



High prevalence of ciprofloxacin and ceftriaxone resistance *Salmonella* in the retail chicken market of Chattogram, Bangladesh

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ABSTRACT

Objective: The present study aimed to investigate the prevalence of *Salmonella* and their antibiotic resistance pattern against two commonly used antibiotics: ciprofloxacin and ceftriaxone in marketed live chickens in a live birds market.

Materials and methods: The study was conducted on live Sonali chickens (crossbred of Fayoumi female and Rhode Island Red male). Cloacal swabs were collected from 50 randomly selected live birds from 5 retail sellers (10 samples/seller) at Jhawtola live birds' market, Chattogram, Bangladesh. Culture and biochemical tests were used to identify *Salmonella*. Positives samples for *Salmonella* were further tested for antibiogram by disc diffusion method.

Results: *Salmonella* was identified from 28 (56%) of samples by culture and biochemical test. Among the positive isolates, 27 (96.42%) samples exhibited resistant to ceftriaxone, and 20 (71.42%) samples were to ciprofloxacin.

Conclusions: The result of this study indicates there is a high prevalence of *Salmonella* in marketed local chicken and are resistant to ceftriaxone and ciprofloxacin which has economic importance as well as a public health concern. Awareness should be increased to prevent random use of antibiotics and provision of strict biosecurity measures.

Keywords: Local chickens, *Salmonella*, Live birds market, Drug resistance, Antibiotics

INTRODUCTION

Food safety is an emerging issue that is often less emphasised in resource-limited countries of the world resulting in reaching of unsafe food from producers to consumers without any intervention. Unwholesomeness of food is one of the significant cause of food-borne illness in human which maybe sometimes life-threatening (McCabe-Sellers and Beattie, 2004). In recent years, cases of food-borne illness particularly the incidence of gastroenteritis are reported greatly in Bangladesh (Ahmed et al., 2018). Health care providers are facing problems for the treatment of these cases, associated with

microbial contamination of foods in particular chicken meat, as those exhibit a high rate of antimicrobial resistance. It plausibly due to lack of awareness of the farmers on indiscriminate use of antimicrobials and direct transmission of pathogens through the live birds or contaminated poultry meat unhygienically processing at the same place where chickens are being kept for selling in live bird market due to lack of legislative actions. The appearance of the ever-increasing range of antimicrobial resistance and high level of disease burden has emerged as one of the greatest

difficulties in ensuring safe food to the consumers (Founou et al., 2016).

Salmonellosis, an important foodborne illness caused by a number of serovars of *Salmonella enterica*, a Gram-negative, flagellated rod-shaped bacterium which is frequently associated with poultry. After *Campylobacter*, *Salmonella* is the leading cause of foodborne gastroenteritis worldwide. Prevalence is believed to be higher in developing countries but is challenging to calculate as many countries have insufficient surveillance data (Majowicz et al., 2010). Pathogenic *Salmonella* colonises in the gut of chicken and produces localised and systemic infection which cause a variable degree of mortality in young chickens and active shedding of bacteria via faecal route (Pande et al., 2016). A human can acquire non-typhoid *Salmonella* infection from chicken through direct contact with an infected bird or indirectly by ingestion of foods or water that are contaminated by infected chickens dropping (Gieraltowski et al., 2016). Apparently healthy but carrier chickens can also shed infection and cause reduce production leading to economic losses to the farmers and act as a source of infection to the susceptible people.

In addition to its pathogenicity, *Salmonella* isolates are also shown resistant to the most commonly used antimicrobials that have become a global health issue in recent years (Eng et al., 2015). In Bangladesh, the use of antibiotics in poultry is not subjected to any strict regulation. Poultry farmers can purchase antimicrobials directly from the drug dealers without consulting a veterinarian. Diagnosis is typically presumptive and indiscriminate use of antibiotics is a common practice (Bhowmik et al., 2017). A common tendency is to use of broad-spectrum antimicrobials at a higher dose. Fluoroquinolone group of drug particularly ciprofloxacin is mostly used against Salmonellosis in poultry. However, irrational use or overuse of such antibiotics increases the chance of resistance to these drugs. On the other hand, in human gastroenteritis cases third generation cephalosporins mainly ceftriaxone is commonly prescribed by the physician (Chowdhury et al., 2008). Taking together the aim of the study was to determine the incidence of *Salmonella* in marketed live birds in Chittagong, Bangladesh and their antibiotic resistance profile against the most specifically used two antibiotics ciprofloxacin and ceftriaxone.

MATERIALS and METHODS

Study area and sampling

Chattogram is the second largest metropolitan city of Bangladesh has a population of more than 2.5 million. Only a few supermarkets are available in the city and the majority of city-dwelling people depend on local market traders who have stalled in particular place in the markets and sell their chickens. For a regular collection of chickens, the live chicken retailers make an agreement with the chicken farmers and purchase from them on a daily basis.

For this study, five chicken retailers were selected in the Jhawtola local market, who used to collect chickens from small scale local poultry farm (<200 chickens/shed) for daily sell.

Sample collections and processing

A total of 50 faecal samples were collected. 10 Sonali chickens (crossbred of Fayoumi female and Rhode Island Red male used mainly for meat purpose in Bangladesh) were selected randomly from each trader. Faecal swab sample was collected from the recto-anal junction in sterile plastic containers at morning and shifted immediately under chilled condition using an ice box to the Poultry Research and Training Institute, CVASU for further investigation.

Isolation and identification of *Salmonella* spp.

Swabs collected were pre-enriched in Buffered peptone water (BPW) (Oxoid Ltd, Basingstoke, Hampshire, England), incubated at 37°C for 18 hours. Selective agar media were used to isolate *Salmonella* from enriched swab samples. A loop full of enriched culture from BPW was inoculated onto Xylose-Lysine-Desoxycholate Agar (XLD. Agar) (Oxoid Ltd, Basingstoke, Hampshire, England) plates and Brilliant Green Agar (BGA) (Oxoid Ltd) plates and incubated at 37°C for 24 hours. The selective differential agar plates were examined for the presence of typical colonies. TSI agar (Oxoid Ltd) was used for biochemical analysis. All media were prepared according to the manufacturers' guideline.

Gram's staining method was also performed to study morphology and staining characters and thus purity of *salmonella* colony was determined. *Salmonella* suspected colony from XLD agar was stained as described by manual of veterinary investigation laboratory technique.

Antibiotic sensitivity test

All positive *salmonella* isolates were subjected to antibiotic susceptibility test using ceftriaxone and ciprofloxacin discs (HIMEDIA Ltd, Mumbai, India) on Mueller-Hinton agar (Oxoid Ltd, Basingstoke, Hampshire, England) by modified Kirby-Bauer disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2013). The M-H agar was prepared as per the manufacturer's instruction. The diameters of the zone of inhibition were measured, including the diameter of the disc by using HI Antibiotics Zone Scale-c. Antibiotic resistance results were classified according to their inhibition zone *Salmonella* relative chart of zone sizes (Inhibited zone in mm), chart reproduced from the sixth report of a WHO expert committee.

RESULTS

Isolation and identification of Salmonella

A total of 50 samples collected from faeces of Sonali (Fayoumi female × Rhode Island Red male)

chickens. Based on the staining, cultural, morphological and biochemical properties of *salmonella*, positive samples were identified. Thus, among 50 samples, 28 samples (56%) were found positive for *Salmonella*. No significant difference was observed in prevalence between male [11 (39.29%)] and female [17 (60.7%)] chickens ($P = 0.28$).

Antimicrobial susceptibility patterns of Salmonella sp

Out of 50 faecal samples, 28 cultural test positive *Salmonella* were tested for susceptibility to two different antimicrobial agents. The frequencies of isolates showing resistant (R), Intermediate (I) and sensitive (S) to the antimicrobials tested are shown in Table 1. Among the positive isolates, 27 (96.42%) samples were resistant to ceftriaxone and 20 (71.42%) samples were to the ciprofloxacin.

Table 1. Antibiotic resistance pattern of marketed live Sonali chickens (♀ Fayoumi × Rhode Island Red ♂) faecal *Salmonella* isolates to ciprofloxacin and ceftriaxone in Chattogram, Bangladesh

| Seller ID | Faecal <i>Salmonella</i> (%) | | Ciprofloxacin (%) | | | Ceftriaxone (%) | | |
|-----------|------------------------------|----------|-------------------|-----------|-------|-----------------|-------|-----------|
| | Positive | Negative | R | I | S | R | I | S |
| 1 | 5 (50) | 5 (50) | 5 (100) | 0 (0) | 0 (0) | 4 (80.00) | 0 (0) | 1 (20.00) |
| 2 | 7 (70) | 3 (30) | 3 (42.86) | 4 (57.14) | 0 (0) | 7 (100) | 0 (0) | 0 (0) |
| 3 | 5 (50) | 5 (50) | 4 (80.00) | 1 (20.00) | 0 (0) | 5 (100) | 0 (0) | 0 (0) |
| 4 | 5 (50) | 5 (50) | 4 (80.00) | 1 (20.00) | 0 (0) | 5 (100) | 0 (0) | 0 (0) |
| 5 | 6 (60) | 4 (40) | 4 (66.67) | 2 (33.33) | 0 (0) | 6 (100) | 0 (0) | 0 (0) |

R: Resistant; I: Intermediate; S: Sensitive

DISCUSSION

The study was conducted on 50 marketed Sonali (Fayoumi female × Rhode Island Red male) chickens at Khulshi area, Chattogram, Bangladesh for the isolation and identification of *Salmonella* and their sensitivity to two major antibiotics. Among the 50 chickens faecal samples, 28 (56.0%) samples were positive for *Salmonella* spp. Similarly, high percentage (55%) of positive samples were stated from apparently healthy ready-to-slaughtered poultry faeces by Kagambèga et al. (2013) in Burkina Faso, West Africa. Slightly lower per cent (47%) of *Salmonella* was isolated from a conventional poultry farm in Wisconsin, USA

(Siemon et al., 2007). In another local birds' market of Mymensingh region of Bangladesh, *Salmonella* was found in 32% of marketed broiler chickens (Aditya, 2015). The occurrences of *Salmonella* contamination in samples from poultry sources have also been reported elsewhere (Akond et al., 2012; Hassan et al., 2015). However, the relatively lower prevalence was observed in marketed broiler chickens in Coimbatore City of southern India, where 1.4-6.9% samples were found positive for *Salmonella* (Suresh et al., 2011). The prevalence of *Salmonella* in chickens depends on multiple factors ranging from the collection of *Salmonella*-free day-old-chicks from hatcheries to biosecurity status of a farm. It has been reported that poor biosecurity of

the farm and access of rodents and unwholesomeness of feed and water is one of the potential risk factors of *Salmonella* prevalence (Lapuz et al., 2012).

Use of antibiotic is a common practice to protect from Salmonellosis in poultry. However, commonly used drugs are becoming resistance due to overuses. Cohen and Tauxe (1986) stated *Salmonella* strains are becoming more resistant to antimicrobial agents which has public health implications as well due to more exposure of drugs to food animals leading to persistence of resistant strains. Resistance to a single or even multiple resistance has also become common among the poultry *Salmonella* in Bangladesh (Hassan et al., 2014). In this study, among positive samples, 96.42% showed resistance to ceftriaxone, and 71.42% were resistant to ciprofloxacin that is resistance to ceftriaxone. Therefore, it is very alarming, and resistance to ciprofloxacin is also at high-risk levels. Increased resistance towards ceftriaxone might be due to the frequent use of this antibiotic in chickens for prevention and treatment of diseases as well as using it as a growth promoter in sub-therapeutic doses at the early stage of production. A high frequency (64%) of resistance to ceftriaxone was reported in China among the chicken *Salmonella* isolates (Cui et al., 2008). In contrast, 97% sensitive to ciprofloxacin has been reported in Kosovo (Hulaj et al., 2016). Wide variation in resistance pattern may be due to various therapeutic practices and implementations of drug administration rules among different countries.

CONCLUSION

The presence of *Salmonella* in marketed live chickens represent the possible transmission of infection from production to consumers which is alarming for food safety issue. From this study, it can be concluded that local chickens are a good carrier of *Salmonella* which not only affects the chickens itself but also has major zoonotic importance. The trends of increasing multi-drug resistance constitute a potential source of transmission of resistant strains to human and pose a problem of public health issue. Therefore awareness should be increased to control the random use of antibiotics that make the organism resistant.

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