Bioresearch Communications
Volume 04, Issue 02, July 2018
Journal Homepage: www.bioresearchcommunications.com

Original Article

Occurrence of Pathogenic and Multidrug Resistant *Salmonella* spp. in Poultry Slaughter-House in Bangladesh

Samina Momtaz[1], Otun Saha[1], Masuma Khatun Usha[1], Munawar Sultana[1], M. Anwar Hossain[1, 2]*

[1]*Department of Microbiology, University of Dhaka, Dhaka, Bangladesh,*
[2]*Vice-Chancellor, Jessore Science and Technology University.*

ABSTRACT: *Salmonella* is a diverse food-borne and zoonotic pathogen. The route of transmission is cardinal for surveillance and epidemiological investigations. The present study was conducted to characterize circulatory genotypes and pathogenicity of MDR *Salmonella* spp. from poultry slaughter house. Three swab samples from slaughterer's hand and poultry residual container were collected from a slaughter-house of Dhaka city. Of these, 7 isolates were confirmed as *Salmonella* spp. by phenotypic and molecular analysis. All seven isolates showed positive result for *inv*A genes. Virulent genes *(stn* and *sop*B)specific PCRpresented13.3% and 6.66% positive isolates, respectively. Genotyping using Random Amplified Polymorphic DNA (RAPD) analysis exhibited six genotypes. Phylogenetic analysis and sequencing from each genotype exhibited close similarity to *Salmonella enterica*, *S.* Anatum and *S. bongori* from the slaughter hand and container swab. Antibiogram profiling presented 100% resistance to ampicillin and 83.33% to imipenem. This study recommends that dispersion of MDR and pathogenic *Salmonella* spp. from poultry slaughter house may pose threat to public health if goes unchecked.

Key words: *Salmonella,* Slaughter-House, RAPD, Virulence, Antibiogram

Article History
Received: 04 March 2018
Accepted: 07 May 2018

Scan the QR code to see the online version or,visit- www.bioresearchcommunications.com

Corresponding author
Mohammed Anwar Hossain, PhD, Professor, Department of Microbiology, University of Dhaka, Dhaka, Bangladesh.
Mobile: 01715-363753,
E-mail: hossaina@du.ac.bd

Citation: Momtaz S, Hossain MA Occurrence of Pathogenic and Multidrug Resistant *Salmonella* spp: Biores Comm. V4-(2) 506-515.

INTRODUCTION

Salmonellosis, caused by *Salmonella* spp., is a matter of public health concern as it is the leading cause of food-borne illness in humans and animals resulting in 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths and great public health concerns of the modern world[1, 2].Sources of *Salmonella* infections include foods of animal origin, dairy products, pet food, fresh produce, and foods contaminated during processing[3]. The genus *Salmonella*, which possesses various virulence genes like *inv*A, *sef* and *pef* are considered for adhesion and invasion of the pathogen in the host system and *spv* gene for systemic disease state in the host cells whereas *stn* virulence gene codes for enterotoxin production and *sop* and *pip* genes are associated with actual expressions of host pathogenic processes[4, 5] which causes disease in most warm-blooded animals. Therefore, *Salmonella* spp. harboring those virulence genes are thus of concern for both the economic production of food from animals and the possible transmission to human consumers, resulting in enteritis. Hypothetically, there are numerous possible ways for the transmission of this organism to human, but slaughterhouse might be the prime one where animals are slaughtered, and the meat are sold, therefore can contaminate the environment, and act as a vehicle for transmission of *Salmonella* species to human[6,7].

Meat contact surfaces in the abattoir such as equipment's (e.g. Meat holding drums, knives, and axes), walls and floors may serve as sources of transmitting infectious agents and are therefore considered important factors in the evaluation of the

level of contamination in such establishment[8]. Contamination occurs during slaughtering, handling, cutting, processing, and storage[9]. Without having practical knowledge, large amount of various antibiotics is used in the poultry sectors that's contributes the antibiotic or multidrug resistance (MDR) in bacteria of animal origin and has gained particular attention[10].Scarcity of information regarding MDR and pathogenic *Salmonella* spp. from the poultry meat processing unit or slaughterhouse in Bangladesh led this study to focus on the prevalence of MDR and pathogenic *Salmonella* spp. isolated from poultry slaughter house.

MATERIALS AND METHODS

Collection of Sample

Three swab samples from poultry residual container side, bottom and slaughterer hand were collected by using cotton swab from the slaughterhouse situated at BUET Market (Location: 23.7270035, 90.3886437) in Dhaka, Bangladesh. The samples were immediately transferred into buffer peptone water and were transported to the laboratory.

Total Heterotrophic Count

Samples were diluted in normal saline by ten-fold dilutions, up-to 10^{-4} and from each dilution 100µl was inoculated on nutrient agar media by spread plate technique followed by overnight incubation at 37˚C.

Enrichment and Isolation of *Salmonella* spp.

For pre-enrichment, collected cotton-swab was inoculated in Rappaport-Vassiliadis (RV) broth and selenite broth and incubated at 37°C for 24 hours[11]. Subsequently, samples were inoculated on Xylose Lysine Deoxycholate (XLD) agar (Oxoid, UK), *Salmonella- Shigella* (SS) agar (Oxoid, UK), and MacConkey agar (Oxoid, UK) plates and incubated at 37°C for 24 hours. Colonies resemble to *Salmonella* spp. were selected for further biochemical tests including indole, methyl red, Voges-Proskauer, citrate (IMViC), triple sugar iron (TSI), Oxidase, Catalase and urease tests as per the protocol[12,13].Colonies showing *Salmonella* specific IMViC (-, +,-, +) pattern were further inoculated on TSI slants. Later, colonies producing alkaline slant (pink color) and acidic butt (yellow color) with or without H_2S production were tested for urease production on urea broth. All biochemical results were summarized using online tool Microrao (http://www.microrao.com/index.html) and Bio Cluster which is used to predict the presumptive organisms on the basis of biochemical results[14].

Preparation of template DNA

Bacterial colonies were freshly grown in nutrient agar plates, suspended in 150µl of sterile distilled water in a micro centrifuge tube, gently vortexed and boiled for 10 min in a water bath followed by centrifugation (10,000 rpm for 5min at 4˚C). Supernatant were carefully collected and used as a source of template DNA. The concentration of template DNA was obtained as ng/µL and the purity was expressed in terms of the ratio of absorbance at 260 nm and 280 nm[15].

Screening of Virulence genes by Gene Specific PCR

Presumptively identified *Salmonella* sp. were screened for the virulence genes like invasion gene (*inv*A), *Salmonella* enterotoxin gene (*stn*) and *Salmonella* host pathogenic processes expression associated gene *sop*B by PCR. Template DNA was prepared by boiling method[15]. The primers used were *inv*A (284bp) [Forward: 5′-GTGAAATTATCGCCACGTTCGGGCAA-3′,Reverse: 5′-TCATCGCACCGTCAAAGGAACC-3′]; *stn* (260bp) [Reverse: 5′-TGCCCAAAGCAGAGAGATTC-3′, Forward: 5′-CTTTGGTCGTAAAATAAGGCG-3′]; *sop*B (1378bp) [Forward: 5′-CAACCGTTCTGGGTAAACAAGAC-3′, Reverse: 5′-AGGATTGAGCTCCTCTGGCGAT-3′][16,17,18]. The reaction was set as initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 64°C for 1 min, extension at 72°C for 1 min and final extension was done at 72°C for 7 min[16].Subsequently, the amplified PCR product was visualized by agarose gel-electrophoresis (1.5% agarose gel).

Molecular Fingerprinting by Random Amplified Polymorphic DNA (RAPD)

Acquired isolates were further grouped by using more confirmatory molecular techniques such as Random Amplified Polymorphic DNA (RAPD).For RAPD genotyping, primers sequence (5'- GCGATCCCCA-3') namely 1283 was tested for their ability to discriminate between species of *Salmonella* and for their reproducibility, reaction conditions included initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 1 minute at 94°C, 1 min at 39°C, and 2 minutes at 72°C. Thirty-five cycles of these segments were repeated with a final extension of 7 minutes at 72°C[19, 20]. After amplification, 10 µl of PCR product was subjected to agarose gel electrophoresis for 120 minutes. Each gel contained both 100bp and 1Kb DNA marker (Bioneer, USA) as molecular weight standards. Subsequently, products were visualized by using Gel Documentation System (AlphaImager HP System Versatile Gel Imaging, USA). UPGMA clustering method using PyElph software 1.4. were used subsequently to delineate the groups.

Phylogenetic analysisbased on 16S rRNA gene Sequence

The nucleotide sequences of 16S rRNA along with reference sequences were aligned and a neighbor-joining analysis was used to reconstruct a phylogenetic tree by using the software named MEGA7[21,22,23].The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches[24].The evolutionary distances were computed using the

Kimura 2-parameter method and is in the units of the number of base substitutions per site[25]. To boost the phylogenetic analysis result, hierarchy analysis of the isolated strains were observed along with by using an online tool called RDP (http://rdp.cme.msu.edu/) which predicts the strain phylum, class, order, family and genus using 16S rRNA gene sequence[26].

Antimicrobial Susceptibility Test

After confirmation of isolates as *Salmonella* spp., antimicrobial susceptibility of all isolates was determined by the micro disc diffusion method using Mueller Hinton agar (Oxoid, UK), according to guidelines established by Clinical and Laboratory Standards Institute[27]. Antibiotics were selected for susceptibility testing corresponding to a panel of antimicrobial agents of interest to the both poultry industry and public health in Bangladesh. A panel of nine antibiotics discs were used for this study: (Ampicillin (10µg); Doxycycline (30µg); Nitrofurantoin (300µg); Imipenem (10µg); Tetracycline (30µg); Meropenem(10µg);Nalidixic Acid (30µg); Colistin-Sulphate(10µg); Gentamycin (10µg).

RESULTS

The present study focused on isolation of multidrug resistant and pathogenic *Salmonella* spp. from poultry waste container and slaughterer hand swab from a representative market at Dhaka city, Bangladesh.

Quantification of Total Heterotrophic Bacteria

To obtain an account of total bacterial population in samples, total heterotrophic plate count was done. The total heterotrophic plate count from Container Bottom, Container Side and Handler samples was 1.575×10^6 (CFU)/ml, 2.84×10^7 CFU/ml and8.1×10^5CFU/ml, respectively.

Isolation and Presumptive Identification of *Salmonella* spp.

From three swab samples, initially 30 isolates with characteristic *Salmonella* like colonies were detected positive based on the colony characteristic on XLD, *SS* and MacConkey agar. For presumptive identification of *Salmonella* spp., a panel of biochemical test (IMViC, TSI, and Urease) was performed. Out of 30 isolates, after completing all biochemical tests, 7 isolates (23.33%) were presumptively identified as *Salmonella* spp. All biochemical results were summarized using online tool Microrao (http://www.microrao.com/index.html) and BioClusterto corroborate the presumptive identification (Table1).

Table 1: Summarization of biochemical results along with Microrao tool for presumptive identification of isolates

SL No.	Biochemical test	Results	Likelihood of the isolates by Microrao tool*(%-Confidence level)
1.	Indole test	Negative	*Salmonella typhi* (97.81%) *Salmonella choleraesuis* (1.85%)
2.	Methyl Red test	Positive	
3.	Voges Proskauer test	Negative	
4.	Citrate utilization test	Negative	
5.	Hydrogen sulfide test	Variable, Positive	
6.	Urea hydrolysis test	Negative	
7.	Gas from glucose	Negative	
8.	Sucrose fermentation test	Negative	

*http://www.microrao.com/index.html and BioCluster

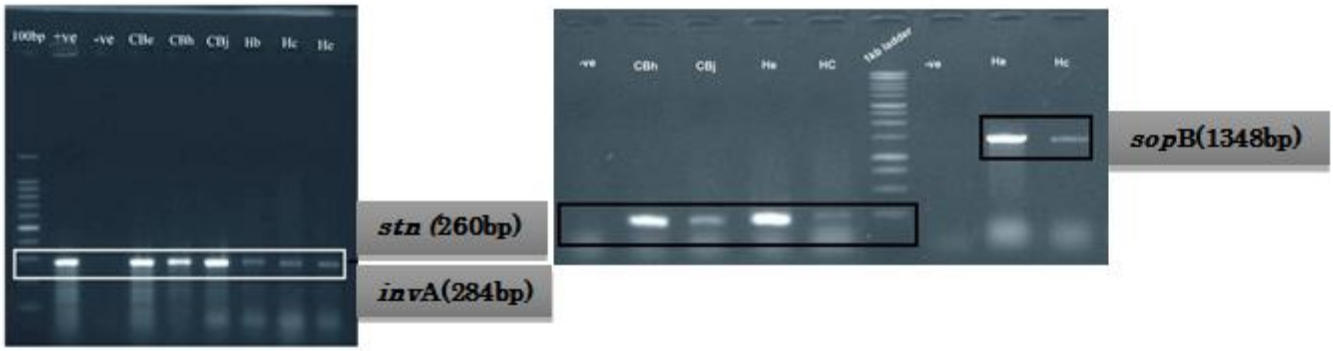
It has been observed that 50 % of isolated *Salmonella* spp. were present in the slaughterer's hand (H) and 25% of contamination was found in waste container bottom (CB).Besides,waste container side (CS) samples were free of *Salmonella* species (Table 2).

Table 2: Incidence of *Salmonella* contamination of the examined poultry slaughterhouse sample (slaughterer hand, poultry residual container bottom and side swab samples)

Sl No.	Isolated strain	Sample source	RAPD group	Presence of virulence gene	Antibiotics resistant profile
1.	*S. enterica*	Slaughterer Hand	I	*invA*$^{+}$, *stn*$^{+}$, *sopB*$^{+}$	Amp,Im,DO, NA, F, T, CN
2.	*S.*Anatum	Waste Container Bottom	II	*invA*$^{+}$ *stn*$^{+}$*sopB*$^{-}$	Amp, NA, CN
3.	*S. bongori*	Waste Container Bottom	III	*invA*$^{+}$ *stn*$^{-}$*sopB*$^{-}$	Amp, Im, DO, NA, T
4.	*S. bongori*	Slaughterer Hand	IV	*invA*$^{+}$ *stn*$^{-}$*sopB*$^{-}$	Amp, Im, CN
5.	*S. enterica*	Waste Container Bottom	V	*invA*+ *stn*$^{+}$*sopB*$^{-}$	Amp, Im, DO, NA, F
6	*S. enterica*	Slaughterer Hand	VI	*invA*$^{+}$ *stn*$^{+}$ *sopB*$^{+}$	Amp, Im, DO, F, T

+ve (Presence of Genes); -ve (Absence of Genes)
Amp= Ampicillin; DO= Doxycycline; F =Nitrofurantoin; Im = Imipenem; CN= Gentamycin; T=Tetracycline; and NA= Nalidixic Acid

Detection of Virulence Genes
All seven isolates presented positive result for i*nvA* (284 bp)gene (Supplementary Figure 1).



A. invA gene fragment of 284bp ***B. Amplified product of stn (260 bp) and sopB (1348 bp) gene fragment***

Supplementary Figure 1: Agarose gel electrophoresis of PCR amplified product of A) *invA* gene (284 bp) for identification and characterization of *Salmonella* species; and B) *stn(260bp)and sopB* gene (1348bp) for detection of pathogenicity *pattern*.

Out of seven isolates, 4 isolates were found positive for the *stn* gene (260bp) and 2isolates exhibited positive result for *sop*B (1348bp) gene (Table2). Additionally, it has been observed that isolates from slaughterer hand contains all three genes (*inv*A, *stn* and *sop*B).

Analysis of genetic diversity of *Salmonella* spp. by RAPD

To investigate the discriminatory power of the fingerprint techniques used in the present study, *inv*A gene positive seven *Salmonella* isolates were subsequently analyzed by RAPD method. Findings of RAPD method presented six distinct genotypes (Figure 1 and Table 2).

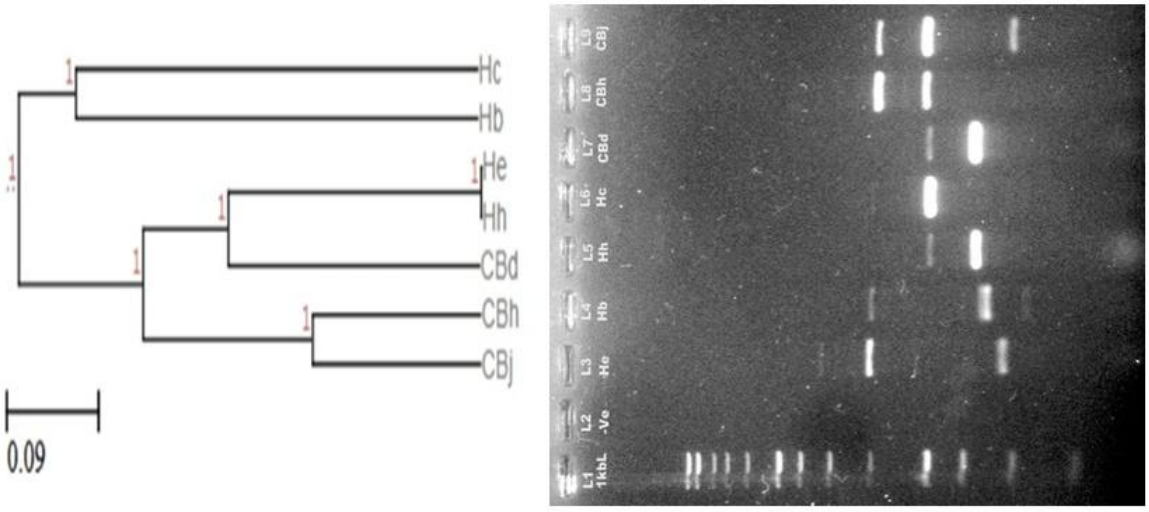


Figure 1: RAPD typing accordance with band differences based on UPGMA clustering method using PyElph software 1.4. Lane 1 contains 1Kb ladder as molecular size DNA marker. Lane 2 contains negative control, Lanes 3, Lane 4, Lane 5, Lane 6, Lane 7, Lane 8and 9 are Positive *Salmonella* spp.

Phylogenetic Determination of *Salmonella* spp.

Representative isolates from each RAPD were selected for 16S rRNA nucleotide sequencing and aligned for phylogenetic analysis. Alignment of the 16S rRNA nucleotide sequence along with reference sequences was performed by the MEGA7 software for constructing phylogenetic tree (Figure 2).

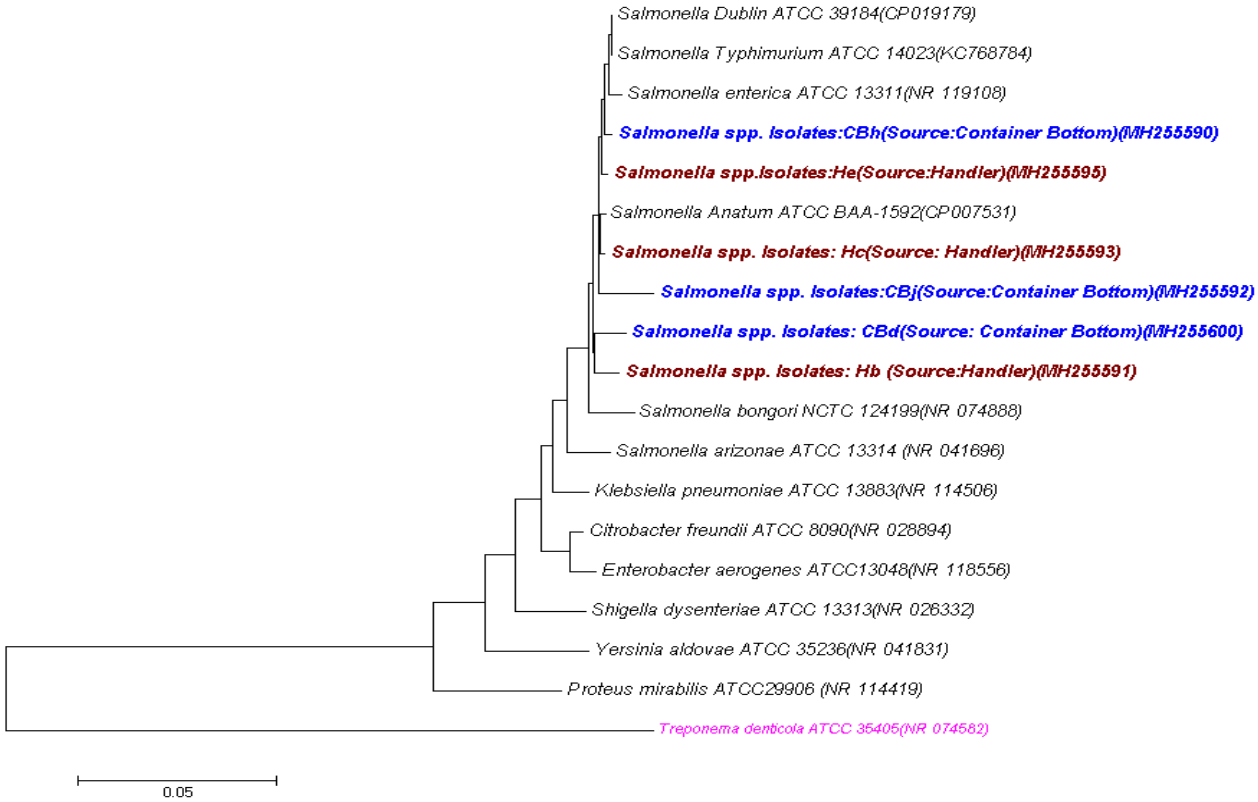


Figure 2: Phylogenetic tree predicted by the neighbor-joining method using 16S rRNA nucleotide sequences

Findings of phylogenetic analysis with RAPD analysis indicated that, RAPD group I harbored *Salmonella enterica*, group II presented resemblance with *S.* Anatum and group III showed *S. bongori* from handler swab; andgroup IV was *S. bongori* andgroup V and VI owns *S. enterica* sampled from container bottom(Table 2).We also found that *S. enterica* contains from slaughterer hand (RAPD I) belongs all three virulence genes (*inv*A, *stn and sop*B) while *S.* Anatum contains *inv*A and *stn* genes. *S. bongori* was found solely with *inv*A gene.

Antibiotic Resistance Profile of Isolated *Salmonella* spp.

Among eleven antibiotics tested against isolated *Salmonella* spp., 100% resistance were observed against ampicillin followed by Imipenem (83.33%), Doxycycline (66.66%), Nalidixic Acid (66.66%), Nitrofurantoin (50%), Tetracycline (50%) and Gentamicin (16.66%). No resistance was noticed against Colistin-Sulphate and Meropenem (Figure 3).

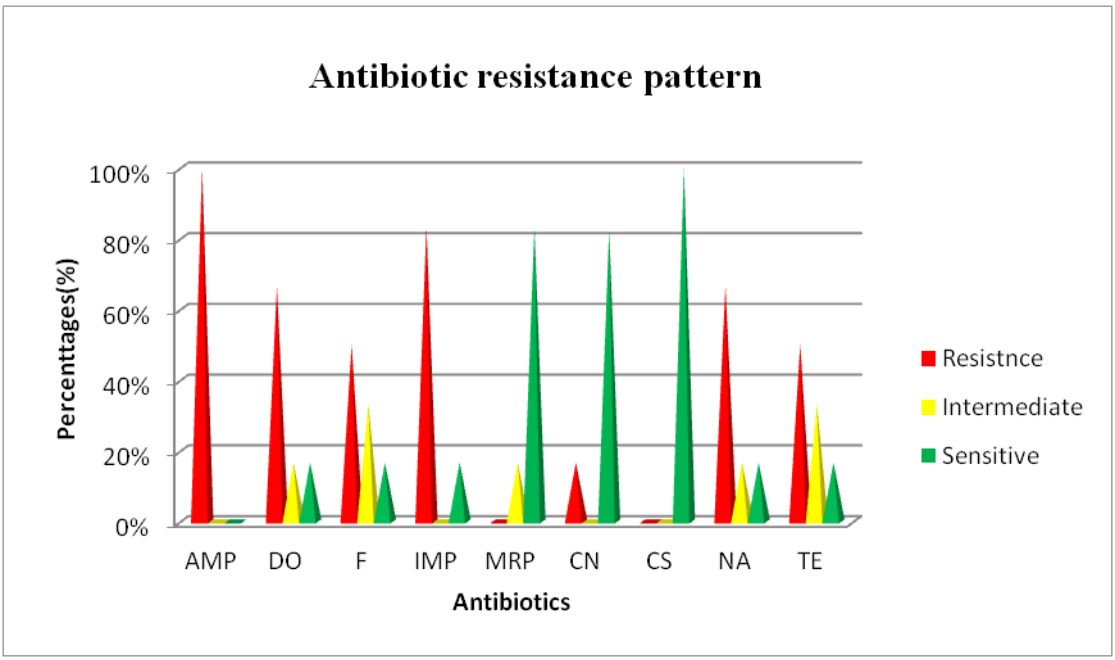


Figure 3: Diagrammatic representation of antibiotic resistance pattern of the isolates: AMP= Ampicillin (10µg); DO= Doxycycline (30µg); F =Nitrofurantoin (300 µg); IMP = Imipenem (10 µg); CS= Colistin Sulphat(10 µg); CN= Gentamicin (10 µg); MRP= Meropenem(10 µg); Tetracycline (30µg); and NA(30µg) = Nalidixic Acid.

Out of the all tested isolates, *S. enterica* from RAPD group-I, V and VI presented 50% Ampicillin resistant, 50% to Doxycycline, 50% to Imipenem, 50% to Nitrofurantoin, 33.33% to Nalidixic Acid, 33.33% to Tetracycline, 16.67% to Gentamycin. Similarly, *S. bongori*from RAPD group III and IV exhibited highest resistance against ampicillin (50%) and imipenem (33.33%). *S.*Anatum from RAPD group-II exhibited resistance against ampicillin (33.33%) and Nalidixic Acid (16.67%)(Table 2).

DISCUSSION

Poultry slaughter house systems are frequently contaminated with microorganisms leading to frequent infections leading to spread out of these infections. We have employed bacteriological and molecular methods for this study. This study focuses on to determine the occurrence of *Salmonella* spp. in slaughterhouse samples along with estimating the virulence and prevalence of antimicrobial resistance against *Salmonella* spp.

In this study, overall prevalence of *Salmonella* spp. was observed 23.33% which is similar to that of previous studies reported by Gunasegaran *et al*.,2011and Naik *et al.*, 2015 but lower than that in previous reports: 36.0% and 42.3% in Korea, 72% in Thailand[28,29,30,31]. We speculated that the difference of *Salmonella* prevalence between reports might be associated with difference in hygiene and sanitation levels of each country and the different detection methods used in each study. Moreover, significantly higher prevalence (50%) of *Salmonella* was occurred in slaughterer's hand. But no *Salmonella* was found in waste container side (CS).This is due to the various reasons behind it. Firstly, when slaughterer slaughter the chicken, they readily throw it to the container bottom. This chicken may or may not touch the side. But there have some possibilities of serving as a source of Salmonella of these samples because it contains some material like blood or stools of chicken during processing. Due to the smooth surface of the container side (mostly plastic container) and gravitational force, it is tough to trap sample

containing *Salmonella* in the container side. Besides, during sampling we observed that slaughterer frequently spray water to the container which may cause to wash out *Salmonella* from the container side and mostly deposit on the container bottom. Further, all *Salmonella* isolates had taken into consideration to detect the presence of three virulence genes *inv*A, *stn* and *sop*B.Of these, *inv*A gene has been widely recommended for the detection of *Salmonella* spp.[32]. All 7 isolates presenting positive results for *inv*A gene(100%) substantiated the presumptive identification of *Salmonella* spp. by biochemical test. Our finding was corroborated by Moussa *et al.* (2010) who suggested that all *Salmonella* serovars were positive for *inv*A genes of 284bp fragment[33]. In this investigation, four of the seven *inv*A positive isolates exhibited positive outcome for *stn* gene (13.3%) and two of them showed positive result for *sop*B gene (6.66%).Previously, Ammar *et al.*, 2016, remarked *Salmonella* isolates as virulent which showed positive result for *inv*A, *stn* and *sop*B[34]. Similar results related high prevalence of some virulence genes in strains isolated from North America and Africa was reported by Dione *et al*., 2011[35]; Shah *et al.*, 2011[36]; Zou *et al*., 2012[37].All those preceding report clearly depict that isolates under this investigation harbors virulence gene and are potential pathogen.

Subsequently, molecular typing methods were employed to identify infections aiming to prevent disease and for the epidemiological study of salmonellosis. RAPD was done to differentiate genotypes of *Salmonella* isolates which is easy to use and has discriminatory power to fingerprint bacteria involved in disease outbreaks and to determine the sources, vectors and vehicles of transmission[38,39, and 40]. RAPD typing with 1283 primer showed sixdistinguishing genotypes clearly indicating that primer for RAPD method was able to generate polymorphism.

In our investigation, representative isolate from each RAPD group belonging *inv*A 284bp fragment were later phylogenetically identified as *Salmonella enterica (*RAPD group I and VI*), S.* Anatum (group II) and *S. bongori (*group III and V*)* and supported PCR result. A study of Lamas *et al.* (2016)presented that there is a high incidence of *Salmonella enterica* in broiler flocks (64.18%)and is responsible for foodborne outbreaks and salmonellosis in humans[38, 41]. We also detected the occurrence of *Salmonella enteric* from both poultry slaughterer hand and container bottom which clearly possesses a high risk of zoonosis. According to Fookes *et al.*, 2011, *S. bongori* is predominantly associated with cold-blooded animals, but it can infect humans[42]. Our work found *S. bongori* from handler swab which can in turn infect human. Moreover, a study carried out by Rodríguez *et al.*, (2006) found a high prevalence (28.36%) of *S.* Anatum in poultry farms belonging to the subspecies *arizonae*, which is typically associated with cold-blooded animals[41, 43]. Although some *S. arizonae* infections were reported in immunocompromised patients, therefore, the prevalence of this subspecies cannot be underestimated[44].

Clinical and Laboratory Standards Institute (CLSI) recommends some classes of drugs to treat *Salmonella* infections which has been taken into consideration into this study[45.] Nalidixic acid, a quinolone that targets DNA gyrase, has been prominently used in human and veterinary treatments[46]. In thisinvestigation, the antibiogram profile exhibited that 100% resistance to ampicillin, and 66.66% resistance to nalidixic acid followed by 83.33%, 66.66%, 50%, 50% and 16.67% against imipenem, doxycycline, nitrofurantoin, tetracycline andGentamicin in consistent with previous reports[30, 47]. No resistance was noticed against Colistin-Sulphate and Meropenem. Similar resistance profiles (100% resistant against amoxicillin and tetracycline, 87% to Ciprofloxacin, and 50% to Doxycycline) were found in strains isolated in Bangladesh[48]. In accordance with the previous study, antibiogram profiling imparts that isolated *Salmonella* spp. possess resistant four or multiple antimicrobial agents. In addition, resistance to the nalidixic acid indicates an increase in the use of quinolones to treat *Salmonella* infections in poultry sectors in Bangladesh.

Our data demonstrate that the prevalence of MDR *Salmonella* isolated from slaughter-House in Bangladesh is high.Prevalence of *Salmonella* spp. and multi drug resistance (MDR) patterns of isolates differ depending on the place of processing, even that process in the same region. Therefore, to effectively reduce *Salmonella* contamination of poultry, the surveillance and intervention strategy must be implemented. Findings of this study suggest that a national surveillance program should be implicated for monitoring the dissemination of multidrug resistant (MDR) pattern of food-borne bacterial pathogens.

CONCLUSIONS

In conclusion, the prevalence of *Salmonella*, reported in this study, was not so high, but the presence of multidrug resistant and potentially pathogenic *Salmonella* cannot be ruled out which can be a matter of concern from public health point of view. Further, there is a need of conducting such study in this region on a regular basis to assess and compare the past and present status of this zoonotic pathogen to secure animal and public health.

Funding Information

This work was supported by the grant from University Grants Commission and Ministry of Science and Technology, Government of People's Republic of Bangladesh. Partial reagent

was supported by grant from Biotechnology Research Centre, University of Dhaka.

Conflict of Interest: The authors declare that they have no conflict of interests.

ACKNOWLEDGEMENT

We thank Syeda Roufun Nesa for her assistance in RAPD gel images analysis.

REFERENCES

1. Bahadur, D. L., Prasad, D. I., Kumar, Y. S., Md, A., and Zohorul, I. M. Prevalence and antibiotic resistance profile of *Salmonella* from livestock and poultry raw meat, Nepal. *International Journal of Molecular Veterinary Research*,2016.6: p.1-23.
2. Abd-Elghany, S. M., Sallam, K. I., Abd-Elkhalek, A., and Tamura, T. Occurrence, genetic characterization and antimicrobial resistance of *Salmonella* isolated from chicken meat and giblets. *Epidemiology & Infection*,2015. 143(5): p. 997-1003.
3. Kasturi, K. N. and Drgon, T. Real-time PCR method for detection of Salmonella spp. in environmental samples. *Appl Environ Microbiol*,2017.83:e00644-17.https://doi.org/10 .1128/AEM.00644-17.
4. Lostroh, C. P. and Lee, C. A. The HilA box and sequences outside it determine the magnitude of HilA-dependent activation of P-*prgH* from *Salmonella* Pathogenicity Island 1. *J. Bacteriol*, 2001.183: p. 4876-4885.
5. Murugkar, H. V., Rahman, H., and Dutta, P. K. Distribution of virulence genes in Salmonella serovars isolated from man & animals. *Indian Journal of Medical Research*, 2003.117: p. 66.
6. Doyle, M. P. and Cliver, D. O. *Salmonella*. In: Food Borne Diseases, Academic Press Inc, San Diego, 1990.p. 185- 204.
7. Griffin, P. M., and Tauxe, R. V. The epidemiology of infections caused by *Escherichia coli* O157: H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiologic reviews*, 1991. 13 (1):p. 60-98.
8. Bello, M., and Son, K. N. Assessment of microbial load from meat contact surfaces and isolation of enteropathogenic *Escherichia coli* at a meat processing plant, Russia. *Nigerian Veterinary Journal*, 2009.30(2):p. 1-8.
9. Okolocha, E.C, Umoh, J.U, and Raji, K.B. The environmental and microbial food contamination: A public Health perspective. In: proceedings of the First National Conference of Environment health society of Nigeria. Zaria, Nigeria, 2002.p.381-387.
10. Schwarz, S., Silley, P., Simjee, S., Woodford, N., van Duijkeren, E., Johnson, A. P., and Gaastra, W. Assessing the antimicrobial susceptibility of bacteria obtained from animals. *Journal of antimicrobial chemotherapy*,2010.65(4): p. 601-604.
11. Rybolt, M. L., Wills, R. W., and Bailey, R. H. Use of secondary enrichment for isolation of Salmonella from naturally contaminated environmental samples. *Poultry science*., 2005.84(7): p. 992-997.
12. Buchanan, R. E. and Gibbons, and N. E., eds. Bergey's Manual of Determinative Bacteriology. 8th ed. Williams & Wilkins Co., Baltimore, Md, 1974.p.21202. Xxvi + 1246.
13. Ewing, W.H. Edwards and Ewing's Identification of *Enterobacteriacea*e. 4th ed. Elsevier Science Publishing Co., Inc., New York.1986.
14. Abdullah, A., Alam, S. S., Sultana, M., and Hossain, M. A. BioCluster: tool for identification and clustering of *Enterobacteriaceae* based on biochemical data. Genomics, proteomics & bioinformatics. 2015.13(3):p. 192-199.
15. Nagappa, K., Tamuly, S., Brajmadhuri, Saxena, M.K. and Singh, S.P. Isolation of *Salmonella typhimurium* from poultry eggs and meat of Tarai region of Uttaranchal. *Indian J. Biotechnol*,2007.6(3): p. 407-409.
16. Rahn, K., De Grandis, S.A., Clarke, R.C., McEwen, S.A., Galan, J.E., Ginocchio, C., Curtiss, R. and Gyles, C.L. Amplification of an *inv*A gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. Mol. Cell. Probes,1992.6 (4): p. 271-279.
17. Makino, S., Kurazono, H., Chongsanguam,M., Hayashi, H., Cheun, H., Suzuki, S. and Shirahata, T. Establishment of the PCR system specific to *Salmonella* spp. and its application for the inspection of food and fecal samples. *J. Vet. Med. Sci*,1999. 61(11):p. 1245-1247.
18. Prager, R., Mirold, S., Tietze, E., Strutz, U., Knüppel, B., Rabsch, W., Hardt, W.D. and Tschäpe, H. Prevalence and polymorphism of genes encoding translocated effector proteins among clinical isolates of *Salmonella enterica*. Int. J. Med. Microbiol.1992.290 (7): p. 605-617.

19. Akopyanz, N., Bukanov, N. O., Westblom, T. U., Kresovich, S., and Berg, D. E. DNA diversity among clinical isolates of Helicobacter pylori detected by PCR-based RAPD fingerprinting. *Nucleic Acids Research*,1992.20(19): p. 5137-5142.
20. Demetrius L. M., Roy D. B., Margie D. L., and John J. M. Variation in *Salmonella Enteritidis* RAPD-PCR Patterns may not be due to genetic differences. *Avian Diseases*,2011.55: p. 620–625.
21. Kumar, S., Stecher, G., and Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular biology and evolution*, 2016.*33*(7): p. 1870-1874.
22. Rahman, A., Islam, M. S. Saha, O. and Saha, T. C. Molecular Evolutionary Analysis of α-Defensin Peptides in Vertebrates. *Rajshahi University Journal of Science & Engineering*, 2016. 44: p. 85-93.DOI 10.3329/rujse.v44i0.30392.
23. Adhikari, U. K., Saha, O., Ahmmed, M., Sultana, A., Rahman, F., Islam, K., and Hasan, M. Characterization and comparative modeling of iron superoxide dismutase B1 (FeSODB1) of *Leishmaniadonovani* using an in-silico approach.*International Journal of Computational Bioinformatics and In Silico Modeling*,2016.5(1): p. 770-779.
24. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*. 1985.39: p. 783-791.
25. Kimura, M. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 1985.16: p. 111-120.
26. Lan, Y., Wang, Q., Cole, J. R. and Rosen, G. L. Using the RDP Classifier to Predict Taxonomic Novelty and Reduce the Search Space for Finding Novel Organisms. *PLoS ONE*, 2012.7(3): p. 32491.
27. Wikler, M. A. Performance standards for antimicrobial susceptibility testing: Seventeenth informational supplement. Clinical and Laboratory Standards Institute.2007.
28. Gunasegaran, T., Rathinam, X., Kasi M, Sathasivam, K., Sreenivasan, S. and Subramaniam, S. Isolation and identification of *Salmonella* from curry samples and its sensitivity to commercial antibiotics and aqueous extracts of Cameliasinensis (L.) and Trachyspermumammi (L.). *Asian Pac J Trop Biomed*,2011.1(4): p. 266-269.
29. Naik, V. K., Shakya, S., Patyal, A., and Gade, N. E. Isolation and molecular characterization of *Salmonella* spp. from chevon and chicken meat collected from different districts of Chhattisgarh, India. Veterinary world,2015.8(6): p. 702-706.
30. Hyeon, J. Y., Chon, J. W., Hwang, I. G., Kwak, H. S., Kim, M. S., Kim, S. K., ... & Seo, K. H. Prevalence, antibiotic resistance, and molecular characterization of *Salmonella* serovars in retail meat products. *Journal of food protection*,2011.*74*(1): p. 161-166.
31. Angkititrakul S. Chomvarin C. Chaita T. Kanistanon K. Waethewutajarn S. Epidemiology of antimicrobial resistance in *Salmonella* isolated from pork, chicken meat and humans in Thailand. *Southeast Asian J. Trop. Med. Public Health*,2005.36: p. 1510–1515.
32. Chen J and Griffiths MW. Detection of *Salmonella* and simultaneous detection of *Salmonella* and Shiga-like toxin-producing Escherichia coli using the magnetic apture hybridization polymerase chain reaction. Letters in Applied microbiology,2001.32: p. 7-11.
33. Moussa IM, Gassem MA, Al-Doss AA, Mahmoud WA and Sadik. Using molecular techniques for rapid detection of *Salmonella* serovars in frozen chicken and chicken products collected from Riyadh, Saudi Arabia. African Journal of Biotechnology, 2010.9: p. 612-619.
34. Ammar, A. M., Mohamed, A. A., El-Hamid, M. I. A., and El-Azzouny, M. M. Virulence genotypes of clinical *Salmonella* serovars from broilers in Egypt. *The Journal of Infection in Developing Countries*, 2016.10(04): p. 337-346.
35. Dione, M.M., Ikumapayi, U., Saha, D. Mohammed, N.I., Adegbola, R.A., Geerts, S., Leven, S., and Antonio, M. Antimicrobial resistance and virulence genes of non-typhoidal Salmonella isolates in the Gambia and Senegal, Journal of Infection in Developing Countries,2011.5: p. 765-775.
36. D.H. Shah, X. Zhou, T. Addwebi, M.A. Davis, L . Orfe, D.R. Call, J. Guardand T.E. Besser. Cell invasion of poultry-associated *Salmonella* serovar Enteritidis isolates is associated with pathogenicity, motility and proteins secreted by the type III secretion system, Microbiology, 2011.p. 1428-1445.
37. Zou, M., Keelara, S., and Thakur, S. Molecular characterization of *Salmonella enterica* serotype Enteritidis isolates from humans by

38. antimicrobial resistance, virulence genes, and pulsed-field gel electrophoresis, Foodborne Pathogens and Disease,2012.p.232-238.
39. Betancor L, Schelotto F, Martinez A, Pereira M, Algorta G, Rodriguez MA, Vignoli R and Chabalgoity JA. Random amplified polymorphic DNA and phenotyping analysis of Salmonella enterica serovar Enteritidis isolates collected from humans and poultry in Uruguay from 1995 to 2002. *Journal of Clincal of Microbiology*,2004.42: p. 1155-1162.
40. Baratto CM, Gelinski JMLN, Bernardi AZ, Marafon A and Braun F. Potential use of molecular- typing methods for the identification and characterization of *Salmonella* enterica serotypes isolated in the poultry production chain. *Brazilian Journal of Poultry Science*,2012.14: p.159-232.
41. Sultana, M., Bilkis, R., Diba, F. and Hossain, M. A. Predominance of Multidrug Resistant Zoonotic *Salmonella* Enteritidis Genotypes in Poultry of Bangladesh. *Japan Poultry Science Associatio*,2014.51: p. 424-434.
42. Lamas, A., Fernandez-No, I. C. Miranda, J. M. Vázquez, B., Cepeda, A., and Franco, C. M.Prevalence, molecular characterization and antimicrobial resistance of *Salmonella* serovars isolated from northwestern Spanish broiler flocks (2011–2015), Poultry Science,2016. 95(9): p.1 2097–2105.
43. Fookes, M., Schroeder, G. N., Langridge, G. C., Blondel, C. J., Mammina, C., Connor, T. R., Thomson, N. R. *Salmonella bongori* Provides Insights into the Evolution of the Salmonellae. *PLoS Pathogens*,2011.7(8), e1002191.
44. Rodriguez, A., Pangloli, P., Richards, H. A., Mount, J. R., and Draughon, F. A. Prevalence of Salmonella in diverse environmental farm samples J Food Protect,2006. 69: p.2576-2580.
45. Kolker S., Itsekzon T., Yinnon A. M.,and Lachish T. Osteomyelitis due to Salmonella enterica subsp arizonae: the price of exotic pets. Clin. Microbiol. Infect,2012. 18: p.167-170.
46. Campioni, F., Bergamini, A.M.M., and Falcão, J.P. Genetic diversity, virulence genes and antimicrobial resistance of *Salmonella* Enteritidis isolated from food and humans over a 24-year period in Brazil. *Food Microbiology*,2012.32(2): p. 254-264.
47. Tamang,M.D., Nam,H.M.,Kim,A., Lee,H.S., Kim,T.S.,Kim,M.J., Jang,G.C.,Jung,S.C., and S.K. Lim. Prevalence and mechanisms of quinolone resistance among selected nontyphoid *Salmonella* isolated from food animals and humans in Korea. Foodborne Pathogens and Disease, 2011.8: p.1199-1206.
48. Chung Y. H., Kim S. Y., and Chang Y. H. Prevalence and antibiotic susceptibility of Salmonella isolated from foods in Korea from 1993 to 2001. *J. Food Prot*,2003.66:p. 1154–1157.
49. Nath, S. K., Akter, S., Dutta, A., Sen, A. B., Chakrabarty, R., and Gupta, M. D. Prevalence and Antibiogram of Salmonella in Hisex Brown Strain at Commercial Poultry Farm in Chittagong, Int. J. Curr. Res. Biol. Med, 2015. 2(3): p. 14-19.