Original Research

Cultural Characterization of *Salmonella* spp Isolated from Poultry Samples

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Abstract

The prevalence of Salmonella spp in poultry (meat, egg and cloacal swabs) samples collected in and around Hyderabad by cultural and the Polymerase Chain Reaction (PCR) methods. A total of 150 poultry samples (50 each of meat, egg and cloacal swabs) were tested of which 45 and 50 samples were positive for Salmonella spp by cultural method and PCR assay accounting for 30% and 33.3% respectively. Out of 50 poultry meat samples 17 samples (34%) were positive for Salmonella spp by cultural method, whereas 19(38%) were positive by PCR assay. Out of 50 each of egg shell and cloacal swab samples 15(30%) and 13(26.0%) respectively were positive by cultural method whereas 17(34%) and 14(28%) respectively were positive by PCR assay.

Key words: Egg Shells and Cloacal Swabs, Prevalence, Poultry Meat, *Salmonella spp*

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Introduction

Salmonella spp is short bacilli, 0.7- 1.5 x 2.5 µm, gram-negative, aerobic or facultative anaerobic, catalase positive, oxidase negative. Genus *Salmonella spp* is subdivided into 2,541 serovars, such as *Salmonella typhimurium* and *Salmonella enteritidis*, which are promiscuous and can cause infections in different species, including poultry and humans. The route of infection is the fecal-oral route via food or water contaminated with faeces or urine of previously infected persons or animals (Orji *et al*., 2004).

Salmonella spp is generally divided into two categories typhoidal and non-typhoidal group. Typhoidal *Salmonella spp*, which causes typhoid fever and is caused by *S.typhi*, *S.paratyphi* A, *S.paratyphi* B and *S. paratyphi* C are carried only by humans. Non-typhoidal *Salmonella spp* is the most common form, responsible for the food borne salmonellosis and is carried by both humans and animals.

The poultry farming in India has been seen as a lucrative business and has emerged as one of the major sources of the much-desired animal protein over the years. But the industry is facing great setbacks due to frequent outbreaks of salmonellosis, causing serious economic loss manifested by reduced egg production and mortality of poultry (Khan *et al*., 2005). Although many developed countries have eradicated the disease from commercial poultry, it has increased incidence in most developing countries leading to gastroenteritis and deaths in both developed and developing countries (Majowicz *et al*., 2010; Rothrock *et al*., 2015). The main cause of *Salmonella spp* infection handling of raw poultry carcasses and products and consumption of undercooked poultry meat. A link between the prevalence of *Salmonella spp* infection in poultry and human cases of Salmonellosis was identified in a risk assessment conducted by the Food and Agriculture organisation of the United Nations and the World Health Organisation (WHO, 2007).

India is one of the important producers and exporting country in the world for both chicken meat and eggs. Taking into consideration of all issues of poultry industry, the present study was undertaken to study the prevalence of *Salmonella spp* in poultry products.

Material and Methods

Sample Collection

A total of 150 samples (50 each of meat, egg and cloacal swabs) were collected from local poultry farms, college farms and markets from in and around Greater Hyderabad Municipal Corporation, Telangana state. All the samples were transported to the Department of Veterinary Public Health and Epidemiology laboratory, PV Narasimha Rao Telangana Veterinary University, Rajendranagar immediately after collection in an insulating foam box with ice maintaining the temperature from 4 ^{0}C to 6 ^{0}C (WHO, 2010) and stored under refrigeration till further analysis.

Bacterial Isolation

For isolation and identification of *Salmonella spp* a modified method followed by Hatha and Lakshmanaperumalsamy (1997) was used. The samples were pre-enriched in Buffered Peptone Water (BPW) at 37 °C for 24 hours followed by selective enrichment in Rappaport Vassidialis broth (RVB) at 37°C for 24h. After 24h of selective enrichment, a loopful of cultures from RV were streaked onto selective media such as Xylose Lysine Deoxycholate agar (XLD) (Hi media, India) plates and incubated at 37 °C for 24 to 48 hours. On XLD, colonies showing typical characteristics, like translucent with typical black center in case of H_2S producers and translucent red colonies in non H_2S producers were presumed to be *Salmonella spp* (OIE,2010). The presumptive *Salmonella spp* colonies were subjected to gram staining and catalase tests. Colonies showing Gram negative coco-bacillary rods morphologically and catalase positive reaction were stored for further confirmation biochemically using KB001 HiIMVic Biochemical test kit from HiMedia laboratories Private Ltd.

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PCR assay

In this study, the template preparation of *Salmonella spp* from culture was done by phenol chloroform method. PCR assay was performed in Eppendorf gradient Thermal cycler with a heated lid. PCR assay was carried out on extracted DNA targeting invasion A gene (*inv*A) which is genus-specific, as per method described by Cocolin *et al.* (1998) with little modification. The primers used from the *inv*A gene for the detection of *Salmonella spp*. respectively were custom synthesized by Xcelris labs limited are given in Table 1.

Table 1: Primers used for identification of *Salmonella spp*.

Primer	Target gene	Length	Primer sequence	Amplification Product (bp)	Reference
Salm-3	*inv*A	20	GCT GCG CGC GAA CGG CGA AG	389	Cocolin *et al.* (1998)
Salm-4	*inv*A	19	TCC CGG CAG AGT TCC CAT T	389	

The PCR protocol was initially standardized by optimizing the concentration of the components of the reaction mixture in the PCR assay involved the empirical variation of annealing temperature (58ºC-66ºC), concentration of primer (5-15pmol), magnesium chloride (1.5mM-3mM), template volume (2µl-8µl) and the cycling conditions. Optimal results were obtained using 5µl of the bacterial lysate or 20ng of purified DNA, 2.5 µl of 10x assay buffer for Taq polymerase containing 1.5 mM $MgCl_2$, 1 µl of 25µM each dNTP mix, 2 µl each of forward and reverse primer (4pmol) and 0.9U/µl of Taq DNA polymerase made up to 25 µl using molecular grade water. Desired PCR product of 389 bp to detect *Salmonella spp* was obtained with cycling conditions presented in Table 2.

Table 2: Cycling conditions used for *Salmonella spp* primers

S. No.	Step	***inv*A (*Salmonella spp.*)**
1	Initial denaturation	95ºC /5min
2	Final denaturation	95ºC/1min
3	Annealing	58ºC/80sec
4	Initial extension	72ºC/45 sec
5	Final extension	72ºC/7min
6	Hold	4ºC

Agarose gel (1.5%) was prepared by boiling agarose in an appropriate volume of 1X TAE buffer. After cooling for about 3 min, ethidium bromide (Biogene, USA) was added to the agarose solution to a final concentration of 0.5µg/ml. The molten agarose was then poured into the tray and the comb was fitted into the slots on the tray. The tray was kept undisturbed till the gel had solidified. The comb was then taken out carefully and the tray containing the gel was then placed in a submarine horizontal electrophoresis unit filled with 1xTAE buffer upto a level of 1mm above the gel surface. About 5 µl of each PCR product was

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mixed with 2 µl of bromophenol blue (6x) loading dye and loaded into each well. Electrophoresis was performed at 5 V/cm and the mobility was monitored by the migration of the dye. After sufficient migration, the gels were observed under UV transilluminator to visualize the bands. The PCR product size was determined by comparing with a standard molecular weight marker and was photographed by the gel documentation system.

Results and Discussion

The prevalence of *Salmonella spp* in poultry samples by cultural and PCR methods are presented in Table 3. Out of 150 poultry samples (50 each of meat, egg and cloacal swabs) 45 and 50 samples were positive for *Salmonella spp* by cultural method and PCR assay accounting for 30% and 33.3% respectively. The efficiency of cultural method compared to PCR assay was 90.2%. Compared to the prevalence of *Salmonella spp* in poultry pooled samples in present study (30.0%) by cultural method, low prevalence of 8%, 23.5% and 26.27% were reported by Sudhantirakodi *et al*. (2016), tran *et al*. (2005) and Suresh *et al*. (2011) respectively.

Out of 50 poultry meat samples 17 samples (34%) were positive for *Salmonella spp*, whereas 19 (38%) were positive by PCR assay. The prevalence of 35.5%, 35.8% and 36.7% in chicken samples reported by Rusul *et al*. (1996), Dominguez *et al*. (2002) and Uyttendaele *et al*. (1998) respectively, were almost similar to the prevalence (34%) by cultural in the present study. A prevalence of 29.4%, 28.3%, 25%, 23.7%, 22.8%, 21.99%, 19.2% and 18% was reported by Elkenany *et al*. (2019), Moon (2011), Kaushik *et al*. (2014), Jorgensen *et al*. (2002), Plummer *et al*. (1995), Rahman *et al*. (2004), Van nierop *et al*. (2005) and Cortez *et al*. (2006) respectively by cultural method, which were less than the prevalence in present study. High prevalence of 66.0%, 60.0%, 57.0%, 53.3%, 52.2%, 49.3%, 49%, 42.3% and 40% was reported by Jerngklinchan *et al*. (1994), Chuananchuen and Padungtod (2009), Antunes *et al*. (2003), Van *et al*. (2007), Yang *et al*. (2011), Vuthy *et al*. (2017), Capita *et al*. (2007), Suresh *et al*. (2006) and Sudhanthirakodi *et al*. (2016) respectively than the prevalence in the present study.

Very high prevalence of *Salmonella spp* i.e. 93% and 83% in chicken samples from South Africa and Brazil respectively was reported by Zishiri *et al*. (2016). A wide range of 10-50% and 6.79-97.6% prevalence of *Salmonella spp* in chicken samples was reported by Uttah *et al*. (2013) and Ramya *et al*. (2012) respectively. The prevalence of *Salmonella spp* in chicken samples by PCR assay was 38% in present study which was less than the prevalence of 51% observed by Zishiri *et al*. (2016). Very low prevalence in the chicken by PCR i.e. 9.2% and 6.5% was reported by Nwiyi *et al*. (2015), Garba *et al*. (2017) respectively. Out of 50 egg shell surface swab samples 15 (30%) and 17(34%) were positive by cultural and PCR respectively in the present study. A prevalence of 9.01%, 9.0%, 7%, 6.1% and 1% were reported by Rock (2000), Gordon (1996), Evans *et al*. (1998), Suresh *et al*. (2006), Perales and Audicana (1989) respectively which are less

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than the prevalence in the present study, whereas no prevalence was reported by Mawer *et al*. (1989) and Sudhanthirakodi *et al*. (2016). Almost similar to the prevalence (30% and 34%) by cultural and PCR respectively in the present study, a prevalence of 33% and 35% was reported by Mai *et al*. (2013) and Ishola *et al*. (1999) respectively on the egg surface.

Table 3: Prevalence of *Salmonella spp* in poultry by cultural and PCR methods

Type of Sample	No. of Samples	Cultural Method	PCR assay
Meat	50	17 (34%)	19 (38%)
Egg shell	50	15 (30%)	17 (34%)
Cloacal swabs	50	13 (26%)	14 (28%)
Total	150	45 (30%)	50 (33.3%)

In the present study out of 50 cloacal swab samples 13(26.0%) and 14(28%) were positive by cultural and PCR respectively. Lower prevalence of 22%, 21.3%, 15.6, 14.7%, 11.0% and 10.9% were reported by Sudhanthirakodi *et al*. (2016), Cheong *et al*. (2007), Fasure *et al*. (2012), Murugkar *et al*. (2005), Fashae *et al*.(2010) and Chiu *et al*.(2010) respectively, whereas very low prevalence of 1.40% was reported by Suresh *et al*. (2011). A prevalence (3-4%) of Salmonella *spp* in cloacal swabs during pre-recommendations of biosecurity measures observed by Velasquez *et al*.(2018) which increased to 5-14% during post recommendations period which was contrary to the sanitary and hygiene principles.

Conclusion

The prevalence of *Salmonella spp* in Poultry samples is high and quite alarming in the present study. Also, the last few decades have witnessed the emergence of highly virulent *Salmonella spp*, due to its extra-animal environmental strongholds causing greater morbidity and mortality in humans. Therefore, necessary corrective measures need to be adapted for prevention and control of *Salmonella spp* by adopting hygienic practices during production and slaughter practices.

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