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Original article

PREVALENCE AND ANTIMICROBIAL RESISTANCE OF *SALMONELLA SPP*. FROM RAW CHICKEN MEAT AND EGGS IN MARKETS OF DINAJPUR DISTRICT, BANGLADESH

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Summary

Aziz, F. B., M. K. Hossain, M. R. Akter, S. Sarkar, K. A. Ferdous, V. Kulkarni & R. Rafia, 2026. Prevalence and antimicrobial resistance of *Salmonella spp*. from raw chicken meat and eggs in markets of Dinajpur district, Bangladesh. *Bulg. J. Vet. Med.,* **29**, No 1, 64–73.

The current study examined the antibiotic resistance of zoonotic *Salmonella spp*. from raw chicken meat and eggs gathered from various live bird markets in Bangladesh Dinajpur region. A total of 250 samples were collected from Sonali (n=100) & Esha Brown layer chicken (n=100) and eggs (n=50) and the presence of *Salmonella* spp in collected samples was assessed by standard bacteriological techniques followed by PCR assay. The primers for the *invA* gene at a specific molecular size of 284 bp were used to detect *Salmonella* spp. The overall prevalence of *Salmonella* spp in Sonali chicken, Esha Brown layer chickens, and eggshells were 74%, 68%, and 0% respectively; 100% motile salmonellae carrying the *invA* gene were found in 22 samples with an 8.8% prevalence out of 250 samples. From a variety of dressed poultry (Sonali and Esha Brown layer chickens) and egg samples, 142 *Salmonella spp*. were identified in which 68 Esha Brown and 74 Sonali tested positive for *Salmonella spp*. Furthermore, the isolates were subjected to antimicrobial resistance studies using the disc diffusion method, which used six commonly used antibiotics. Antibiograms revealed that 16.7% of *Salmonella spp*. were sensitive to gentamicin whereas most of the isolates were resistant to chloramphenicol (75%), cotrimoxazole (58.3%), cefixime (58.3%), streptomycin (8.3%) and tetracycline (75%). Out of all isolates, 12 *Salmonella spp*. (58.3%) were multidrug-resistant. The study revealed the presence of multidrug-resistant *Salmonella spp*. in raw chicken meat sold in different live bird markets in Dinajpur, Bangladesh. It was concluded that bacterial contamination in raw chicken meat might be the cause of indiscriminate use of antibiotics which is very common in live bird markets in this area, and that bacterial contamination poses an important threat to human health. The results of this study would be helpful for the prevention and control of zoonotic salmonellosis.

Key words: antibiotic resistance, *invA* gene, motile, *Salmonella*, zoonotic

INTRODUCTION

Salmonellosis is the main source of global food-borne zoonoses (Hohmann, 2023; Marcus *et al.*, 2007). *Salmonella* is more likely to be present in poultry and poultry products (Braden, 2006; Stilz *et al.*, 2022), and the *invA* gene is a crucial diagnostic indicator for salmonellosis (Li *et al.*, 2012; Wang *et al.*, 2024). Recently, *Salmonella* prevalence and serotyping from poultry products have been extensively studied (Parvej *et al.*, 2016; Zhang *et al.*, 2021; Farahani *et al.*, 2023;). *Salmonella enterica* is the isolate that most frequently displays multidrug resistance, and it poses a danger to the commercial chicken industry in Bangladesh (Sultana *et al.*, 2014). *Salmonella enterica* is the main causal agent of salmonellosis. Every year, almost 20 million people and animals become infected with *Salmonella*. It reduces the productivity of animals and results in 150,000 animal and human deaths annually (Abd El-Ghany, 2020).

Salmonella spp. was isolated and molecularly characterised in faecal samples of pigs and cows, and the results revealed that the overall incidence of salmonella was 3.8% and 5.9%, respectively (Nyabundi *et al.*, 2017). Thirty *Salmonella* Typhimurium isolates of chicken and human origin showed the highest resistance rates to tetracycline and ampicillin (53.3% each), gentamicin (30%), streptomycin (56.7%), and trimethoprim-sulfamethoxazole (73.3%) (Ahmed *et al.*, 2016). In a recent study that determined the prevalence of potential bacterial pathogens, including *Salmonella spp*. and their associated risk factors for zoonotic transmission, the isolates were mostly sensitive to gentamicin (83.1%), followed by levofloxacin (81.5%), ciprofloxacin (80.4%), kanamycin (77.8%), and cefixime (59.3%). Additionally, they exhibited 100 percent resistance to bacitracin, amoxicillin, and penicillin G. (Nupur *et al.*, 2023). *Salmonella* is frequently found in eggs and is vulnerable to antibiotics, as shown in several works worldwide (Ghoddusi *et al.*, 2015; Tessema *et al.*, 2017; Arkali & Çetinkaya, 2020). Polymerase chain reaction (PCR) technology is used as a rapid detection tool for *Salmonella* (Abdel-Aziz, 2016). Fresh markets and supermarkets are two different sources where poultry products can be purchased for human consumption in Bangladesh. Fresh markets are conventional outdoor marketplaces where farmers or lone vendors sell chicken, frequently offered and kept at room temperature. Naturally, there are several potential sources of contamination in these markets (rodents, insects, sewage) (Rabby *et al.*, 2021). Research has shown that *Salmonella* is resistant to various antibiotics, and the findings provide insight into working with raw chicken meat and tools used in Bangladeshi retail chicken meat shops for public health concerns. The increasing likelihood that this zoonotic pathogen would encounter humans is a major factor impacting the effectiveness of therapy, and the emergence of antimicrobial resistance results in drug-resistant strains (Prestinaci *et al.*, 2015; Baloch *et al.*, 2020; Shami, 2020; Vercelli *et al.*, 2022). Rahman *et al.* (2016) isolated *Salmonella* spp. from 39.58% of ducks and 28.57% of pigeons in the Dinajpur district of Bangladesh and concluded that they may play a significant role in carriers of zoonotic *Salmonella* spp. transmission in the poultry in that area (Rahman *et al.*, 2016). The current investigation on risk variables is prompted by the fact that very few studies have been done on raw

chicken meat and retail shop accessories in Bangladesh.

MATERIALS AND METHODS

Collection and processing of samples

Using aseptic cotton stick swabs, a total of 250 samples (200 raw chicken flesh and 50 eggs) were taken from various live bird markets with diverse levels of sanitary measures in Dinajpur district (Chirirbandar, Birganj, Kaharole, Khansama, and Birol) (Table 1). In the case of meat samples, 8 cm^2 from each raw chicken and 2 cm^2 for egg samples were swabbed (Russell *et al.*, 1997). The laboratory work was carried out in the bacteriology laboratory from October 2019 to January 2020 in the Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science & Technology University (HSTU), Bangladesh.

Isolation and identification

Collected samples were transferred to the laboratory using a cool box (4 °C) and then enriched in Buffer Peptone Water (HiMedia, India) for 24 h at 37 °C. Inoculations of 0.1 ml of enriched samples were made in nutrient agar (HiMedia, India), McConkey agar (HiMedia, India), Salmonella-Shigella agar (HiMedia, India), Brilliant Green agar (HiMedia, India), and Deoxycholate Citrate agar (HiMedia, India) and incubated at 37 °C for 24 h for colony formation. *Salmonella spp*. was identified according to the colony characteristics on the agar plate. A morphological study was done with Gram's staining technique following Marchant & Packers (1967). The isolate was then cultured on TSI, MIU, MR, VP, and Simmon's citrated medium (HiMedia, India) for biochemical examination. The motility test was done using MIU media and hanging drop preparation. After confirmation, the rest of the solutions were kept at –20 °C as a stock culture for further research.

Molecular characterisation of bacterial isolates

DNA extraction. Biochemically identified *Salmonella* spp. colonies were cultured overnight on nutrient broth. Then DNA was extracted following the protocol of Pure Link TM Genomic DNA Mini Kit, England (Psifidi *et al.*, 2010).

Primers and PCR amplification program. The specific forward and reverse primers for the *invA* gene are selected according to the recommendation followed by Rahn *et al.* (1992). The sequences are presented in Table 2.

The *invA* gene was amplified via PCR employing 12.5 µL of Tag Green Master Mix 2×DNA polymerase, 1 µL forward and 1 µL reverse specific primers, 2 µL of DNA extract as a template, and 8.5 µL of nuclease-free water to increase the total reaction volume to 25 µL. The initial de-

Table 1. Summary of samples (n=250) collected from the live bird market

Sample collection area	Number of samples		
	Eggs	Sonali chicken	Esha Brown layer chicken
Chirirbondor	10	20	20
Birganj	10	20	20
Kaharole	10	20	20
Khansama	10	20	20
Birol	10	20	20

Table 2. Primers used for the detection of *Salmonella spp*. (Rahn *et al.*, 1992)

Specificity	*Salmonella spp.*
Gene	*invA*
Primers' sequence (5` to 3`)	F: TCATCGCACCGTCAAAGGAACC
	R: GTGAAATTATCGCCACGTTCGGGCAA
Amplicon size (bp)	284

naturation at 95 °C for 1 minute was followed by 35 cycles of denaturation at 95 °C for 1 minute, annealing at 52 °C for 1 minute, extension at 72 °C for 1 minute, and one cycle of final extension at 72 °C for 1 minute in the thermocycler (Eppendorf) programme.

PCR product electrophoresis. *Salmonella*-specific PCR amplified DNA products were analysed by electrophoresis on 1.2% agarose w/v gels stained with ethidium bromide and observed by UV illumination (Fig. 2). Each gel received a 120 V current application. Onto an agarose gel, 8 µL of PCR product and 3 µL of 6× loading dye (Genei, Bangalore) were loaded. The PCR products were marked using a 100 bp DNA ladder, formulated to run accurately and to result in sharp band patterns. It contains two premixed dyes (bromophenol blue and xylene cyanol) for easy loading and migration tracking during gel electrophoresis.

Antibiotic sensitivity tests

Different widely accessible antimicrobial discs (Mast Diagnostics Mersey Side, UK) were utilised to determine the sensitivity and resistance trends of the isolated *Salmonella* spp. (Morgan *et al.*, 2023). Taking advantage of the Kirby-Bauer disk diffusion method by the Clinical and Laboratory Institute's 2018 standards, the antibiotic sensitivity profile of isolated *Salmonella* was evaluated. A panel of six antibiotics including gentamicin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg), streptomycin (10 µg), cefixime (5 µg), and co-trimoxazole (25 µg) were chosen for antibiotic sensitivity testing. These drugs are frequently used in the field to treatment of salmonellosis. A

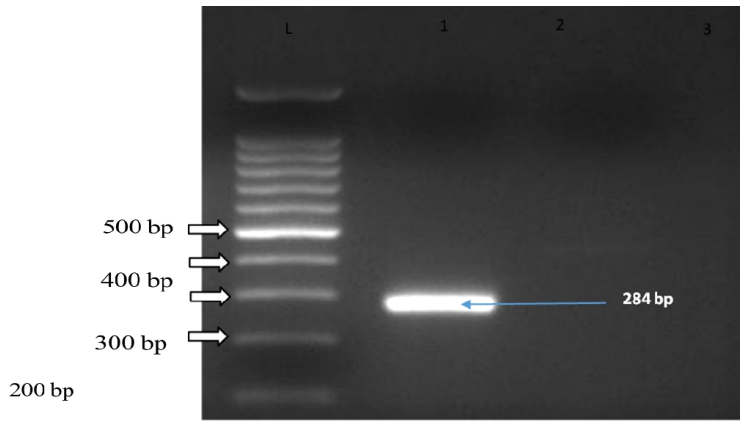

Fig. 1. Result of amplification of *invA* gene region of *Salmonella spp*. by PCR. L= 100 bp DNA ladder, 1= 284 bp *invA* gene for Sample 1, Sample 2, and 3 were negative.

fresh culture of the bacteria was usually grown in a suitable broth medium before being suspended in sterile saline or a similar solution to achieve a specific turbidity, usually matched to a 0.5 McFarland standard, which corresponds to a bacterial concentration of about 1×10^8 colony forming units (CFU) per milliliter. The Mueller Hinton agar-fixed antimicrobial discs were incubated in the plates for 24 to 48 hours at 37 °C.

RESULTS

From a variety of dressed poultry (Sonali and Esha Brown layer chicken) and egg samples, 142 *Salmonella spp*. were identified in which 68 Esha Brown and 74 Sonali tested positive for *Salmonella spp*. In the market, *Salmonella* was found in 74% of cases in retail Sonali chicks, and in 68% of cases in Esha Brown layer chicken (Table 3). No positive cases of *Salmonella spp*. were found in the egg samples. All 142 isolates were positive for catalase and oxidase, and they all grew on brilliant green agar and deoxycholate citrate agar media without changing the medium colour. Twenty-two isolates (8.8% prevalence) showed 100% motility. The tests for indole and methyl-red were positive, while the Voges-Proskauer test was negative. These results indicated citrate utilisation, while the urease test was negative. The bacteria were non-lactose fermenters on MacConkey agar, fermented glucose, fructose, and maltose. TSI was found to be K/AG+H_2S+. It was determined that each of the 142 isolates was a *Salmonella* organism.

Table 3. Summary of isolation of *Salmonella* spp. bacteria from chicken meat and egg samples.

	Isolate number (%)
Sonali chicken (n=100)	74 (74%)
Esha Brown Layer chicken (n=100)	68 (68)
Eggs (n=50)	0

Six different antibiotics were used to examine the antibiotic susceptibility of 142 *Salmonella* isolates found in poultry samples. Co-trimoxazole and cefixime-resistant bacteria were the most prevalent

Table 4. Antimicrobial resistance profile of isolated *Salmonella spp.* (n=142) to different antimicrobials

Number and percentage of isolates resistant to antimicrobials					
Gentamicin	Chloramphenicol	Tetracycline	Streptomycin	Cotrimoxazole	Cefixime
2 (16.7)	9 (75)	9 (75)	1 (8.3)	7 (58.3)	7 (58.3)

Table 5. Profiles of multidrug-resistant (MDR) *Salmonella* spp. (n=12) isolated from raw chicken meat

Antimicrobial compound	Antibiotic class	Number (%) of MDR isolates
Tetracycline, chloramphenicol, cotrimoxazole, cefixime	Tet-Chl-Cep	5 (41.7)
Tetracycline, cotrimoxazole, cefixime	Sul-Cep-Tet	2 (16.7)

Cep = cephalosporins, Sul = sulphonamides, Tet = tetracyclines, Chl = chloramphenicol.

(58.3%) (Table 4). Of all isolates from raw chicken meat, 12 were multidrug-resistant (Table 5).

DISCUSSION

Food contamination by microorganisms is a well-established global public health hazard. Raw poultry products are thought to be the cause of a significant number of human food poisoning incidents due to the relatively high frequency of meat contamination by *Salmonella* (Hobbs, 1968). The present research work was conducted to determine the antimicrobial resistance of zoonotic *Salmonella* spp. from raw chicken meat and eggs which cause infections in poultry and humans, being responsible for a considerable loss in poultry industry and a threat to human health (Habrun *et al.,* 2006).

In retail Sonali, 74% positive *Salmonella* cases were found which was very similar to a previous study by Yang *et al.* (2011) reporting an overall *Salmonella* prevalence of 52.2%. *Salmonella* prevalence was the highest in the wet markets (54.4%) compared with the large markets (50.3%) and small markets (52.1%). Rahman *et al.* (2016) researched the prevalence of *Salmonella spp*. in ducks and pigeons in the Dinajpur region, Bangladesh, and found 39.58% and 28.57% prevalence respectively.

In this study, the *Salmonella* isolates demonstrated positive reactions in the MR test but negative reactions in the indole, VP, and SC tests in line with the findings of Buxton & Fraser (1977). The DNA was extracted through PCR, very similar according to the results of Parvej *et al.* (2016). *Salmonella* genus-specific primer (*invA*) was used for molecular characterisation of *Salmonella* spp. in the PCR. The amplified size of the PCR product of 284 bp indicated or reconfirmed that *Salmonella* isolates were present in the chicken meat samples similar to a previous study (Asif *et al.*, 2017). In 22 out of 250 samples (8.8%), 100% motile *Salmonella* with the *invA* gene were found in agreement with previously reported findings (Shanmugasamy *et al.*, 2011; Sharma & Das, 2016; Tiwari *et al.*, 2022).

The antibiogram study was performed by the disc diffusion method with 6 different antibiotics. In this study, *Salmonella* isolates were resistant to gentamicin (16.7%), chloramphenicol (75%), tetracycline (75%), cefixime (58.3%), streptomycin (8.3%), and co-trimoxazole (58.3%). The prevalence and antibiotic susceptibility of *Salmonella* isolates from fresh raw chicken meat were studied in Malaysia, Ethiopia, and some other countries. According to Tessema *et al.* (2017), Thung *et al.* (2016), and Ghoddisi *et al.* (2015), the isolates were resistant to vancomycin, erythromycin, and penicillin, while they were found to be sensitive to amoxicillin/clavulanic acid, gentamicin, tetracycline, and trimethoprim. These findings showed that retail chicken meat may be a source of *Salmonella* spp. resistant to several antibiotics and could be harmful to public health.

This study demonstrated a total of 58.3% multidrug-resistant (MDR) isolates were found in chicken meat. Saud *et al.* (2019) investigated MDR bacteria from raw buffalo and chicken meat and reported an overall rate of 32.7% MDR isolates, of which 50.0% from chicken raw meat and 21.9% from buffalo raw meat. One possible explanation for *Salmonella* spp.'s resistance could be that the organisms developed this trait because of careless use of antibiotics. The isolation of bacterial pathogens from chicken meat samples should be considered hazardous

to health. In this study, no positive cases of *Salmonella* infection were found in egg samples. *Salmonella* is a bacterium that can be present inside eggs, but it doesn't always infect them. It is typically transmitted when hens lay eggs that are contaminated with *Salmonella* either from the hen reproductive tract or external surfaces. Garcia *et al.* (2011) demonstrated that feces were 92% positive for *Salmonella*, followed by eggshells (34%) and cloacal swabs (4%). No *Salmonella spp*. was detected in the egg contents.

CONCLUSION

Salmonellosis plays an important role in zoonotic infections. The results of this study indicated that raw chicken meats are susceptible to several bacterial agents common to other poultry species. Prudent use of antibiotics should be considered in poultry production (where permissible) since many strains are resistant to common antibiotics, and some were multidrug resistant as described in this study. Potential drug-resistant pathogens in otherwise normal poultry may be a serious public health concern. Therefore, this study calls for immediate attention to introduce and continuous monitoring of *Salmonella* infection by randomised detection of antibodies by serological test, identifying the infected bird, follow hygienic measures in the local market since the diseases have great public health importance. Current findings warrant further studies with the isolated strains of bacteria that are responsible for the resistance to antibiotics. Based on the results of the study it may be concluded that *Salmonella* spp. contamination plays an important role in raw chicken meat samples in Bangladesh. The identified multidrug-resistant bacteria from raw meat samples that might have resulted from the indiscriminate use of antibiotics for treatment in poultry farms. Streptomycin was the most effective antibiotic among the antimicrobial agents used in this study. In further study, we need advanced study with species identification of the *Salmonella* isolates.

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