmonella* colonies were then sub-cultured by streaking over the fresh SS agar using a sterile loop and incubated at 37 ^{o}C for 48 h. The suspected colonies as Salmonella are identified by confirmatory biochemical tests.

Isolation and identification of *Staphylococcus aureus*

Mannitol salt agar (MSA), a selective medium is used for the isolation of staphylococci. A loopful of inoculum was taken and streaked on the media and incubated for 24-48 h at 37 ^{o}C. Medium yellow colored colonies were seen on the media. Suspected colonies were then subjected to Gram's staining where they appeared as irregular, grape-like clusters. Further catalase test was performed where the colonies are taken using a sterile loop and placed in the test tube containing hydrogen peroxide. Evolution of oxygen bubbles indicate the presence of *S. aureus*.

Results

Total sample size is 75 including leg, wing and breast each of 25 samples respectively.

Table 1: Statistics of Total viable count in various parts of chicken raw meat

Type of sample	No of samples	Total viable count		
		Range (log_{10} CFU/g)	Mean (log_{10} CFU/g)	Number (%)
Leg	25	4.03 -10.41	7.36	15 (60%)
Wing	25	0.94 – 7.43	3.26	10 (40%)
Breast	25	2.73 – 8.62	5.62	20 (80%)

Table 2: Statistics of *Escherichia coli* in various parts of chicken raw meat

Type of sample	No of samples	*Escherichia coli*		
		Range (log_{10} CFU/g)	Mean (log_{10} CFU/g)	Number (%)
Leg	25	2.95 – 9.75	7.19	3 (12%)
Wing	25	1.96 – 9.76	5.18	5 (20%)
Breast	25	0.76 – 5.01	2.93	10 (14%)

Table 3: Statistics of *Salmonella* in various parts of chicken raw meat

Type of sample	No of samples	*Salmonella*		
		Range (log_{10} CFU/g)	Mean (log_{10} CFU/g)	Number (%)
Leg	25	5.09 – 12.98	10.37	7 (28%)
Wing	25	2.15 – 9.54	6.21	4 (16%)
Breast	25	4.96 - 12.87	8.99	9 (36%)

Table 4: Statistics of *Staphylococcus aureus* in various parts of chicken raw meat

Type of sample	No of samples	*Staphylococcus aureus*		
		Range (log_{10} CFU/g)	Mean (log_{10} CFU/g)	Number (%)
Leg	25	1.96 – 10.67	5.93	3 (12%)
Wing	25	0.47 – 4.86	1.92	1 (4%)
Breast	25	0.65 – 5.64	2.64	2 (8%)

Out of 25 samples of leg, 60% of samples were found to be contaminated i.e. 15 samples with the count ranging from 4.03 -10.41 log_{10} CFU/g with mean value of 7.36 log_{10} CFU/g. Among 25 samples of wing, 40% were contaminated i.e. 10 samples and their count ranged from 0.94 – 7.43 log_{10} CFU/g with mean value of 3.26 log_{10} CFU/g. In 25 samples of breast, 80% of the samples were infected which is the highest among the three parts that were assessed for TVC i.e. 20 samples and the count ranged from 2.73 – 8.62 log_{10} CFU/g with mean count of 5.62 log_{10} CFU/g. Among the three parts, the highest mean value is that of leg with 7.36 log_{10} CFU/g.

Escherichia coli were found in 12% of leg samples i.e. 3 out of the total 25 samples. Total count of *Escherichia coli* ranged from 2.95 – 9.75 log_{10} CFU/g with a mean value of 7.19 log_{10} CFU/g in leg. While in wing, the presence of *E.coli* was only 20% i.e. 5 out of the 25 samples with count ranging from 1.96 – 9.76 log_{10} CFU/g with mean value of 5.18 log_{10} CFU/g. In breast, 14% of the samples were contaminated i.e. 10 out of the 25 samples and the count ranged from 0.76 – 5.01 log_{10} CFU/g with the mean count of 2.93 log_{10} CFU/g.

Salmonella spp., were found in 28% of leg samples i.e. 7 out of the total 25 samples. Total count of *Salmonella* spp.ranged from 5.09 – 12.98 log_{10} CFU/g with a mean value of 10.37 log_{10} CFU/g in leg. While in wing, the presence of *Salmonella* spp. was only 16% i.e. 4 out of the 25 samples with count ranging from 2.15 – 9.54 log_{10} CFU/g with mean value of 6.21 log_{10} CFU/g. In breast, 36% of the samples were contaminated i.e. 9 out of the 25 samples and the count ranged from 4.96 - 12.87 log_{10} CFU/g with the mean count of 8.99 log_{10} CFU/g.

Staphylococcus aureus were found in 12% of leg samples i.e. 3 out of the total 25 samples. Total count of *Staphylococcus aureus* ranged from 1.96 – 10.67 log_{10} CFU/g with a mean value of 5.93 log_{10} CFU/g in leg. While in wing, the presence of *Staphylococcus aureus.* was only 4% i.e. 1 out of the 25 samples with count ranging from 0.47 – 4.86 log_{10} CFU/g with mean value of 1.92 log_{10} CFU/g. In breast, 8% of the samples were contaminated i.e. 2 out of the 25 samples and the count ranged from 0.65 – 5.64 log_{10} CFU/g with the mean count of 2.64 log_{10} CFU/g.

Discussion

Bacterial proliferation leads to the meat spoilage, thus resulting in decrease in meat's quality and therefore becoming un-edible for the consumers.

According to the studies conducted in Pakistan, Salmonella was more prevalent in chicken samples as 6 out of 10 samples were positive (Ayesha Zafar *et al*., 2016) which is similar to the result in the study done in Hyderabad. Observations showed heavy bacteriological load in the chicken meat with total plate count ranging from 3.26 to 7.36 log_{10} CFU/g. The results indicated the predominance of Gram-negative bacteria such as *E.coli* and *Salmonella* with few Gram negative bacteria such as *S. aureus. Salmonella* spp., were found in 40% of the breast samples where as the samples examined by KOZAČINSKI *et al*., (2006) [7] had only 10.60% contamination by *Salmonella* spp., Total count of *S. aureus* ranged from 1.70 to 3.69 log_{10} CFU/g (KOZAČINSKI *et al*., 2006) [7] which is lower compared to this study that ranged between 1.92 to 5.93 log_{10} CFU/g. According to ALVAREZ-ASTORGA *et al*., (2002) [8, 11], the presence of *S. aureus* is the main reason for the inadequate bacteriological quality of the chicken meat sold in Spanish market (2.47 log_{10} CFU/g in drumsticks and 3.48 log_{10} CFU/g in wings).Total bacterial count of 4.4 log_{10} CFU/g was reported in chicken breast meat by SALEH *et al*., (1997) [9] which is lower than the bacterial count present in the breast meat samples found in the areas of Hyderabad. The average *E.coli* and coliform counts for all samples were observed to be above the acceptable range for E.coli counts as set by Hazard Analysis and Critical Control Point System (HACCP), developed by Food and Agriculture Organization (FAO) and adopted by the Codex Alimentarius Commission (3.911 and 5.0261 log_{10} CFU/g respectively). According to this system, the acceptable food safety range is 2.000 log_{10} CFU/g or less, the intermediate range is over 2.000 log_{10} CFU/g but not above 3.000 log_{10} CFU/g. The unacceptable range is above 3.000 log_{10} CFU/g. Only 20 out of the total 75 samples purchased fell under the acceptable range of E.coli counts. The 25% of the chicken samples collected from retail outlets of Lahore were positive for *Salmonella* (M.U.D. Ahmad *et al*., 2013) which is similar to the present study in which 28% of the samples were contaminated. The contamination level of legs and wings with coli-forms was 3.56 log_{10} cfu/g and 4.27 log_{10} cfu/g respectively. For E.coli, average counts were 2.60 log_{10} cfu/g or 1.78 log_{10}CFU/cm^2 in legs and 3.68 log_{10} cfu/g or 2.86 log_{10} cfu/cm^2 in wings (ALVAREZ –ASTORGA *et al*., 2002) [8, 11]. The mean count of E.coli in leg and wing is 7.19 and 5.18 log_{10} CFU/g which is much higher than the results found in Spain where E.coli average counts were 2.60 log10 cfu/g or 1.78 log10 cfu/cm^2 in leg and 3.68 log10 cfu/g or 2.86 log10 cfu/cm^2 in wings (Maite A´ lvarez-Astorga *et al*., 2002) [8, 11].

Conclusion

It is concluded that the microbial load in raw chicken in areas in and around Hyderabad can be attributed to unhygienic conditions. Results of the present study suggest that a significant risk of the spoilage of meat and an increase in the number of bacteria depend on the specific part of the meat that is analyzed, and also the meat processing and packaging. The study show high incidence of pathogenic microorganisms which is a concern for consumers, suppliers and public health officials.

Conflict of Interest

All the authors don't have any conflict of interest

Acknowledgement

The authors are very much thankful to the University authorities for providing necessary facilities to carry out the present research work.

References

1. Cohen N, Ennaji H, Bouchrif B, Hassar M, Karib H Comparative Study of Microbiological Quality of Raw

Poultry Meat at Various Seasons and For Different Slaughtering Process in Casablanca (Morroco). J Appl Poult Ress. 2007;16:502-508
2. Simmons M, Fletcher DL, Cason JA, Berrang ME. Recovery of Salmonella from retail broilers by a whole-carcass enrichment procedure. J Food Prot. 2003;66:446-450.
3. Van TH, Moutafis G, Tran LT, Coloe PJ. Antibiotic Resistance in Food -Borne Bacterial Contaminants in Vietnam. Appl Environ Microbiol. 2007;73(24):7906-7911.
4. ICMSF (International Commission on Microbiological Specifications for Foods). Microorganisms in Foods 7. Microbiological Testing in Food Safety Management. New York: Kluwer Academic/Plenum Publishers, 2002.
5. Food Safety and Inspection Service: Guidelines for Escherichia coli testing for process control verification in poultry slaughter establishment: HACCP guidelines and policies. 1996. http://www.fsis.usda.gov/wps/portal/fsis/topics/regulatorycompliance/compliance-guides-index#Ecoli.
6. Bhandare SG, Sherikar AT, Paturkar AM, Waskar VS, Zende RJ. A comparison of microbial on sheep/ goat carcasses in a modern Indian abattoir and traditional meat shops. Food Control. 2007;18(7):854-858.
7. Microbiological Evaluation of Raw Meat Products Available in Local Markets of Karachi, Pakistan : Ayesha Zafar, Erum Ahmed, Hafiza Wajiha, and Abdul Basit Khan Kozačinski, l., m.hadžiosmanovič, n.zdolec: Microbiological quality of poultry meat on the Croatian market. Vet. Arhiv. 2006;76:305-313.
8. Alvarez-Astorga M, Capita R, Alonso-calleja C, Moreno B, Del Camoni Garcia-Fernandez M. Microbiological quality of retail chicken by-products in Spain. Meat Sci. 2002;62:45-50.
9. Saleh, Abd EL Atty E, Bauer F, Paulsen P. Shelf life of poultry: Chemical and microbiological changes during storage and spoilage. World congress on food hygiene The Hague, The Netherlands. Proceedings, 1997, 227.
10. Ahmad MUD, Sarwar A, Najeeb MI, Nawaz M, Anjum AA, Ali MA, Mansur N. Assessment of microbial load of raw meat at abattoirs and retail outlets. The Journal of Animal & Plant Sciences, 2013;23(3):745-748 ISSN: 1018-7081.
11. Maite´lvarez-Astorga A, Rosa Capita, Carlos Alonso-Calleja, Benito Moreno, Mari´a del Camino Garci´a-Ferna´ndez: Microbiological quality of retail chicken by-products in Spain, 2002.
12. Abu-ruwaida AS, Sawaya WN, Dashti BH, Murard M, Al-Othman HA. Microbiological quality of broilers during processing in a modern commercial slaughterhouse in Kuwait. J Food Protect. 1994;57:887-892.
13. Capita R, Alonso-Calleja C, Garcia-Arias MT, Moreno B, M. Del Camino Garcia-Fernandez. Methods to detect the occurrence of various indicator bacteria on the surface of retail poultry in Spain. J Food Sci. 2002b;67:765-771.
14. El-Khateib T. Microbiological status of Egyptian salted meat (Basterma) and fresh sausage. J Food Safety. 1997;17:141.
15. Fries R. Reducing Salmonella transfer during industrial poultry meat production. World Poultry Sci. J. 2002;58:527-540.
16. Jablonski LM, Bohach GA. *Staphylococcus aureus* In: Food microbiology: fundamentals and frontiers. (Doyle, M. P., L. R. Beuchat, T. J. Montville, Eds.), American Society for Microbiology, Washington, D. C. 1997, 353-375.
17. Kreyenschmidt J, Peters N, Petersen B, Kunz B. Characterisierung des Verderbs von Frischfleisch - Veränderung mikrobiologischer und biochemischer Parameter von Geflügelfleisch bei unterschidlichen Lagertempraturen. Fleischwirtschaft. 2002;82:108-111.
18. Mead GC, Hudson WR, Hintonm MH. Microbiological survey of five poultry processing plants in the UK. Brit. Poultry Sci. 1993;34:497-503.
19. Mead GC. Hygiene Problems and Control of Process Contamination. In: Processing of poultry. (Mead G. C., Ed.), Elsevier Science Publishers Ltd. 1989, 183-220.
20. Mulder RWAW. Hygiene during transport, slaughter and processing. In: Poultry Meat Science. Poultry Science Symposium Series Volume Twenty-five. Eds. Richardson and Mead. CABI Publishing, 1999, 277-285.
21. Nortjé GI, Nel L, Jordan E, Badenhorst K, Goedhart E, Holzapfel H. The aerobic psychotrophic populations on meat and meat contact surfaces in a meat production system on meat stored at chill temperatures. J Appl. Bacteriol. 1990;68:335-344.
22. Rashad FM. Microbiological studies of Egyptian fresh sausage. Archiv Lebensmittelhyg. 1990;41:11.
23. Stern NJ, Jones DM, Weslex IV, Rolins DM. Colonisation of chick by non-culturable Campylobacter spp. Lett. Appl. Microbiol 1994;18:33-36.
24. Stolle FA. Establishing microbiological surveillance programmes at slaughter-lines - new concept of meat hygiene. Meat Sci. 1988;22:203-211.
25. Živković J. Novi postupci veterinarsko-sanitarne kontrole u proizvodnji mesa peradi. Tehnologija mesa 1990;31:32-36.
26. Živković J, Jakšić S, Mioković B. Salmonella enteritidis in chicken meat and chicken meat products in Zagreb, Croatia. Vet. Arhiv. 1997a;67:167-175.
27. Cohen N, Ennaji H, Bouchrif B, Hassar M, Karib H Comparative Study of Microbiological Quality of Raw Poultry Meat at Various Seasons and For Different Slaughtering Process in Casablanca (Morroco). J Appl Poult Ress. 2007;16:502-508.
28. Van TH, Moutafis G, Tran LT, Coloe PJ. Antibiotic Resistance in Food -Borne Bacterial Contaminants in Vietnam. Appl Environ Microbiol. 2007;73(24):7906-7911.