



## Detection of Virulence Genes in *Salmonella* Species Isolated from Chevron and Chicken Meat

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### ABSTRACT

The aim of present study was to investigate the detection of virulence factors encoded *Salmonella* enterotoxin (*stn*) and plasmid encoded fimbriae (*pef*) genes in isolates of *Salmonellae* isolated from chevon and chicken meat samples collected from different districts of Chhattisgarh. A total of 32 *Salmonella* isolates were recovered, among them 18 and 14 isolates were recovered from chevon and chicken meat samples, respectively. All the *Salmonella* isolates were tested for the presence of virulence associated genes viz., *stn* and *pef* by using polymerase chain reaction (PCR) protocols, standardized separately for each gene. All 32 (100%) *Salmonella* isolates were found to carry the enterotoxin determinant *stn* gene where as none of the *Salmonella* isolate was found positive for *pef* gene, indicating that the *stn* gene is widely distributed among the *Salmonella* isolates irrespective of source of sample, species, serovars and location. The *stn* gene in *Salmonella* was found to be highly conserved. Thus, *stn* gene may be used as a target gene for detection of *Salmonellae* in different types of field samples.

**Keywords:** Salmonellosis, *stn*, *pef*, meat, virulence

In India, *Salmonella* has become leading cause of food borne illnesses viz., food poisoning, acute gastroenteritis, dysentery and other diseases even to death with considerable economic impact. Eggs, chicken, meat and meat products are the most common vehicles of salmonellosis to humans (Maharjan *et al.*, 2006). There are more than 2500 serotypes of *Salmonella* and new serotypes are emerging every year, most of them have strong pathogenicity to humans and animals (Fang *et al.*, 2010). The outcome of a *Salmonella* infection is determined by the status of the host and of the bacterium. Age, genetic and environmental factors mainly determine the status of the host whereas virulence factors determines the status of the bacterium (Van Asten and Van Dijk, 2005). Virulence factors responsible for pathogenicity in enteric bacteria are often plasmid encoded, as in *E. coli*, *Yersinia* spp. and *Shigella* spp. However, current evidences suggest that the contribution

of virulence plasmids to pathogenesis in *salmonella* is less important (Muthu *et al.*, 2014) and dictated by an array of factors that act in tandem and ultimately manifest in the typical symptoms of salmonellosis. Virulence genes encode products that assist the organisms in expressing its virulence in the host cells. Some genes viz., *sef*, *pef*, *spv* or *inv* are known to be involved in adhesion and invasion of *Salmonella* into the host cell; whereas other *mgtC* genes are associated with the survival in the host system and *sop*, *stn*, *pip* A, B, D genes are involved in the actual manifestation of pathogenic processes. Other virulence factors of *Salmonella* include production of endotoxins and exotoxins, and presence of fimbriae and flagella (Van Asten and Van Dijk, 2005).

Therefore, present study was planned to determine the virulence factors encoded by *Salmonella* enterotoxin (*stn*)

**Table 1.** Primer sequences for polymerase chain reaction

Target Virulence Gene	Primer Sequence (5'-3')	Amplicon Size (bp)	Reference
<i>Stn</i>	(F*): TTGTGTCGCTATCACTGGCAACC	617	Murugkar <i>et al.</i> (2003)
	(R**): ATTCGTAACCCGCTCTCGTCC		
<i>Pef</i>	(F*): TGTTTCCGGGCTTGTGCT	700	
	(R**): CAGGGCATTGCTGATTCTTCC		

\* Forward Primer, \*\* Reverse Primer

and plasmid encoded fimbriae (*pef*) genes of *Salmonella* in isolates recovered from chevon and chicken meat samples collected from different districts of Chhattisgarh.

## MATERIALS AND METHODS

### Bacterial isolation

During the study period from September 2013 to August 2014, a total of 32 *Salmonella* isolates were recovered from chevon and chicken meat samples collected from different districts of Chhattisgarh. Among the 32 *salmonella* isolates, 18 and 14 isolates were recovered from chevon and chicken meat samples, respectively. All the isolates were available and maintained with the Department of Veterinary Public health and Epidemiology, College of Veterinary Science and A.H., Anjora, Durg (C.G.).

### Polymerase Chain Reaction (PCR) for *stn* and *pef* genes

All the *Salmonella* isolates were tested for the presence of virulence associated *stn* and *pef* genes using PCR protocols standardized separately for each gene. Template DNA of *Salmonella* isolates incorporated in PCR reactions was

prepared by boiling and snap chill method (Nagappa *et al.* 2007). Briefly, all *Salmonella* isolates were grown in 10 ml Luria Bertani (LB) broth (HiMedia, India) and incubated at 37°C for 24 hrs. Thereafter, one ml of the test culture were taken in a 1.5 ml microcentrifuge tube and centrifuged at 8000 rpm for 10 min. The pellet was washed twice with sterile saline solution and finally re-suspended in 300 µl sterilized DNase and RNase-free milliQ water (Millipore, USA). All the *Salmonella* isolates were vortexed and boiled for 10 min and then were immediately kept on ice. Suspensions were centrifuged at 12000 rpm for 10 min and 3µl of the supernatant was used as a DNA template in PCR mixtures. The PCR analysis for *stn* and *pef* genes was carried out as per the protocol described by Murugkar *et al.* (2003) with suitable modifications. The primers sets of *stn* and *pef* genes used in this study were synthesized from Imperial Life Sciences (P) Limited, Gurgaon, Haryana, India. The primer sequence of target virulence genes used in this study are presented in Table 1 and PCR cycling conditions are mentioned in Table 2.

The PCR was performed using thermocycler (Mastercycler, Eppendorf, Germany) in a final reaction volume of 25 µl containing 2.5 µl of 10X Taq Buffer, 1.5 mM MgCl<sub>2</sub>, 50 µM of each deoxyribonucleotide triphosphate (dNTP), 10

**Table 2.** Polymerase chain reaction conditions

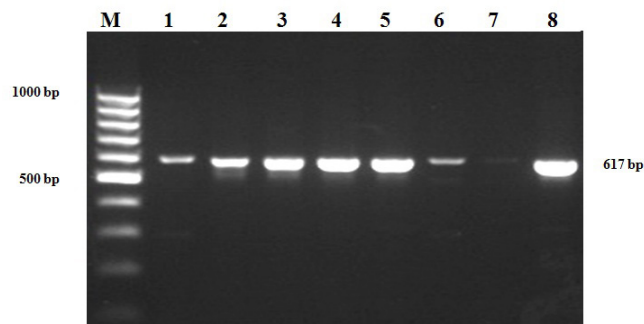
Primers	Initial Denaturation	Cycling Conditions			Final Extension
		Denaturation	Annealing	Extension	
<i>Stn</i> (F*)	94°C for 5 min	94°C for 1min	59°C for 1 min	72°C for 1 min	72°C for 10 min
<i>Stn</i> (R**)		Repeated for 30 Cycles			
<i>pef</i> (F*)	94°C for 5 min	94°C for 1min	55°C for 1 min	72°C for 1 min	72°C for 10 min
<i>pef</i> (R**)		Repeated for 25 Cycles			

\* Forward Primer, \*\* Reverse Primer

**Table 3.** Distribution of virulence genes among *Salmonella* isolated from chevon and chicken meat samples.

Source/Sample type	Number of isolates tested	No. of isolates positive (%) for virulence genes	
Chevon	18	18 (100%)	-
Chicken Meat	14	14 (100%)	-
Total	32	32 (100%)	-

pmol of each primers, 1U Taq Polymerase, 3 µl of template DNA and nuclease free water to make up the total volume 25 µl. After completion of PCR reaction cycles, amplified products were analysed using submarine agarose gel electrophoresis by running along with DNA ladder. The final PCR product was electrophoresed on 1.5% (w/v) agarose gel prepared in 1X Tris- Borate EDTA (TBE) buffer, visualized by ethidium bromide (0.5µg/ml) stain under UV transilluminator (Biometra) and documented by Gel Documentation System (Gel Doc<sup>tm</sup> XR, Biorad, USA). All the biologicals required for molecular work were procured from Thermo Scientific (USA), Genetix (India) and Bangalore Genei (India).



**Figure 1.** Agarose gel electrophoresis showing amplified PCR product of *stn* gene. Lane M: 100 bp DNA Ladder, Lane 1-8: *Salmonella* isolates with *stn* positive amplicons (617 bp)

## RESULTS AND DISCUSSION

In the present study, all 32 (100%) *Salmonella* isolates were found to carry the enterotoxin determinant *stn* gene (Table 3). The amplified PCR products of all *salmonella* isolates on agarose gel electrophoresis yielded a 617 bp product in the *stn* gene segment (Figure1).

Present study revealed that *stn* gene was present in all the isolates (100%) irrespective of source of sample and region

of sampling. *Salmonella* induced diarrhoea is a complex phenomenon involving several pathogenic mechanisms including production of enterotoxin (Baloda *et al.*, 1983) which is mediated by the *stn* gene (Chopra *et al.*, 1987). The findings of present study are in agreement with Ezzat *et al.* (2014), wherein they reported the presence of *stn* gene in all *salmonella* isolates (100%) isolated from broiler chicken's liver, caecum, heart blood, spleen & kidney samples. The *stn* gene was found in 41 faecal isolates of *Salmonella* including 34 isolates of *S. Typhimurium* and 7 isolates of *S. Enteritidis* of calves, piglets and poultry (Barman *et al.*, 2013) and in 95 *Salmonella* strains belonging to *Salmonella enterica*, isolated from man and animals (Murugkar *et al.*, 2003). Prager *et al.* (1995) reported that *stn* gene could be detected in all the strains of *Salmonella* Enterica but not in *Salmonella* Bongori. The gene *stn* is reported to be absent in *S. bongori* strains as well as in other members of *Enterobacteraceae* or *Vibrio* families harbouring enterotoxigenic potential (Murugkar *et al.*, 2003). Makino *et al.* (1999) also reported that the presence of even a single organism per gram of meat sample was detectable by PCR on the basis of detection of *stn* gene. Shi *et al.* (2013) reported the presence of *stn* gene in 45 out of 47 strains of *salmonella* had a chicken source origin and revealed that the enterotoxin *stn* gene carrying rate among *Salmonella* strains was 95.7%. Findings of present study revealed that the *stn* gene is widely distributed among the *Salmonella* isolates irrespective of source of sample, species, serovars and region of sampling. *stn* gene in *Salmonella* is highly conserved and it is expected to be a new target gene for detection of Salmonellae in field samples.

In the present study, none of the *Salmonella* isolates were found to be positive for *pef* gene. Results of our study are in agreement with Muthu *et al.* (2014) who also couldn't find *pef* gene among any of the *Salmonella* isolates recovered from human clinical samples. In contrary, Murugkar *et al.* (2003) reported the presence of *pef* gene in 85 of the 95 *Salmonella* isolates and revealed that there was no serotype specific presence or absence of this gene. The *pef* gene expresses plasmid-encoded fimbriae that contribute in adhesion and invasion of host cells.

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