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Full Length Research Paper

Drug resistant *Salmonella* in broiler chicken sold at local market in Bangladesh and its public health significance

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***Salmonella* is a globally widespread food-borne pathogen having major impact on public health. Salmonellosis is an infection with bacteria called *Salmonella*. Most persons infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 12 to 72 h after infection. This study was designed to isolate and identify *Salmonella spp*. from cloacal swabs of apparently healthy broiler chickens in Bangladesh. *Salmonella* was characterized culturally, biochemically and also via PCR method. Among 50 isolates, 16 were found to be positive for *Salmonella.* PCR using *16S rRNA* gene primers produced a 496 bp band indicating positive result for *Salmonella spp*. Antibiotic sensitivity test using six commonly used antibiotics in Bangladesh named colistin sulphate, erythromycin, cloxacillin, ciprofloxacin, neomycin and amoxicillin demonstrated that 5 (31.25%) strains were resistant towards ciprofloxacin. Fourteen (87.5%) isolates were resistant to amoxicillin, while fourteen (87.5%) were found intermediate towards neomycin. The study revealed that, healthy broiler chicken sold at local markets of Bangladesh transmit drug resistant *Salmonella* to the environment, therefore, use of antibiotics can be monitored in food producing animals since drug resistance could be a major public health problem in developing countries like Bangladesh.**

Key words: Antibiogram, Salmonellosis, PCR, broiler chicken, drug resistance.

INTRODUCTION

Salmonella is considered the most important food-borne bacterial pathogen in the world (Foley et al., 2011), this is a Gram negative, short rod-shaped, non-spore forming, non-capsulated, facultative anaerobic bacterium (OIE, 2006). The reasonably surveillance for *Salmonella* is to reduce human Salmonellosis of poultry origin across the world (Keery, 2010). *Salmonella* is grouped into three main classes based on their association with human and animal hosts. The first group is specific for human host, the second consists of serotypes which are adapted to

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Abbreviations: MIU, Motility indole urea; **I,** indole; **MR,** methyl red; **VP,** Voges-Proskauer; **MHA,** Mueller-Hinton agar.

specific animal hosts and the third consists of serotypes causing diseases in human and a variety of animal species. *Salmonella* infection causes several clinical conditions those are harmful to human such as: enteric fever, enterocolitis and systemic infections (Piyush and Anju, 2008). The most common source of human Salmonellosis is food of poultry origin since poultry are the most crucial reservoirs of *Salmonella*. Poultry and poultry products are often involved in sporadic cases and in outbreaks of human Salmonellosis (Humphrey, 2000). As *Salmonella* is a pathogen resides in the gut of poultry, there is scope of transmission of *Salmonella* infection from poultry to human through food chain. Cross-contamination happens especially at scalding, defeathering, evisceration and giblet operations (Bryan and Doyle, 1995). The Salmonella spreading with the presence of antimicrobial resistance genes have some global public health impacts, because of their trans-mission to other countries, by travelers or by trade are impossible to prevent (Collard et al., 2007) but differences in hygiene practices and handling between slaughterhouses, could significantly eliminate the risk of *Salmonella* contamination of broiler meat (Heyndrickx et al., 2002). In developed countries it is becoming more and more accepted that a majority of resistant strains are of zoonotic origin and have gained their resistance in an animal host before being moved to humans through the food chain (Molbak et al., 2002; Threlfall et al., 2002). This resistance occurs due to the indiscriminate and improper use of sub-therapeutic doses of antibiotics. Present study was thus designed to characterize *Salmonella spp*. as well as determination of prevalence and antibiogram analysis in apparently healthy chickens sold directly to the consumers. Thus, it is inevitably of great importance for both animal and public health.

MATERIALS AND METHODS

Collection and transportation of samples

A total of 50 samples were collected from cloaca of commercial broiler from local market, at Mymensingh region of Bangladesh during the study period of January to June 2014. The swabs were collected in nutrient broth and brought to Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh. The samples were used to isolate, identify and characterize the salmonella by cultural, biochemical and molecular methods.

Isolation of *Salmonella*

The samples were cultured into SS agar (Figure 2). An amount of 100 µL nutrient broth from test tube were inoculated into SS agar and incubated at 35°C for 24 h. Characteristics salmonella colony were sub cultured into SS agar. Suspected colony was selected and performed gram staining. Isolates with supporting growth characteristics of *Salmonella* were subjected to 3 standard biochemical tests, indole (I), methyl red (MR), Voges-Proskauer (VP). Methyl red (MR) test was performed by inoculating a single colony of the test organism into 0.5 ml sterile glucose phosphate broth. The motility test was performed to differentiate motile bacteria from non-motile one using motility indole urea (MIU) medium. All media and reagents were purchased from (HiMedia, India).

Molecular characterization by PCR

DNA Extraction: Bacterial DNA was extracted by Wizard® Genomic DNA Purification Kit (Promega, USA) and followed the instruction from the manufacturer. All samples were examined by a pair of primers to detect *16s rRNA* gene, Sequence of PCR primer is (5´-3´), upper strand ACTGGCGTTATCCCTTTCTCTGGTG, lower strand- ATGTTGTCCTGCCCCTGGTAAGAGA and the amplicon Size was 496 bp (Noah et al., 1993). A volume of 1 µL of prepared DNA (0.5 µg) was added to a final volume of 25 µL PCR mixture containing 12.5 µL of 2X Master Mix (Promega, USA), 1 µL of 10 pmol/µL each primer and 9.5 µL of sterile distilled water. Thermal cycler was programmed with 30 cycles of 94°C for 30 s, 65°C for 30 s, 72°C for 45 s and an initial denaturation at 94°C for 5 min with final extension at 72°C for 7 min. PCR amplification was performed on a thermo cycler (Eppendorf Personal, Germany). PCR products were separated on 1.5% agarose gel, stained with ethidium bromide and photographed using a Gel documentation system (BioRad, Germany). Salmonella reference strain was used as positive control. Ten microliters of each PCR products were analyzed in agarose (Sigma, USA) gel, stained with ethidium bromide (0.5 µg/ml) and examined under US Solo TS (BioRad, Germany). The positive sample was monitored based on the expected size of band in the gel.

Antimicrobial susceptibility test

In vitro antibiotic sensitivity test was performed according to the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2012). Colistin sulphate (25 µg/disc), erythromycin (15 µg/disc), cloxacillin (5 µg/disc), ciprofloxacin (5 µg/disc), neomycin (30 µg/disc) and amoxicillin (10 µg/disc) were used in this study. Briefly, isolated colonies of the same morphological type were selected and grown in 5 ml of a nutrient broth. Sterile cotton swab was dipped into the broth suspension. The turbidity of broth was adjusted with 0.5 McFarland turbidity standards before swab on MHA. The swab was inoculated into Mueller-Hinton agar (MHA) plate using standard microbiological protocol throughout the agar surface. The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs were placed down individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from centre to centre. The plates were inverted and placed in an incubator set to 37°C within 15 min after the disc were applied.

Reading plates and interpreting results

After incubation each plate is examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies were apparent, the inoculums were too light and the test must be repeated. The diameters of the inhibition of zones were measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted Petri plate. The Petri dishes is held a few inches above a non reflecting background and illuminated with reflected light. The results were recorded at 16 to 18 h post

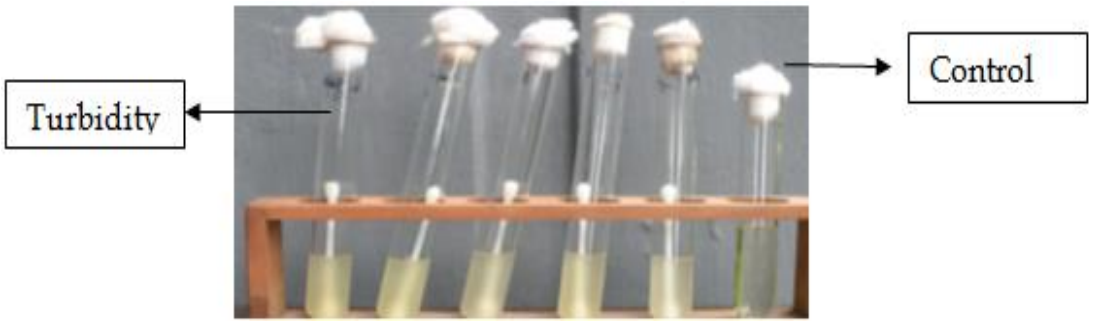

Figure 1. Growth of bacteria in nutrient broth indicated by turbidity.

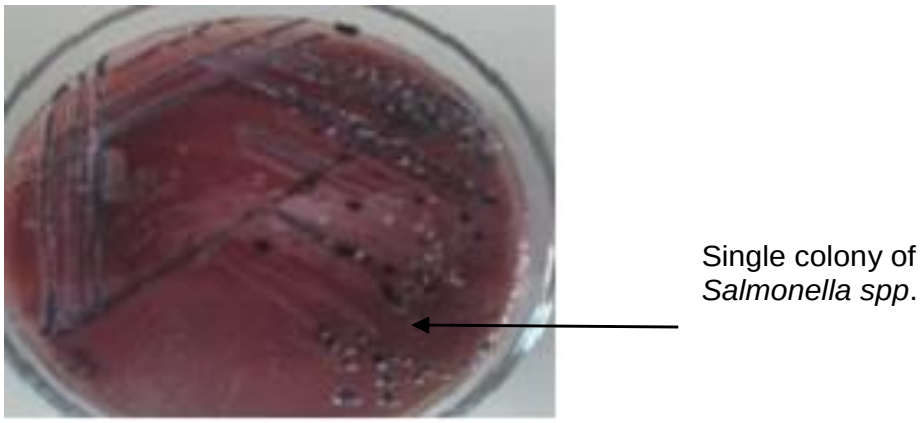

Figure 2. *Salmonella* colony on SS agar.

incubation. Transmitted light is used to examine the zone of inhibition. The zone margins were taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. The sizes of the zones of inhibition were interpreted according to Zone of Diameter Interpretative Standards of CLSI (2012).

RESULTS

Prevalence of salmonella in poultry

A total of 50 samples were collected during the study period. Among them, 16 samples (32%) were positive for salmonella.

Culture in nutrient broth

Nutrient broth was used to collect samples. NB inoculated with the swab samples were incubated for primary culture and revealed the growth of bacteria after 24 h of incubation at 37°C. The proliferation of bacteria was indicated by the presence of turbidity (Figure 1).

Gram's staining technique

The microscopic examination of Gram's stain revealed Gram-negative, pink colored, short rod shaped bacteria, arranged in single and paired (Figure 3).

Results of motility test

Among 16 isolates 10 isolates were found to be non motile characterized by forming the stab line without producing turbidity in the Motility Indole Urea (MIU) medium and another 6 isolates were found motile characterized by changing of colour of MIU medium.

PCR to detect *16S rRNA* gene

Polymerase chain reaction with *16S rRNA* gene detecting primer showed positive band at 496 bp which indicates all

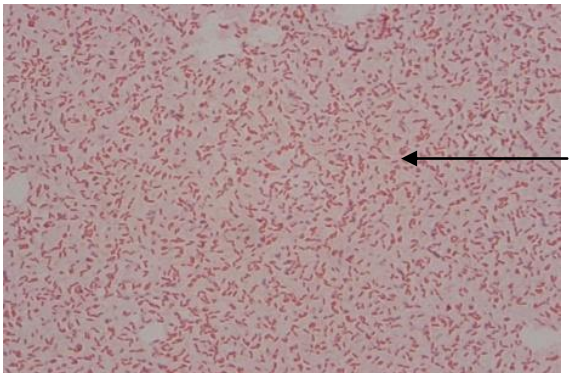

Figure 3. Gram's staining of Salmonella isolates (scale bar 100X10).

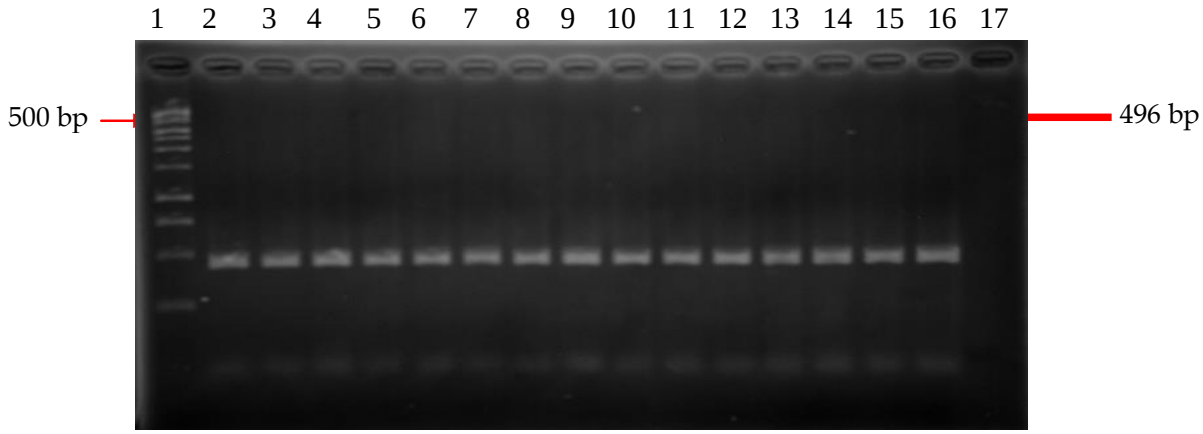

Figure 4. PCR image of Salmonella, Lane 1: 1 kb DNA ladder (Promega, USA), Lane 2-15: Isolated sample of *Salmonella*, lane 16: Positive control, Lane 17: Negative control.

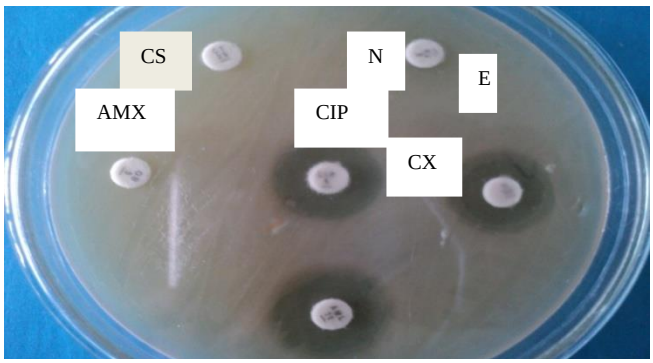

Figure 5. Antimicrobial susceptibility test for a representative Salmonella strain. Legends: E= erythromycin, CIP= ciprofloxacin, CS= colistine sulphate, N= neomycin, AMX= amoxicillin, CX= cloxacilin.

(Figure 4) isolates was found to be positive for Salmonella spp.

Antibiotic sensitivity test

Antibiogram using six drugs revealed 5(31.25%) strains to be resistant and 5(31.25%) intermediate towards Ciprofloxacin. Fourteen (87.5%) isolates were resistant to Amoxicillin, while fourteen (87.5%) were found intermediate resistance towards Neomycin. However, none of the isolates were sensitive to Cloxacilin and Erythromycin, two (12.50%) were intermediate and 14 (87.50%) were resistant. In case of Colistine sulphate, 8(50%) isolates were resistant while the remaining 8 (50%) were intermediate (Figure 5).

DISCUSSION

This research work was conducted to isolate and characterize *Salmonella spp*. of apparently healthy broiler chickens from different areas around Bangladesh Agricultural University Campus, Mymensingh, Bangladesh.

The isolates were confirmed as *Salmonella* by cultural staining, motility, biochemical and PCR. Finally, antibiotic sensitivity and resistance patterns of the isolates of broiler chickens identified in this study. Among 50 isolates, 16 isolates were positive for *Salmonella.* All isolates were cultured in S-S agar and produce single colony of *Salmonella spp*. Then, isolates were inoculated in EMV agar and give pink color colony which helps to distinguish the positive isolates. Meanwhile, *Salmonella spp.* produce pink color with black center after inoculating the isolates into XLD agar medium. The microscopic examination of Gram's stain revealed Gram-negative, pink colored, short rod shaped bacteria, arranged in single and paired (Freeman, 1985; Jones et al., 1997; Gene, 2002). All of the isolates were positive for Methyl Red test. Among 16 isolates 10 isolates were found to be non motile characterized by forming the stab line without producing turbidity in the Motility Indole Urea (MIU) medium and another 6 isolates were found motile characterized by changing of colour of MIU medium. In motility test, motile isolates showed swinging movement which correlated with the results of Buxton and Fraser (1977) and Merchant and Packer (1967). PCR with 16sRNA gene detecting primer produce 496 bp band directs the positive result for *Salmonella spp*. Six drugs were used for antibiogram study. These six drugs were Colistine sulphate, Erythromycine, Cloxaciline, Ciprofloxacin, Neomycin and Amoxyciline. Antibiogram analysis revealed 5(31.25%) strains to be resistant towards Ciprofloxacin. Fourteen (87.5%) isolates were resistant to Amoxicillin, while fourteen (87.5%) were found intermediate resistance towards Neomycin. However, none of the isolates were sensitive to Cloxacilin and Erythromycin, two (12.50%) were intermediate and 14 (87.50%) were resistant. These results were more or less similar to other researchers (Haque, 2011; Rayamajhi et al., 2010; Dallal et al., 2009). In case of Colistine sulphate, 8(50%) isolates were resistant while the remaining 8 (50%) were intermediate. The results directed that broiler chickens play important role as reservoirs of multi-drug resistant *Salmonella*.

Infection by *Salmonella* is a common cause of food poisoning in humans (Hobbs and Robert, 1993). Human might get *Salmonella* infection easily during processing of poultry carcass and close contact with poultry in live bird markets and farms. Therefore, it is of great public health significance to study the prevalence of *Salmonella* in the apparently healthy broiler chickens available in the live bird markets and farms to assess and eliminate the risk originated from those. Thereby, this

research was aimed to create public consciousness revealing the risks of *Salmonella* infection with the healthy broiler chickens. Data of this study suggests that, broiler chickens at farms and live bird markets are the major reservoirs of *Salmonella spp*. that might be potential cause of food poisoning if proper hygienic measure is not undertaken during rearing, handling and processing of poultry and

poultry by products.

Conclusion

The research work was undertaken with a view to isolating *Salmonella spp.* from cloacal samples of broiler chickens. Results of the study direct that isolates from broilers are being multi-drug resistant which may be due to indiscriminate and continuous use of sub therapeutic doses of antibiotics during rearing in commercial production system. Therefore, more sensible use of antibiotics can be strongly suggested for the veterinarians since drug resistance could be a major public health. These findings would certainly help the veterinarians to select the correct antibiotics against *Salmonella* infections. However, fluoroquinolones are important antimicrobial compounds in the treatment of Salmonellosis in humans. Overall, the prevalence of *Salmonella* in broiler chickens and their drug resistance is obviously a threat to public health. Moreover, the disease caused by *Salmonella* has a great public health importance. Therefore, broiler sector should be provided with immediate attention by the government to maintain strict hygienic measurement in farm and live bird markets all over the country. This study will be helpful in selecting appropriate antibiotics against *Salmonella* infection.

Conflict of interests

The author(s) did not declare any conflict of interest.

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