Plasmid Characterization of *Salmonella* Isolated from Foods of Animal Origin


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**ABSTRACT**

Eleven isolates of *Salmonella* comprising five pork, three raw fish, one fish product, one raw milk and one pork product were processed for antibiogram, plasmid profiling and curing. Antibiogram study revealed 100% resistance to penicillin-G, followed by ampicillin, amoxyclav and trimethoprim. Moderate degree of sensitivity was observed towards cephotaxim, neomycin, nalidixic acid and tetracycline and highest degree of sensitivity towards amikacin and gentamicin. Ten of eleven isolates harboured one to four plasmid fragments with molecular weight ranging between 1.509 kb and 70.838 kb. Curing study of plasmid bearing nine amoxyclav resistant isolates with potassium nitrate as a curing agent revealed elimination of a plasmid band from four isolates. Further processing of these cured isolates for antibiogram study revealed sensitivity to amoxyclav indicating resistance for this drug as plasmid mediated character.

**Keywords:** Antibiogram study, curing, plasmid profiling, *Salmonella*

**Introduction**

Salmonellae are widely distributed in nature, infecting man and animals and are major public health concern. *Salmonella enterica* serovar Typhi is the etiological agent of typhoid fever in humans with an annual global burden of approximately 16 millions cases, leading to 6,00,000 fatalities. Indiscriminate and unwarranted use of antimicrobials and chemotherapeutic agents in the food animals has resulted in emergence of multiple drug resistance in the bacterial isolates. Plasmid, an extra chromosomal DNA molecule is responsible for development of drug resistance in the bacterial species. The presence of plasmids in bacterial species pose a potential public health hazard, since plasmid harbouring multiple antimicrobial resistance determinants (R-plasmid) are transferred in simulated natural micro-environments from various bacterial pathogens of human, animal or fish origin to susceptible strains from different ecological niche (Kruse and Sorum, 1994). Thus plasmid profile along with feasibility of elimination of plasmid using potassium nitrate (class-I preservative) as a curing agent was undertaken.

**Materials and Methods**

**Isolates**

A total of 11 *Salmonella* isolates obtained from foods of animal origin comprising five from pork, three from raw fish and one each from fish fry, raw milk and back bacon available with Department of Veterinary Public Health, Nagpur Veterinary College, Nagpur were included in the study (Table 1).

**Antibiogram study**

Antibiotic sensitivity pattern of isolates was studied by agar diffusion method as suggested by
Table 1: Plasmid profile study of *Salmonella* isolates

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th><em>Salmonella</em> isolate number</th>
<th>Source</th>
<th>No. of plasmids</th>
<th>Molecular size (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PK36 Pork</td>
<td>01</td>
<td>49.970</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PK38 Pork</td>
<td>00</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>PK39 Pork</td>
<td>01</td>
<td>36.601</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PK40 Pork</td>
<td>01</td>
<td>38.351</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>PK41 Pork</td>
<td>02</td>
<td>70.8389.188</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>FR10 Raw fish</td>
<td>02</td>
<td>67.90537.566</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>FR25 Raw fish</td>
<td>02</td>
<td>66.48529.147</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>FR34 Raw fish</td>
<td>01</td>
<td>1.509</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>F40 Fish product (Fish fry)</td>
<td>04</td>
<td>65.09426.7841.9971.940</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>274 Raw milk</td>
<td>01</td>
<td>50.754</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>PKP5 Pork product (Back bacon)</td>
<td>04</td>
<td>66.13029.8951.9971.934</td>
<td></td>
</tr>
</tbody>
</table>

Bauer *et al.* (1966) using standard antibiotic discs. The commonly used antibiotic discs such as ampicillin, amoxyclav, amikacin, azithromycin, ceftazidime, cephotaxim, doxycycline hydrochloride, erythromycin, gentamicin, neomycin, nalidixic acid, penicillin-G, streptomycin, tetracycline and trimethoprim were included in study.

**Multiple antibiotics resistance (MAR) index**

The multiple antibiotics resistance (MAR) index was calculated as per Krumpelman (1985), by applying $a/b$ where “$a$” is the number of antibiotics to which an isolate was resistant and “$b$” is the number of antibiotics to which the isolate was exposed.

**Plasmid profile analysis**

Isolates were processed for plasmid DNA profile study by alkaline lysis method suggested by Sambrook *et al.* (1989).

**Curing of drug resistance plasmids**

Amoxyclav resistant nine isolates harbouring plasmids were processed for curing study by employing potassium nitrate (class-I preservative) as a curing agent. Twenty µl of enriched culture in BHI under aeration for 8 h was inoculated into 5 ml of BHI broth containing 150 µg/ml of potassium nitrate. These were further incubated at 37°C under aeration for 8 h. The cultures thus obtained were streaked onto master plates without antibiotics (prepared using *Salmonella Shigella* agar) to obtain single isolated colonies.

The colonies were transferred onto replica plates (prepared using *Salmonella Shigella* agar containing amoxyclav @ 30 mg/ml) with Whatman filter paper No. 42 soaked in sterile distilled water. The plates were then incubated at 37°C for 24 and 48 h.

A colony was considered to be cured, when it failed to grow on antibiotic containing agar plates, but grew on antibiotic free agar plates.

The cured colonies were then processed for plasmid profile study as stated earlier.

**Correlation study**

The correlation between the plasmid content and the antibiotic resistance was studied by ascertaining the antibiogram of cured isolates as described earlier.
Results and Discussion

Antibiogram study

All isolates exhibited resistance to penicillin-G, followed by ampicillin, amoxyclav and trimethoprim (81.81% each); erythromycin and tetracycline (63.63% each); doxycycline hydrochloride (54.54%); cefazidime (45.45%); streptomycin (18.18%); azithromycin, cephotaxim and nalidixic acid (9.09% each). Moderate degree of sensitivity was revealed towards cephotaxim (45.45%); neomycin and nalidixic acid (27.27% each); tetracycline (18.18%); azithromycin, cefazidime, erythromycin and trimethoprim (9.09% each). Highest degree of sensitivity was recorded towards amikacin and gentamicin (100% each); azithromycin and streptomycin (81.81% each) and neomycin (72.72%) (Table 1).

Results of antibiogram indicated the potential importance of foods of animal origin as source of multiple antimicrobial-resistant Salmonella for human infections. The presence of antimicrobial-resistant Salmonella strains in foods of animal origin may be associated with the use of medicated feeds in animal husbandry systems, subtherapeutic doses and indiscriminate uses of antimicrobials in treatments.

Susceptibility of Salmonella isolates for amikacin/gentamicin in the present study can be explained by the limited availability and high cost of these groups of antimicrobials that might have reduced their frequent utilization. However, further epidemiological and molecular studies are essential on the frequency, sources of acquisition of resistant genes and distribution of antimicrobial resistant Salmonella among food animals, food products and humans. The presence of multiple antimicrobial resistant Salmonella indicates the need for the prudent drugs usage to diminish the development and spread of antimicrobial resistance.

Multiple antimicrobial-resistant Salmonella organisms have also been reported by other workers including Molla et al. (2003), Larkin et al. (2004) and Bada et al. (2006).

In the present study the MAR index of Salmonella ranged from 0.06 to 0.53. Out of 11 isolates, 10 were found to have MAR index more than 0.2, thus indicating injudicious use of antibiotics. The findings of present study are comparable with the work of Hatha and Lakshmanaperumalsamy (2005), who reported 95% of strains originated from risk source of contamination such as poultry, swine, cattle and human environment where antibiotics are often used.

Plasmid profiling

The plasmid profiling revealed 10 Salmonella isolates harbouring one to four plasmid fragments with molecular weight ranging between 1.509 kb and 70.838 kb (Fig. 1 and 2).

The banding pattern of one to four plasmid fragments in Salmonella spp. has been reported to be a common phenomenon (Pawar, 2004, Shome et al., 2006).
These findings are supported by the works of Anjanappa et al. (1993) and Luque et al. (1994). Treatment of isolates for curing/elimination of R-plasmid by use of class-I preservative is an useful finding, which may have practical applications.

**References**


**Curing and correlation study**

Overall, four (three from pork and one from fish) isolates responded to curing. A plasmid band bearing molecular size of 36.601 kb was eliminated from isolate PK39; 38.351 kb from PK40, two bands bearing 70.838 kb and 39.188 kb from PK41; and a plasmid with molecular weight 1.509 kb from an isolate from fish (FR34) were eliminated after curing. The further evaluation of cured isolates against amoxyclav revealed sensitivity to amoxyclav thus confirming plasmid mediated drug resistance.

Plasmid profile could be a means of identifying either related or unrelated isolates in a particular geographical area. Once the origin is located the spread of organism can easily be tracked for epidemiological study of outbreaks (Jarvis, 1984). However the plasmid profile technique needs to be employed for large number of isolates for its proper exploitation.

![Fig. 2. Plasmid profile of *Salmonella* isolates. SM - Standard molecular weight marker, PK41- Pork isolate, FR10, FR25- Raw Fish isolates, F40- Fish fry isolate](image-url)