Plasmid Profile Analysis of *Salmonella* spp. from Man, Animals and Foods of Animal Origin

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**ABSTRACT**

The present study was carried out for plasmid profile analysis of *Salmonella* spp. from man, animals and foods of animal origin. A total of 50 *Salmonella* isolates from 1,132 different sources viz., poultry meat (212), poultry eggs (49), poultry droppings (60), autopsied poultry tissues (80), pork (156), pig faeces (189), cattle dung (105), buffalo dung (103), sheep faeces (11), goat faeces (31), deer faeces (2) and human stool (154) from Pantnagar, Nagla, Jawahar nagar, Lalkuan and Rudrapur were included in this study. Plasmid profile analysis of all *Salmonella* isolates revealed that majority of *Salmonella* isolates harboured 2 plasmids with size of 23.13 kb and above 23.13 kb. Four isolates of *Salmonella* harboured similar pattern of 5 plasmids of different sizes, in which each isolate harboured the plasmids of 2.027 kb, 2.322 kb, 4.361 kb, 23.13 kb and more than 23.13 kb size. However, a different banding pattern, i.e. presence of single band was also exhibited by some *Salmonella* isolates in the present study. The plasmid profile analysis of *Salmonella* isolates indicated genetic similarities as well as genetic variations among some of these isolates.

**Keywords:** Plasmid profile, plasmid size, *Salmonella* serovars

*Salmonella* has been implicated as a major cause of foodborne illness throughout the world. It constitutes a major public health burden and represents a significant cost in many countries. Millions of human cases of salmonellosis are reported world-wide every year resulting into thousands of deaths. This disease has become an important public health problem throughout the world (Workman *et al.*, 1999 and Srifuengfung *et al.*, 2005), which also contributes to negative economic impacts due to the cost of surveillance investigation, treatment and prevention of illness.

Plasmid profile (PP) analysis is a method of determining a number and size of plasmids in bacterial isolates. Plasmids of *Salmonella enterica* vary in size from 2 to more than 200 kb (Rychlic *et al.*, 2006). Plasmids found in *Salmonella* may be classified from the functional point of view into two groups viz., the serovar specific virulence plasmids and other high molecular weight plasmids including those transferring resistances to antibiotics (Rychlic *et al.*, 2006). Plasmids are therefore important not only for storage of genetic information but also for dissemination of genetic information including the antibiotic resistances.

The present study was conducted for isolation, identification and plasmid profile analysis of *Salmonella* serovars form man, animals and foods of animal origin in order to know the similarities or variations among them.

Plasmid DNA was isolated from 50 *Salmonella* isolates of an earlier study by alkaline lysis with SDS as per the method described by Sambrook and Russell (2001).
Table 1. Plasmid profile of *Salmonella* isolates

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Number of <em>Salmonella</em> isolates (n=50)</th>
<th>Number of plasmid in each isolate</th>
<th>Size of plasmid (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>1</td>
<td>4.361, 23.130 and &gt;23.130</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>2</td>
<td>23.130 and &gt;23.130</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>3</td>
<td>6.557, 23.130 and &gt;23.130</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>4</td>
<td>1.050, 2.027, 2.322, 4.361, 9.416, and &gt;23.130</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>5</td>
<td>2.027, 2.322, 4.361, 23.130 and &gt;23.130</td>
</tr>
</tbody>
</table>

Plasmid profile analysis was carried out as per the method described by Sambrook and Russell, (2001) with suitable modifications to determine the number and size of plasmids as well as to know the commonality in the *Salmonella* isolates. The agarose gel electrophoresis of plasmid DNA was carried out in 0.8% agarose gel using horizontal submarine gel electrophoresis system. A separate well was also loaded with a plasmid marker (0.5 µg/lane of 5 mm width). The plasmid marker consisted of a mixture of λ DNA digested with Hind III and pUC 18 DNA digested with Sau3AI and Taq I separately, phenol extracted and supplied in 10 mM Tris-Cl (pH 8.0) and 10 mM EDTA. λ DNA/ Hind III - pUC 18/ Sau3AI - pUC 18/ Taq I digest consists of 27 double stranded DNA fragments of 23130, 9416, 6557, 4361, 2322, 2027, 1444, 943, 754, 585, 564, 458, 341, 258, 153, 125, 105, 78, 75, 46, 36, 30, 18, 17, 12, 11 and 8 base pairs. Agarose gel electrophoresis was carried out at 50 V for 1 h and visualized on gel documentation system. The relative molecular size of plasmid DNA was calculated against plasmid marker.

Plasmid profile could be a means of identifying both related and unrelated isolates in a particular geographical area. Once the origin is located, the spread of the organism can easily be tracked during epidemiological study of outbreaks (Jarvis, 1984). Therefore, plasmid profile analysis was carried out in the present study to determine the number and size of plasmids and also to know the commonality or difference among the *Salmonella* isolates. The number of plasmids was found in a range of 1-5 with the size varying from 1.050 kb to more than 23.130 kb (Table 1). Banding pattern of 1-4 plasmids in *Salmonella* spp. has been reported to be a common phenomenon (Pawar, 2004; Shome *et al.*, 2006), which is almost similar to the banding pattern of plasmids of *Salmonella* observed in the present study.

The plasmid profile analysis revealed that 4 isolates of *Salmonella* harboured similar pattern of 5 plasmids of different size, in which each isolate contained the plasmids of 2.027...
kb, 2.322 kb, 4.361 kb, 23.130 kb and more than 23.130 kb size (Fig. 1). This pattern of plasmid profile indicates genetic similarities among these isolates. Since these 4 isolates were recovered from different geographical areas of the present study, therefore, it may be concluded that the isolates might be of similar origin.

Moreover, different banding patterns of Salmonella isolates were also found in the present study. Some Salmonella serovars exhibited single band pattern of different size, which shows their genetic unrelatedness. Most of the isolates harboured 2 plasmids with the size of 23.130 kb and above 23.130 kb. Similar findings with of larger size of plasmids in the Salmonella isolates have also been described by Rychlik et al. (2006). Plasmids of Salmonella enterica vary in size from 2 to more than 200 kb. The best described groups of plasmids are the virulence plasmids (50–100 kb in size) present in serovars Enteritidis, Typhimurium, Dublin, Cholerae-suis, Gallinarum, Pullorum and Abortus-ovis (Rychlik et al., 2006).

It may be concluded that the plasmid profile analysis of Salmonella serovars in the present study indicated the genetic similarities among some serovars as well as genetic un-relatedness among some of these serovars. Similar banding pattern of plasmids that is same number and size of plasmids exhibited by some Salmonella isolates recovered from different geographical areas of the present study is suggestive of prime importance of plasmid profile analysis in tracking the outbreaks of disease during epidemiological investigations. Moreover, the findings of the present study also revealed the presence of large size of plasmids in anti-microbial resistant Salmonella serovars.

References