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Detection of virulence genes of *Salmonella* by polymerase chain reaction

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ABSTRACT

Present investigation was conducted to screen the presence of virulence genes *stn, invA, sopB* and *sefC* from 41 *Salmonella* isolates isolated from diarrheic as well as apparently healthy animals and birds. Thirty four (82.92%) belonged to *Salmonella enterica* serovar Typhimurium and 7 (17.07%) belonged to serovar *S*. Enteritidis. All the isolates detected positivity to *stn* gene, however *invA, sopB* and *sefC* were 63.41, 87.80 and 14.63% respectively. The *sef*C gene was present only in serovar *Salmonella* Enteritidis. More numbers of virulence genes could be detected among the isolates recovered from clinically affected animals/birds (34 isolates) in compare to the isolates from apparently healthy (7) animal /birds.

Key words: PCR, *Salmonella*, Virulence genes

Salmonella species are widely distributed in the environment and cause salmonellosis in human and animals. The virulence factors responsible for the induction of gastroenteritis and systemic infection by *Salmonella* are still poorly understood (Rahman 2006). More than 30 *Salmonella* specific genes have been amplified by polymerase chain reaction (PCR) to detect and characterize *Salmonella.* Use of the standard PCR technique to study the presence of genes amongst the field isolates can be an important tool in molecular characterization of field isolates of *Salmonella*. In the present study, detection of virulence factors encoded by virulence genes viz. *stn, invA, sopB* and *sefC* of *Salmonella* strains isolated from diarrhoeic and apparently healthy animals and birds were investigated using polymerase chain reaction.

MATERIALS AND METHODS

Salmonella isolates (41)belonging to *Salmonella* Typhimurium (34) and *S.* Enteritidis (7) isolated from faecal samples, rectal and cloacal swabs of diarrhoeic as well as apparently healthy calves (13), piglets (6) and poultry (22) were subjected to analysis by polymerase chain reaction (PCR) for detection of genes encoding virulence factors, viz. *stn*, *inv*A, *sop*B and *sef*C using specific primers.

Polymerase chain reaction (PCR): Salmonella isolates were grown in 2 ml Luria Bertani (LB broth) and incubated 18 h at 37°C on a shaking incubator. One ml of the culture were taken in a 1.5 ml microcentrifuge tube and centrifuged at 8000 rpm for 10 min. The pellet was washed for twice with sterile saline solution and finally re-suspended in 300 µl nuclease free water and boiled for 10 min and immediately kept in ice. Suspensions were centrifuged at 12 000 rpm for

Table 1. Polymerase chain reaction primers and conditions used

Toxin gene	Sequence	Amplicon size (bp)	Annealing temp.	Reference
stn	(F) TTG TGT CGC TAT CAC TGG CAA CC (R) ATT CGT AAC CCG CTC TCG TCC	617	59°C	Prager *et al.* (1995)
invA	(F) ACC ACG CTC TTT CGT CTG G (R) GAA CTG ACT ACG TAG ACG CTC	941	60°C	Galan *et al.* (1992)
sopB	(F) CAA CCG TTC TGG GTA AAC AAG AC (R) AGG ATT GAG CTC CTC TGG CGA T	1348	55°C	Prager *et al.* (1995)
sefC	(F) GCG AAA ACC AAT GCG ACT GTA (R) CCC ACC AGA AAC ATT CAT CCC	1103	55°C	Clouthier *et al.* (1994)

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10 min at 4°C and the supernatant was used as template. PCR analysis for the *stn, inv*A and *sop*B gene was carried out as per Prager *et al*. (1995) and *sef*C gene by Clouthier *et al.* (1994). The PCR mixture included a final volume of 25 µl containing 12.5µl of 2X master mix containing 4mM $MgCl_2$, 0.4 mM of each dNTPs, 0.05 U/ml Taq DNA polymerase, 150Mm Tris HCl PCR buffer, 1µl (20 pmol) each of forward and reverse primer and 2.5µl of template DNA (culture lysate) and nuclease free water to make up the volume 25µl. Reference strain of *Salmonella* (MTCC *Salmonella* Typhimurium culture) was used as positive and *E.* coli (C 600) was used as negative control. Amplification was performed in a cycler. PCR amplification was performed in 25 cycles of denaturation for *stn* and *sef*C and 30 cycles for *inv*A and *sop*B gene, primer annealing and primer extension followed by incubation at 72°C for 10 min. The primer sequence of targeted virulence genes and the PCR conditions are presented in Table 1.

RESULTS AND DISCUSSION

The PCR amplification products were separated by horizontal submarine electrophoresis with 1% (w/v) agarose gel in 1X TAE buffer (Tris acetate 0.04 M, EDTA 0.001M and pH adjusted to 8.0) as per Sambrook *et al*. (1989). About 5µl of PCR product mixed with 2 µl of gel loading dye was loaded on to the gel with standard markers (100 bp DNA ladder, Bioenzyme and 1kb DNA ladder). The ethidium bromide stained amplified product was visualized under gel documentation system.

In the present study, the *stn* gene was present in all the 41 (100%) isolates of *Salmonella* including *S*. Typhimurium (34) and *S.* Enteritidis (7) (Table 2). All these strains yielded a 617 bp product in the *stn* gene segment (Fig. 1). The study revealed that *stn* gene was present in all the strains (100%) irrespective of species, serovars and location of the isolates.

Table 2. Detection of virulence genes in *S*. Typhimurium and *S.* Enteritidis strains isolated from cattle, pig and poultry

Source	Serovars	No. of isolates	No. of strains positive for virulence gene			
			stn	*inv*A	*sef*C	*sop*B
Calves	*S*. Typhimurium	13	13	10	-	13
	S. Enteritidis	-	-	-	-	-
Piglets	*S*. Typhimurium	6	6	4	-	5
	S. Enteritidis		-		-	-
Poultry	*S*. Typhimurium	15	15	8	-	12
	S. Enteritidis	7	7	4	6	6
Total		41	41 (100)	26 (63.41)	6 (14.63)	36 (87.80)

Figures in parentheses indicate percentages.

Prager *et al.* (1995) reported that *stn* gene could be detected in all the strains of *Salmonella* Enterica but not in *Salmonella* Bongori. Similar observations were also made by Majtán *et al.* (2005) who could also detect the presence of *stn* gene in all the strains (100%) tested by PCR. Makino *et al.* (1999) reported that the presence of even a single organism per gram of faeces and meat sample was detectable by PCR on the basis of detection of *stn* gene.

Twenty-six (63.41%) of the 41 isolates of *Salmonella* including 10 (38.46%), 4 (15.38%), 12 (48%) from cattle, pig and poultry, respectively, showed presence of *inv*A gene which yielded 941 bp (Fig. 2). The per cent positivity for *inv*A gene in *Salmonella* in the present study was closely similar to that observed by Isogai *et al.* (2005) who reported detection of *inv*A gene in 89.83% of poultry samples and cattle faeces by PCR. However, Chiu *et al.* (1996) reported the detection of *inv*A gene in 10% *Salmonella* isolates.

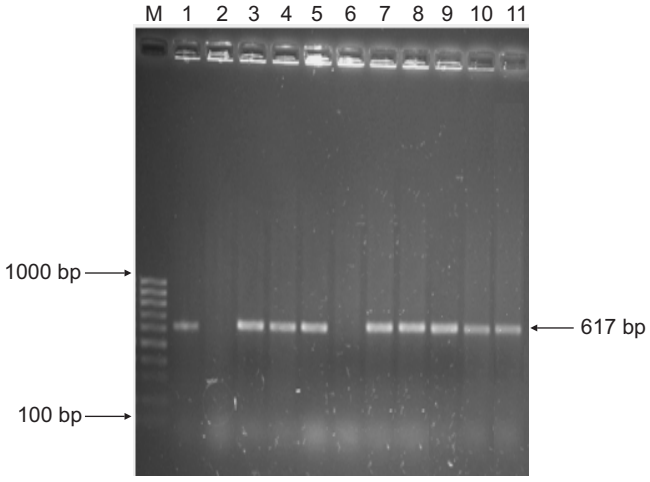

Fig. 1. Detection of *stn* gene by polymerase chain reaction. M, Marker; lane 1, positive control; lane 2, negative control; lane 3 to 11, test isolates.

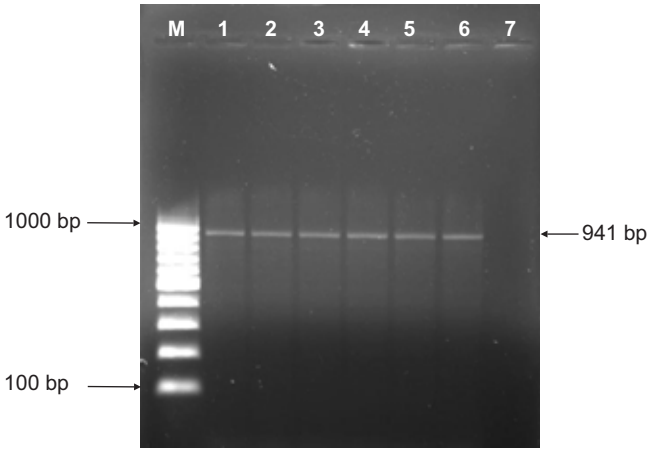

Fig. 2. Detection of *inv*A gene by polymerase chain reaction. M, Marker; lane 1, positive control; lane 2 to 6, test isolates; lane 7, negative control (*E. coli* C600).

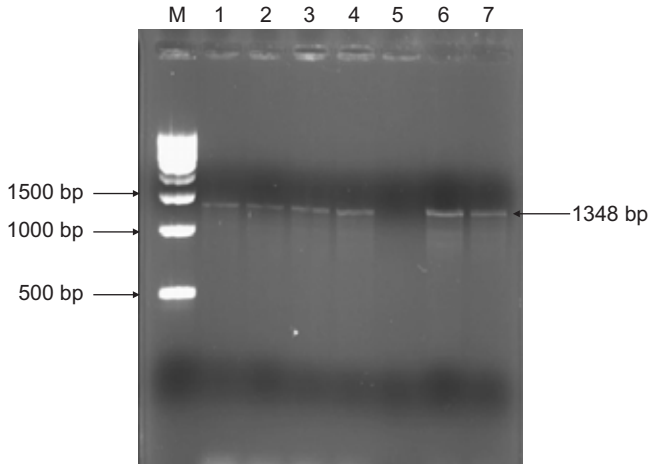

Fig. 3. Detection of *sop*B gene by polymerase chain reaction. M, marker; lane 1, positive control; lane 5, negative control; lane 2 to 4 and 6 to 7, isolates.

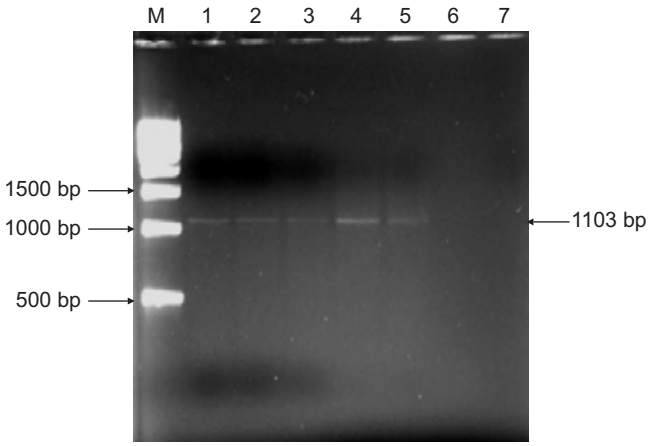

Fig. 4. Detection of *sefC* gene by polymerase chain reaction. M, marker; lane 1, positive control,lane 7, negative control; lane 2 to 6, isolates.

Similarly, *sop*B gene of 1348 bp product was present in 36 (87.80%) including 13 (100%), 5 (83.33%) and 18 (81.81%) out of 41 *Salmonella* isolates from cattle, pig and poultry respectively (Fig. 3). This observation was more or less similar to that reported by Rahman (2006), who detected *sop*B gene by PCR in all *Salmonella* isolates (100%) irrespective of serovar and source. Streckel *et al.* (2004) and Nógrády *et al.* (2010) also reported the detection of *sop* B gene in *S. enterica* strains.

The *sef*C gene was present only in 6 (14.63%) strains of *Salmonella* Enteritidis isolated from poultry. They yielded a 1103 bp product (Fig. 4). This gene was reported to be present only in *S*. Enteritidis strains by many workers (Murugkar 2003 and Castilla *et al.* 2006). More number of isolates (34) recovered from clinically affected animals showed presence of virulence genes than the isolates from apparently healthy (7) animals and birds. This finding assumed special significance in *Salmonella* pathogenicity for animals and birds as the all the genes are present in all the species with different percentage except *sefC* gene. Cytosin gene, *stn* and virulent fimbriae *sef*C and *pef*A were recorded among *Salmonella* Enteritidis isolates from acute turkey gastroenterititis (Dutta *et al.* 2010).

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