CIBTech Journal of Microbiology ISSN: 2319-3867 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjm.htm
2014 Vol. 3 (1) January-March, pp.11-14/Muthu et al.
Research Article

DETECTION OF VIRULENCE GENES FROM *SALMONELLA* SPECIES IN CHENNAI, INDIA

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ABSTRACT

The present study was undertaken to detect the two genes, namely, Salmonella enterotoxin (*stn*) and plasmid encoded fimbrial (*pef*) genes, among clinical isolates of three Salmonella species from humans. A total of 176 isolate belonging to *Salmonella enterica serovar Typhi* (133), *Salmonella enterica serovar paratyphi A* (41) and *Salmonella enterica serovar Typhimurium* (2) serovars were analyzed by Polymerase Chain Reaction (PCR) using their specific primers for the detection of *stn,* and *pef* genes. Varying pattern of *stn,* and *pef* genes were observed amongst the isolates. While, *stn* was found in 140/176 (79.5%) Salmonella strains, the *pef* gene was not found in any of the tested strains. PCR findings indicated that the *stn* gene is widely distributed among Salmonella irrespective of the serovars and source of isolation.

Keywords: *Salmonella, Enterotoxin gene, Polymerase Chain Reaction (PCR)*

INTRODUCTION

Salmonella is a facultative intracellular pathogen that causes a variety of infectious diseases like typhoid, septicemia and gastroenteritis owing to bacterial multiplication in intestinal submucosa and production of enterotoxins which elicits the inflammatory response of the host (Salyers and Whitt, 1994). Virulence factors responsible for pathogenicity in enteric bacteria are often plasmid encoded, as in *Escherichia coli*, *Yersinia* spp. and Shigella spp. In Salmonella, the existence of plasmid-borne virulence genes was first suggested in 1982, but current evidence suggests that the contribution of virulence plasmids to pathogenesis in Salmonella is less important than in the above mentioned bacteria. Virulence plasmids have been found only in a few serovars of Salmonella (Guiney *et al.,* 1994, Bäumler *et al.,* 1998).

Epidemiology and pathogenic process in salmonellosis are 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 are known to be involved in adhesion and invasion *viz.*, *sef, pef, spv* or *inv*; (Clouthier *et al.,* 1993, Bäumler *et al.,* 1996, Krause *et al.,* 1992, Galán *et al.,* 1992) others are associated with the survival in the host system- *mgt*C (Blanc-Potard and Groisman, 1997)or in the actual manifestation of pathogenic processes *viz.*, *sop, stn, pip* A, B, D (Wallis and Galyov, 2000, Chopra *et al.,* 1994, Wood *et al.,* 1998). Nucleic acid based diagnostic techniques are being employed for the detection of various gene -encoded virulence factors *viz.*, Salmonella enterotoxin (*stn*), (Prager *et al.,* 1995, Rahman, 1999) Salmonella Enteritidis fimbriae (*sef*) and plasmid encoded fimbriae (*pef*) genes (Rahman *et al.,* 2000). However, the distribution of these genes among the various isolates obtained from biological source is yet to be elucidated. The present study reports that the distribution of *stn,* and pef genes among the *Salmonella* strains isolated from clinical cases of infections in human.

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MATERIALS AND METHODS

Bacterial strains:

During the study period (August 2007 - Jan2010), a total of 176 Salmonella isolates were collected and characterized from different hospitals and Diagnostic laboratories in Chennai.

Out of the 176 Salmonella isolates, 133 were *Salmonella enterica serovar Typhi*, 41 were *Salmonella enterica serovar paratyphi A* and 2 were *Salmonella enterica serovar Typhimurium*. Among the 176 clinical isolates, 172 were from blood, 3were from stool and one was from bone marrow.

Out of 133 *Salmonella enterica serovar Typhi*, 131 were blood isolates, 1 was a stool isolate and 1 was a bone marrow isolate and out of 41 *Salmonella paratyphi A*, 40 were blood isolates and 1 was stool isolate. Out of 2 *Salmonella Typhimurium*, 1 isolate each was from blood and stool respectively.

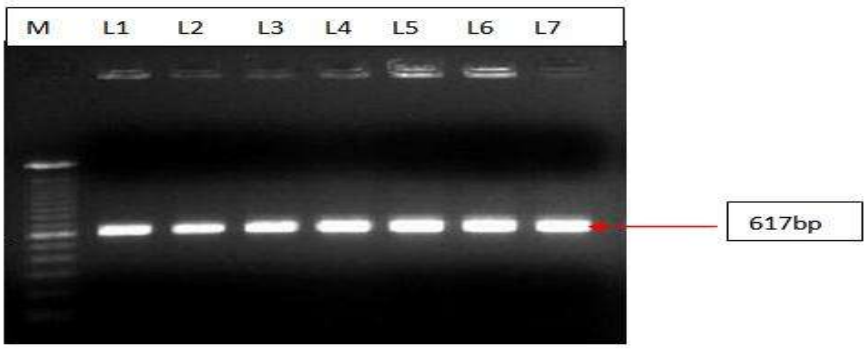
Amplification of stn and pef genes:

All the 176 Salmonella isolates were tested for the presence of *stn* and *pef* genes by modified PCR analysis (Murugkar *et al.,* 2003). Template DNA isolated from bacterial strains as described above was amplified by PCR using gene specific primers for *stn* Forward P1 5'- TTG TGT CGC TAT CAC TGG CAACC – 3' and Reverse M13 5' - ATT CGT AAC CCG CTC TCG TCC – 3' and *pef* Forward A1 5' TGT TTC CGG GCT TGT GCT – 3' and Reverse A2 5' CAG GGC ATT TGC TGA TTC TTC C – 3' procured from Sigma- Oligos, India. The PCR was performed using Gene Amp gold 9700 (ABI USA) in a total reaction volume of 10µl containing 1µl of 10x PCR buffer with 25mM $MgCl_2$, 1µl of 2.5mM dNTP mix, 0.4µl of 2µm primer stocks each, 0.25µlof 5U/µl *Taq* DNA polymerase (NEB, USA) and 1µl of template DNA.

The thermal cycling protocol for amplification is programmed in GeneAmp gold 9700 (ABI, USA) as follows; initial denaturation at 94ºC for 10min followed by 35 cycles, i) Denaturation at 94ºC for 45sec; ii) primer annealing at 58ºC for 45secand iii) primer extension at 72ºC for 45sec followed by final extension at 72ºC for 7min. The PCR product was electrophoresed in 1%(w/v) agarose gel (Sigma -USA) prepared in 0.5x Tris-Acetate EDTA (TAE) buffer at 100V for 15min using Mupid-Ex (Takara, Japan) and visualized by ethidium bromide staining using Gel documentation system (Bio Rad -USA).

RESULTS AND DISCUSSION

All the 176 isolates of Salmonella were subjected to virulence characterization by PCR technique to detect *stn* and *pef* genes. Prevalence of *stn* and *pef* genes among *Salmonella, isolated* from clinical sources is indicated in (Table 1). 140/176 (79.5%) Salmonella isolates were positive for enterotoxin (*stn)* gene and none of them were positive for Plasmid encoded fimbrial (*pef*) gene. PCR revealed that 81.2% (108 isolates) of *Salmonella Typhi* were positive for *stn*, the gene encoding Enterotoxin, while 18.8% (25 isolates) were negative (Figure 1).



M=100bp ladder; L1= Postive control S. typhi for stn gene; L2-L7= Test strains stn gene positive (617bp)

Figure 1: Gel picture showing stn gene positivity in Salmonella spp.

Table 1: Prevalence of *stn* and *pef* genes among *Salmonella* isolated from clinical sources.

S.No	Source/serotype Human	No. of isolates	No. positive (%) for virulence genes *stn*	*pef*
1	*S. typhi*	133	108 (81.20)	-
2	*S. paratyphi A*	41	32 (78.04)	-
3	*S. typhimurium*	2	-	-

None of the isolates were positive for *pef* gene. Among the *Salmonella paratyphi A* isolates, 78.04% (32 isolates) were positive for *stn* gene and the remaining were negative (21.96% or 9 isolates) (Table-1). All the *Salmonella paratyphi A* isolates were negative for *pef*. The two isolates of *Salmonella typhimurium* turned up to be negative for both the Enterotoxin gene as well as plasmid encoded fimbrial gene.

Salmonella induced diarrhoea is a complex trend concerning numerous pathogenic mechanisms including production of enterotoxin (Baloda *et al.,* 1983). This enterotoxin production is mediated by the *stn* gene (Chopra *et al.,* 1987). Our findings are in concordant with earlier reports (Prager *et al.,* 1995, Rahman, 1999). Observations from the present study indicated that the *stn* gene is widely distributed among the Salmonella irrespective of their serovars and source of isolation. This *stn* gene has been reported to be absent in *S. typhi* (25), *S.paratyphi A* (9) and *S.typhimurium* (2) and the other members of *Enteriobacteriaceae* or *Vibrio*, which have enterotoxigenic potential (Rahman, 1999).

Development of a PCR system for diagnosis of Salmonella on the basis of *stn* detection has been reported, (Makino *et al.,* 1999)wherein a single organism could be detected per gram of faecal or meat sample by the application of this method. The role of *Stn* gene has not been clearly understood (Nakano *et al.,* 2012). However *Stn* from *Salmonella typhimurium* strain Q1 showed enterotoxic and cytotoxic activities (Chopra *et al.,* 1999). Amino acid sequence of *Stn* (amino acid residues 127–142) showed some similarity to the active site of cholera toxin (CT) and heat-labile enterotoxin (LT) ADP-ribosyltransferases which suggest that *Stn* could act as a key factor in acute gastroenteritis and diarrhea and contribute to *Salmonella* virulence (Chopra *et al.,* 1994).

A complete study involving all the major serotypes needs to be carried out to obtain a definite status regarding the presence of this gene among various serotypes of Salmonella and the role of these genes on the actual pathogenesis of Salmonellosis in man and animals.

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