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Prevalence of *Salmonella* Serovars and Antimicrobial Resistance Profiles in Poultry of Savar Area, Bangladesh

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Abstract

Salmonellosis is one of the major concerns in the poultry industry and some serovars of *Salmonella* involve in zoonosis. This study determines the seroprevalence of *Salmonella* in poultry and their drug-resistant patterns, variability in infectivity and mortality rate of birds, and predilection of some serovars to cause zoonoses. The average seroprevalance of *Salmonella* in three different age groups was found to be 37.9%. A total of 503 samples were examined over a period of 1 year from five different poultry farms of a semiurban area of Savar, Dhaka, Bangladesh. The prevalence of *Salmonella* was recorded to be 21.1%. *Salmonella* was found high in dead birds (31.2%) than live birds (18.1%). *Salmonella* infection was higher (23.6%) in summer than in winter (12.9%) season. Among the 106 isolates, 46 belong to serogroup B (43%) and 60 isolates to serogroup D (57%). The highest *Salmonella* infection was recorded as 47.9% on the 30–35-week-old birds. A total of 106 *Salmonella* isolates were used for antimicrobial susceptibility test against 10 common antibiotics and 17 multiple drug resistance patterns were found. Among the isolates, 69 (65%) harbored plasmids 1–4 with size variation between >1.63 and >40 kb and rest 37 (35%) isolates were plasmid free but showed resistance against 5–10 antibiotics. The results of the present investigation suggested that multiple drug resistance is common among the *Salmonella* isolates of poultry and some of these isolates may have zoonotic implications.

Introduction

ENTERIC SALMONELLA INFECTION is a global concern in both human and animals and has been attributed to be the most important bacterial etiology for enteric infections worldwide (Bhat and Macaden, 1983). The World Health Organization has estimated that annually 1.3 billion cases of acute gastroenteritis or diarrhea occur because of nontyphoid salmonellosis, causing 3 million deaths. In addition to human health implications, Salmonella is a pathogen of significant importance in worldwide animal production. The emergence of antibiotic-resistant strains is a further threat to human and animal health, which occurs principally because of the therapeutic use of antimicrobials in animals. There are >2500 Salmonella serovars distributed throughout the world (L Plym and Wierup, 2006).

Poultry is known to be the largest single reservoir of *Salmonella* (Gupta *et al.*, 1999). Salmonellosis is one of the most important diseases in poultry that cause serious economic loss because of mortality and reduced egg production (Khan *et al.*, 1998). The major host-adapted serovars of *Salmonella* in poultry are *Salmonella* Gallinarum and *Salmonella* Pullorum,

which are responsible for fowl typhoid and Pullorum disease, respectively (Snoeyenbos, 1994; Khan et al., 1998). In addition to avian salmonellosis (Palaniswamy et al., 1989; Verma and Gupta, 1997), Salmonella Typhimurium and Salmonella Enteritidis are unambiguously categorized as zoonotic hazards, because poultry are known to be the major transmitter of nonhost-adapted salmonellosis in humans (Rahman et al., 1997).

Salmonella infection is one of the major constraints of poultry farming that hindered its development in Bangladesh (Kamaruddin and Giasuddin, 2003; Das et al., 2005). In recent days, the prevalence of salmonellosis in breeder flock, commercial broiler, and layer flocks is increasing day by day. However, very limited research works had been carried out in Bangladesh concerning salmonellosis in poultry so far. Therefore, salmonellosis status of a farm needs to be determined for its proper control and management (Ahmed et al., 2008). The objective of this study is (1) to determine the seroprevalence of Salmonella in poultry, (2) antibiogram of isolated salmonella strains, (3) variability in infectivity and mortality rate in differing season, and (4) predilection of some serovars to cause zoonoses.

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Materials and Methods

Study area

This study was conducted in five different layer poultry farms located in semiurban area of Savar region of Dhaka, Bangladesh (Fig. 1), during the period May 2009 to June 2010. The samples were collected from the birds of selected poultry farm and brought to the Department of Microbiology, University of Dhaka, for laboratory analysis.

Seroprevalence study

Sample collection. A total of 346 blood samples of the wing vein of individual birds of 20th–25th, 30th–35th, and >40th weeks of age were collected from four selected poultry farm (10% of the total flock). Blood samples were aseptically collected in sterile vial with sterile 5-mL syringe and the samples were allowed to clot in the syringe and kept for 1–2 hours at room temperature. After clotting, sera were separated, centrifuged (12,000 rpm, 1 minute) at room temperature and poured in sterile vials, individually labeled, and stored at –20°C until further use.

Salmonella antigen. Standard *Salmonella* Pullorum antigen manufactured by Lohman Animal Health Ltd. was used for serum plate agglutination (SPA) test for the detection of *Salmonella* antibodies in the sera samples.

Detection of *Salmonella* infection by SPA test. The SPA test was performed according to the procedure described in OIE Manual, 2000, with crystal violet-stained *Salmonella* antigen (Pullorum antigen; Lohman Animal Health Ltd.). For this test, 0.02 mL of antigen and 0.02 mL of chicken sera were placed side by side with a micropipette on a glass plate. Then, the antigen and the sera were thoroughly mixed by stirring with a small tooth pick followed by rocking. Results of SPA test were read within 2 minutes. In positive case, granules

were slowly formed, indicating that sera samples contained antibody against *Salmonella* infection. In negative case, granules were not formed within 2 minutes, indicating that antibody against *Salmonella* were absent in the sera samples. The results of SPA test were recorded.

Isolation and identification of Salmonella

Sample collection. A total of 391 cloacal swabs were collected from live birds and a total of 112 samples were collected from liver, intestine, ovary, oviduct, and spleen swab after postmortem examination of dead birds. Sterile cotton swab sticks were used for sample collection and collected samples were directly brought to the laboratory in an insulated ice box with minimum delay and bacteriologically examined immediately. Isolation and identification of salmonellae were done according to the procedure described by OIE (2000), Merchant and Packer (1967), and Cowan (1985).

Cultivation of the sample. The collected swab containing samples were grown into tetrathionate broth (Oxoid Ltd.) at 37°C for 18–24 hours. Tetrathionate broth-grown cultures were grown in MacConkey, brilliant green, and Salmonella–Shigella agar to get pure and putative *Salmonella* culture. The organisms were further characterized as *Salmonella* species according to their morphology, Gram staining, mortality, and biochemical properties (Merchant and Packer, 1967; Cowan, 1985; OIE, 2000).

Serogrouping of *Salmonella* isolates. Serogrouping of *Salmonella* isolates was performed by slide agglutination test using commercial *Salmonella*-specific polyvalent O (A-I) antisera, *Salmonella* O group B (Factor O: 4, 5, 27) antisera, and *Salmonella* O group D (Factor O: 9, 46) antisera (S&A Reagent Lab). The test was performed according to the protocol supplied by the manufacturer.

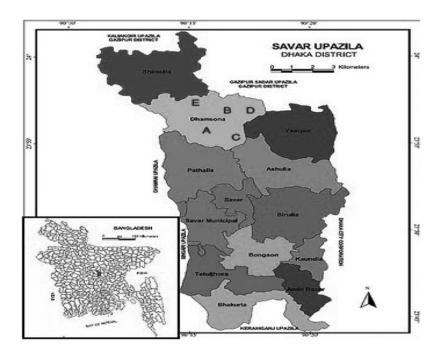


FIG. 1. Sample collection location of five poultry farms in Savar area indicated as A, B, C, D, and E.

Farm code	Age (weeks)	Serum sample tested	Positive cases	Prevalence (%)	Total serum sample tested	Total positive cases	Total prevalence (%)
A	20–25	12	3	25	64	22	34.4
	30-35	34	14	41.18			
	>40	18	5	27.78			
В	20-25	10	2	20	46	14	30.4
	30-35	22	9	40.9			
	>40	14	3	21.43			
С	20-25	27	11	40.74	111	50	45
	30-35	48	23	47.92			
	>40	36	16	44.44			
D	20-25	20	5	25	52	19	36.5
	30-35	17	8	47.05			
	>40	15	6	40			
E	20-25	18	5	27.78	73	26	35.7
	30-35	31	13	41.93			
	>40	24	8	33.33			
Overall					346	131	37.9

Table 1. Seroprevalence of Salmonella in Birds of Different Ages in Five Different Locations at Savar Area

Antibiogram study of Salmonella isolates. In vitro antibiotic sensitivity test of the isolated Salmonella was performed through disc diffusion method (Bauer et al., 1966) with the standard commercial discs manufactured by Oxoid Ltd. In this method, Salmonella isolates were grown overnight on brilliant green agar (Oxoid Ltd.) at 37°C and the overnight grown isolates were then inoculated into nutrient broth and incubated at 37°C for 18-24 hours. After incubation, one loopful of inocula was poured onto 9 mL of Mueller Hinton broth (Oxoid Ltd.) and incubated at 37°C for 5-6 hours. The bacterial cultures were compared with McFarland (Jorgensen et al., 1999) turbidity standard (108 CFU/mL) and streaked evenly in three planes onto the surface of the Mueller Hinton agar plate (5×40) with a sterile cotton swab. After the inocula dried, antibacterial discs such as ampicillin (A) 10 µg, amoxycillin (Am) 10 μg, ciprofloxacin (C) 5 μg, erythromycin (E) 15 μg, gentamycin (G) 10 μg, penicillin-G (P) 10 units, streptomycin (S) $10 \,\mu g$, sulfamethoxazole (Su) $25 \,\mu g$, tetracycline (T) $30 \mu g$, and nitrofurantoin (F) $300 \mu g$ were placed on the agar aseptically and kept at 4°C for 30-60 minutes for better diffusion. The inoculated plates containing the discs were incubated in an upright position at 37°C overnight and/or 24-48 hours (if necessary). The results were expressed as the diameter of inhibition zone around the paper disk (8 mm).

Maintenance of stock culture. The stock cultures of the Salmonella isolates were preserved in TSB medium containing 20% glycerol and kept at -86° C. Working cultures were kept at 4° C on tryptic soy agar slants (Nissui) and were periodically transferred at 15 days interval to fresh slants.

Isolation of plasmid DNA. A single bacterial colony was transferred into screw-capped tubes containing Luria broth medium 1:5 (v/v) containing appropriate antibiotics and incubated at 37°C overnight with shaking. The culture was pelleted by centrifugation (12,000 rpm, 30 seconds) at 4°C. The supernatant was removed and the pellet was homogeneously suspended in buffer and plasmids were isolated according to Birnboim and Doly (1979) method or using PureLinkTM Quick Plasmid Miniprep kit (Invitrogen). A 5 μ L plasmid DNA was

loaded on to a 1.0% agarose gel containing $0.5 \,\mu g$ mL⁻¹ ethidium bromide and electrophoresed in $1 \times Tris$ –boric acid–EDTA buffer. The plasmid DNA were visualized by an ultraviolet transilluminator and recorded using the Alfa Digital documentation imaging system (Alfa Innotech Corporation).

Statistical analysis

The serological test results were statistically analyzed based on farm location and age and sex of the birds. Data were subjected to chi-square statistics using Microsoft Excel program. Significant differences of the data were established by least significant difference at the 5% level of significance.

Results and Discussion

The SPA test revealed that the average seroprevalance of *Salmonella* in three different age groups was 37.9% (Table 1).

Table 2. Prevalence of *Salmonella* in Live and Dead Birds Detected in Five Different Locations at Savar Area

Farm code	Birds	Sample no.	Isolates no.	Percentage (%)
A	Live	20	5	25
	Dead	4	2	50
	Total	24	7	29.2
В	Live	38	7	18.42
	Dead	3	1	33.33
	Total	41	8	19.5
C	Live	169	26	15.38
	Dead	35	11	31.43
	Total	204	37	18.1
D	Live	11	5	45.45
	Dead	3	2	66.67
	Total	14	7	50
E	Live	178	28	15.73
	Dead	42	19	45.24
	Total	220	47	21.4
Overall	Live	416	71	17.1
	Dead	87	35	41.2

Table 3. Prevalence of *Salmonella* at Savar Area During Summer, Winter, and Rainy Seasons

Time	Season	Sample no.	No. of isolates	%
April–June July–September December–February	Summer Rainy Winter	263 155 85	62 33 11	23.6 21.3 12.9
	Total	503	106	

The average seroprevalence was lowest between 20 and 25 weeks and recorded as 27.7%, whereas the highest reached 43.8% of 30-35-week-old birds. No significant difference of the prevalence among five farms and three groups of ages were observed in chi-square test. Seroprevalence study under various conditions of varying season and age and sex of birds have been carried out by different researchers. The overall prevalence of Salmonella was reported to be as low as 14.1% to as high as 45.9% (Islam et al., 2006; Ahmed et al., 2008; Hossain et al., 2010). In another study, the mean seropositivity of different farms in three different age groups was found to be $18.9\% \pm 2.3\%$, $33.2\% \pm 3.53\%$, and $27.8\% \pm 2.67\%$ on 10th, 24th, and 40th week of age (Hossain and Islam, 2004). Similar results by other workers also found that the prevalence of Salmonella infection increases with increasing age (Sikder et al., 2005; Islam et al., 2006).

A total of 503 poultry samples were analyzed; of them, 416 were live poultry and 87 were dead poultry. Of 416 live poultry samples, 71 (18%) were found to be Salmonella positive, whereas from 87 dead poultry, 35 (31%) poultry was Salmonella positive (Table 2). Significant difference of the prevalence between live poultry and dead poultry (p < 0.01) was observed by chi-square test. It should be noted that the live poultry purchased for this study was not clinically diseased; on the other hand, the dead poultry purchased for this study were case fatalities. The isolation rate of Salmonella had been reported to be 24.5%, 28%, 47.6%, and 14.3%, respec-

tively, from the liver of dead poultry (Rusul and Yassin, 1996; Habib-ur-Rahman *et al.*, 2003; Lee *et al.*, 2003; Tibaijuka *et al.*, 2003). These differences in isolation rate may be due to the geographical variation.

The prevalence of *Salmonella* isolates within a 1-year period has been presented in Table 3. The average prevalence of *Salmonella* infection was found higher (23.6%) in summer season than in rainy (21.3%) and winter (12.9%) seasons (Table 3). These results are in agreement with the result reported by Saleque *et al.* (2003), Rahman *et al.* (2004), and Sikder *et al.* (2005). Recently, Hossain *et al.* (2010) reported that the prevalence of *Salmonella* infection was the highest $(18.5\%\pm11.9\%=30.4\%)$ in summer, followed by winter $(11.6\%\pm12.1\%=23.7\%)$, rainy season $(14.2\%\pm10.8\%=25.0\%)$, and autumn $(13.3\%\pm10.0\%=23.3\%)$.

From the 503 poultry samples, 106 *Salmonella* were isolated (Table 4). Among the 106 isolates, 46 belonged to serogroup B (43%) and 60 isolates to serogroup D (57%) (Table 5). The most prevalent serogroup identified in this study was serogroup D. These findings are in agreement with the result reported by Arroyo and Arroyo (1995).

Antibiogram of the 106 isolates against 10 commonly used antibiotics revealed 17 different phenotypic expressions acquiring 10 to 5 drugs resistance concurrently. The prevalence of the multiple drug resistance (MDR) (resistance to greater than or equal to three classes of antimicrobial agents) Salmonella isolates against 10 to 5 antibiotics were 26.4%, 17%, 20.7%, 2.8%, 27.4%, and 5.7% (Table 4). Interestingly, same serogroup with identical MDR phenotypes and plasmid profiles containing Salmonella isolates were found in five farms, indicating the possibility of intrafarm transmission of this bacterium. The relative susceptibility of commonly used antibiotics revealed that the highest percentage of antibiotic resistance was found against penicillin-G and ampicillin, amoxicillin, tetracycline, nitrofurantoin, sulfamethaxol, gentamycin, and ciprofloxacin, with 100%, 99%, 98%, 93%, 78%, 60%, 46%, and 40%, respectively (data not shown). Similar resistance patterns to common antibiotics and con-

Table 4. Multiple Drug Resistance Patterns of Salmonella Isolated from Poultry

Total isolates (isolate no.)	Multiple drug resistance pattern	Percentage (%)
28 (2, 21, 22, 23, 24, 25, 26, 27, 28, 60, 61, 62, 63, 64, 65, 82, 74, 76, 78, 88, 89, 98, 99, 101, 103, 104, 105, 106)	A, Amp, C, E, F, G, P, S, Su, T	26.4
18 (68, 80, 69, 72, 81, 84, 75, 77, 79, 92, 95, 100, 102, 93, 94, 86, 87, 73)	A, Amp, C, E, F, G, P, S, T A, Amp, C, E, F, P, S, Su, T A, Amp, E, F, G, P, S, Su, T A, Amp, C, E, F, G, P, Su, T Amp, C, E, F, G, P, S, Su, T	17
22 (4, 5, 6, 7, 8, 9, 17, 18, 19, 20, 57, 58, 59, 66, 83, 91, 85, 90, 67, 96, 97, 1)	A, Amp, E, F, P, Strep, Su, T A, Amp, E, F, G, P, Su, T A, Amp, E, F, G, P, Su, T A, Amp, C, E, F, P, S, T A, Amp, F, G, P, S, Su, T A, Amp, C, E, G, F, P, T	20.7
3 (3, 70, 71)	A, Amp, C, E, G, P, F, T A, Amp, C, E, F, P, T A, Amp, E, G, P, S, Su, A, Amp, C, E, F, P, S,	2.8
29 (29, 30, 31, 32, 10, 11, 12, 13, 14, 15, 16, 39, 40 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56)	A, Amp, E, F, P, S, A, Amp, E, P, S, T	27.4
6 (33, 34, 35, 36, 37, 38)	A, Amp, E, P, S,	5.7

P, penicillin 10 units; Amp, ampicillin $10 \mu g$; A, amoxicillin $25 \mu g$; E, erythromycin $15 \mu g$; S, streptomycin $10 \mu g$; T, tetracycline $10 \mu g$; F, nitrofurantoin $300 \mu g$; Su, sulfamethaxol $10 \mu g$; G, gentamycin $10 \mu g$; C, ciprofloxacin $5 \mu g$. Antibiotic per disc.

Table 5. Serotyping and Plasmid Profile of the *Salmonella* Isolates Isolated from Five Different Poultry Farms^a

Farm code	Isolate no.	Serotyping	Plasmid no. (size in kb)
E	60	Group-B	Nil
E	61	Group-B	One (>40)
E	2	Group-D	Nil
C	22	Group-D	Three (5, 3, 2)
E	23	Group-D	Nil
E	74	Group-D	Two (4, 3.5)
A	76	Group-D	Nil
A	78	Group-D	One (>40)
C	89	Group-D	One (3)
C	101	Group-D	Three (5, 3, 2)
В	104	Group-D	Three (6, 4, 3)
В	68	Group-B	Three (5, 3, 2)
В	80	Group-B	Two (4, 3)
A	69	Group-B	Three (5, 3, 2)
E	81	Group-B	2 (4, 3)
E	84	Group-B	Nil
E	75	Group-B	1 (>40)
E	92	Group-B	Nil
E	95	Group-B	2 (4, 3.5)
E	93	Group-D	Three (4, 3, 2)
C	94	Group-D	4 (>40, 4, 3, 2)
C	86 72	Group-D	Nil
B B	73 4	Group-D	Nil Nil
В	6	Group-D	
D	18	Group-D	2 (4, 3) Nil
C	66	Group-B	Nil
C	83	Group-B	2 (4, 3.5)
E	91	Group-B Group-B	Three (5, 3, 2)
Ē	85	Group-D	Nil
Ā	90	Group-B	Four (>40, 4, 3, 2
C	67	Group-D	2 (5, 3)
Č	96	Group-D	Nil
Ē	97	Group-D	2 (4.05, 2)
E	1	Group-D	Nil
E	3	Group-D	1 (>40)
E	70	Group-B	Three (4, 3, 2)
E	71	Group-B	Three (4, 3, 2)
C	29	Group-B	2 (4.07, 3.5)
C	30	Group-B	Nil
E	32	Group-B	1 (>40)
C	13	Group-D	1 (>40)
E	14	Group-D	1 (>40)
E	15	Group-D	Nil
E	16	Group-D	1 (>40)
E	40	Group-D	2 (5 kb, 3)
E	41	Group-B	1 (>40)
E	43	Group-B	Nil
E	49	Group-B	Nil
E	50 51	Group-B	1 (3)
C C	51 53	Group-B	Nil
C	53 54	Group-B	1 (>40)
E	54 55	Group-B	Nil 1 (>40)
C	33	Group-B	1 (>40) 1 (>40)
E	34	Group-D Group-D	1 (>40) Nil
	J-1	Group D	7 411

^aSalmonella isolates that showed at least one variable (farm, serogroup, and plasmid profile) different are listed in table.

current increase in multiple resistances in Salmonella isolates worldwide had been reported elsewhere (Verma et al., 1993; Anjanappa et al., 1994; Hui and Das, 2001) for the last three antibiotics (Molla et al., 2003; Carraminana et al., 2004; Muhammad et al., 2009; Maripandi and Al-Salamah, 2010). The multiple antibiotics resistances observed in the present study is an alarming sign for public health concern, because these antibiotics are frequently used for the treatment of human infection diseases. Increasing resistance to important antimicrobials used for human therapy, such as cephalosporins and fluoroquinolones, as well as increasing multiple resistance often linked with virulence determinants is an increasing concern (Su et al., 2008; Garcia-Fernandez et al., 2009). The high MDR index (1–0.5) found for 10 commonly used antibiotics (Table 4) indicated the alarming situation and implicated that the Salmonella isolated sites were highly contaminated with large numbers of these antibiotics.

To verify the MDR properties whether chromosomal or plasmid borne, the plasmid profiles of 106 Salmonella isolates were examined and the results are shown in Table 5 and Figures 2 and 3. Plasmid profile analysis revealed that 69 isolates among the total 106 contained 1 to 4 plasmids of varying sizes (>1.63 to >40 kb) (Table 5); but rest of the 37 isolates were plasmid free and showed resistance against 5–10 antibiotics with 12 different resistance patterns (Tables 4 and 5). Plasmid profiles of 46 isolates belonging to serogroup B revealed that 76% (35 of 46) of the isolates contained plasmid, whereas this value was 56.67% (34 of 60) for serogroup D (Figs. 2 and 3). Surprisingly, 34 isolates belong to both B and D serogroups, contained a single large plasmid of >40 kb, and showed six resistance patterns. Isolate number 90 and 94 harbored the large plasmid, >40 kb, along with three other small plasmids of 4, 3, and 2kb, although these two isolates show different antibiograms and belong to different serogroups. In contrast, isolate number 72 belonging to same serogroup and with similar MDR pattern with the isolate number 90 harbored different plasmid profiles (>40, 3, and 2 kb). On the other hand, Salmonella isolated from same farm, belonging to same serogroup, and having similar plasmid profile also showed different antibiograms. Salmonella isolated from same or different farm belong to same or different serogroup and had not showed same plasmid or different antibiograms. In contrast, it was observed that Salmonella isolated from same farm that belonged to the same serogroup and showed same antibiograms varied in plasmid profiles or even did not contain any plasmid. The same was also true for cross-serogroups.

The present work was designed to investigate salmonellae involved in poultry infection on the basis of five variables farm code (situated within 5 sq. miles), serotypes, resistotypes, and plasmid profiles. Depending on at least single variable variation, 56 *Salmonella* isolates were identified with 17 different resistotypes harboring 1–4 plasmid or none (Table 5). The very high unanticipated levels of commonly used therapeutic antibiotics resistance observed in *Salmonella* isolates is probably due to the indiscriminate and widespread use of these antibiotics in the poultry, veterinary, and public health practices, because people have very easy access to various antimicrobials in Bangladesh.

Salmonella harbored multiple R-plasmids as reported by Maripandi and Al-Salamah (2010), Morshed and Peighambari (2010), Singh *et al.* (2010), and Threlfall *et al.* (2005). The study

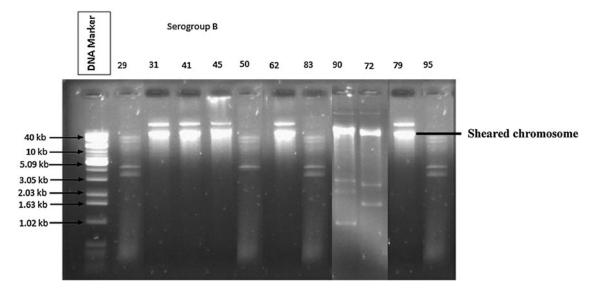


FIG. 2. Plasmid profile of representative 11 *Salmonella* isolates of serogroup B. Plasmid pattern of 11 isolates (29, 31, 41, 45, 50, 62, 83, 90, 72, 79, 95) of *Salmonella* are shown in lanes 2–12, and lane 1 is the marker used.

indicated that the presence or number of plasmids is not associated with resistant phenotype of *Salmonella* isolates (Table 5). The absence of correlation between MDR patterns and the plasmid profiles in *Salmonella* isolates indicates that the origin of the resistance properties might be on chromosome. Similar results of *Salmonella* isolates from veterinary origin in India were reported by Singh *et al.* (2010). Alarmingly, 35% of the *Salmonella* isolates were plasmid free, but had resistance to 5–10 antibiotics and belonged to 3–7 different antimicrobial groups. So far, to the best of our knowledge, this is the first study reporting *Salmonella* resistant to 10 antibiotics and belonging to 7 antimicrobial groups. *Salmonella* Tyhimurium DT104 resistance to five antibiotics and belonging to four antimicrobial groups had been documented (Threlfall *et al.*, 2005). Very recently, a plasmid-free *Salmonella*

Anatum strain had been isolated from buffalo sources in India, which is resistant to seven antibiotics and belongs to four antimicrobial groups (Singh *et al.*, 2010). Chromosome-borne bacterial resistance mostly occurs because of alteration of antimicrobial drug target and alteration and/or limit permeation/efflux of the drugs. The alteration of target and drug efflux mechanism had been documented for *Salmonella* (Threlfall *et al.*, 2005; Nair *et al.*, 2006).

In conclusion, *Salmonella* isolates from poultry farm of Savar area, Dhaka, Bangladesh, showed MDR properties at alarming levels. Further, a wide range of MDR has implications for veterinary and human therapy, as their misuse in poultry could lead to emergence of resistant zoonotic pathogens. Therefore, MDR strains of *Salmonella* of poultry origin might be important for public and personal health, as well as

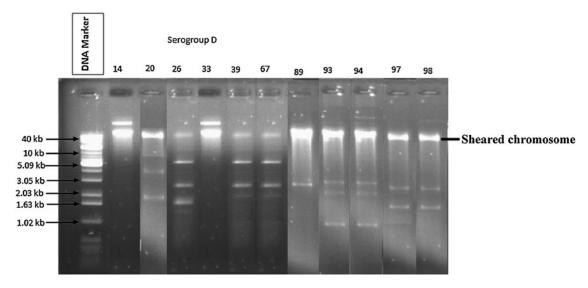


FIG. 3. Plasmid profile of representative 11 isolates of serogroup D. Plasmid pattern of 11 isolates (14, 20, 26, 33, 39, 67, 89, 93, 94, 97, 98) of *Salmonella* are shown in lanes 2–12, and lane 1 is the marker used.

for epidemiologists monitoring the spread of MDR in zooonotic *Salmonella* pathogens in Bangladesh and beyond the borders.

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Disclosure Statement

No competing financial interests exist.

References

- Ahmed AKM, Islam MT, Haider MG, and Hossain MM. Seroprevalence and pathology of naturally infected Salmonellosis in poultry with isolation and identification of causal agents. J Bangladesh Agril Univ 2008;6:327–334.
- Anjanappa M, Harbola PC, and Verma JC. Plasmid profile analysis of field strain of Salmonella gallinarum. Indian Vet J 1994;71:417–421.
- Arroyo G and Arroyo JA. Detection of *Salmonella* serotypes in edible organ meats from markets in Madrid, Spain. Food Microbiol 1995;12:13–20.
- Bauer AW, Kirby WM, Secherris JC, and Turek M. Antibiotic susceptibility testing by standard single disc method. Am J Clin Pathol 1966;45:493–496.
- Bhat P and Macaden R. Outbreak of gastroenteritis due to multidrug resistant *Salmonella typhimurium* phage type 66/122/UT in Bangalore. Indian J Med Res 1983;78:454–458.
- Birnboim HC and Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 1979;7:1513–1523.
- Carraminana JJ, Rota C, Agustin I, and Herrera A. High prevalence of multiple resistance to antibiotics in *Salmonella* serotypes isolated from a poultry slaughterhouse in Spain. Vet Microbial 2004;104:133–139.
- Cowan ST. Cowan and Steel's Manual for the Identification of Medical Bacteria, 2nd edition. New Delhi, India: New Age Int. (P) Ltd., 1985.
- Das PM, Rajib DMM, Noor M, and Islam MR. A retrospective analysis on the proportional incidence of poultry diseases in greater Mymensingh district of Bangladesh. *Fourth International Poultry Show and Seminar Held on 10–12 March in Dhaka*, Bangladesh, 2005, pp. 33–37.
- Garcia-Fernandez A, Fortini D, Veldman K, Mevius D, and Carattoli A. Characterization of plasmids harbouring Qnrs1, Qnrb2 and Qnrb19 genes in *Salmonella*. J Antimicrob Chemother 2009;63:274–281.
- Gupta V, Ray P, and Sharma M. Antimicrobial resistance pattern of *Shigella* & non-typhi *Salmonella* isolated from patients with diarrhoea. Indian J Med Res 1999;109:43–45.
- Habib-ur-Rahman S, Sirzanin S, Hamayun K, Saleem K, Nazir A, and Bhatti WM. Incidence and gross pathology of *Salmonellosis* in chicken in Hyderabad. Jasso Vet Adv 2003;2:581–584.
- Hossain KMM, Hossain MT, and Yamato I. Seroprevalence of *Salmonella* and *Mycoplasma gallisepticum* infection in chickens in Rajshahi and surrounding districts of Bangladesh. Int J Biol 2010;2:74–80.

- Hossain MA and Islam MA. Seroprevalence and mortality in chickens caused by pullorum disease and fowl typhoid in certain government poultry farms in Bangladesh. Bangladesh J Vet Med 2004;2:103–106.
- Hui AK and Das R. Studies on isolation, serotyping and antibiotic sensitivity of Salmonellae isolated from ducks. Indian Vet J 2001;78:1058–1059.
- Islam MM, Haider MG, Chowdhury EH, Kamruzzaman M, and Hossain MM. Seroprevalance and pathological study of salmonella infection in layer chickens and isolation and identification of causal agents. Bangladesh J Vet Med 2006; 4:79–85.
- Jorgensen JH, Turnide JD, and Washington JA. Antibacterial susceptibility tests: dilution and disk diffusion methods. In: *Manual of Clinical Microbiology*, 7th edition. Murry PR, Pfaller MA, Tenover FC, Baron EJ, and Yolken RH (ed.). Washington, DC: ASM Press, 1999, pp. 1526–1543.
- Kamaruddin KM and Giasuddin M. Poultry disease and its diagnostic facilities. Growth of poultry industry in Bangladesh with poverty alleviation and employment opportunity. *Proceedings of the Third International Poultry Show and Seminar*, February 28–March 2, Dhaka, Bangladesh, 2003, pp. 141–148.
- Khan A, Bari ASM, Islam MR, Das PM, and Ali MY. Pullorum disease in semi mature chickens and its experimental pathology. Bangladesh Vet J 1998;32:124–128.
- L Plym F and Wierup M. *Salmonella* contamination: a significant challenge to the global marketing of animal food products. Rev Sci Tech Off Int Epiz 2006;25:541–554.
- Lee YJ, Kim KS, Kwon YK, Kang MS, Mo IP, Kim JH, and Tak RB. Prevalent characteristics of *fowl typhoid* in Korea. J Vet Clin 2003;20:155–158.
- Maripandi A and Al-Salamah AA. Multiple-antibiotic resistance and plasmid profiles of *Salmonella enteritidis* isolated from retail chicken meats. Am J Food Technol 2010;5:260–268.
- Merchant IA and Packer RA. *Veterinary Bacteriology and Virology*, 7th edition. Ames, IA: The Iowa State University Press, 1967.
- Molla B, Mesfin A, and Alemayehu D. Multiple antimicrobial-resistant *Salmonella* serotypes isolated from chicken carcasses and giblets in Debre Zeit and Addis ababa, Ethiopia. Ethiop J Health Dev 2003;17:131–149.
- Morshed R and Peighambari SM. Drug resistance, plasmid profile and random amplified polymorphic DNA analysis of Iranian isolates of *Salmonella Enteritidis*. New Microbiol 2010;33:47–56.
- Muhammad M, Muhammad LU, Mani AU, Azard S, and Barco L. Prevalence of *Salmonella* associated with chick mortality at hatching and their susceptibility to antimicrobial agents. Vet Microbiol 2009;140:131–135.
- Nair S, Unnikrishnan M, Turner K, Parija SC, Churcher C, Wain J, and Harish BN. Molecular analysis of fluoroquinolone-resistant *Salmonella* paratyphi A isolate, India. Emerg Infect Dis 2006;12:489–491.
- [OIE] Office International Des Epizooties. Manual of standards for diagnostics test and vaccines. OIE Guide-2; 2000. Available at http://www.oie.int/eng/normes/mmanual/A_00021.htm (Chapter 2.1.4), accessed May 29, 2011.
- Palaniswamy KS, Masillamony PR, and Purushothaman V. Paratyphoid infection in chicks due to Salmonella Typhimurium. Indian Vet J 1989;66:84–85.
- Rahman H, Barman NN, Patgiri GP, and Kalita N. Outbreak of Salmonellosis in broiler flocks in Assam. Indian J Comp. Microbiol Immunol Infect Dis 1997;18:56–58.
- Rahman MA, Samad MA, Rahman MB, and Kabir SML. Bacterio-pathological studies on salmonellosis, colibacillosis and

pasteurellosis in natural and experimental infections in chickens. Bangladesh J Vet Med 2004;2:1–8.

- Rusul GK and Yassin RM. Prevalence of *Salmonella* in broilers at retail outlets, processing plants and farm in Malaysia. Int J Food Microbiol 1996;33:183–194.
- Saleque MA, Rahman MH, and Hossain MI. Seasonal variation in the prevalence of poultry diseases in Bangladesh. 9th BSVER Annual Scientific Conference held at BAU, Mymensingh, January 6–7, 2003. BSVER Publication 2003;24:23–24.
- Sikder AJ, Islam MA, Rahman MM, and Rahman MB. Seroprevalance of *Salmonella* and *Mycoplasma gallisepticum* infection in the six model breeder farms at Patuakhali district of Bangladesh. Int J Poult Sci 2005;4:905–910.
- Singh BR, AgarwalM, Chandra M, Verma M, Sharma G, Verma JC, and Singh VP. Plasmid profile and drug resistance of zoonotic *Salmonella* isolates from India buffaloes. J Infect Dev Ctries 2010;4:477–483.
- Snoeyenbos GH. Pullorum disease. In: *Diseases of Poultry*, 9th edition. Calnek BW, Barnes HJ, Beard CW, Reid WM, and Yoder HW Jr. (eds.). Ames, IA: Iowa State University Press, 1994, pp. 73–86.
- Su LH, Chu C, Cloeckakaert A, and Chiu CH. An epidemic of plasmids? Dissemination of extendedspectrum cephalospor-

- inases among *Salmonella* and other Enterobacteriaceae. FEMS Immunol Med Microbiol 2008;52:155–168.
- Threlfall J, Hopkins KL, and Ward LR. Diversification in Salmonella Typhimurium DT104. Emerg Infect Dis 2005;11: 980–981.
- Tibaijuka B, Molla B, Hildebrandt G, and Kleer J. Occurrence of Salmonellae in retail raw chicken products in Ethiopia. Berliner. und Munchener. Tierar Fiche Wochen 2003;116:55–58.
- Verma JC and Gupta BR. Prevalence of Salmonella serotypes of avian origin. Indian J Comp Microbiol Immunol Infect Dis 1997;18:52–55.
- Verma JG, Gupta BR, and Ghosh SS. Studies on *Salmonella* virchow: *in vitro* sensitivity. Indian Vet J 1993;70:572–573.

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