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Short Communication

Characterization of Virulence Factors among Diverse *Salmonella* Serotypes and Sources

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ABSTRACT

A total of 37 *Salmonella* isolates of 11 different serotypes and rough type from human and animal clinical cases and meat samples were studied for the presence of 8 virulence determinants including 4 virulence genes and 4 toxic factors. All the *Salmonella* isolates harboured *inv*A and *stn* genes, whereas 94.59% of isolates had the presence of *fim*H and *hil*A genes indicating colonizing, invasive and enterotoxic potential of the pathogen. Among the four toxic factors studied, highest percentages of the isolates were positive for cytotoxicity (72.97%), followed by DNAse (70.27%), enterotoxic (51.35%) and hemolysin (13.51%) activities. All the isolates were found to be highly pathogenic as indicated by 100% mortality in mouse model. Among the different serotypes studied, Typhimurium and Paratyphi B var Java showed the presence of all the virulence determinants in varying degree. The 2 rough strains demonstrated least number of virulence determinants. Tshiongwe and Labadi serotypes were also devoid of enterotoxigenic, DNAse and hemolytic activities. Other serotypes also lacked 1-2 virulence factors. *Salmonella* isolates exhibited diverse virulence determinants and were highly pathogenic.

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Salmonellosis is a major infectious disease of humans and animals globally. *Salmonella* infections cause heavy economic losses in United States amounting to $ 2-4 billion due to loss of life, work and medical care (Buzby et al., 1996; Frenzen et al., 1999). Different disease syndromes of salmonellosis are gastroenteritis, typhoid, bacteremia, and local infections (Darwin and Miller 1999). Nontyphoidal salmonellosis is manifested by mild to moderate gastroenteritis (CDC 2001). In very few casesa small percentage of cases, septicemia and invasive infections of organs and tissues can occur, which may result in diseases such as pneumonia, osteomyelitis, and meningitis (Cohen et al., 1987).

The ability of *Salmonella* spp. to cause a disease largely relies on a repertoire of elaborate virulence determinants including toxins fimbriae, flagella and virulence-plasmids (Van Asten and Van Dijk 2005). *Salmonella* produces both endotoxins and exotoxins (Houston et al. 1981, Ray et al. 1987). The endotoxin is lipid A of the outer membrane lipopolysaccharide (LPS) of *Salmonella*. The exotoxins are of two type *viz*., cytotoxins and the enterotoxins. Differences in virulence among *Salmonella* serovars have been attributed to the variable acquisition and evolvement of virulence genes (Falkow 1996). Several *Salmonella* specific virulence genes which takes an important role in the pathogenicity have been identified (Baumler et al., 2000). In Typhimurium serovar, at least 80 different virulence genes have been identified. Some genes are known to be involved in adhesion and invasion, like *sef* (Clouthier et al., 1993), *Fim*H (Duncan et al., 2005), *invA* (Galan et al., 1992) and other genes associated with toxin production *viz*., stn (Makino et al., 1999).

The present study was undertaken to determine some of the important virulence factors of *Salmonella* isolated from human, animals and meats.

A total of 37 *Salmonella* belonging to 11 serovars (Paratyphi B var java - 14, Typhimurium - 8, Enteritidis - 3, Isangi - 3, Virchow -1, Heidelberg -1, Berta -1, Tshiongwe -1, Saintpaul -1, Bsilla -1, Labadi -1 and 2 rough strains isolated from human and animal clinical cases and meat samples were included in the study. The isolates were confirmed through biochemical, followed by serological tests (Agarwal et al., 2003, Farmer 1995) and maintained on nutrient agar slants.

Primers used in the study were custom synthesized from Genuine Chemical Corporation (GCC), New Delhi (Table 1) for the detection of various virulence genes.

All the isolates were grown in brain heart infusion broth at 37^{0}C for 18 hrs. One ml of BHI grown sample was drawn and subjected to template DNA preparation by boiling and snap chilling method (Singh et al., 2009). Briefly, cultures were centrifuged at 10000 rpm for 2 min at room temperature, collected pellet was dissolved in 200 µl distilled water and put in boiling water bath for 10 min and chilled immediately on crushed ice for 10 min. For PCR 5 µl of supernatant was used as template from this preparation.

All the isolates of *Salmonella* were subjected to PCR for the detection of *inv*A (Galan et al., 1992), *hil*A (Guo et al., 2000), *fim*H (Menghistu, 2010) and *stn* (Makino et al., 1999) genes. The reaction mix invariably consisted of 5 µl of the bacterial lysate, 5 µl of 10x assay buffer for Taq polymerase containing 1.5 mM $MgCl_2$, 2 µl of 10mM dNTP mix 1 µl each of forward and reverse primer (10 pmol) and 1.25 U of Taq DNA polymerase made upto 50 µl using sterile distilled water. The optimal cycling conditions used for different genes are mentioned in Table 1. At the end of reaction the amplified product was electrophoresed on agarose gel (1.5%), visualized under UV light.

Table 1: Oligo-nucleotide primers used in the study

No.	Primer Sequences	Gene	Size	Reference
1.	F:GTGAAATTATCGCCACGTTCGGGCA R: TCATCGCACCGTCAAAGGAACC	*inv*A	284 bp	Galan et al. (1992)
2.	F: CTTTGGTCGTAAAATAAGGCG R: TGCCCAAAGCAGAGAGATTC	*Stn*	260 bp	Makino et al. (1999).
3.	F: CTGCCGCAGTGTTAAGGATA R: CTGTCGCCTTAATCGCATGT	*hil*A	497 bp	Guo et al. (2000)
4.	F: GGA TCC ATG AAA ATA TAC TC R: AAG CTT TTA ATC ATA ATC GAC TC	*Fim*H	1008 bp	(Menghistu, 2010)

F = Forward primer, R = Reverse primer

Cell free supernatant of *Salmonella* isolates was prepared as per Agarwal, 1997. The isolates were grown in brain heart infusion (BHI) broth fortified with 3% (w/v) cassamino acid and 0.6% (w/v) yeast extract, incubated in a shaker (200 rpm) at 37^0C for 12 h, centrifuged at 10000 g for 30 min at 4^0C and supernatant was collected. The cell free supernatants (CFS) were filtered through 0.22 µm filters and stored at -20^0C till further use.

Cytotoxic potential of the isolates were tested on African green monkey kidney cells (vero) lines grown in minimal essential medium (MEM) with Eagle's salts containing 10% fetal bovine serum, 100 U of benzylpenicillin/ml, 100 µg of streptomycin/ml, amphotericin-B 100 µg/ml, 2 mM L-glutamine and 150 µg of G 418/ml.

The Vero cells were grown to confluent monolayer in 96 well tissue culture plate (Greiner, Germany). Two fold dilution of each CFS were prepared and inoculated in triplicate in separate wells, incubated at 37^0C in 5% CO_2 atmosphere and the cytopathic changes (CPE) were recorded at different intervals up to 48 h PI.

The enterotoxigenic activity was determined by vascular permeability reaction (VPR) assay according to the procedure of Sandefur and Peterson (1976).The toxin preparations were injected intra dermal in 0.1 ml quantity in rabbit. After 2 and 18 h of inoculation for rapid permeability factor (RPF) and delayed permeability factor (DPF), respectively. The skin at the site of inoculation was examined for erythema, induration, necrosis and any other changes.

Mouse lethality assay was carried out as per Agarwal et al. (2003). Mice weighing about 16-20 g was injected with 0.2 ml of 20 h broth culture (approx $\leq 10^7$ cells) of test organism intra-peritonealy and observed for 5-7 days for the death of the mouse. Each isolate was injected in to 4 mice. The organism was then recovered from liver, spleen and heart blood. Gross pathological lesions were also recorded.

DNAse activity of all the *Salmonella* isolates was studied on DNAse test agar (Himedia, Mumbai) as per Kavitha et al., (2007). After spot inoculation plates were incubated at 37^OC for 48 h to observe clearance around colonies for the detection of extracellular DNAse activity. Plates were exposed to ultraviolet rays (150 to 3900A^O) for half an hour and incubated at 37^OC for 24 h. A strain of *Staph aureus* was included as positive control, which consistently produced purple zone around the colonies.

The haemolysin production was determined by inoculating the sheep blood agar plates by stab method to create an anaerobic pocket. After incubation for 48 h at 37^0C, the plates were examined for any discoloration or haemolysis and kept at 4^0C for hot and cold lysis activity, if any (Okazaki et al. 2003).

Thirty seven isolates from human, animal and meat sources were characterized for the presence of 4 virulence determining genes (*invA*, *hilA*, *fimH* and *stn*) and 4 phenotypic virulence characteristics viz. (cytotoxicity, entertoxigenicity, hemolysin and DNaes activity). Screening of 37 *Salmonella* isolates for *fim*H and *hil*A gene revealed 35 (94.597%) to be positive (Table 2 and 3). Two isolates negative for both these genes were of rough type and were isolated from animal diarrheal cases. All the isolates were positive for *inv*A and *stn* genes.

Enterotoxic potential of *Salmonella* isolates was carried out by vascular permeability assay in rabbit skin. Both rapid permeability factor (RPF) and delayed permeability factor (DPF) were observed. The test revealed that of the 37 *Salmonella* isolates, only 17 (45.9%) and 19 (51.35%), respectively, showed RPF and DPF activity. The positive reaction was indicated by haemorrhagic zone of varying degree and in some cases enduration and necrosis. Among the different clinical conditions, 100% isolates from human diarrhea and cow wound followed by human pyrexia (71.4%) and animal diarrhea (50%) cases were positive for the enerotoxic activity (Table 2). Furthermore, only 2 (28.5%) of meat isolates were found to be enterotoxigenic. Analysis according to serotype indicated that none of the isolates belonging to Enteritidis, Berta, Saintpaul, Labadi and Virchow serotypes were positive for VPR. Other serotypes produced variable reaction (Table 3).

Cytotoxic potential of all the 37 *Salmonella* isolates were studied on Vero cell line. Microscopic examination of different cells following exposure to CFS (1:2 and 1:4 dilution) prepared from *Salmonella* isolates revealed number of changes including rounding and shriveling of cells, loss of cytoplasmic membrane, extension, disorganization of cell sheets, shrinking of cytoplasm etc. Of the 37 isolates tested, 27 (72.97%) isolates were found to

be cytotoxic in nature at 1:2 dilutions, whereas at 1:4 dilutions only 12 (32.43%) isolates were cytotoxic (Table 2). Highest (85.71%) cytotoxic potential was demonstrated by isolates of meat, followed by the isolates of animal clinical origin (75%) and human clinical (60%) origins. Among different clinical cases, highest cytotoxic activity was shown by isolates of reproductive disorder and cow pyrexia origin (100% each), followed by human pyrexia (85.71%) and wound (50%) isolates. The 2 isolates from human diarrhea cases and 1 UTI case did not demonstrate any cytotoxic potential.

Table 2: Presence of virulence determinants gene among *Salmonella* isolates according to source

Source	Cytotoxicity		Enterotoxigenicity		DNase activity		Hemolysin	Virulence genes*	
	1:4	1:2	RPF	DPF	24 h	48 h		*hil*A	*Fim*H
Animal clinical isolates									
Diarrhea (16)	6 (37.5%)	12 (75%)	8 (50%)	8 (50%)	6 (37.5%)	6 (37.5%)	0	14 (87.5%)	14 (87.5%)
Wound (2)	0	1 (50%)	2 (100%)	2 (100%)	1 (50%)	1 (50%)	0	2 (100%)	2 (100%)
Reproductive disorder (1)	0	1 (100%)	0	0	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)
Cow pyrexia (1	1 (100%)	1 (100%)	0	0	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)
Subtotal (20)	**7 (35%)**	**17 (85%)**	**10 (50%)**	**10 (50%)**	**9 (45.0%)**	**9 (45.0%)**	0	**18 (90%)**	**18 (90%)**
Human clinical isolates									
Pyrexia (7)	2 (28.57%)	6 (85.71%)	3 (42.8%)	5 (71.4%)	6 (85.71%)	7 (100%)	2 (28.57%)	7 (100%)	7 (100%)
Diarrhea (2)	0	0	2 (100%)	2 (100%)	2 (100%)	2 (100%)	0	2 (100%)	2 (100%)
Urine tract infection (1)	0	0	0	0	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
Subtotal (10)	**2 (20%)**	**6 (60%)**	**5 (50%)**	**7 (70%)**	**9 (90%)**	**10 (100%)**	**3 (30%)**	**10 (100%)**	**10 (100%)**
Meat isolates (7)	4 (57.14%)	6 (85.71%)	2 (28.5%)	2 (28.5%)	6 (85.71%)	7 (100%)	2 (28.57%)		
Total (37)	**12 (32.43%)**	**27 (72.97%)**	**17 (45.9%)**	**19 (51.35%)**	**24 (64.86%)**	**26 (70.27%)**	**5 (13.51%)**	**35 (94.59%)**	**35 (94.59%)**

RPF- Rapid permeability factor, DPF- Delayed permeability factor, * All the isolates were positive for *inv*A and *stn* genes

CSF of all the isolates of serotypes Eneritidis, Saintpaul, Berta, Heidelberg, Virchow, Tschicngwe, Labadi, as well as Rough strains were found to be cytotoxic at 1:2 dilution, however, a few of these strains were not toxic at 1:4 dilution. Interestingly, only 11 of 14 isolates of S. Paratyphi B var Java, 2 of 3 isolates of Isangi and 4 of 8 (50%) of S. Typhimurium serotypes were cytotoxic at 1:2 dilution (Table 3).

Screening of all the 37 isolates on sheep blood agar for hemolytic potential revealed that only 5 (13.51%) isolates were positive for β hemolysis. Two of the isolates were from human pyrexia cases, 1 from UTI and 2 were from meat. Moreover, 3 of the positive isolates were Paratyphi B var Java and 1 each were Typhimurium and Berta serotypes.

Examination for DNAse activity of all the *Salmonella* isolates indicated that after 24 h of incubation 24 (64.86%) of the 37 strains were positive. After 48 h of incubation, 2 more strains i.e. a total of 26 (70.27%) strains turned out to be positive. On further incubation no change in the size of zone or in the number of strains giving positive reaction was seen. Among the different sources of the isolates, only 6 of 16 isolates from animal diarrheal cases and 1 of 2 isolates from wounds produced positive reaction. The DNAse negative strains belonged to S. Paratyphi B var Java (4/14), S. Typhimurium (1/8), S. Isangi (2/3), S. Tschicngwe (1/1), S. Labadi (1/1) serotypes and rough type (2/2).

Mouse pathogenicity assay revealed 100% mortality for all the 37 isolates within 24 h. The organisms were re-isolated from liver, spleen and heart of all the dead mice. On gross examination visceral organs were found to be highly congested, there were hemorrhages in intestine and fluid accumulation in peritoneal cavity.

Salmonella are able to colonize multiple sites including the small intestine, colon, and cecum. Intestinal adhesion in *Salmonella* infection is mediated by fimbriae present on the bacterial cell surface. Type 1 fimbriae (Fim), thin aggregative, long polar fimbriae (Lpf), and plasmid-encoded fimbriae (Pef) are some among the many types of fimbriae that are involved in *Salmonella* colonization (Darwin and Miller 1999). FimA, and FimH are the major structural subunit of the 7 genes (*fimAICDHF*) of Type 1 fimbriae, which interacts with host cell to facilitate attachment (Darwin and Miller 1999; Duncan et al., 2005). *Fim*H gene was selected in this study to assess the ability of *Salmonella* isolates to colonize the intestine as it could be critical to microbial pathogenesis (Duncan et al., 2005). All the isolates of *Salmonella* in the study except the two rough strains were found to posses *fim*H gene (Table 2 and 3) indicating their ability to colonize the intestine.

Invasiveness is the next step in *Salmonella* pathogenesis determined by several genes located in *Salmonella* pathogenicity island 1 (SPI1) (Mills et al., 1995). *HilA* (hyperinvasive locus) is

one of the virulence genes located in SPI1. (Bajaj et al., 1995). This study revealed 94.59% isolates to be positive. The 2 negative isolates were of rough type and were isolated from animal diarrheal cases.

Table 3: Presence of virulence determinants gene among *Salmonella* isolates according to serotype

Serotype	Cytotoxicity		Enterotoxigenicity		DNase activity		Hemolysin	Virulence genes*	
	1:4	1:2	RPF	DPF	24h	48 h		*hil*A	*Fim*H
S. bsilla (1)	0	0	1 (100%)	1 (100%)	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)
S. typhimurium (8)	1 (12.5%)	3 (37.5%)	5 (62.5%)	5 (62.5%)	6 (75%)	7 (87.5%)	1 (12.5%)	8 (100%)	8 (100%)
S. eneritidis (3)	2 (66.6%)	3 (100%)	0	0	2 (66.6%)	3 (100%)	0	3 (100%)	3 (100%)
S. isangi (3)	0	2 (66.6%)	1 (33.3%)	1 (33.3%)	1 (33.33%)	1 (33.33%)	0	3 (100%)	3 (100%)
S. saintpaul (1)	0	1 (100%)	0	0	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)
S. berta (1)	1 (100%)	1 (100%)	0	0	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
S. heidelberg (1)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)
S. virchow (1)	0	1 (100%)	0	0	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)
S. paratyphi B var Java (14)	4 (28.5%)	11 (78.5%)	7 (50%)	7 (50%)	10 (71.43%)	10 (71.43%)	3 (21.42%)	14 (100%)	14 (100%)
S. tshiongwe (1)	0	1 (100%)	1 (100%)	1 (100%)	0	0	0	1 (100%)	1 (100%)
Rough strain (2)	2 (100%)	2 (100%)	1 (50%)	2 (100%)	0	0	0	0	0
S. labadi (1)	1 (100%)	1 (100%)	0	0	0	0	0	1 (100%)	1 (100%)
Total	12 (32.43%)	27 (72.97%)	17 (45.9%)	19 (51.35%)	24 (64.86%)	26 (70.27%)	5 (13.51%)	35 (94.6%)	35 (94.59%)

R PF- Rapid permeability factor, DPF- Delayed permeability factor; * All the isolates were positive for *inv*A and *stn* genes

Guo et al. (2000) tested 83 *Salmonella* strains of different serotypes and found all of them to be positive for this gene. However, their study did not include rough variants, which were negative in this study. Pathmanathan et al., (2003) also reported its presence in a total of 33 *Salmonella* strains of 27 serovars. In an interesting study, Hu et al., (2008) reported that 2 *Salmonella* isolates of serovar Senftenberg from human clinical cases lacked some genes including *hil*A critical to *Salmonella* pathogenicity island 1 (SPI-1). Absence of *hil*A gene has also been reported in Orion serovar (Trafny et al., 2006). Thus our study supports the fact that some strains of *Salmonella* may not posses' *hil*A gene.

We also examined all the isolates for the presence of *inv*A gene, which is also important for entry into the intestinal mucosa. All the isolates were found to be positive for this gene. The result is in conformity to earlier reports (Galan et al., 1991; Swamy et al. 1996; Abouzeed et al., 2000).

Cytotoxic and enterotoxic activities in salmonellae have been described (O'Brien and Holmes 1996). *Salmonella* induced diarrhoea is a complex phenomenon involving several oxins. Enterotoxin, a 29-kDa protein encoded by *stn* gene is most commonly studied (Chary et al., 1993). This *stn* gene has been observed in all *Salmonella* serovars investigated except *S. bongori* (Prager et al., 1995). The present report also found *stn* gene in all the 37 isolates studied. Murugkar et al., (2003) studied the distribution of *stn* gene in 95 *Salmonella* isolates from five different serovars and four different sources and found that it was present in all the isolates. Rahman (1999) also reported the prevalence of *Salmonella* enterotoxin (*stn*) gene in all the 26 strains of *Salmonella enterica* studied by him. It is evident from our study that *stn* gene is commonly found among different *Salmonella* serovars.

It is suggested that enterotoxigenic properties are vital for pathogenesis of *Salmonella* diarrhea. Vascular permeability reaction in rabbit skin has been shown to demonstrate enterotoxigenic potential of *Salmonella* by several workers (Kaura et al. 1982; Chary et al., 1993). Culture filtrates of *Salmonella* have been shown to produce two skin permeability factors (PF). A heat stable rapid acting factor causes bluing of the skin. The second one is a heat labile delayed factor, which results in marked indurations of the rabbit skin within 18 h (Sandefur and Peterson 1976). Chary et al. (1993) demonstrated

enterotoxic activity by VPR altered vascular permeability in rabbit skin of a 25 kDa protein expressed from *stn* gene. In this study, 45.9% and 51.35% isolates showed RPF and DPF activity, respectively. Earlier reports have shown that 55% to 72% (Kuhn et al., 1978; Jiwa 1981) of *Salmonella* isolates to produce enterotoxin. Slightly lower enterotoxigenic activity recorded in our study may be due to variations in strain, source of isolation or bioassay model used. It is also interesting to note that all the isolates were positive for *stn* gene but less than half produced enterotoxin. Thus, it appears that mere presence of gene may not necessarily result in enterotoxin production and even *stn* mutants of S. Dublin and S. Typhimurium in comparision to wild-type strains shows no difference in fluid secretion (Watson et al., 1998). Another interesting finding was that only 50% of animal diarrheal isolates were positive for enterotoxic activity. This indicated that there might be some other mechanism involved in diarrheal pathogenesis (Giannella et al., 1975). Moreover, Wallis et al., (1986) found no correlation between enterotoxigenicity *in vitro* and the ability of the organisms to produce disease *in vivo*. Some of the isolates from other clinical conditions such as cow wound (100%), human pyrexia (71.4%) and as well as meat isolates (28.5%) were also found to be enterotoxigenic. None of the isolates belonging to Enteritidis, Berta, Saintpaul, Labadi and Virchow serotypes were positive for VPR. Other serotypes produced variable reactions (Table 2).

In recent years cytotoxin production by enteric pathogens has been studied. These toxins are defined by their ability to kill mammalian cells (van Asten and van Dijk 2005). In this study, overall 72.97% isolates were found to be cytotoxic. The cytotoxicity ranged from 60% to 85.71% in isolates from diverse origin. Among different serotypes studied, serotypes Eneritidis, Saintpaul, Berta, Heidelberg, Virchow, Tshiongwe, Labadi and Rough strains were found to be cytotoxic. Several serotypes including Typhimurium, Enteritidis Choleraesuis have been shown to have cytotoxic potential (O'Brien et al. 1982; Ketyi et al., 1979; Chiu and Ou 1996; Prager et al., 1995; Baloda et al., 1983), but the amount of toxin produced by the different serovars may vary (Ashkenazi et al., 1988).

In search of other virulence factors, all the isolates were also screened for hemolysin and DNAse activities. Hemolysin is one of the virulence factors identified in *Salmonella*, which is useful in invasion of epithelial cell and cell-surface factor induction It also helps in survival in macrophages and the counteraction of host immune defenses (Uppal et al., 1998). Although *sly*A gene encoding for hemolysin is present in all *Salmonella*, the organism is not usually hemolytic on culture media. In low-passage clinical isolates, weak hemolysis may be occasionally observed (Libby et al., 1994). In the present study also, only 13.51% isolates demonstrated the presence of hemolysin on blood agar. Tiwari et al. (2002) found only some of the 29 strains of salmonellae to be weakly haemolytic.

Analysis for DNAse activity indicated 70.27% isolates to be positive. In an earlier study, 53 of 60 *Salmonella* strains were found to be DNAse positive and it was opined that the test may elserve as virulence and epidemiological marker (Chandra et al., 2007). Lysov and LuK (1991) found hospital originated strains to be highest DNAse producer.

DNase activity has been reported in S. Paratyphi B, S. Abortusequi and S. Weltevreden (Agarwal 2002; Sharma 2002; Kavitha et al., 2007). Our study also revealed that most of the serotypes isolated from diverse sources were positive for this activity in varying degree, indicating it to be wide spread phenomena. Role of DNAse in *Salmonella* pathogenesis is not clear but evidence from other bacteria indicates that DNAse has clear evidence in pathogenesis (Sumby et al., 2005)

The mouse pathogenicity test by intra-peritoneal administration is an extensively used method for testing the virulence of *Salmonella* (Saxena et al., 2004) and considered superior over oral administration (Karim et al., 2008). In the present study, 100% mortality within 24 h was recorded for all the isolates tested indicating high virulence nature of the isolates. Variable mortality has been reported in earlier reports, which have been attributed to differential rate of clearance of pathogen (Saxena et al., 2004; Karim et al., 2008).

The study demonstrated that all the *Salmonella* isolates harboured *inv* and *stn* genes, whereas 94.59% of isolates had the presence of *fim*H and *hil*A genes. The isolates were also studied for cytotoxic, enterotoxic, Dnase and hemolysin activities which were found to be 72.97%, 51.35%, 70.27% and 13.51%, respectively. Besides, all the isolates were found to be highly pathogenic in mouse model. Among the different serotypes studied, Typhimurium and Paratyphi B var Java showed the presence of all the virulence determinants in varying degree. The 2 rough strains demonstrated least number of virulence determinants. They were devoid of *fim*H and *hil*A genes but harboured *stn* and *inv*A genes. They were also positive for cytotoxic and enterotoxic activities. We could not trace any literature to compare our findings on rough strains. Tshiongwe and Labadi serotypes were also devoid of enterotoxigenic, DNAse and hemolytic activities. Other serotypes also lacked 1-2 virulence factors. In conclusion it can be said that *Salmonella* isolates studied in this report possessed diverse type of virulence determinants and were highly pathogenic. We also affirm that *Salmonella* pathogenecity is a complex mechanism and the organism uses different virulence determinants to inflict disease on the host.

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