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Original Article

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*1 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*1 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[1]. 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[2-3]. 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[4]. Furthermore, *S. enteritidis* has been isolated from chicken feces and chicken meat in Thailand[5-6]. 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[7-9].

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[10-11]. 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*1 to compare genomic organizations of different serotypes of different species. PFGE using *Xba*1 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

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(BAU), Mymensingh and Enteric Microbiology Laboratory, International Centre for Diarrhoeal Diseases Research of Bangladesh (ICDDR,B) in Dhaka. A total of 65 cloacal swab samples from apparently healthy and diarrhoeic ducks were tested for the examination. In addition, 27 isolates of *Salmonella* from chicken, cattle, sheep and goat collected from the repository of the Department of Microbiology and Hygiene, BAU, Mymensingh were used to compare with those of ducks.

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 37ºC 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 37ºC 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[12]. 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[13]. 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[14].

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 *Xba*1 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[15]. *Salmonella* is a pathogen of both humans and animals. This organism has caused outbreaks of human disease both in developed[16-17] and developing[18] 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[19]. 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[20]. 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 exception 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*

Source of *Salmonella*	Highly sensitivity	Moderately sensitivity	Less sensitivity	Resistance
Duck	CIP, K, NA, SxT, CL	K, NA, SxT, CL, E, AML	E, AML, C	C
Chicken	CIP, K, NA	SxT, CL	E	AML, C
Ruminant	CIP, K, NA, SxT	CL	E, AML	C

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 salmonellosis[21]. In this study, PFGE analysis of the *Xba*1 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.

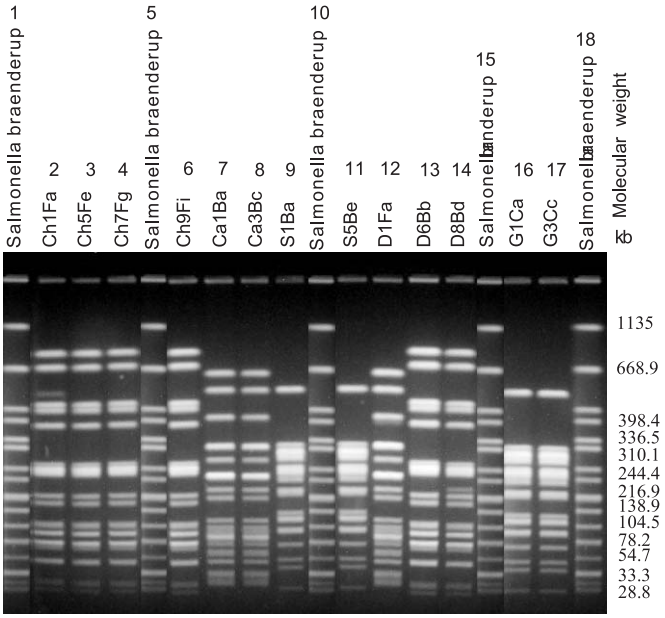

Figure 1. *Pulsed-field gel electrophoresis (PFGE) with Xba1 of Salmonella isolates from chicken, cattle, sheep, duck and goat. Lane 1, 5, 10, 15 and 18 = Genomic organization of Salmonella braenderup used as a marker. Lane 2, 3, 4 and 6 = Genomic organizations of chicken isolates collected from BAU Poultry Farm; Lane 7 and 8 = Genomic organizations of cattle isolates collected from Boyra, Mymensingh; Lane 9 and 11 = Genomic organizations of sheep isolates collected from Boyra, Mymensingh; Lane 12 = Genomic organization of duck isolates collected from BAU Poultry Farm; Lane 13 and 14 = Genomic organization of duck isolates collected from Boyra, Mymensingh; Lane 16 and 17 = Genomic organizations of goat isolates collected from Char Nilokkhiya.*

The present study showed, interestingly, that the *Salmonella* isolates of different lifestock 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 humans[24-25]. 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.

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