

Isolation and molecular confirmation of Non-Typhoidal Salmonella (NTS) from desi chicken farms in Tamil Nadu: Significance in public health

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ABSTRACT

The present research work was conducted to study the prevalence of NTS (Non-typhoidal Salmonella) in desi chicken farms in Namakkal district of Tamil Nadu state, India. For the study, fresh faecal samples were collected from 30 desi chicken farms located in Namakkal district. The samples were subjected to cultural examination for isolation and identification of Non-typhoidal *Salmonella*. The samples found to be positive in cultural isolation were subjected to molecular screening by PCR for confirmation. In the present study, out of 30 farms investigated, samples from seven farms were found to be positive for Non-typhoidal *Salmonella* and all the positive samples were confirmed to be *S. typhimurium*. The study indicates that small scale poultry production with desi chicken is also becoming source of NTS infection in human beings apart from organized commercial poultry sector. Creating awareness on prevalence of NTS in desi chicken and giving trainings to meat handlers and butchers regarding hygienic slaughter and food safety will minimize contamination of desi chicken meat with non-typhoidal *Salmonella*.

Keywords: Desi chicken, India, NTS, Public health, *Salmonella*

Salmonella are the enteric pathogens belonging to the family Enterobacteriaceae. *Salmonella* are classified into typhoidal and non-typhoidal *Salmonella* (NTS). Poultry can get infected either with specific *Salmonella* serovars, like *S. pullorum* and *S. gallinarum*, which cause a typhoid-like systemic disease or by wide range of NTS.

Foodborne and waterborne diarrhoeal illnesses kill an estimated population of 2.2 million people every year globally and most of them are children (Nata Menabde 2015). Non-typhoidal *Salmonella* are neglected group of enteric pathogens whose prevalence is increasing at alarming rates across India. The major serovars involved in NTS are *S. Typhimurium* and *S. enteritidis*, particularly *Salmonella enteric* var *typhimurium* is the most frequently isolated serovar from chicken meat (Nagappa *et al.* 2007). Non-typhoidal *Salmonella* normally localize in the intestinal tract of livestock and poultry. The birds infected with NTS shed the organism into environment through faeces without showing any clinical signs.

Human infections with NTS is being associated with gastro enteritis, food poisoning, bacteremia and extra intestinal focal infection in infants such as meningitis and may also result in serious complications among the elderly and immunocompromised patients (Bhowmick

et al. 2012). Meat and poultry products are recognized as the major sources for transmission of *Salmonella* spp. The chicken meat can be contaminated with *Salmonella* from intestinal contents, faecal material or from cross-contamination during slaughtering process. Human beings get infection through ingestion of undercooked meat and raw eggs contaminated with NTS. More than 2500 serotypes of *Salmonella* have been reported, but only about 10% of these have been isolated from poultry (Grimont and Weill 2007). *Salmonella* isolates are also obtained from meat of desi chicken apart from commercial layers and broilers which go unnoticed most of the times. Since there is increasing demand for desi chicken meat nowadays, it is essential to ensure that the meat obtained from desi chicken is hygienic.

The cross-contamination between meat, personnel and equipment during the processing of meat due to ineffective cleaning and disinfection particularly with chopping boards, knives and tables were the risk factors for *Salmonella* contamination (Upadhyaya *et al.* 2012). Microbial load of raw meat can be attributed to unhygienic conditions in slaughter houses and transportation (Ahmad *et al.* 2013, Bhandhari *et al.* 2013).

Hence, the present work on isolation, identification and molecular confirmation of Non typhoidal *Salmonella* (NTS) in desi chicken will enlighten the serotypes of *Salmonella* involved in food borne infection and thus the ways and means for effective containment of such infection in food chain can be worked out.

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MATERIALS AND METHODS

Collection of faecal samples from desi chicken: Fresh faecal samples (20 g of pooled samples) were collected from 30 desi chicken farms located in and the samples were processed.

Cultural isolation and identification: The samples were collected in brain heart infusion (BHI) broth and incubated at 36°C for 16 h (non-selective pre-enrichment). 100 µL broth was inoculated on Modified Semisolid Rappaport Vassiliadis (MSRV) agar supplemented with novobiocin (selective media for motile *Salmonella*) and incubated at 42-43°C for 24 h. A loopful of inoculum from the periphery of suspected *Salmonella* growth on MSRV agar was inoculated in Xylose-Lysine Deoxycholate (XLD) agar and the plates were incubated at 37°C for 24 h. Characteristic red colonies with black centre was observed in XLD agar plates indicative of *Salmonella* spp. Individual colonies from XLD agar plates were inoculated in blood agar and incubated at 37°C for 24 h.

Biochemical tests: The colonies from XLD agar plates were stabbed into TSI (Triple sugar iron) slant and incubated at 37°C for 24 h and observed for acid, gas and H₂S production. Colonies from XLD agar plates were also stabbed in to urease slant and incubated at 37°C for 24 h to eliminate non-Salmonella organisms if any in the study.

Molecular confirmation: Molecular confirmation of Non-typhoidal *Salmonella* by polymerase chain reaction (PCR) was carried out in samples found to be positive in cultural isolation. Serotype-specific PCR was used for specific identification of *S. enteritidis* and *S. typhimurium*.

PCR for confirmation of *Salmonella* spp.: DNA was extracted from the colonies in blood agar plates and PCR was conducted with primers targeting ST 11 and ST 15 gene at 429 bp for confirmation of *Salmonella* spp., The sequences of the primer pair used for targeting random sequence for confirmation of *Salmonella* spp., were 5' AGCCAACCATTTGCTAAATTGGCGCA-3' and 5'-GGTAGAAATTCCCAGCGGGTACTGG-3' (Soumet *et al.*, 1999). The primer pair used for targeting the *sdfI* gene at 304 bp for confirmation of *S. enteritidis* was 5'-TGTGTTTTATCTGATGCAAGAGG-3' and 5'-TGAACACGTTTCGTTCTTCTGG-3' (Alvarez *et al.* 2004). The primer pair used for targeting the *fliC* gene at 620 bp for confirmation of *S. typhimurium* was 5'CGGTGTTGCCAGGTTGGTAAT-3' and

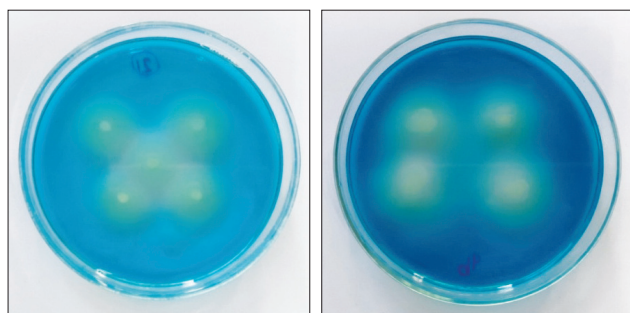


Fig. 1. *Salmonella* suspected growth in MSRV media.

5'-ACTGGTAAAGATGGCT-3' (Soumet *et al.* 1999). PCR amplification was performed with the following conditions: Initial denaturation for 2 min. at 95°C; 30 cycles of: 1 min. at 95°C, 1 min. at annealing temp. 54°C, 2 min. at 72°C; 1 cycle for 5 min. at 72°C for *Salmonella* spp. In PCR for confirmation of *Salmonella* serotypes, the conditions for PCR amplifications were initial denaturation at 2 min at 94°C; 30 cycles of: 1 min at 94°C, 1 min. at annealing temperature 53°C, 1 min at 72°C; 1 cycle for 7 min at 72°C.

RESULTS AND DISCUSSION

Cultural isolation and identification: On cultural isolation, out of 30 samples collected, white zone of growth was noticed in MSRV media (Fig. 1) for eight samples indicative of motile *Salmonella* in positive cases. Characteristic red colonies with black centre were noticed in XLD agar plates (Fig. 2 A) in positive samples and no growth was observed in control plates (Fig. 2 B). White translucent colonies (Fig. 3) were observed in blood agar plates in positive samples.

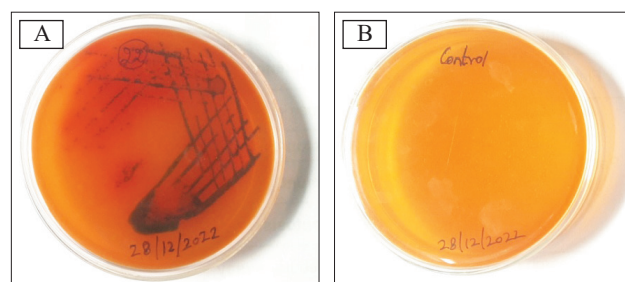


Fig. 2. (A) Growth of *Salmonella* (red colonies with black centre) in XLD agar media; (B) Control plate with no growth.

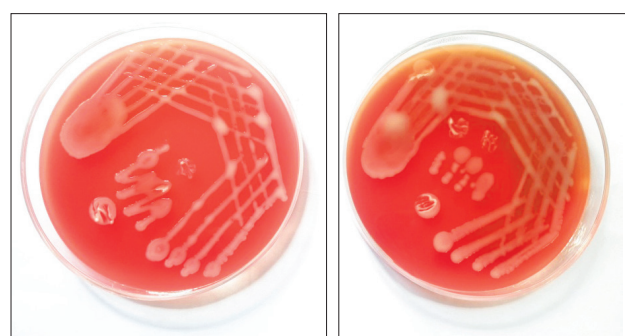


Fig. 3. White translucent colonies in blood agar plates.

Biochemical tests: *Salmonella* positive samples showed positive result in TSI slant with growth of black colonies, acid butt, alkaline slant and negative result for urease test.

Molecular confirmation by PCR

Confirmation of *Salmonella* spp.: All the samples which were positive in cultural isolation were confirmed positive for *Salmonella* spp., in PCR by amplification product of 429 bp targeting ST 11 and ST 15 gene in agarose gel electrophoresis (Fig. 4).

Serotyping of non-typhoidal *Salmonella*: Out of 8 samples confirmed positive for *Salmonella* spp., by PCR, 7 samples were found positive for *S. typhimurium* that

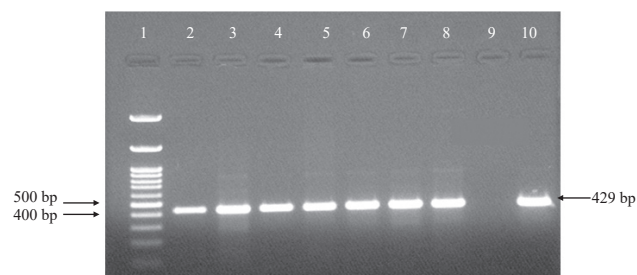


Fig. 4. *Salmonella* spp., - ST 11 and ST 15 gene - 429 bp on agarose gel electrophoresis [Lane -1, 100 bp ladder; Lane -2 to 8, Positive samples; Lane 9, Negative control; Lane 10, Positive control].

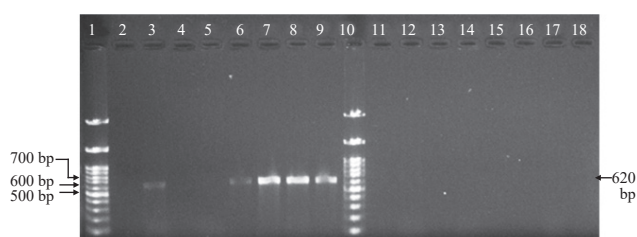


Fig.5. *S. typhimurium* - fliC gene - 620 bp on agarose gel electrophoresis [Lane - 1,10, 100 bp ladder; Lane - 2 to 9, 11 to 18, Samples].

amplified at 620 bp targeting fliC gene on agarose gel electrophoresis and none of the samples showed positive result for *S. Enteritidis* in PCR (Fig. 5).

Incidence of NTS in desi chicken: In the present study, out of 30 farms investigated, eight farms were found to be positive for *Salmonella* spp., and out of eight positive samples seven samples were confirmed to be *S. typhimurium* (Table 1). Highest rate of prevalence was found in the age group of more than 52 weeks followed by prevalence in the age group of 9-20 weeks and 21-40 weeks (Table 2).

In the present study, 30 desi chicken farms were

screened for NTS and samples from 7 farms were found to be positive for NTS and all of them were *S. typhimurium*.

Poultry and poultry products are the most frequently implicated reservoirs of NTS in human food chain. Unhygienic handling of birds during slaughtering process, using unclean equipment and contaminated water results in contamination of desi chicken meat from birds's intestinal contents (Balakrishnan *et al.* 2018). Saravanan *et al.* (2015) reported that the percentage of NTS isolates obtained from intestinal contents and faecal samples of poultry were 1.94 and 0.62, respectively.

Senthil *et al.* (2012) reported a moderate prevalence of *Salmonella* infection in backyard chickens with an overall isolation rate of 13.4% (22 isolates from 164 samples) and therefore prophylactic programmes must be undertaken in the backyard chickens to control *Salmonella* infections.

Among the Non-typhoidal *Salmonella* infections, *S. typhimurium* has contributed significantly and it has become the most common cause of bacteraemia in children (Verma *et al.* 2014). Bangera *et al.* (2019) reported that 58 NTS serovars were isolated from 396 samples including poultry meat, intestinal contents and faecal samples of which *S. typhimurium* accounted for 12.07% of isolates. In another study, the relative occurrence of *S. enteritidis* and *S. typhimurium* in disease outbreaks in broilers was 14.13 and 6.52%, respectively (Kumar *et al.* 2012).

Samanta *et al.* (2014) reported that out of 360 samples, 22 isolates (6.1%) of *Salmonella* were identified from cloacal swabs, drinking water, feed and eggs of backyard poultry. Out of 22 isolates *S. Enteritidis* was isolated from six samples and *S. Typhimurium* was isolated from two samples which is in contrast with the findings of the present study. Kumar *et al.* (2019) studied the distribution of *Salmonella* serovars in poultry (India) and reported that out of seven *Salmonella* serovars identified, *S. typhimurium* accounted for 21.9% of isolates.

Table. 1. NTS isolation percentage in desi chicken

Type of sample	Total no. of samples	Total no. of farms	No. of <i>Salmonella</i> positive farms	No. of NTS isolates (<i>S. typhimurium</i>) obtained	NTS isolation percentage (%)
Pooled fresh faecal samples from desi chicken farms (6 samples were collected from each farm)	180	30	8	7	23.33

Table 2. NTS – Age group wise isolation percentage in desi chicken

Age group	No. of farms investigated	No. of farms positive for <i>S. typhimurium</i>	Flock size in affected farms		Age group wise isolation %
			No. of farms	Flock size (No.) / Age	
0 – 8 weeks	2	0	0	-	-
9- 20 weeks	8	2	1	74 (17 weeks)	25.0
			1	65 (15 weeks)	
21- 40 weeks	12	3	1	60 (28 weeks)	25.00
			1	68 (32 weeks)	
			1	72 (37 weeks)	
41- 52 weeks	5	1	1	55 (46 weeks)	20.00
More than 52 weeks	3	1	1	43 (58 weeks)	33.33
Total	30	7	7		

The level of prevalence of NTS can be reduced by adopting hygienic practices during poultry slaughter (Manoj *et al.* 2015, Sudhanthirakodi *et al.* 2016) to ensure food safety.

It can be concluded that the present study reveals that small scale poultry production with desi chicken is also becoming source of NTS infection in human beings apart from organized commercial poultry sector. The level of NTS prevalence in desi chicken can be reduced by adopting hygienic practices during poultry slaughter.

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