Molecular Characterization of *Salmonella* Isolates of Duck in Comparison to *Salmonella* Isolates of Chicken and Ruminants

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*Salmonella* species are recognized as a major cause of food borne illness that are closely associated with the consumption of contaminated poultry and egg products. The present study was conducted to compare the cultural, biochemical characteristics, antibiotic sensitivity pattern and the patterns of genomic organization of duck *Salmonella* isolates associated with chicken, cattle, sheep and goat using pulsed-field gel electrophoresis (PFGE) with *Xba*I restriction enzyme. The comparative antibiogram study among duck, chicken and ruminants showed variable results in antibiotic sensitivity and similar results in resistance pattern. Genome analysis using PFGE with *Xba*I restriction enzyme revealed that the *Salmonella* isolates of the same species collected from same areas to be of same genomic pattern, although a great genomic diversity could be found among duck, chicken, sheep, goat and cattle *Salmonella* strains. It may be concluded from the result of this research work that the heterogeneity in genomic organization among different isolates of different species collected from different areas occurred greatly and for this reason.

Keywords: Salmonellosis, *Salmonella*, Pulsed-field gel electrophoresis (PFGE), Duck, Chicken, Ruminants

**Introduction**

Salmonellosis is a disease of human beings, cattle, sheep, goat, pigs and chicken including duck and is manifested clinically in all hosts by one of three major syndromes: a peracute systemic infection, acute enteritis or chronic enteritis. But the clinical signs may vary from species to species. Human infections with *Salmonella* have been increasing worldwide since 1980 and have been shown to be related mainly to consumption of eggs and egg products. On the other hand, *S. blockley*, *S. weltevreden*, and *S. amsterdam* have been identified as common serovars found in broilers, layers, and breeder parent stock, respectively, and *Salmonella* has been detected in eggs from layers, according to a Thai report. Furthermore, *S. enteritidis* has been isolated from chicken feces and chicken meat in Thailand. However, the relationship between human infections and isolates of *S. enteritidis* from broiler chicken meat remains obscure.

The members of the genus *Salmonella* are being isolated, identified and characterized by using various cultural, biochemical, serological and molecular studies. The reliable methods for isolation require the use of media, which encourage the growth of *Salmonella* and inhibit that of other enteric organisms. A great variety of fluid and solid enrichment and selective media such as selenite broth, *Salmonella*-Shigella (SS) agar, MacConkey agar, brilliant green agar (BGA) have been used for this purpose. Antibiogram study, serum agglutination test, pathogenicity test, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), DNA-DNA hybridization, pulsed-field gel electrophoresis (PFGE) are widely being used to identify and characterize *Salmonella* spp. in the laboratories.

For the molecular characterization, PFGE is an important tool for the analysis of the genomic organization of different species of *Salmonella*, which is used to explain the genomic basis of the epidemic. In this study, isolation, identification and characterization of duck *Salmonella* in comparison to chicken, cattle, sheep, and goat *Salmonella* isolates from various locations were analyzed via PFGE with *Xba*I to compare genomic organizations of different serotypes of different species. PFGE using *Xba*I restriction provided a possible alternative method for screening and identifying duck *Salmonella* serotypes in comparison to other serotypes previously isolated from chicken, cattle, sheep and goat.

**Materials and Methods**

**Laboratory and specimens**

The experiment was conducted between November 2006 and October 2007 in the Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University.
Cultivation and isolation of Salmonella

Cloacal swabs were collected and each of swabs was inoculated into freshly prepared selenite broth. Then the tubes were marked properly and incubated at 37°C for 24 h aerobically in bacteriological incubator. The tubes were then examined for growth of bacteria. Smears were prepared for each culture and the Gram-stained examined under microscope. Gram-negative rod isolates were streaked on MacConkey agar, Salmonella-Shigella agar and brilliant green agar separately. The plates were then incubated at 37°C for 24 h. The plates containing characteristic colonies of Salmonella were selected. Motility test and Gram-reaction were performed to identify Salmonella. Subculturing in Salmonella-Shigella agar was performed from the suspected plates containing Salmonella to obtain a pure culture. These pure isolates obtained in this way were used for further study.

Biochemical characterization

Organisms showing cultural characteristics of Salmonella on various media were maintained on SS and BGA and were subjected to biochemical tests such as sugar fermentation test, MR-VP reaction and indole reaction.

Antibiogram study of Salmonella isolates

Susceptibility of the Salmonella isolates to different antibacterial agents was performed through disc diffusion method. In this method Salmonella isolates were grown overnight on BGA. The overnight cultured isolates were inoculated into NB and poured on BGA and spread uniformly with the help of sterile glass spreader. Antibacterial discs were applied aseptically to the surface of the plate at an appropriate arrangement with the help of sterile forceps and incubated aerobically at 37°C for 24 h.

Maintenance of stock culture

Salmonella isolates from duck and the laboratory isolates of Salmonella from chicken, cattle, sheep and goat were preserved in 20% glycerine and soft agar method. Salmonella isolates preserved in 20% glycerine were placed in ice box and transported to ICDDR,B, Dhaka for performing molecular characterization.

Molecular typing of Salmonella by pulsed-field gel electrophoresis (PFGE)

Molecular typing of Salmonella isolates by PFGE was done using preparation of PFGE agarose plugs from cell suspensions and lysis of cells in agarose plugs. After cell lysis agarose plugs were washed and then subjected to restriction digestion of DNA in agarose plugs with XbaI followed by casting agarose gel and loading restriction plug slices on the comb. Electrophoresis was performed with the contour clamped homogenous electric field (CHEF-DRII) apparatus from the Bio-Rad (Richmond, USA).

Results and Discussion

Around the world, Salmonella is the most important agent causing food-borne illness, with Salmonella enterica serovar Enteritidis and Salmonella serovar Typhimurium predominating. Salmonella is a pathogen of both humans and animals. This organism has caused outbreaks of human disease both in developed and developing countries. A Danish surveillance program for Salmonella in fresh meat, instituted after an epidemic of Salmonella, found 3.1% of pork cuts were contaminated with Salmonella serotype Infantis. Isolates from a cattle outbreak of Salmonella associated with contaminated feed in Finland were analyzed by pulsed-field gel electrophoresis (PFGE), plasmid analysis, ribotyping, and IS200 typing. The disease burden in terms of sporadic cases due to Salmonella of animal origin to human disease is not known in Bangladesh. In this study an attempt was made to compare Salmonella isolates of livestock including duck, chicken, cattle, sheep and goat.

In this study, all Salmonella isolates from duck, chicken, cattle, sheep and goat showed similar colony characteristics on Salmonella-Shigella, MacConkey and BrilliantGreen agar media. All isolates showed similar staining characteristics, i.e., Gram-negative, short rod shaped organisms. All of the isolates except for the chicken isolates showed positive motility test. After biochemical examination it was observed that all the isolates fermented dextrose, maltose and mannitol and produced acid and gas but did not ferment lactose and sucrose. They were negative to VogesProskauer test and indole test with the exception of a few isolates (viz., D1Fa, D6Bb and Ch7Fg) but all isolates showed positive result to methyl red test.

Comparative antibiogram results of duck Salmonella isolates and chicken and ruminants isolates are summarized in Table 1. The isolates of duck and ruminants were found resistant to chloramphenicol, whereas the isolates of chicken were found resistant to chloramphenicol and amoxicillin.

### Table 1. Comparative antibiotic sensitivity pattern of Salmonella isolates from various sources

<table>
<thead>
<tr>
<th>Source of Salmonella</th>
<th>Highly sensitivity</th>
<th>Moderately sensitivity</th>
<th>Less sensitivity</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duck</td>
<td>CIP, K, NA, SxT, CL</td>
<td>K, NA, SxT, CL, E, AML</td>
<td>E, AML, C</td>
<td>C</td>
</tr>
<tr>
<td>Chicken</td>
<td>CIP, K, NA</td>
<td>SxT, CL</td>
<td>E</td>
<td>AML, C</td>
</tr>
<tr>
<td>Ruminant</td>
<td>CIP, K, NA, SxT</td>
<td>CL</td>
<td>E</td>
<td>AML, C</td>
</tr>
</tbody>
</table>

AML = Amoxicillin, 10 µg; C = Chloramphenicol, 30 µg; CIP = Ciprofloxacin, 5 µg; CL = Cephalexin, 30 µg; E = Erythromycin, 10 µg; K = Kanamycin, 30 µg; NA = Nalidixic acid, 30 µg; SxT = Cotrimoxazole, 25 µg.
Pulsed-field gel electrophoresis (PFGE) based on analysis of the whole genome by restriction endonuclease digestion might also be useful for investigation of sources of salmonellosis21. In this study, PFGE analysis of the XbaI digested chromosomal DNA of the Salmonella isolates yielded 12 to 17 reproducible DNA fragments ranging in size of approximately from <20 to <668.9 kbp (Figure 1). PFGE analysis revealed that Salmonella isolates (n = 13) from the same origin displayed very similar restriction fingerprint pattern, while the isolates of different species of different places yielded diverse and heterogeneous banding pattern.

The present study showed, interestingly, that the Salmonella isolates of different livestock produced different PFGE pattern. However, Thong et al.22 and Boonmar et al.23 observed that the isolates of different phage types produced the same PFGE pattern. Some studies have implicated poultry and poultry product (e.g., egg) contamination as the primary cause of increased Salmonella infection in humans24-25. It has also been reported that the phage type distribution in isolates originating from the meat of broiler chickens is similar to that in human isolates. The evidence presented here indicates that meat products could be a potential source of human infection by Salmonella. PFGE is required for an adequate description of the strain characteristics of Salmonella from various sources. Continued epidemiological and laboratory monitoring of changes in the background of sporadic human cases, especially of uncommon strains, will further define the scope of the problem.

References


