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DETECTION OF FOOD-BORNE PATHOGENS IN CHICKEN MEAT SOLD IN RETAIL OUTLETS OF CHENNAI CITY

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Abstract- Contaminated poultry meat is regarded as a major source of food-borne pathogens, so the present study was under taken to screen the raw broiler chicken breast meat samples for the presence of food-borne pathogens viz. *Salmonella* spp., *Campylobacter jejuni* and *Staphylococcus aureus* collected from different retail outlets in Chennai city, India. The presence of *Salmonella* spp., *C. jejuni* and *S. aureus* was assessed by cultural method and species-specific PCR assay. The polymerase chain reaction was carried out by targeting the *invA*, *flaA* and *nuc* gene for *Salmonella* spp., *C. jejuni* and *S. aureus* respectively. Out of 60 samples, the frequency of isolation of *S. aureus* and *C. jejuni* was 16.6 and 11.6% respectively by both the methods. However, in case of *Salmonella* spp. the isolation rate was noted to be 10% and 15% by cultural method and PCR assay respectively. The PCR assay was found to be more specific for identification of *Salmonella* spp. Cultural method combined with PCR assay would provide a holistic approach in terms of sensitivity and specificity with regards to identification of these food-borne pathogens. The above findings also indicate the need of creating awareness among general public and butchers operating retail meat shop regarding good hygienic practice before, after cooking and during processing of carcass. Identifying the source of these organisms at the farm level will also help to control the entry of these organisms in food.

INTRODUCTION

Food-borne disease or illness are caused by microorganisms like bacteria, virus, protozoa etc. It occurs either due to ingestion of pathogens that colonise and establish itself in the host's system or due to consumption of food materials where the pathogenic organisms have already established itself and started producing toxins. Based on these, food-borne illness is generally classified into a) Food-borne infection and b) Food-borne intoxication (Bintsis, 2017). Food-borne disease outbreak is defined as the occurrence of two or more similar cases of illness due to consumption of common food (CDC, 2012). With the ever-increasing human population and their frequent movement around the world coupled with movement of foods as a commodity from one place to another presents a new challenge related with food safety (Pal, 2014). The concern about food safety has gained importance mostly in affluent parts of the societies worldwide, although majority of the food-borne diseases are observed in developing parts of the world where it affects the individuals and also retards the economic development of that region (WHO, 2015). In India, majority of the food-borne diseases goes unreported except in urban areas and in place with high morbidities. Microorganisms like *Salmonella* spp., *E coli*, *S. aureus*, *Vibri*o sp., *Yersinia enterocolitica* has been implicated with food-borne illness occurring in India. (NCDC, 2017). Non-typhoidal Salmonellosis and Campylobacteriosis occurs due to consumption of contaminated food and water with these organisms, poultry and its products being regarded as the main source for introduction of these organisms in the human food chain (FAO 2009, Dhama *et al.*, 2013) when compared with other terrestrial animals (CDC, 2017). Staphylococcus are mammalian commensal and opportunistic pathogenic organisms (Haag *et al.*, 2019) which are ubiquitous in nature, although they are mostly found in human and animal's body. They are susceptible to heat but their enterotoxins may retain their biological activity even at 100 °C for 30 minutes (Collins and Huey, 2015). Infected animals can act as a reservoir for humans and it can

also switch its host which is aggravated due to intensive farming of specific animal species (Haag *et al.,* 2019).

Food-borne disease has become a global issue and it requires continuous surveillance to identify the pathogens associated with different types of food in a region. The culture method for detection of food-borne pathogens may require almost days for completion (Bintsis, 2017). Recently *in vitro* amplification of DNA by the PCR method has become a powerful tool in microbiological diagnostics (Malorny *et al.,* 2003). The PCR assay targeting specific gene for different bacteria has been found to be effective and sensitive method of identification, the *invA*gene of *salmonella* contains sequence unique to this genus and has been proved as suitable PCR target with potential diagnostic applications (Rahn *et al.,* 1992), the *nuc* gene for *S. aureus* (Hedge, 2013) and *flaA* gene for *C. jejuni* identification (Dhanalaxmi, 2011).

MATERIALS AND METHODS

Collection of samples

A total of 60 nos. of raw broiler chicken breast meat from retail outlets in Chennai city, India, were randomly collected in a sterile containerfor isolation and identification of *Salmonella, S. aureus* and *C. jejuni.* The collected chicken meat samples were brought to the Meat Science Laboratory, Department of Livestock Products Technology, Madras Veterinary College and were analyzed.

DNA extraction kit and primers

Qiagen® bacterial DNA extraction mini kit for extraction of DNA and both forward and reverse primers targeting genes specific to organisms investigated in this study were custom designed and obtained from M/S Synergy Scientific Services.

Isolation and identification of *Salmonella*

Isolation and identification of *Salmonella spp.*was done as per the procedure mentioned in ISO standard 6579:2002. Non-selective enrichment of *Salmonella* from the meat sample was done in sterile buffered peptone water followed by selective enrichment in Rappaport-Vassiliadis (RV) broth medium (HiMedia). Selective plating of the culture from RV broth medium was done on Xylose deoxycholate agar (HiMedia) and incubated in inverted position for 24 hours at 37 °C. The randomly selected single colonies were used for biochemical analysis for confirmation. The confirmation of the isolates was based on the following tests: - indole, methyl red, Voges-Proskauer, citrate utilization, triple sugar iron and ONPG.

Isolation and identification of *S. aureus*

Isolation and identification of *S. aureus* was done as per the procedure mentioned in ISO standard 6888/1:1999 and 6888/2: 1999. Initially enrichment of *S. aureus* from meat sample was done in buffered peptone water followed by second enrichment in Brain Heart Infusion (BHI) broth (HiMedia) supplemented with 6% Sodium chloride. Selective plating of the culture from BHI broth was done on Mannitol salt agar (HiMedia) and Baird-parker agar (HiMedia). The plates were incubated for 24 hours at 37 °C and randomly selected single colonies were used for biochemical analysis for confirmation. The confirmation of the isolates was based on the following tests: - indole, methyl red, Voges-Proskauer, citrate utilization, urease activity and ONPG.

Isolation and identification of *C. jejuni*

Isolation and identification of *C. jejuni* was done as per the procedure mentioned in ISO standard 10272:1995. Initially enrichment of *C. jejuni* from meat sample was done in buffered peptone water followed by second enrichment in Bolton broth (HiMedia) with selective supplement. Selective plating of the culture from Bolton broth was done on the blood free Campylobacter selective agar (HiMedia) and the plates were incubated at 42 °C under micro aerophilic condition for 24-48 hours. The confirmation of the isolates was based on Hippurate hydrolysis test, urease activity and indoxylacetate hydrolysis test.

PCR Amplification and Gel electrophoresis

The DNA was extracted by boiling method using overnight broth cultures of *Salmonella, Staphylococcus* and *Campylobacter*. The PCR was carried out by targeting bacterial species-specific primers as mentioned in Table 1. A 20 µl of the PCR reaction mixture was made up in 0.2 ml PCR tube with components such as master mix - 10µl, forward primer-1 µl, reverse primer-1 µl, template DNA-3 µl and nuclease free water-5 µl. The PCR amplification was carried out in Master Cycler Gradient Thermo cycler (M/s. Eppendorf, Germany) with the cycling conditions of specific primers mentioned in Table 2.

Table 1. Primer sequence for PCR

Sl.No	Target gene	Primer sequence	Product size (bp)	References
1.	*invA*	***Salmonella spp.* Forward Primer** 5′GTG AAA TTA TCG CCA CGT TCG GGC AA3′ ***Salmonella spp.* Reverse Primer** 5′ TCA TCG CAC CGT CAA AGG AACC 3′	284	Salehi *et al.*, (2005)
2.	*nuc*	***S. aureus* Forward Primer** 5′ GTGCTGGCATATGTATCGCAATTGT3′ ***S. aureus* Reverse Primer** 5′ TACGCCCTTATCTGTTTGTGATGC3′	181	Hedge (2013)
3.	*flaA*	***C.jejuni* forward primer** 5′TCTGCTAAGGCTCCAAGT3′ ***C. jejuni* forward primer** 5′CTCAAGCGGCTCAAGATG3′	367	Dhanalakshmi (2011)

The PCR product obtained was subjected to electrophoresis in 1.5% agarose gel and was documented.

RESULTS AND DISCUSSION

Prevalence of *Salmonella* spp. in raw chicken meat

Poultry meat is the second highest meat consumed worldwide and its consumption has been predicted to increase more rapidly as compared to other meat and it has been attributed as an important source of food-borne illness caused by microorganisms like *Salmonella* (Wessels *et al.,* 2021), *Campylobacter* spp. (Kumar *et al.,* 2020) etc.In this study, we found that the prevalence of *Salmonella* spp. in raw chicken meat by cultural method and PCR assay was 10% and 15% respectively [Table 3 and Fig. 1 (PCR product)]. PCR assay detected all the *Salmonella* which were previously found positive by cultural methods and additionally it detected *Salmonella* in 3 more samples which were earlier not detected by the culturalmethod. Similar results were reported by Whyte *et al.,* 2002 for broiler carcass with 16% and 19% of identification through cultural method and PCR assay. However, frequency of isolation of *Salmonella* was higher when compared to previous findings of Shanmugaswamy *et al.*, 2010 (8.3%) and

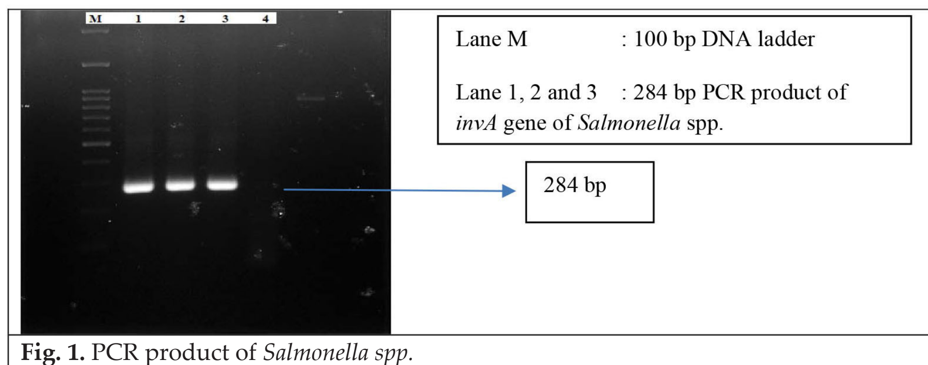

Fig. 1. PCR product of *Salmonella spp.*

Table 2. Reaction conditions for PCR

Sl. No	Parameters	*Salmonella* spp.	*S. aureus*	*C. jejuni*
1.	Initial denaturation	94 °C for 5 min	94°C for 5 min	94 °C for 5 min
2.	Denaturation	94 °C for 1 min	94 °C for 1 min	94 °C for 1min
3.	Annealing	55 °C for 1 min	58 °C for 45 sec	52 °C for 45 sec
4.	Extension	72 °C for 2 min	72 °C for 30 sec	72 °C for 1min
5.	Final Extension	72 °C for 5 min	72 °C for 10 min	72 °C for 10 min
	No of cycles	Sl. No. 2, 3 and 4 were repeated for 35 cycles	Sl. No. 2, 3 and 4 were repeated for 29 cycles	Sl. No 2, 3 and 4 were repeated for 34 cycles

Table 3. Prevalence of food-borne pathogens

Food-borne pathogen	Number of samples screened	Number of positive samples	
		Cultural method	PCR
Salmonella spp.	60	6(10%)	9 (15%)
S. aureus	60	10(16.6%)	10 (16.6%)

Zhao *et al*., 2001 (4.2%) for broiler chicken and lesser than the findings of Saikia and Joshi, 2010 (20%) and Yulistiani *et al*., 2019 (85%). In the current study for identification of *Salmonella*, PCR assay proved to be better than cultural method which might be due to the fact that PCR can detect target sequence in test organisms irrespective of the number of cells present compared to cultural methods. Similar findings were reported by Bennett *et al*., 1998; Amavisit *et al*., 2001 and Whyte *et al*., 2002 for identification of *Salmonella* by PCR. The prevalence of *Salmonella* in raw chicken meat as observed in our study might be due to cross contamination of carcass with intestinal contents and adhered faecal materials on bird's wings, vent during processing (Abd El-Aziz, 2013), the use of contaminated rinse water and reusing the same knife during slaughtering and processing of the meat without sterilization (Yulistiani *et al*., 2019). Additionally in our study, it was also observed that the butchers don't use bleeding cones for bleeding of chicken, rather they use a conventional drum or container for bleeding which are not cleaned regularly for bleeding of birds and the water in the scalding tank is also not changed frequently, thus increasing the chances of cross-contamination ofmeat with the microorganisms (FAO, 2009).

Prevalence of *S. aureus* in raw chicken meat

Identification of *S. aureus* is one of the paramount importance due to its exoprotein and enterotoxin producing nature which may cause various disease symptoms and food poisoning when ingested (Zouharova and Rysanek, 2008) and it is also one of the important community acquired infection (Kourtis *et al*., 2019). In our study, the prevalence rate of *S. aureus* in raw chicken meat by cultural method and PCR assay was found to be 16.6% by

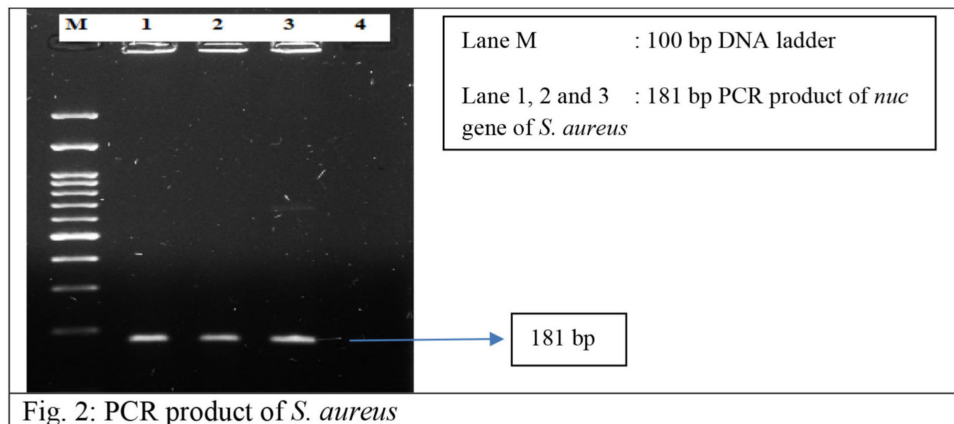

Fig. 2: PCR product of *S. aureus*

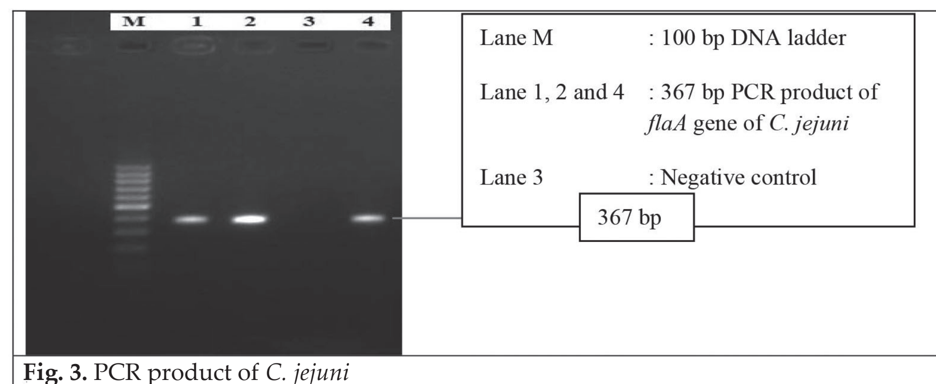

Fig. 3. PCR product of *C. jejuni*

both the methods [Table 3 and Fig. 2 (PCR product)]. There was no difference in percentage of identification of *S. aureus* by both methods. Similar result was reported by Dutta *et al.,* (2020) for the identification of *S. aureus* by cultural method and PCR. The isolation frequency of *S. aureus* observed in this study was higher than the findings of Arul and Sarvanan, 2011 (6.67%) and was lower than findings of Wang *et al*., (2013), Herve and Kumar, 2017 and Wu *et al.,* (2018) who reported incidence of 24.2%, 46.61% and 67.9% respectively. The prevalence of *S. aureus* observed in in our study might be due to cross-contamination from faeces of birds (Syed *et al.,* (2020), humans, cages where the birds are stocked in retailer's shop (Wu *et al*., (2018) as it was noted during the study period that the butchers don't wash their hands regularly after handling live birds and also subsequently handles the carcass with the same hands.

Prevalence of *C. jejuni* in raw chicken meat

Raw chicken meat and derived products are considered to be major source of Campylobacter and rate of contamination can be as high as 100% (Atanassova *et al.,* 1999). *C. jejuni* is mainly isolated from poultry samples however it is the leading cause of Campylobacteriosis in humans (Nastos *et al.,* 2019). In this study, no difference was observed in between cultural method and PCR in rate of isolation, indicating equal reliability of both techniques and this was in accordance with findings of Singh *et al.,* 2011 and Denis *et al.,* 2001. The prevalence of Campylobacter in raw chicken meat in our study was 14.4% by both culturalmethod and PCR assay [Table 3 and Fig 3 (PCR product)]. The findings in the present study were lower than the findings of Khan *et al.,* 2018 in Bareilly city, India, Granic*et al.,* 2009 in Medimurje County and Abd El-Azizand Abd-Allah, 2017 in Assiut city, Egyptwith 38.6%, 66.6% and 87.5% prevalence respectively.The prevalence of *C. jejuni* as observed in our study might be due to contamination of the carcass with the intestinal contents during evisceration process (FAO 2009) or through butcher's hand (Sindhi *et al.,* 2020). Scalding, defeathering and poor hygiene during transportation has also been attributed to be the routes of cross contamination in case of *C. jejuni* in case of chickens (Shane, 1992). From the current study it can be appreciated that PCR assay provides rapid response for identification of bacteria with better specificity and becomes more reliable method for identification of food-borne pathogens (Chen *et al.,* 1997; Rychlik *et al.,* 1999) than cultural method. Although cultural techniques are universally recognised as standard method for detection of food-borne pathogens, however major limitations of culture-based method are its inability to detect sub lethally injured or viable non culturable cells in foods and its time taking nature which may range from 4 to 7 days (Whyte *et al.,* 2002).

CONCLUSION

The presence of human enteropathogens viz. *Salmonella, S. aureus* and *C. jejuni* in raw chicken meat is of major public health concern and it indicates that raw chicken meat can act as a vehicle for transmission of these food-borne pathogens to humans unless the product is cooked well before consumption. In order to prevent this, suitable strategies like creating awareness among the general public and butchers operating retail meat shop regarding good hygienic practice before, after cooking and during processing level has to be created. Also, regular monitoring of microbiological quality and hygiene of retail meat shops by the concerned authority would be a welcome step in identifying food-borne pathogens and check their entry in food. Moreover, the method of identification will depend on the level of identification required with PCR assay providing greater sensitivity and specificity in identification of food-borne pathogens. Further studies like antimicrobial resistance pattern of these organisms may be conducted.

REFERENCES

Abd El-Aziz, D. and Abd-Allah, S. 2017. Incidence of Campylobacter species in wholesale chicken carcasses and chicken meat products in Assiut city, Egypt. *Int. Food Res. J*. 24(6): 2660-2665.

Abd El-Aziz, D.M. 2013. Detection of Salmonella typhimurium in retail chicken meat and chicken giblets. *Asian Pacific Journal of Tropical Biomedicine*. 3(9): 678-681.

Amavisit, P., Browning, G.F., Lightfoot, D., Church, S., Anderson, G.A., Whithear, K.G. and Markham, P.F. 2001. Rapid PCR detection of Salmonella in horse faecal samples. *Veterinary Microbiology*. 79(1): 63-74.

Arul, K.T. and Saravanan, S., 2011. Assessment of contamination in chicken meat by food-borne *Staphylococcus aureus*. *Int J Res Pure Appl Microbiol*. 1(3): 59-60.

Atanassova, V. and Ring, C. 1999. Prevalence of *Campylobacter* spp. in poultry and poultry meat in

Germany. *International Journal of Food Microbiology*. 51: 187-190.

Bennett, A.R., Greenwood, D., Tennant, C., Banks, J.G. and Betts, R.P. 1998. Rapid and definitive detection of *Salmonella* in foods by PCR. *Letters in Applied Microbiology*. 26(6): 437-441.

Bintsis, T. 2017. Foodborne pathogens. *AIMS Microbiology*. 3(3): 529-563.

CDC 2012. Surveillance for Foodborne Disease Outbreaks United States, 2012: Annual Reporthttps://www.cdc.gov/foodsafety/pdfs/foodborne-disease-outbreaks-annual-report-2012 508c.pdf

CDC 2017. Surveillance for foodborne disease outbreaks United States, 2017: Annual report. https://www.cdc.gov/fdoss/pdf/2017_FoodBorneOutbreaks_508.pdf

Chen, S., Yee, A., Griffiths, M., Larkin, C., Yamashiro, C.T., Behari, R., Paszko-Kolva, C., Rahn, K. and Stephanie, A. 1997. The evaluation of a fluorogenic polymerase chain reaction assay for the detection of *Salmonella* species in food commodities. *International Journal of Food Microbiology*. 35: 239-250.

David S. Collins and Robert J. Huey, 2015. Preservation of meat. In: *Gracey's Meat Hygiene.* 11th Edition, John Wiley & Sons, Ltd. The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK. pp. 67-88.

Denis, M., Refrégier Petton, J., Laisney, M.J., Ermel, G. and Salvat, G. 2001. Campylobacter contamination in French chicken production from farm to consumers. Use of a PCR assay for detection and identification of *Campylobacter jejuni* and Camp. coli. *Journal of Applied Microbiology*. 91: 255-267.

Dhama, K., Rajagunalan, S., Chakraborty, S., Verma, A.K., Kumar, A., Tiwari, R. and Kapoor, S. 2013. Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: a review. *Pakistan Journal of Biological Sciences*. 16(20): 1076-1085.

Dhanalakshmi, M. 2011. *Zoonotic prevalence of Campylobacter by molecular methods*, M.V.Sc., Thesis submitted to Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-7.

Dutta, N., Banga, H.S., Deshmukh, S., Leishangthem, G.D. and Singh, N.D. 2020. 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. *Int. J. Curr. Microbiol. App. Sci*. 9(7): 2095-2101.

FAO 2009. *Salmonella* and *Campylobacter* in chicken meat. https://www.fao.org/3/i1133e/i1133e.pdf

Graniæ, K., Krèar, D., Uhitil, S. and Jakšiæ, S. 2009. Determination of *Campylobacter* spp. in poultry slaughterhouses and poultry meat. *Veterinarski Arhiv*. 79(5): 491-497.

Haag, A.F., Fitzgerald, J.R. and Penadés, J.R. 2019. *Staphylococcus aureus* in Animals. *Microbiology Spectrum*. 7(3) : 1-19.

Hedge, 2013. *Identification and characterization of Staphylococcus aureus and Streptococcus in bovine mastitis milk*. Thesis submitted to Karnataka Veterinary Fishery and Animal Sciences University, Bidar, Karnataka.

Herve, D.T. and Kumar, G. 2017. Prevalence of *Staphylococcus aureus* in retail chicken meat samples in Jalandhar, Punjab. *Research Journal of Pharmacy and Technology*. 10(1) : 281-285.

ISO 10272:1995. Microbiology of food and animal feeding stuffs. Horizontal method for detection of thermotolerant Campylobacter. International Standardization Organization ed., Geneva, Switzerland.

ISO 6579:2002. Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Salmonella* spp. International Standardization Organization ed., Geneva, Switzerland.

ISO 6888/1:1999. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive Staphylococci (*Staphylococcus aureus* and other species)-Part 1: Technique using Baird-Parker agar medium. International Standardization Organization ed., Geneva, Switzerland.

ISO 6888/2:1999. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive Staphylococci (*Staphylococcus aureus* and other species)-Part 2: Technique using rabbit plasma fibrinogen agar medium. International Standardization Organization ed., Geneva, Switzerland.

Khan, J.A., Rathore, R.S., Abulreesh, H.H., Qais, F.A. and Ahmad, I. 2018. Prevalence and antibiotic resistance profiles of *Campylobacter jejuni* isolated from poultry meat and related samples at retail shops in Northern India. *Foodborne Pathogens and Disease*. 15 : 1-8.

Kourtis, A.P., Hatfield, K., Baggs, J., Mu, Y., See, I., Epson, E., Nadle, J., Kainer, M.A., Dumyati, G., Petit, S. and Ray, S.M. 2019. Vital signs: epidemiology and recent trends in methicillin-resistant and in methicillin-susceptible *Staphylococcus aureus* bloodstream infections—United States. *Morbidity and Mortality Weekly Report*. 68(9) : 214-219.

Kumar, H., Bhardwaj, K., Kaur, T., Nepovimova, E., Kuèa, K., Kumar, V., Bhatia, S.K., Dhanjal, D.S., Chopra, C., Singh, R. and Guleria, S. 2020. Detection of Bacterial Pathogens and Antibiotic Residues in Chicken Meat: A Review. *Foods*. 9(1504): 1-35.

Malorny, B., Hoorfar, J., Bunge, C. and Helmuth, R. 2003. Multicenter validation of the analytical accuracy of *Salmonella* PCR: towards an international standard. *Applied and Environmental Microbiology*. 69(1) : 290-296.

Natsos, G., Mouttotou, N.K., Ahmad, S., Kamran, Z., Ioannidis, A. and Koutoulis, K.C. 2019. The genus Campylobacter: detection and isolation methods, species identification and typing techniques. *Journal of the Hellenic Veterinary Medical Society*. 70(1) : 1327-1338.

NCDC 2017. CD alert. Food-borne diseases and food safety in India. https://ncdc.gov.in/WriteReadData/linkimages/CD%20Alert4053017156.pdf

Pal, M., Demissie, D., Demissie, K. and Abera, F., 2014. Microbial Food Safety: A Challenge to Public Health. *Beverage and Food World*. 41(11): 39-41.

Rahn, K., De Grandis, S.A., Clarke, R.C., McEwen, S.A., Galan, J.E., Ginocchio, C., Curtiss Iii, R. and Gyles, C.L. 1992. Amplification of an invA gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of Salmonella. *Molecular and Cellular Probes*. 6(4): 271-279.

Rychlik, I., Van Kesteren, L., Cardova, L., Švestková, A., Martinkova, R. and Šišák, F. 1999. Rapid detection of *Salmonella* in field samples by nested polymerase chain reaction. *Letters in Applied Microbiology*. 29 : 269-272.

Saikia, P. and Joshi, S.R. 2010. Retail market poultry meats of North-East India–a microbiological survey for pathogenic contaminants. *Res J Microbiol*. 5(1): 36-43.

Salehi, T.Z., Mahzounieh, M. and Saeedzadeh, A. 2005. Detection of invA gene in isolated *Salmonella* from broilers by PCR method. *Int. J. Poult. Sci*. 4(8): 557-559.

Shane, S.M. 1992. The significance of Campylobacter jejuni infection in poultry: a review. *Avian Pathology*. 21 : 189-213.

Shanmugasamy, M., Velayutham, T. and Rajeswar, J. 2011. Inv A gene specific PCR for detection of *Salmonella* from broilers. *Veterinary World*. 4(12): 562-564.

Sindhi, S.H., Mathapati, B.S., Parmar, V.L., Marandi, S., Sumankumar, M., Javia, B.B., Ghodasara, S.N., Kathiriya, J.B., Bhedi, K.R. and Patel, J.S. 2020. Prevalence of *Campylobacter* spp. Isolated from Poultry, Human and Environment in Junagadh District of Gujarat, India. *Int. J. Curr. Microbiol. App. Sci*. 9(9) : 3319-3326.

Singh, H., Rathore, R.S., Singh, S. and Cheema, P.S. 2011. Comparative analysis of cultural isolation and PCR based assay for detection of *Campylobacter jejuni* in food and faecal samples. *Brazilian Journal of Microbiology*. 42 : 181-186.

Syed, M.A., Ullah, H., Tabassum, S., Fatima, B., Woodley, T.A., Ramadan, H. and Jackson, C.R. 2020. Staphylococci in poultry intestines: a comparison between farmed and household chickens. *Poultry Science*. 99: 4549-4557.

Wang, X., Tao, X., Xia, X., Yang, B., Xi, M., Meng, J., Zhang, J. and Xu, B. 2013. *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in retail raw chicken in China. *Food Control*. 29(1): 103-106.

Wessels, K., Rip, D. and Gouws, P. 2021. *Salmonella* in chicken meat: Consumption, outbreaks, characteristics, current control methods and the potential of bacteriophage use. *Foods*. 10(1742): 1-20.

WHO, 2015. Who estimates of the global burden of foodborne diseases: Foodborne disease burden epidemiology reference group 2007-2015.https://apps.who.int/iris/bitstream/handle/10665/199350/?sequence=1

Whyte, P., Mc Gill, K., Collins, J.D. and Gormley, E., 2002. The prevalence and PCR detection of *Salmonella* contamination in raw poultry. *Veterinary Microbiology*. 89(1): 53-60.

Wu, S., Huang, J., Wu, Q., Zhang, J., Zhang, F., Yang, X., Wu, H., Zeng, H., Chen, M., Ding, Y. and Wang, J., 2018. *Staphylococcus aureus* isolated from retail meat and meat products in China: incidence, antibiotic resistance and genetic diversity. *Frontiers in Microbiology*. 9,2767: 1-14.

Yulistiani, R. and Praseptiangga, D. 2019. Occurrences of *Salmonella* spp. and *Escherichia coli* in chicken meat, intestinal contents and rinse water at slaughtering place from traditional market in Surabaya, Indonesia. In *IOP Conference Series: Materials Science and Engineering*. 633,012007: 1-6.

Zhao, C., Ge, B., De Villena, J., Sudler, R., Yeh, E., Zhao, S., White, D.G., Wagner, D. and Meng, J. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella serovars* in retail chicken, turkey, pork, and beef from the Greater Washington, DC, area. *Applied and Environmental Microbiology*. 67(12): 5431-5436.

Zouharova, M. and Rysanek, D. 2008. Multiplex PCR and RPLA Identification of *Staphylococcus aureus* enterotoxigenic strains from bulk tank milk. *Zoonoses and Public Health*. 55 : 313-319.