Prevalence & phenotypic expression of sopB gene among clinical isolates of Salmonella enterica

H. Rahman*

Department of Microbiology, College of Veterinary Sciences, Assam Agricultural University, Guwahati, India

Received December 28, 2004

Background & objectives: Salmonella induced enteritis is a complex phenomenon involving a number of pathogenic factors. Type III secretions play a central role in the development of Salmonella induced enteritis. One such Type III secretion protein is Salmonella outer proteinB (SopB). Reports on the prevalence of this gene among different serovars of Salmonella of Indian origin appears to be lacking. The present investigation reports on the prevalence of sopB gene and its phenotypic expression (SopB protein) among different serovars of Salmonella enterica isolated from man and animals.

Methods: A total of 50 isolates of S. enterica belonging to 11 serovars isolated from cases of enteric infection in man, birds and animals were tested for the presence of sopB gene by polymerase chain reaction (PCR) using its specific primers. The in vitro phenotypic expression of SopB protein was detected by dot-ELISA using anti-SopB serum.

Results: All the 50 isolates of S. enterica belonging to 11 were found to carry sopB gene irrespective of their serovars like Typhimurium, Enteritidis, Gallinarum, Choleraesuis, Virchow, etc., and source of isolation. Of these, 41 isolates were found to express sopB gene phenotypically as detected by dot-ELISA using anti-SopB serum. Of the different serovars, all but Gallinarum expressed SopB protein phenotypically in vitro.

Interpretation & conclusion: Our findings indicated that sopB gene was widely distributed and conserved among Salmonella irrespective of their serovars and source of isolation. Further work need to be done to study the factors associated with the phenotypic expression of this gene.

Key words Animals - man - polymerase chain reaction (PCR) - Salmonella - sopB gene - SopB protein - type III secretion

Salmonellae are widely distributed in nature and cause a spectrum of diseases in man and animals but their virulence factors responsible for the induction of gastroenteritis and/or systematic infection are still poorly understood. Moreover, the different subspecies and serovars of Salmonella

*Present address: Principal Scientist & Head, Division of Veterinary Public Health, Indian Veterinary Research Institute Izatnagar, Bareilly 243122 (U.P.), India
differ considerably in their virulence for man and animals. *S. enterica* serovar Typhi (*S. Typhi*) is highly pathogenic for humans but never associated with animals, while *S. Gallinarum* is a pathogen for poultry but a rare cause of infections in humans. *Salmonella* possesses a dedicated protein secretion system denoted as type III secretion system (TTSS) which is involved in the early stage of *Salmonella* infection. This sophisticated system is found to contribute to the pathogenesis by directing secretion and translocation of several virulence-associated proteins (effector proteins) directly into the cytoplasm of host cells. In recent years several translocated effector proteins have been identified that might be involved in the pathogenesis of salmonellosis. One such group of effector proteins of *Salmonella* is *Salmonella* outer proteins (Sop). These proteins are encoded by *sop* genes and several polymorphisms of these genes have been identified (*sopA-E*). Of the different types of Sop proteins, SopB protein (a 60 kDa), initially identified in the culture filtrate of *S. enterica* serovar Dublin was found to be associated with *Salmonella* induced diarrhoea and gastroenteritis. Though reports on the prevalence of this gene among different serovars of *Salmonella* isolated in different countries are available, such information on *Salmonella* from India appears to be lacking. The present investigation was therefore carried out to report on the prevalence of *sopB* gene and its phenotypic expression among different serovars of *S. enterica* isolated from man and animals in India.

**Material & Methods**

**Bacterial isolates:** A total of 50 isolates of *S. enterica* belonging to 11 different serovars isolated from human patients with gastroenteritis and birds and animals suffering from enteric infection were included in this study (Table). All these were isolated in the Department of Microbiology, College of Veterinary Science, Assam Agricultural University, Guwahati, Assam, India and serotyped at National Salmonella and Escherichia Centre, Central Research Institute, Kasauli (HP), India. A strain of *Salmonella Dublin* (2229) and a strain of *Escherichia coli* (C-600) used as positive and negative controls, respectively for *sopB* gene, were obtained from Dr H. Tschape, Director, Robert Koch Institute, Germany. The strains were maintained on nutrient agar slants before use.
Detection of sopB gene by PCR amplification: Bacterial cells from overnight cultures were suspended in sterile distilled water and boiled at 100°C for 10 min. After boiling, the cell suspensions were cooled in ice bath and were immediately tested for the presence of sopB gene by PCR amplification technique. Primers used for PCR reaction were sopB PRSB1 5-CAA CCG TTC TGG GTA AAC AAG AC-3 (upper primer) and sopB PRSB2 5-AGG ATT GAG CTC CTC TGG CGA T-3 (lower primer) (GENSET, Singapore). The nucleotide sequence corresponds to the respective gene from S. Dublin (sopB: AF060858). These primers flank a 1348 bp segment in the sopB gene sequence (Fig.). The PCR mixture (25 ml) contained 10x PCR buffer (Perkin-Elmer, USA), 1 µM of each primer, 200 µM each of dATP, dGTP, dCTP and dTTP (Perkin-Elmer, USA), 1 mM of MgCl₂ solution, 0.25 U of AmpliTaq Gold Polymerase (Perkin-Elmer, USA) and 2.5 µl of template (target) DNA preparation from test organism. The PCR incubation was performed in a thermal-cycler (Perkin-Elmer, USA) in 30 cycles of denaturation (94°C, 1 min), primer annealing (55°C, 1 min) and primer extension (72°C, 2 min). This was followed by incubation at 72°C for 10 min and storage at 4°C. Then a 15 µl aliquot of each PCR product was electrophoretically separated in agar gel (1%) containing 0.5 µl ethidium bromide per ml (Serva, Germany). The separated bands were visualized and analysed under a UV transilluminator (300 nm) and photographed using Gel Doc 2000 documentation system (Bio-Rad, USA).

Table. Prevalence of sopB gene and its phenotypic expression among different serovars of Salmonella enterica isolated from human, birds and animals

<table>
<thead>
<tr>
<th>Salmonella enterica serovars</th>
<th>Source</th>
<th>No. of isolates tested</th>
<th>No. of isolates positive in PCR</th>
<th>No. of isolates positive in Dot-ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhimurium</td>
<td>Human</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Pigs</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Birds</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>Human</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Pigs</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Birds</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Choleraesuis</td>
<td>Pigs</td>
<td>8</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Agona</td>
<td>Human</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Gallinarum</td>
<td>Birds</td>
<td>7</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Java (Paratyphi B)</td>
<td>Birds</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Virchow</td>
<td>Pig</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Richmond</td>
<td>Buffalo</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Newport</td>
<td>Cattle</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bareilly</td>
<td>Bird</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Salmonella</em> ssp I (rough)</td>
<td>Pig</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>50</td>
<td>50</td>
<td>41</td>
</tr>
</tbody>
</table>

PCR, Polymerase chain reaction
+, positive; -, negative
Isolation of SopB protein secreted by different strains of Salmonella: Bacteria were grown on Luria broth (LB) agar overnight at 37°C. One colony from agar plate was inoculated in 5 ml of LB broth containing 0.3 M NaCl and incubated at 37°C for 6 h on a rotary shaker (100 rpm). The culture was then diluted 4 times in fresh LB broth (final volume 20 ml) and incubated at 37°C for 18 h on a rotary shaker (100 rpm). Then the culture was cooled in an ice-bath for 30 min and centrifuged (20000xg, at 4°C for 1 h). The culture supernatant was collected and filtered (0.45 mm, Sartorius, Germany). The protein present in the supernatants was precipitated with 10 per cent trichloroacetic acid (Serva, Germany). The sediments were dissolved in 0.4 ml of NaOH (0.1 M) to which 2.0 ml ice cooled acetone (-20°C) was added and incubated at -20°C for 20 min. The suspension was centrifuged (20000xg, at 4°C for 15 min). The sediments were redissolved in 2.0 ml of acetone (-20°C) and incubated and centrifuged as above. The sediments were dried at room temperature and dissolved in 0.1 ml of PBS (pH 7.2).

Detection of SopB protein by Dot-ELISA using anti-SopB serum: Protein prepared from each isolate was subjected to Dot-ELISA using anti-SopB serum for the detection of SopB protein. Two µl of each protein preparation and its dilution were dotted on nitrocellulose (NC) membrane strips (Sigma, USA) and dried at 37°C for 1 h. The unsaturated sites were blocked by immersing the strips in 1 per cent solution of skimmed milk powder in PBS (0.01 M, pH 7.2) for 1 h at 37°C. The strips were washed three times in PBS-T (0.01 M PBS, pH 7.2 with 0.5% Tween-20) for five min each. The strips were dipped in the anti-SopB serum diluted 1: 20,000 (predetermined) in Tris-buffer (0.02 M, pH 7.2) and incubated at 37°C for 1 h (antiserum to SopB protein was obtained through the courtesy of Dr H. Tschape, Director, Robert Koch Institute, Germany). After incubation, the strips were washed thrice in PBS-T and incubated with anti-rabbit IgG-horse radish peroxidase (HRPO) conjugate (Boehringer, Germany) at a dilution of 1:1000 for 1 h at 37°C. Finally, the strips were washed thrice in PBS-T and immersed in freshly prepared substrate solution (1 chloro-4 naphthol, Sigma, USA) containing 30 per cent H₂O₂. The enzymatic reaction was stopped by washing the strips in running tap water and a positive reaction was indicated by the presence of deep purple dot against a white background within 10 min.

Results & Discussion

The prevalence of Salmonella outer protein B (sopB) gene among different serovars of Salmonella enterica isolated from man, birds and animals was investigated by PCR amplification technique and its in vitro phenotypic expression was detected by dot-ELISA. The organisms that gave rise to 1348 bp in the sopB gene sequence as did by the reference strain S. Dublin (2229) were taken as positive for the presence of sopB gene (Fig.). All the 50 isolates of S. enterica belonging to 11 serovars tested for the presence of sopB gene were found to carry sopB gene irrespective of their serovars like Typhimurium, Enteritidis, Gallinarum, Virchow, Agona, Choleraesuis, Paratyphi B, Bareilly, Newport, etc., and source of isolation (Table). The data obtained indicated that sopB gene was widely distributed and conserved among all the serovars of Salmonella and present in all clinical isolates. These observations are in agreement with findings of other workers.

The SopB protein secreted by different serovars of S. enterica and the reference strain S. Dublin (2229) was isolated and analyzed by dot-ELISA. The isolates that produced 60.0 kDa proteins, which specifically reacted with SopB antibody were taken as the producer of SopB protein. Of the 50 isolates, 41 belonging to serovars Typhimurium, Enteritidis, Virchow, Agona, Choleraesuis, Paratyphi B,
Bareilly, Newport, etc., were found to produce SopB protein (Table). Although all the isolates of serovar Gallinarum were found to harbour sopB gene, they did not express it phenotypically in vitro. The missing phenotypic expression among genetically sopB gene positive strains indicated that some environmental signal might be necessary for induction of sopB gene in S. Gallinarum. Earlier studies revealed that some signals are required for in vitro induction of proteins by genes\textsuperscript{14}. The optimum in vitro expression of Salmonella enterotoxin (stn) gene was found to be associated with a number of factors which include co-cultivation of the organisms with host cell\textsuperscript{15}, autoinducers like norepinephrine available in the intestine\textsuperscript{16}, other environmental conditions like cultivation of organism under shaking condition, additional growth factors\textsuperscript{17}, etc. These factors might have also been required for induction of sopB gene. Further work on this regard is envisaged.

The SopB protein is one of the important type III secretions and associated with enteritis\textsuperscript{10}. SopB is an inositol phosphate phosphatase capable of hydrolyzing several inositol phosphates resulting in elevated cellular level of InS(1,4,5,6)P4 which in turn induces electrolytes and fluid secretion and recruitment of polymorphnuclear (PMN) cells in Salmonella infected intestinal mucosa\textsuperscript{18,19}. Thus, the SopB is regarded as a novel bacterial enterotoxin\textsuperscript{6}. Further research on the mechanism of phenotypic expression of this gene would be helpful in the control of Salmonella induced enteritis.

Acknowledgment

The author thanks to Dr H. Tschape, Director, Robert Koch Institute, Germany for providing anti-SopB serum.

References


Reprint requests: Dr H. Rahman, Head, Division of Veterinary Public Health, Indian Veterinary Research Institute Izatnagar, Bareilly 243122, India
e-mail: hr19@rediffmail.com