

Identification and Antimicrobial Susceptibility of *Salmonella* species Isolated from Washing and Rinsed Water of Broilers in Pluck Shops

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Abstract: The study was designed with a view to isolate, identify and characterize *Salmonella* species from washing and rinsed water of broilers in pluck shops at Sreepur of Gazipur district in Bangladesh during the period from December 2011 to May 2012. A total of 30 samples collected from the different layers of drums of pluck shops were subjected to bacterial isolation and identification by using cultural and biochemical techniques. Furthermore, the isolated *Salmonella* species were characterized by antimicrobial susceptibility testing. Among the 27 positive *Salmonella* isolates 11.11% (n = 3) were *Salmonella pullorum*, 29.83% isolates (n = 8) were *Salmonella gallinarum* and the rest 59.26% isolates (n = 16) were *Salmonella typhimurium*. In case of motility test performed by MIU media and hanging drop slide method, 40.74% isolates were non-motile and 59.26% isolates were motile. *Salmonella* spp. were resistant to doxycycline and erythromycin. However, most of the *Salmonella* spp. were susceptible to sulfamethoxazole-trimethoprim and gentamicin. Out of 27 *Salmonella* isolates, 75% *Salmonella typhimurium*, 100% *Salmonella gallinarum* and 100% *Salmonella pullorum* were detected as multidrug resistant. The findings of the study revealed the presence of multidrug resistant *Salmonella* species in washing and rinsed water of broilers in pluck shops at Sreepur of Gazipur district in Bangladesh.

Keywords: Antimicrobial susceptibility, pluck shops, *Salmonella* species, washing and rinsed water of broilers

INTRODUCTION

Salmonellosis is a food borne disease of primary concern in developed and developing countries. It is one of the major public health problems in terms of socio-economic impact (Gracia and Finlay, 1994). A wide array of animal reservoir and commercial distribution of both animals and food products favor the spread of the disease. Salmonellosis is more common in summer than winter. The lower incidence of the disease during the winter could be explained by seasonal variations in consumer behavior, such as changes in the types of food that are consumed. However, it is likely that the higher incidence of salmonellosis during the summer is also the result of higher temperatures increasing the incidence of infections in animals and favoring the multiplication of the pathogenic microorganism (Bentham and Langford, 2001; Hobbs and Roberts, 1987).

Poultry birds have frequently been incriminated as a mean of *Salmonella* contamination and consequently act as major source of the pathogen in humans. This organism has been isolated from a range of foods in

almost every country (Rumeu *et al.*, 1997). The level of contamination dramatically increases during the containment of the animals in holding pens before slaughter; besides this, the increasing incidence of salmonellosis is due to a number of technical practices (D'Aoust, 1994). After slaughter, the subsequent dressing of meat increases the spread of *Salmonella* on meat surfaces, and by the time the meat is in retail outlets, contamination levels may be increased by 20% (Forsythe and Hayes, 1998). Most *Salmonella* infections in humans result from the ingestion of contaminated poultry, beef, pork, eggs, and milk (Gomez *et al.*, 1997).

Use of antimicrobials in any environment creates selection pressures that favor the survival of antibiotic-resistant pathogens. The routine practice of giving antimicrobials to domestic livestock for growth promotion and prophylaxis is an important factor in the emergence of antibiotic-resistant bacteria in the food chain (Tollefson *et al.*, 1997). The emergence of multidrug resistance among *Salmonella* spp. is an increasing concern. Information concerning bacterial loads during slaughtering, washing, handling and

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packaging by the retail shopkeepers, renders or the consumers are not available to get the safe consumption of poultry meat. Therefore, the present study was undertaken with a view to isolate, identify and characterize *Salmonella* species from washing and rinsed water of broilers in pluck shops at Sreepur of Gazipur district in Bangladesh.

MATERIALS AND METHODS

Study area: The present research was conducted during the period of December 2011 to May 2012 in the Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh. The samples (Broiler washing and rinsed water) which were collected from Pluck shops (cottage poultry processors) at Gorgoria-masterbari, Sreepur of Gazipur district and transported through ice flasks to the laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh for isolation, identification, biochemical and antibiogram characterization.

Collection and transportation of samples: A total of 30 samples were collected from source water and 3 layers of washing and rinsed water of drums of pluck shops' at Gorgoria-masterbari, Sreepur, Gazipur and immediately brought to Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh through cool chain maintaining. After that, the samples were inoculated into the nutrient broth for better nourishment of the desirable organisms.

Cultural characterization and isolation of *Salmonella* spp.: The longitudinal surface of each sample was seared (cauterized) with hot spatula and incised with sterile scalpel followed by introduction of a loop in the cut surface and materials brought with loop was inoculated into Selenite broth and SS agar plates. These were then incubated at 37°C for 24 h in bacteriological incubator. After 24 h the incubated media were then examined for growth of bacteria. Colorless or translucent colony and sometimes black color colony were observed on SS agar. The colony was then subjected to Gram's Method of staining and observed under microscope for Gram negative rods. The organisms from the agar media were sub-cultured into SSA, MCA and BGA with the help of inoculating loop in case of gram negative rods in the smears. In case of SS agar colorless, translucent and black colony were observed. In case of MC agar colorless and translucent colony were observed. In case of BG agar, light pink colony against a rose pink background was observed. Thus single pure colony was obtained. These pure isolates obtained in this way were used for the further study (Cheesebrough, 1985).

Gram's staining: The *Salmonellae* colonies were characterized morphologically using Gram's stain according to the method described by Merchant and Packer (1967). Briefly, a small colony was picked up from SS, MC and BG agar plates with a bacteriological loop, smeared on separate glass slide with a drop of distilled water and fixed by gentle heating. Crystal violet was then applied on each smear to stain for two minutes followed by washing with running water. Few drops of Gram's Iodine was then added which acted as mordant for one minute and then washed with running water. Acetone alcohol was then added (acts as decolorizer) for few seconds. After washing with water, safranin was added as counter stain and allowed to stain for two minutes. The slides were then washed with water, blotted, dried in air and then examined under microscope with high power objective (100X) using immersion oil.

Motility test: The motility test was performed to differentiate motile bacteria from non-motile one (Cheesbrough, 1985). This test was performed in Motility Indole Urea (MIU) medium where a sterile straight wire used to inoculate 5 mL of sterile MIU medium taken earlier in a screw capped test tube with a smooth pure colony of the test organism. When inoculating the MIU medium, a stab was made with a sterile straight wire and stoppered the tube followed by incubation at 35-37°C overnight. Motility is shown by a spreading turbidity from the stab line or turbidity throughout the medium (compared with an uninoculated tube).

Differentiation of isolated *Salmonella* using biochemical test: For this study isolated organisms with supporting growth characteristics of *Salmonella* were subjected to sugar (Carbohydrate) fermentation test, TSI agar slant reaction, MR-VP reaction, indole reaction, urease reaction, citrate utilization and Lysine decarboxylation reaction according to the procedures as described by Cheesbrough (1985).

Sugar (carbohydrate) fermentation test: The carbohydrate fermentation test was performed by inoculating a loopful of thick test bacterial culture into the individual tubes containing sugars like dextrose, maltose, sucrose, lactose, mannitol, dulcitol and rhamnose and incubated at 37°C for 48 h. Acid production was indicated by the change of media from pink to yellow color while gas production was indicated by the appearance of gas bubbles in the inverted Durham's fermentation tubes.

Triple Sugar Iron (TSI) agar slant reaction: TSI agar was used to detect the late or nonlactose fermenters and the dextrose fermenters. The medium also helped to determine the ability of the organisms to produce

Hydrogen Sulfide (H₂S). The organisms under study were heavily seeded with a platinum needle over the surface of the slant and stabbed into the butt of the tubes of TSI agar. After an aerobic incubation period of 24 h at 37°C, aerobically the tubes were examined for all changes in the slant or in the butt. In TSI agar slant, the presence of yellow color and gas bubbles in the media were considered as production of acid and gas respectively in slants or in butt as the case may be. The red or dark pink coloration of the media in slant or in butt was considered as alkaline reaction. The black coloration in any part of media was considered as the production of H₂S (Hydrogen Sulfide).

Methyl-Red (MR) test: The indicator phenyl red solution was prepared by dissolving 0.1 gm of Bacto methyl-red in 300 mL of 95% alcohol and diluting to 500 mL with the addition of 200 mL of distilled water. The test was performed by inoculating a colony of the test organism in 0.5 mL sterile glucose phosphate broth. After overnight incubation at 37°C, a drop of methyl red solution was added. A positive methyl red test was shown by the appearance of bright red color indicated acidity while a yellow or orange color was considered as negative.

Voges-Proskauer (VP) test: Two mL of sterile glucose phosphate peptone water were inoculated with the 5 mL of test organisms and incubated at 37°C for 48 h. A very small amount (knife point) of creatine was added and mixed and 3 mL of sodium hydroxide were added and shaken well. The bottle cap was removed and left for an hour at room temperature. It was observed closely for the slow development of a pink color for positive cases.

Indole test: Two mL of peptone water was inoculated with 5 mL of bacterial culture under observation and incubated for 48 h after which 0.5 mL Kovac's reagent was added, shaken well and examined after 1 min. A red colour in the reagent indicated positive test.

Citrate utilization test: Using a sterile straight wire, inoculated 3-4 mL of sterile Koser's citrate medium with a broth culture of the test organism. Care was taken not to contaminate the medium with carbon particles, such as from a frequently flamed wire. Incubate the inoculated broth at 37°C for up to 4 days, checking daily for growth. Turbidity and blue color indicates positive for citrate utilization test and no color change indicates negative test.

Lysine decarboxylase test: Inoculation was given into the broth media by transferring one or two colonies from the surface of a fresh test culture with an inoculating loop or needle and mixed to distribute the culture throughout the medium. Overlayed the medium in each tube with 1 mL sterile mineral oil. Incubated the

tubes with caps tightened at 35±2°C, examined for growth and decarboxylase reactions after 18-24, 48, 72 and 96 h, respectively. The medium become yellow initially, if the dextrose is fermented, and then gradually turn purple to violet if the decarboxylase or dihydrolase reaction occurs Positive and elevates the pH. A yellow color indicated Negative test.

Antimicrobial susceptibility test: Susceptibility and resistance of different antibiotics was measured in vitro by employing the modified Kirby-Bauer (Bauer *et al.*, 1966; Quinn *et al.*, 2000) method. This method allowed for the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that resulted from diffusion of the agent into the medium surrounding the disc.

A suspension of test organism was prepared in nutrient broth by overnight culture for 24 h at 37°C. The broth were streaked using by sterile glass spreader homogenously on the medium. Antibiotic disc were applied aseptically to the surface of the inoculated plates at an appropriate special arrangement with the help of a sterile forceps on Mueller-Hinton agar plates. The plates were then inverted and incubated at 37°C for 24 h. The diffusion discs with antimicrobial drugs were placed on the plates and incubated for 24 h at 37°C. The antibiotics discs (Oxoid, Basingstoke, Hampshire, England) used were: amoxycillin (30 µg), neomycin (30 µg), amikacin (30 µg), erythromycin (15 µg), gentamicin (10 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), doxycycline (30 µg), erythromycin (10 µg), and sulphamethoxazole-trimethoprim (25 µg). Sterile glass spreader was used to spread the culture homogenously on the medium. Antibiotic disc were applied aseptically to the surface of the inoculated plates at an appropriate special arrangement with the help of a sterile forceps. The plates were then inverted and incubated at 37°C for 24 h. After incubation, the plates were examined and the diameters of the zone of complete inhibition were observed. The zone diameters for individual antimicrobial agents were translated into susceptible, intermediate and resistant categories by referring to an interpretation (Table 1).

Maintenance of stock culture: During the experiment it was necessary to preserve the isolated *Salmonella* spp. for longer period. For this purpose pure culture of isolated *Salmonella* spp. were kept in stock culture. The organisms isolated and identified as *Salmonella* were inoculated into the SS and TSI agar slants and incubated at 37°C for 24 h in bacteriological incubator and then examined for luxuriant growth. Then the sterile mineral oil was poured into the tubes having luxuriant growth until the colonies were covered completely. The tubes were sealed off with paraffin wax and kept at room temperature for future use. By this method, bacteria can be preserved with no deviation of their original characters for few months.

Table 1: Interpretive standards for disc diffusion susceptibility testing of enterobacteriaceae (NCCLS, 2000; Bauer *et al.*, 1966)

Name of antimicrobial disc	Disc concentration	Diameter of zone of inhibition (mm)		
		Susceptible	Intermediate	Resistant
Neomycin (N)	30 µg	≥17	13~16	≤12
Amikacin (AK)	30 µg	≥17	15~16	≤14
Erythromycin (E)	15 µg	≥23	14~22	≤13
Gentamicin (CN)	10 µg	≥15	13~14	≤12
Ciprofloxacin (CIP)	5 µg	≥21	16~20	≤15
Doxycyclin (DO)	30 µg	≥16	13~15	≤12
Norfloxacin (NOR)	10 µg	≥17	13~16	≤12
Amoxycillin (AMC)	30 µg	≥18	14~17	≤13
Enrofloxacin (ENR)	5 µg	≥20	17~19	≤16
Sulfamethoxazole-trimethoprim (SxT)	25 µg	≥16	11~15	≤10

Table 2: Results of cultural, morphological and motility characteristics of the isolates of *Salmonella* spp. at a glance

Sources of isolates	Colony morphology					Staining characteristics	Motility (both in MIU medium and hanging drop method)
	Nutrient agar	SS agar	BG agar	TSI agar	MC agar		
S1 to S30 (Except :S- 8, 17, 23)	Translucent, opaque, smooth colonies	Translucent black smooth, small round colonies	Light pink or pink colony against pink background	Black color colonies against a yellowish background	Color less smooth, transparent raised colonies	Pink short rod, gram negative bacteria arranged in single or paired	+ve (<i>S. typhimurium</i>) -ve (<i>S. gallinarum</i> , <i>S. pullorum</i>)

N.B: SS = *Salmonella*-shigella, BG = Brilliant green, EMB = Eosine-methylene blue, TSI = Triple sugar iron, MC = MacConkey, + = Positive, S = Sample (1.....30); MIU: Motility indole urea

Table 3: Results of percentages (%) of desirable microorganisms available in source samples

Name of microorganisms	% of the present microorganisms			Size (n) in total samples
	Upper layer of drums	Middle layer of drums	Lower layer of drums	
<i>Salmonella typhimurium</i>	37.50	31.25	31.25	(n = 16)
<i>Salmonella gallinarum</i>	25	37.50	25	(n = 8)
<i>Salmonella pullorum</i>	33.33	33.33	33.33	(n = 3)

RESULTS

A total of 27 bacterial isolates out of 30 samples were identified as *Salmonella* spp. by using cultural and biochemical techniques. The results of cultural, morphological and motility characteristics of the isolates of *Salmonella* spp. are presented in Table 2.

Among all of the isolates obtained from this study *Salmonella typhimurium* produced turbidity from line of inoculation in Motility Indole Urea media (MIU) which is a sign of motility and alternatively found to be motile with hanging drop slide (Table 2).

The organisms were checked and confirmed by their purity using selected media (SS agar). Then they were grown in nutrient broth for biochemical test. For identification, a series of selective biochemical tests for *Salmonella* spp. were performed with the culture. All the isolated organisms fermented five sugars like dextrose, sucrose, fructose, glucose, maltose produced acid with gas. Acid production was indicated by the color change from reddish to orange yellow color and the gas production was manifested by the appearance of gas bubbles in the inverted Durham's tubes.

Salmonella typhimurium was detected as 37.5% in upper layer of drums, 31.25% in middle layer of drums and 31.25% in lower layer of drums (Table 3). *Salmonella gallinarum* was detected as 25% in upper layer of drums, 37.5% in middle layer of drums and 25% in lower layer of drums (Table 3). *Salmonella pullorum* was detected as 33.33% in upper layer of drums, 33.33% in middle layer of drums and 33.33% in lower layer of drums (Table 3).

The results of the antimicrobial susceptibility testing by disc diffusion method with 10 chosen antimicrobial agents are presented in Table 4. Out of 16 *Salmonella typhimurium* isolates, 16 (100%) were resistant to erythromycin, 13 (81.25%) were resistant to doxycyclin and 11 (68.75%) were resistant to enrofloxacin. Furthermore, 8 (50%) were intermediate resistant to neomycin. On the other hand, 16 (100%) were susceptible to sulfamethoxazole-trimethoprim. Out of 8 *Salmonella gallinarum* isolates, 8 (100%) were resistant to doxycyclin, each 7 (87.5%) were resistant to erythromycin and enrofloxacin, 5 (62.5%) were resistant to amikacin and 4 (50%) were resistant to amoxycillin. On the other hand, each 6 (75%) were

Table 4: Results of antimicrobial susceptibility of *Salmonella* spp.

Name of isolates	No. (%)									
	N	AK	E	AMC	ENR	CN	DO	CIP	NOR	SxT
<i>Salmonella typhimurium</i> (n = 16)										
S	6 (37.5)	11 (68.75)	0 (0)	9 (56.25)	11 (68.75)	15 (93.75)	1 (6.25)	13 (81.25)	0 (0)	16 (100)
I	8 (50)	3 (18.75)	0 (0)	5 (31.25)	2 (12.50)	1 (6.25)	2 (12.50)	3 (18.75)	1 (6.25)	0 (0)
R	2 (12.5)	2 (12.50)	16 (100)	2 (12.50)	3 (18.75)	0 (0)	13 (81.25)	0 (0)	15 (93.75)	0 (0)
<i>Salmonella gallinarum</i> (n = 8)										
S	0 (0)	0 (0)	0 (0)	2 (25)	0 (0)	8 (100)	0 (0)	1 (12.5)	1 (12.5)	0 (0)
I	6 (75)	3 (37.5)	1 (12.5)	2 (25)	1 (12.5)	0 (0)	0 (0)	4 (50)	6 (75)	0 (0)
R	2 (25)	5 (62.5)	7 (87.5)	4 (50)	7 (87.5)	0 (0)	8 (100)	3 (37.5)	1 (12.5)	8 (100)
<i>Salmonella pullorum</i> (n = 3)										
S	3 (100)	1 (33.33)	0 (0)	1 (33.33)	0 (0)	3 (100)	0 (0)	3 (100)	0 (0)	2 (66.67)
I	0 (0)	2 (66.67)	0 (0)	2 (66.67)	0 (0)	0 (0)	0 (0)	0 (0)	2 (66.67)	1 (33.33)
R	0 (0)	0 (0)	3 (100)	0 (0)	3 (100)	0 (0)	3 (100)	0 (0)	1 (33.33)	0 (0)

N: Neomycin; AK: Amikacin; E: Erythromycin; CN: Gentamicin; CIP: Ciprofloxacin; DO: Doxycyclin; NOR: Norfloxacin; AMC: Amoxycillin; ENR: Enrofloxacin; SxT: Sulfamethoxazole-trimethoprim

Table 5: Results of antimicrobial resistance pattern of *Salmonella* spp.

Isolates	Resistance profiles	No. of isolates (%)	
<i>S. typhimurium</i> (n = 16)	No resistance demonstrated	-	
	a. Resistant to 1 agent (N)	1 (6.25)	
	b. Resistant to 1 agent (E)	3 (18.75)	
	Resistant to 2 agents (E-DO)	6 (37.50)	
	a. Resistant to 3 agents (AK-E-DO)	1 (6.25)	
	b. Resistant to 3 agents (AMC-E-DO)	2 (12.50)	
	c. Resistant to 3 agents (ENR-E-DO)	1 (6.25)	
	a. Resistant to 4 agents (AK-E-ENR-DO)	1 (6.25)	
	b. Resistant to 4 agents (N-E-ENR-DO)	1 (6.25)	
	Resistant isolates	n = 16 (100%)	
	<i>S. gallinarum</i> (n = 8)	No resistance demonstrated	-
		a. Resistant to 4 agents (E-ENR-DO-CIP)	1 (12.5)
b. Resistant to 4 agents (N-E-DO-CIP)		1 (12.5)	
c. Resistant to 4 agents (E-ENR-DO-AMC)		1 (12.5)	
d. Resistant to 4 agents (AK-ENR-DO-AMC)		1 (12.5)	
a. Resistant to 5 agents (AK-E-AMC-ENR-DO)		2 (25)	
b. Resistant to 5 agents (AK-E-ENR-DO-CIP)		1 (12.5)	
Resistant to 6 agents (N-AK-E-NOR-ENR-DO)		1 (12.5)	
Resistant isolates	n = 8 (100%)		
<i>S. pullorum</i> (n = 3)	No resistance demonstrated	-	
	Resistant to 4 agents (E-ENR-DO-CIP)	2 (66.66)	
	Resistant to 5 agents (E-ENR-DO-CIP-NOR)	1 (33.33)	
	Resistant isolates	n = 3 (100%)	

Table 6: Frequency distribution of multidrug resistant *Salmonella* isolates from broiler washing and rinsed water (when considered resistant to 2 or more drugs)

Name of isolates	No. (%)
<i>Salmonella typhimurium</i> (n = 16)	12 (75)
<i>Salmonella gallinarum</i> (n = 8)	8 (100)
<i>Salmonella pullorum</i> (n = 3)	3 (100)

intermediate resistant to neomycin and norfloxacin, while 4 (50%) were intermediate resistant to ciprofloxacin. On the contrary, each 8 (100%) were susceptible to gentamicin and sulfamethoxazole-trimethoprim. Out of 3 *Salmonella pullorum* isolates, each 3 (100%) were resistant to erythromycin, enrofloxacin and doxycyclin. With along this, each 2 (66.67%) were intermediate resistant to amikacin, norfloxacin and amoxycillin. On the other hand, each 3 (100%) were susceptible to gentamicin, neomycin and ciprofloxacin; while 2 (66.67%) were susceptible to sulfamethoxazole-trimethoprim.

The results of antimicrobial resistance patterns of *Salmonella typhimurium* are summarized in Table 5. Out of 16 *Salmonella typhimurium* isolates, 6 (37.5%) were resistant to 2 antibiotics. Furthermore, 1 (6.25%), 2 (12.5%) and 1 (6.25%) were resistant to each of 3

antibiotics respectively. Moreover, 1 (6.25%) and 1 (6.25%) were resistant to each of 4 antibiotics. Out of 8 *Salmonella gallinarum* isolates, each 1 (12.5%) was resistant to each of 4 antibiotics. Furthermore, 2 (25%) and 1 (12.5%) were resistant to each of 5 antibiotics. On the other hand, 1 (12.5%) was resistant to 6 antibiotics. Out of 3 *Salmonella pullorum* isolates, 2 (66.66%) were resistant to 4 antibiotics and 1 (33.33%) was resistant to each of 5 antibiotics. On the other hand, 12 (75%) *Salmonella typhimurium* (n = 16); 8 (100%) *Salmonella gallinarum* (n = 8) and 3 (100%) *Salmonella pullorum* (n = 3) were detected as multidrug resistant isolates (Table 6).

DISCUSSION

This study was aimed at isolation, identification and biochemical differentiation of *Salmonella* spp. from the washing and rinsed water collected from Pluck shops (cottage poultry processors) at Gorgoria-masterbari, Sreepur, Gazipur district and antibiogram characterization of the isolated *Salmonella* strains were also accomplished.

Of the bacteria, since the isolation and correct identification of *Salmonella* are very crucial for the characterization, the colonies having typical cultural characteristics were selected as presumptive for *Salmonella* serovers. For this several differential selective media such as SSA, BGA, MCA, TSIA etc., were used simultaneously to culture the organism because all of them are not equally suitable for all the serovars of *Salmonella*. In the present study, specific enriched media and biochemical tests were used for the isolation and identification of Salmonellae which was also used by a number of researchers (Habrun and Mitak, 2003; Hossain, 2002; Cheesbrough, 1985; Buxton and Fraser, 1987; Amin, 1969). The colony characteristics of *Salmonella* spp. exhibited colorless, smooth, transparent and raised colonies on MacConkey agar; translucent, black or colorless, smooth, small round colonies on SS agar; translucent, opaque, smooth colonies on nutrient agar; black color colonies on TSI agar and light pink or pink colony against a rose pink background on brilliant green agar were similar to the findings of other authors (Muktaruzzaman *et al.*, 2010; Sujatha *et al.*, 2003; Hossain, 2002; Buxton and Fraser, 1987; Amin, 1969).

In Gram's staining, the morphology of the isolated Salmonellae from samples exhibited Gram negative, small rod shaped, single or paired in arrangement under microscope which was reported by other researchers (Cheesbrough, 1985; Freeman, 1985). In case of motility test, performed by MIU media and hanging drop slide method, 40.74% (where n = 11) isolates were non-motile and 59.26% isolates (where n = 16) were motile. Motility test was fundamental basis for the detection of motile and non-motile *Salmonella* organisms (Hossain, 2002; Freeman, 1985; Buxton and Fraser, 1987). In sugar fermentation test, all the isolates (n = 27) fermented glucose, maltose and lysine decarboxylase and produced acid and gas but did not ferment sucrose and lactose those indicated positive for Salmonellae which is in agreement the statement of Buxton and Fraser (1987).

Among the 27 positive *Salmonella* isolates, 11.11% (where n = 3) fermented glucose, maltose, rhamnose and produced both acid and gas but did not ferment dulcitol and also produced H₂S in TSI agar indicating that there were positive for *S. pullorum*. Only 29.83% isolates (where n = 8) fermented glucose, maltose, dulcitol without producing acid and gas and did not ferment rhamnose with variable H₂S production in TSI agar indicating typical for *S. gallinarum*. The rest 59.26% isolates (where n = 16) not only fermented glucose, maltose, rhamnose and dulcitol with or without gas but also produced H₂S in TSI agar demonstrated an indication of being *S. typhimurium*. These results are strongly correlated with the observations of Kwon *et al.* (2010), Sujatha *et al.* (2003) and Crichton and old (1990).

A total of 27 isolates were positive for Methyl Red test (the appearance of the red color in the media was observed after the addition of the appropriate reagent with the cultural growth and thus indicating the isolated Salmonellae were positive for MR test) but in case of Voges-Proskauer (VP) test all of the isolates (where n = 27) did not develop any pink color after the addition of the appropriate reagents, indicating that the isolates of *Salmonella* spp. were negative for VP test which is similar with the statement of Muktaruzzaman *et al.* (2010). In indole test, all the test isolates (where n = 27) did not develop any red color that indicated the *Salmonella* isolates were negative to indole test and no participation on Urease activity (Lee *et al.*, 2003). In case of reaction in TSI agar slant, 70.37% of the isolates (where n = 19) produced red slant, yellow butt with the production of H₂S gas which strongly supported the observations of Selvam *et al.* (2010), Shah *et al.* (2001) and Merchant and Packer (1967). All the isolates (where n = 27) of *Salmonella* spp. were positive for Lysine Decarboxylation test and negative for Citrate Utilization test which satisfied the statement of Lee *et al.* (2003). Organisms isolated from the collected samples under test, revealed unequivocal morphological, cultural and biochemical properties resembling *Salmonella* spp. as was recorded by Hossain (2002), Freeman (1985), Buxton and Fraser (1987) and Amin (1969). Among the 27 isolates of *Salmonella*, 16 *Salmonella* were more or less similar to *Salmonella typhimurium*, 3 *Salomonella* were similar to *Salmonella pullorum* and only 8 isolates showed the characteristics of *Salmonella gallinarum*.

In antimicrobial susceptibility testing, out of 16 *Salmonella typhimurium* isolates, 16 (100%) were resistant to erythromycin, 13 (81.25%) were resistant to doxycyclin and 11 (68.75%) were resistant to enrofloxacin. Furthermore, 8 (50%) were intermediate resistant to neomycin. On the other hand, 16 (100%) were susceptible to sulfamethoxazole-trimethoprim. Out of 8 *Salmonella gallinarum* isolates, 8 (100%) were resistant to doxycyclin, each 7 (87.5%) were resistant to erythromycin and enrofloxacin, 5 (62.5%) were resistant to amikacin and 4 (50%) were resistant to amoxycillin. On the other hand, each 6 (75%) were intermediate resistant to neomycin and norfloxacin, while 4 (50%) were intermediate resistant to ciprofloxacin. On the contrary, each 8 (100%) were susceptible to gentamicin and sulfamethoxazole-trimethoprim. Out of 3 *Salmonella pullorum* isolates, each 3 (100%) were resistant to erythromycin, enrofloxacin and doxycyclin. With along this, each 2 (66.67%) were intermediate resistant to amikacin, norfloxacin and amoxycillin. On the other hand, each 3 (100%) were susceptible to gentamicin, neomycin and ciprofloxacin; while 2 (66.67%) were susceptible to sulfamethoxazole-trimethoprim; these findings are also very close to Shah and Korejo (2012) and Kabir (2010).

Salmonella resistance at varying concentrations of penicillin, chloramphenicol, neomycin and erythromycin has also been reported by Sultana *et al.* (1992).

Out of 27 *Salmonella* isolates, *Salmonella typhimurium* 12 (75%), *Salmonella gallinarum* 8 (100%) and *Salmonella pullorum* 3 (100%) were detected as multidrug resistant. Manie *et al.* (1998), also found several strains of multiple antimicrobial resistant *Salmonella* spp. in chicken. Recently, some authors have reported an increase in quinolone (enrofloxacin) resistance in *Salmonella* (Molbak *et al.*, 2002) which also partially supports the findings of this study.

Salmonella species were isolated and characterized successfully from broiler washing and rinsed water using different cultural, morphological examination, biochemical and antimicrobial susceptibility tests. The findings of the present study revealed the presence of multidrug resistant *Salmonella* species in washing and rinsed water of broilers in Pluck shops at Sreepur of Gazipur district in Bangladesh. However, elucidation of the molecular mechanisms of resistance in our strains will require further study.

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