Prevalence and antimicrobial resistance phenotypes of *Salmonella* species recovered at various stages of broiler operations in Hathazari, Bangladesh

Ferdausi Ali[1], Tazriyan Noor Silvy[1], Tanim Jabid Hossain[2], Md. Kamal Uddin[1] and Mohammad Seraj Uddin[1]

1. Department of Microbiology, University of Chittagong, Chattogram, Bangladesh; 2. Department of Biochemistry and Molecular Biology, University of Chittagong, Chattogram, Bangladesh.
Corresponding author: Tanim Jabid Hossain, e-mail: tanim.bmb@gmail.com
Co-authors: FA: seema@cu.ac.bd, TNS: tazriyannoor.mbio14@gmail.com, MKU: kamalmbio@gmail.com, MSU: seraj.mbio@cu.ac.bd

Abstract

Background and Aim: Dissemination of multidrug-resistant (MDR) *Salmonella* through food chains has serious health implications, with higher rates of morbidity and mortality. Broiler meat remains a major reservoir of *Salmonella* contamination. The lack of proper hygiene in local broiler operations has, therefore, prompted this research into the assessment of *Salmonella* contamination in local shops and associated antimicrobial resistance (AMR) phenotypes.

Materials and Methods: A total of 55 broiler samples including skin, meat, and swab samples from chopping and dressing sites were included in the study. The samples were collected from broiler shops in Hathazari, Bangladesh, and screened for the presence of *Salmonella* strains using culture-based methods. The isolates were biochemically characterized and further tested for AMR to eight common antibiotics using the disk diffusion technique.

Results: *Salmonella* contaminations were identified in 29% (16/55) of the broiler samples. Swab samples collected from the chopping sites appeared to be contaminated in higher proportions (33%) than those collected from the dressing areas (25%). On the other hand, the skin samples (50%) were detected with a higher percentage of contamination than the meat samples (25%). All *Salmonella* isolates showed resistance toward at least one of the eight antibiotics used. Although none of the isolates was resistant to all antibiotics, 18.75% showed resistance to a maximum of seven antibiotics. Resistance to nalidixic acid was most prevalent (87.5%), followed by sulfamethoxazole-trimethoprim (81.25%), erythromycin (81.25%), tetracycline (75%), streptomycin (56.25%), ampicillin-clavulanic acid (50%), chloramphenicol (43.75%), and cefotaxime (18.75%). The resistance patterns of the isolates were found to be highly diverse. The most frequently observed pattern was the following: Ampicillin-clavulanic acid-sulfamethoxazole-trimethoprim-nalidixic acid-tetracycline-chloramphenicol-streptomycin-erythromycin.

Conclusion: The relatively high prevalence of MDR strains in the samples underlies an urgent need for surveillance and control measures concerning hygiene and antibiotic use in local broiler operations.

Keywords: antimicrobial resistance, chicken meat, multidrug-resistant, *Salmonella*, salmonellosis.

Introduction

Salmonella species are opportunistic intracellular pathogens responsible for severe foodborne infections that cause substantial morbidity and mortality worldwide [1]. *Salmonella* infections account for 93.8 million cases of foodborne illnesses every year, 155,000 of which lead to death [2]. Gastroenteritis is the most common of these illnesses, followed by bacteremia and other severe systemic diseases such as typhoid and typhoid fever [3,4]. The major vehicles for the transmission of *Salmonella* pathogens to humans are contaminated foods, particularly those of animal origin, such as poultry, swine, cattle, eggs, and dairy products [5,6]. Of these, poultry accounts for a considerably higher percentage of *Salmonella* outbreaks than any of the other food commodities [7]. Because the consumption of broiler chicken has been on the rise in Bangladesh since the past decade [8], the risk for exposure to *Salmonella* through the direct contact and consumption of the contaminated meat is a public health concern that requires rigorous surveillance.

The emergence of antimicrobial resistance (AMR) in *Salmonella* serovars has been another major concern over the past two decades that further worsens the associated health problems. Increasing reports on the identification of multidrug-resistant (MDR) *Salmonella* isolates from broilers have raised concerns that the treatment of salmonellosis may be compromised. High rates of MDR *Salmonella* have already been documented in several of the South Asian countries [9,10]. The main factor contributing to the emergence of *Salmonella* with AMR is the use of antibiotics in broiler feed to promote growth of the chickens and in veterinary medicine to treat

bacterial infections in those chickens [2]. The AMR obtained therefrom is acquired through several mechanistic and epidemiological events, including random mutation, plasmid exchange, horizontal gene transfer, and clonal spread of the resistant isolates [11,12]. At present, antibiotic-based treatment for human salmonellosis is the most effective method in clinical practice [13]. However, the continuous exposure and even abuse of antibiotics in broilers have facilitated the emergence and dissemination of antibiotic resistance in *Salmonella*, which may have serious health implications, considering the potential for treatment failure when cases of salmonellosis require medical intervention, especially in children, older adults, and immunocompromised individuals [14]. The screening of *Salmonella* for AMR in broiler operations is, therefore, essential for providing information on the magnitude of resistance to plan for the prevention and control of future emergence and spread.

Poultry chicken is the cheapest and most consumed meat product in Bangladesh [15]. However, like several other developing countries, the commercial broiler industry in Bangladesh remains underdeveloped. Chickens raised in broiler farms are sent alive to local retail outlets, wherein several chickens are kept in a relatively small space for days to weeks until sold. After being slaughtered and processed, whole chickens are instantly handed over to the customers in polypacks. All processing in the slaughtering house is generally performed on the same processing line without any cleaning, leading to a possible transfer of bacteria from carcass to carcass. In fact, proper hygiene is rarely maintained in the total process, which poses a high risk of zoonotic disease through the ingestion of food or water contaminated with the animals' feces, direct contact, or consumption of the contaminated meat. Furthermore, the uncontrolled use of antimicrobials, which is relatively a common practice in Bangladesh intended for growth stimulation and disease treatment, further increases the risk for emergence and spread of MDR strains of *Salmonella*.

Several studies have been conducted on the prevalence of *Salmonella* in poultry processing plants in a few locations in Bangladesh [16-18]. However, a continuing need for increased surveillance on the prevalence and antimicrobial susceptibility of *Salmonella* strains at local broiler operations in all areas exists. Moreover, for proper understanding and control of contamination, it is important to determine the relative contributions of various contamination points, including handling, chopping, and dressing. Such information is scarce with respect to the local broiler shops in Bangladesh. Hathazari is one of the major towns in Chattogram district, having a moderately high population density (1700 individuals/km^2) [19] and several retail broiler shops. However, information regarding the occurrence of *Salmonella* in these broiler retailers is not available.

Therefore, this study aimed to determine the prevalence of *Salmonell*a at different stages of broiler operations in retail shops in the Hathazari region of Bangladesh and their resistance profiles toward frequently used antibiotics and to provide essential information for reducing the level of contamination with antibiotic-resistant *Salmonell*a.

Materials and Methods

Ethical approval

Ethical approval was not required for this study. The samples were collected from the retail market.

Study period and location

This study was conducted from March 2018 to January 2019. The samples were collected from retail shops in Hathazari, Bangladesh, and processed for bacteriological examinations in the laboratory of Department of Microbiology, University of Chittagong.

Sample collection

A total of 55 samples, including swab samples from chopping (9) and dressing (4) sites, skin (6) samples, and meat (36) samples, were collected from local broiler shops in Hathazari, Chattogram, Bangladesh. Swab samples were collected in 0.1% buffered peptone water. All samples were transported to the laboratory, maintained at 4°C, and immediately used for further analysis. Meat samples were homogenized using mortar and pestle and dissolved in 90 mL of 0.1% buffered peptone water before enrichment.

Enrichment, isolation, and identification

For the selective enrichment of *Salmonella*, 100 μL of the processed samples in buffered peptone water was inoculated to Rappaport-Vassiliadis *Salmonella* Enrichment Medium (HiMedia, Mumbai, India) and incubated at 42°C for 24 h. The enrichment culture was further streaked on Xylose Lysine Deoxycholate selective growth medium (HiMedia) and incubated at 37°C for 24-48 h [20]. Presumptive *Salmonella* colonies were identified based on morphological and biochemical characterizations, such as colony appearances, Gram staining, indole, catalase, methyl red, Voges–Proskauer, urease, and citrate utilization tests, and growth characteristics on triple sugar iron agar.

Antimicrobial susceptibility determination

The antimicrobial susceptibility of the isolates was determined using the agar disk diffusion method on Mueller-Hinton agar (HiMedia) as described in the Clinical and Laboratory Standards Institute guidelines [21]. The following were the antimicrobial disks (Oxoid, Basingstoke, United Kingdom) used: Ampicillin-clavulanic acid (30 μg), cefotaxime (30 μg), sulfamethoxazole-trimethoprim (25 μg), nalidixic acid (30 μg), streptomycin (10 μg), erythromycin (15 μg), tetracycline (30 μg), and chloramphenicol (30 μg). The diameter of the zones of inhibition was measured, and the response of the isolates was interpreted as susceptible, intermediate, or resistant

according to the Clinical and Laboratory Standards Institute guidelines.

Results

Prevalence of *Salmonella* contamination

To determine the prevalence of *Salmonella* contamination at various stages of broiler processing and in skin and meat, a total of 55 samples, including 19 swab samples from the skin, chopping bench, and dressing area, and 36 meat samples (Table-1), were collected from local broiler shops in Hathazari, Bangladesh, and screened for the presence of *Salmonella* using culture-based techniques by growing successively in two selective media, such as the Rappaport-Vassiliadis broth and Xylose Lysine Deoxycholate agar, which are regularly used for *Salmonella* isolation from food samples. Sixteen of the 55 samples (29%) were found positive for the presence of *Salmonella* (Table-1), as confirmed by the biochemical characterization of the isolated strains showing positive results in catalase, methyl red, and citrate utilization tests; negative results in Gram staining, indole, Voges–Proskauer, and urease tests; and distinctive growth characteristics on triple sugar iron agar.

Considering the distribution of *Salmonella* contamination in processing operations, the samples collected from chopping sites appeared to be contaminated in higher proportions, with *Salmonella* recovered from 33% of these samples (Table-1), but one-fourth of the samples collected from dressing areas showed *Salmonella* contamination, suggesting that the dressing sites may carry a comparatively lower risk for pathogenesis. On the other hand, considering the skin and meat samples, a higher risk appeared to be associated with broiler skin, with half of the samples contaminated with *Salmonella*, whereas a relatively lower percentage of *Salmonella* contamination was detected in the meat samples, with only 25% found to have positive results.

Antimicrobial susceptibility

The susceptibility and resistance of the *Salmonella* isolates were tested against eight frequently used antibiotics, and the results are summarized in Table-2. All isolates showed resistance toward at least one of the antibiotics used, whereas all, except the isolate CS_6, showed multiple resistance (resistance to $\geq$ three antibiotics). On the other hand, 18.75% of the isolates demonstrated resistance to a maximum of

seven of the eight antibiotics, and 25% of the isolates were resistant to six antibiotics. Intermediate susceptibility toward a minimum of one antibiotic was found in 68.75% of isolates. However, none of the *Salmonella* isolates showed resistance or susceptibility to all eight antibiotics used.

With regard to microbial response to a particular antibiotic, the most frequently observed resistance was recorded against nalidixic acid (87.5%), followed by sulfamethoxazole-trimethoprim (81.25%), erythromycin (81.25%), tetracycline (75%), streptomycin (56.25%), ampicillin-clavulanic acid (50%), chloramphenicol (43.75%), and cefotaxime (18.75%) (Figure-1), indicating that cefotaxime might be more effective for the treatment of broiler-associated salmonellosis because the highest number of isolates (62.5%) was found susceptible to it.

The antimicrobial resistance patterns (ARPs) exhibited by the isolates were found to be highly diverse, with all isolates exhibiting unique ARPs (Table-3). Because microbes showing intermediate susceptibility can also be considered "susceptible" under increased exposure ("EUCAST") [22], 12 different ARPs were observed among the 16 isolates after counting both susceptible and intermediate microbes in the same category. The most prevalent ARP was AMC-SXT-NA-TE-C-S-E (XII) found in a maximum of three isolates (25%), followed by SXT-NA-TE-C-S-E (VIII) and AMC-SXT-NA-TE-S-E (XI), each found in two of the isolates (16.67%). Although the two isolates showing ARP XI were both isolated from the meat samples, those showing ARP VIII and XII were all isolated from different types of samples, such as the skin, chopping site, and meat, and skin and meat, respectively.

Table-1: Distribution of *Salmonella* contaminations across the broiler samples.

Sample	No. of sample analyzed	No. of *Salmonella*-positive samples	% of *Salmonella*-positive samples
Chopping site	9	3	33
Dressing site	4	1	25
Skin	6	3	50
Processed meat	36	9	25
Total	55	16	29

Table-2: Profile of antimicrobial susceptibility and resistance of the isolated *Salmonella* spp.

Isolates	Antibiotics*, **							
	AMC	**CTX**	**SXT**	**NA**	**TE**	**C**	**S**	**E**
SS_2	R	S	R	R	R	R	R	R
SS_3	I	S	R	R	R	R	R	R
SS_6	I	S	S	R	R	I	R	I
CS_4	R	I	R	R	R	R	R	R
CS_6	S	R	S	S	S	S	I	I
CS_8	R	S	S	R	S	S	I	R
DS_2	R	R	R	I	R	S	I	I
MS_3	S	S	R	R	R	R	R	R
MS_8	S	S	R	R	R	I	R	R
MS_9	S	S	R	R	R	I	R	R
MS_{14}	S	S	R	R	R	R	R	R
MS_{24}	R	I	R	R	R	R	R	R
MS_{26}	I	S	R	R	R	S	I	R
MS_{30}	R	I	R	R	R	I	R	R
MS_{32}	R	R	R	R	S	S	S	R
MS_{36}	R	S	R	R	R	S	R	R

*AMC=Ampicillin-clavulanic acid, CTX=Cefotaxime, SXT=Sulfamethoxazole-trimethoprim, NA=Nalidixic acid, TE=Tetracycline, C=Chloramphenicol, S=Streptomycin, E=Erythromycin. **S=Susceptible, I=Susceptible, increased exposure, R=Resistant
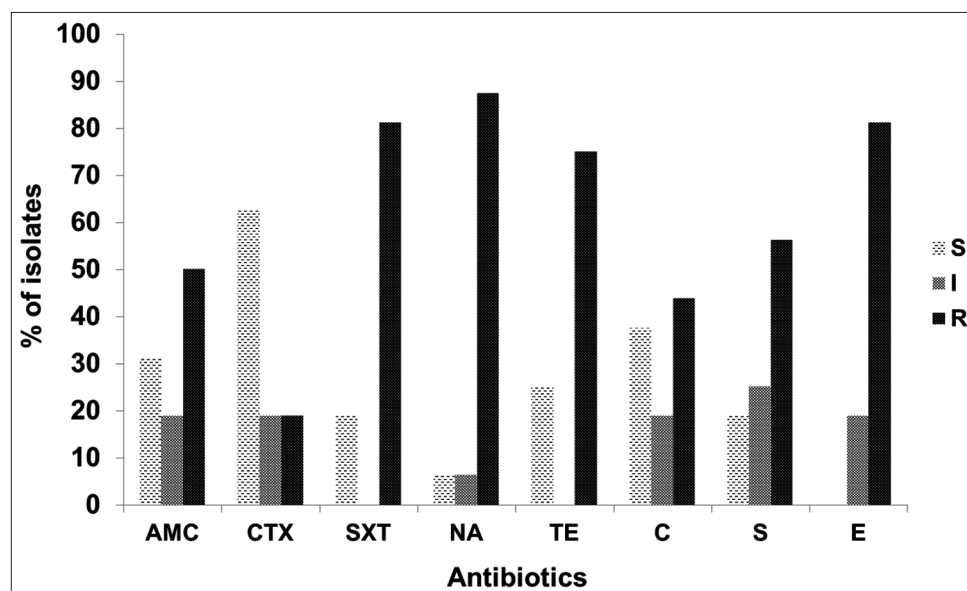
Figure-1: Proportion of *Salmonella* isolates with respect to their responses (susceptibility – S, intermediate susceptibility – I, and resistance – R) toward eight antibiotics including ampicillin-clavulanic acid (AMC), cefotaxime (CTX), sulfamethoxazole-trimethoprim (SXT), nalidixic acid (NA), tetracycline (TE), chloramphenicol (C), streptomycin (S), and erythromycin (E).

Table-3: Antimicrobial resistance pattern of the isolates.

Pattern no.	Antimicrobial resistance pattern*, **	No. of isolates
I	CTX	1
II	NA-TE-S	1
III	AMC-NA-E	1
IV	SXT-NA-C-E	1
V	SXT-NA-TE-E	1
VI	SXT-NA-TE-S-E	1
VII	SXT-NA-TE-C-E	1
VIII	SXT-NA-TE-C-S-E	2
IX	AMC-CTX-SXT-TE	1
X	AMC-CTX-SXT-NA-E	1
XI	AMC-SXT-NA-TE-S-E	2
XII	AMC-SXT-NA-TE-C-S-E	3

*Considering S and I both as susceptible.
**AMC=Ampicillin-clavulanic acid, CTX=Cefotaxime, SXT=Sulfamethoxazole-trimethoprim, NA=Nalidixic acid, TE=Tetracycline, C=Chloramphenicol, S=Streptomycin, E=Erythromycin

Discussion

The findings of this study indicate a moderately high prevalence (29%) of overall *Salmonella* contamination at the local broiler retailers in the Hathazari area of Bangladesh. The frequency of *Salmonella* contamination, in fact, varies over a wide range from one country to another, from place to place in the same country, or even from one farm to another in the same city. Several reports described a prevalence of only 10% or even less in samples associated with broiler operations [23-30]. Some studies even found a prevalence as low as ~1% in broiler samples [31-33]. By contrast, the occurrence of a very high rate of *Salmonella* contamination was likewise found. An overall prevalence of 50% or more was documented in several investigations [34-38]. With respect to studies on Bangladeshi broiler farms, results similar to those

reported herein were also described by a few other groups. For example, the prevalence of *Salmonella* was reported to be between 20% and 40% in samples obtained from commercial broiler farms [16-18]. However, other groups also reported contamination that was much lower or higher than this range [39,40]. The variations in *Salmonella* prevalence between the reports might result from differences in hygiene practice and surveillance programs in each country or differences in experimental design and detection methods employed in the individual investigation.

In the present study, chopping sites appeared to be associated with a higher proportion of contamination than dressing sites. Moreover, skin samples were found with a higher frequency of contamination than processed meat samples. However, studies particularly focusing on dressing or chopping sites are scarce, and more research is needed to confirm if it is a general trend of *Salmonella* contamination in broiler shops. About 25% of the meat samples examined in this study were detected with *Salmonella*. Although the contamination frequency is still an issue of concern, a higher prevalence was found in several other countries. For example, 72%, 53.3%, 52.2%, 35.5%-47.7%, 36.0% and 42.3%, and 36.5% contamination of chicken meat were found in Thailand, Vietnam, China, Austria, Korea, and Belgium, respectively [41].

All *Salmonella* isolates recovered from the broiler samples in this study were resistant to at least one of the 10 antibiotics; except for one isolate, all showed resistance to two or more antibiotics. The average number of antibiotics to which the isolates were resistant is 4.94, which is consistent with the findings of other studies [42,43]. More than 80% of these isolates showed resistance to nalidixic acid (87.5%), sulfamethoxazole-trimethoprim (81.25%), and tetracycline

(81.25%). A similar resistance pattern to these antibiotics is often reported in *Salmonella* species isolated from chicken and other foodstuffs. Sodagari *et al.* [44], for example, found 92.8%, 81%, and 61.2% prevalence of resistance to nalidixic acid, tetracycline, and sulfamethoxazole-trimethoprim, respectively, in *Salmonella* associated with retail chicken meat and giblets. In a study on foodstuff and related sources, 70%, 90%, and 80% of *Salmonella enterica* isolates showed resistance to nalidixic acid, tetracycline, and sulfamethoxazole-trimethoprim, respectively [45]. Indeed, multiple resistance is more or less frequently observed among *Salmonella* species analyzed in most of the studies performed today [2,46]. The high proportion of *Salmonella* isolates of animal origin with resistance to multiple antimicrobial agents indicates excessive or uncontrolled use of antimicrobials in animal feed and medicine.

The 16 *Salmonella* isolates of this study showed 12 different ARPs. Hernandez *et al.* [47] also found the same number of patterns in *S. enterica* serovars isolated from chickens in Spain, although the type of pattern was relatively different. A higher or lower number of patterns were, however, observed in other studies. For example, Antunes *et al.* [48] reported only two resistance patterns in *Salmonella* from poultry products. The following were the ARPs that were most frequently encountered in this study: Ampicillin-clavulanic acid-sulfamethoxazole-trimethoprim-nalidixic acid-tetracycline-chloramphenicol-streptomycin-erythromycin, demonstrated by three isolates followed by the resistance patterns: Sulfamethoxazole-trimethoprim-nalidixic acid-tetracycline-chloramphenicol-streptomycin-erythromycin and ampicillin-clavulanic acid-sulfamethoxazole-trimethoprim-nalidixic acid-tetracycline-streptomycin-erythromycin. The relatively high occurrence of the "sulfamethoxazole-trimethoprim, nalidixic acid, tetracycline, streptomycin, and erythromycin combination" in the resistance patterns may suggest the presence of gene transfer systems, such as bacterial conjugative plasmids or transposable elements, carrying the respective resistant genes [42,49].

Conclusion

The following are the major findings of this study: (i) A moderately high prevalence of *Salmonella* contamination in the broiler samples, (ii) a high percentage of AMR of the *Salmonella* isolates, and (iii) a relatively greater frequency of resistance of the isolates to nalidixic acid, sulfamethoxazole-trimethoprim, erythromycin, and tetracycline. These findings suggest the risk of salmonellosis resulting from local broiler operations and from cross-contamination during the purchase of the broiler products, meal preparation, or consumption of undercooked contaminated meat. Therefore, an urgent need to reduce the risk for development and dissemination of *Salmonella* contamination through regular surveillance and the prudent use of antimicrobial agents during broiler production and processing, in particular, are required.

Authors' Contributions

FA and MSU: Supervised the study and contributed to conception and design; TNS and MKU: Carried out the laboratory experiments; FA and TJH: Performed data analysis; and TJH and FA: Wrote the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

Publisher's Note

Veterinary World (Publisher of International Journal of One Health) remains neutral with regard to jurisdictional claims in published institutional affiliation.

References

1. Cheng, R.A., Eade, C.R. and Wiedmann, M. (2019) Embracing diversity: Differences in virulence mechanisms, disease severity, and host adaptations contribute to the success of nontyphoidal *Salmonella* as a foodborne pathogen. *Front. Microbiol.*, 10: 1368.
2. Eng, S.K., Pusparajah, P., Mutalib, N.S.A., Ser, H.L., Chan, K.G. and Lee, L.H. (2015) *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. *Front. Life Sci.*, 8(3): 284-293.
3. Fernandes, D.V.G., Castro, V.S. and da Cunha Neto, A. (2018) *Salmonella* spp. in the fish production chain: A review. *Ciênc. Rural*, 48(8): 141.
4. Obaro, S.K., Tam, P.Y.I. and Mintz, E.D. (2017) The unrecognized burden of typhoid fever. *Expert Rev. Vaccines*, 16(3): 249-260.
5. Ferrari, R.G., Rosario, D.K., Cunha-Neto, A., Mano, S.B., Figueiredo, E.E.S. and Conte-Junior, C.A. (2019) Worldwide epidemiology of *Salmonella* serovars in animal-based foods: A meta-analysis. *Appl. Environ. Microbiol.*, 85(14): e00591-19.
6. Heredia, N. and García, S. (2018) Animals as sources of foodborne pathogens: A review. *Anim. Nutr.*, 4(3): 250-255.
7. Mani-López, E., García, H.S. and López-Malo, A. (2012) Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. *Food Res. Int.*, 45(2): 713-721.
8. Haque, M.H., Sarker, S., Islam, M.S., Islam, M.A., Karim, M.R., Kayesh, M.E.H., Shiddiky, M.J.A. and Anwer, M.S. (2020) Sustainable antibiotic-free broiler meat production: Current trends, challenges, and possibilities in a developing country perspective. *Biology*, 9(11): 411.
9. Chuang, C.H., Su, L.H., Perera, J., Carlos, C., Tan, B.H., Kumarasinghe, G., So, T., Van, P.H., Chongthaleong, A., Hsueh, P.R., Liu, J.W., Song, J.H. and Chiu, C.H. (2009) Surveillance of antimicrobial resistance of *Salmonella*

enterica serotype Typhi in seven Asian countries. *Epidemiol. Infect.*, 137(2): 266-269.

10. Ochiai, R.L., Acosta, C.J., Danovaro-Holliday, M.C., Baiqing, D., Bhattacharya, S.K., Agtini, M.D., Bhutta, Z.A., Canh, D.G., Ali, M., Shin, S., Wain, J., Page, A.L., Albert, M.J., Farrar, J., Abu-Elyazeed, R., Pang, T., Galindo, C.M., von Seidlein, L., Clemens, J.D. and Domi Typhoid Study Group. (2008) A study of typhoid fever in five Asian countries: Disease burden and implications for controls. *Bull. World Health Organ.*, 86(4): 260-268.

11. Hughes, D. and Andersson, D.I. (2015) Evolutionary consequences of drug resistance: Shared principles across diverse targets and organisms. *Nat. Rev. Genet.*, 16(8): 459-471.

12. Zhao, X., Yang, J., Zhang, B., Sun, S. and Chang, W. (2017) Characterization of integrons and resistance genes in *Salmonella* isolates from farm animals in Shandong Province, China. *Front. Microbiol.*, 8: 1300.

13. Li, S., Zhou, Y. and Miao, Z. (2017) Prevalence and antibiotic resistance of non-typhoidal *Salmonella* isolated from raw chicken carcasses of commercial broilers and spent hens in Tai'an, China. *Front. Microbiol.*, 8: 106.

14. Liljebjelke, K.A., Hofacre, C.L., White, D.G., Ayers, S., Lee, M.D. and Maurer, J.J. (2017) Diversity of antimicrobial resistance phenotypes in *Salmonella* isolated from commercial poultry farms. *Front. Vet. Sci.*, 4: 96.

15. Rahman, M.S., Jang, D.H. and Yu, C.J. (2017) Poultry industry of Bangladesh: Entering a new phase. *Korean J. Agric. Sci.*, 44(2): 272-282.

16. Barua, H., Biswas, P.K., Olsen, K.E., Shil, S.K. and Christensen, J.P. (2013) Molecular characterization of motile serovars of *Salmonella enterica* from breeder and commercial broiler poultry farms in Bangladesh. *PLoS One*, 8(3): e57811.

17. Mahmud, M.S., Bari, M.L. and Hossain, M.A. (2011) Prevalence of *Salmonella* serovars and antimicrobial resistance profiles in poultry of Savar area, Bangladesh. *Foodborne Pathog. Dis.*, 8(10): 1111-1118.

18. Mridha, D., Uddin, M.N., Alam, B., Akhter, A.H.M., Islam, S.S., Islam, M.S., Khan, M.S.R. and Kabir, S.M.L. (2020) Identification and characterization of *Salmonella* spp. from samples of broiler farms in selected districts of Bangladesh. *Vet. World*, 13(2): 275-283.

19. Wikipedia. (2021) Hathazari Upazila. Wikipedia. https://en.wikipedia.org/wiki/Hathazari_Upazila. Retrieved on 19-01-2021.

20. Makwana, P.P., Nayak, J.B., Brahmbhatt, M.N. and Chaudhary, J.H. (2015) Detection of *Salmonella* spp. from chevon, mutton and its environment in retail meat shops in Anand city (Gujarat), India. *Vet. World*, 8(3): 388-392.

21. Weinstein, M.P. and Lewis, J.S. 2nd. (2020) The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: Background, organization, functions, and processes. *J. Clin. Microbiol.*, 58(3): e01864-19.

22. EUCAST: New S, I and R Definitions. (2021) Available from: https://www.eucast.org/newsiandr. Retrieved on 20-01-2021.

23. Bailey, J.S., Stern, N.J., Fedorka-Cray, P., Craven, S.E., Cox, N.A., Cosby, D.E., Ladely, S. and Musgrove, M.T. (2001) Sources and movement of *Salmonella* through integrated poultry operations: A multistate epidemiological investigation. *J. Food Prot.*, 64(11): 1690-1697.

24. Chambers, J.R., Bisaillon, J.R., Labbe, Y., Poppe, C. and Langford, C.F. (1998) *Salmonella* prevalence in crops of Ontario and Quebec broiler chickens at slaughter. *Poult. Sci.*, 77(10): 1497-1501.

25. Duarte, D.A.M., Ribeiro, A.R., Vasconcelos, A.M.M., Santos, S.B., Silva, J.V.D., de Andrade, P.L.A. and da Costa de Arruda Falcão, L.S.P. (2009) Occurrence of *Salmonella* spp. in broiler chicken carcasses and their susceptibility to antimicrobial agents. *Braz. J. Microbiol.*, 40(3): 569-573.

26. Giombelli, A. and Gloria, M.B.A. (2014) Prevalence of *Salmonella* and *Campylobacter* on broiler chickens from farm to slaughter and efficiency of methods to remove visible fecal contamination. *J. Food Prot.*, 77(11): 1851-1859.

27. Hue, O., Allain, V., Laisney, M.J., Le Bouquin, S., Lalande, F., Petetin, I., Rouxel, S., Quesne, S., Gloaguen, P.Y., Picherot, M., Santolini, J., Bougeard, S., Salvat, G. and Chemaly, M. (2011) Campylobacter contamination of broiler caeca and carcasses at the slaughterhouse and correlation with *Salmonella* contamination. *Food Microbiol.*, 28(5): 862-868.

28. Medeiros, M.A.N., de Oliveira, D.C.N., dos Prazeres Rodrigues, D. and de Freitas, D.R.C. (2011) Prevalence and antimicrobial resistance of *Salmonella* in chicken carcasses at retail in 15 Brazilian cities. *Rev. Panam. Salud Pública*, 30(6): 555-560.

29. Poppe, C. (1994) *Salmonella* Enteritidis in Canada. *Int. J. Food Microbiol.*, 21(1-2): 1-5.

30. Wong, T.L., Nicol, C., Cook, R. and MacDiarmid, S. (2007) *Salmonella* in uncooked retail meats in New Zealand. *J. Food Prot.*, 70(6): 1360-1365.

31. Lamas, A., Fernandez-No, I.C., Miranda, J.M., Vázquez, B., Cepeda, A. and Franco, C.M. (2016) Prevalence, molecular characterization and antimicrobial resistance of *Salmonella* serovars isolated from northwestern Spanish broiler flocks (2011-2015). *Poult. Sci.*, 95(9): 2097-2105.

32. Sexton, T.Y., Geornaras, I., Belk, K.E., Bunning, M. and Martin, J.N. (2018) *Salmonella* contamination in broiler synovial fluid: Are we missing a potential reservoir? *J. Food Prot.*, 81(9): 1425-1431.

33. Suresh, T., Hatha, A.A.M., Harsha, H.T. and Lakshmanaperumalsamy, P. (2011) Prevalence and distribution of *Salmonella* serotypes in marketed broiler chickens and processing environment in Coimbatore city of Southern India. *Food Res. Int.*, 44(3): 823-825.

34. Choi, S.W., Ha, J.S., Kim, B.Y., Lee, D.H., Park, J.K., Youn, H.N., Hong, Y.H., Lee, S.B., Lee, J.B., Park, S.Y., Choi, I.S. and Song, C.S. (2014) Prevalence and characterization of *Salmonella* species in entire steps of a single integrated broiler supply chain in Korea. *Poult. Sci.*, 93(5): 1251-1257.

35. Donado-Godoy, P., Gardner, I., Byrne, B.A., Leon, M., Perez-Gutierrez, E., Ovalle, M.V., Tafur, M.A. and Miller, W. (2012) Prevalence, risk factors, and antimicrobial resistance profiles of *Salmonella* from commercial broiler farms in two important poultry-producing regions of Colombia. *J. Food Prot.*, 75(5): 874-883.

36. Mellor, G.E., Duffy, L.L., Dykes, G.A. and Fegan, N. (2010) Relative prevalence of *Salmonella* Sofia on broiler chickens pre-and postprocessing in Australia. *Poult. Sci.*, 89(7): 1544-1548.

37. Nidaullah, H., Abirami, N., Shamila-Syuhada, A.K., Chuah, L.O., Nuru, H., Tan, T.P., Abidin, F.W.Z. and Rusu, G. (2017) Prevalence of *Salmonella* in poultry processing environments in wet markets in Penang and Perlis, Malaysia. *Vet. World*, 10(3): 286-292.

38. Sasaki, Y., Ikeda, A., Ishikawa, K., Murakami, M., Kusukawa, M., Asai, T. and Yamada, Y. (2012) Prevalence and antimicrobial susceptibility of *Salmonella* in Japanese broiler flocks. *Epidemiol. Infect.*, 140(11): 2074-2081.

39. Alam, S.B., Mahmud, M., Akter, R., Hasan, M., Sobur, A., Nazir, K.N.H., Noreddin, A., Rahman, T., El Zowalaty, M.E. and Rahman, M. (2020) Molecular detection of multidrug-resistant *Salmonella* species isolated from broiler farm in Bangladesh. *Pathogens*, 9(3): 201.

40. Parvej, M.S., Nazir, K.N.H., Rahman, M.B., Jahan, M., Khan, M.F.R. and Rahman, M. (2016) Prevalence and characterization of multi-drug resistant *Salmonella enterica* serovar Gallinarum biovar Pullorum and Gallinarum from chicken. *Vet. World*, 9(1): 65-70.

41. Kim, M.S., Lim, T.H., Jang, J.H., Lee, D.H., Kim, B.Y., Kwon, J.H., Choi, S.W., Noh, J.Y., Hong, Y.H., Lee, S.B., Yang, S.Y., Lee, H.J., Lee, J.B., Park, S.Y., Choi, I.S. and Song, C.S. (2012) Prevalence and antimicrobial resistance of *Salmonella* species isolated from chicken meats produced

by different integrated broiler operations in Korea. *Poult. Sci.*, 91(9): 2370-2375.

42. Álvarez-Fernández, E., Alonso-Calleja, C., García-Fernández, C. and Capita, R. (2012) Prevalence and antimicrobial resistance of *Salmonella* serotypes isolated from poultry in Spain: Comparison between 1993 and 2006. *Int. J. Food Microbiol.*, 153(3): 281-287.

43. Logue, C.M., Sherwood, J.S., Olah, P.A., Elijah, L.M. and Dockter, M.R. (2003) The incidence of antimicrobial-resistant *Salmonella* spp. on freshly processed poultry from US Midwestern processing plants. *J. Appl. Microbiol.*, 94(1): 16-24.

44. Sodagari, H.R., Mashak, Z. and Ghadimianazar, A. (2015) Prevalence and antimicrobial resistance of *Salmonella* serotypes isolated from retail chicken meat and giblets in Iran. *J. Infect. Dev. Ctries.*, 9(5): 463-469.

45. Ribeiro, V.B., Lincopan, N., Landgraf, M., Franco, B.D.G. and Destro, M.T. (2011) Characterization of Class 1 integrons and antibiotic resistance genes in multidrug-resistant *Salmonella enterica* isolates from foodstuff and related sources. *Braz. J. Microbiol.*, 42(2): 685-692.

46. Cosby, D.E., Cox, N.A., Harrison, M.A., Wilson, J.L., Buhr, R.J. and Fedorka-Cray, P.J. (2015) *Salmonella* and antimicrobial resistance in broilers: A review. *J. Appl. Poult. Res.*, 24(3): 408-426.

47. Hernandez, T., Rodriguez-Alvarez, C., Arevalo, M.P., Torres, A., Sierra, A. and Arias, A. (2002) Antimicrobial-resistant *Salmonella enterica* serovars isolated from chickens in Spain. *J. Chemother.*, 14(4): 346-350.

48. Antunes, P., Réu, C., Sousa, J.C., Peixe, L. and Pestana, N. (2003) Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. *Int. J. Food Microbiol.*, 82(2): 97-103.

49. Capita, R. and Alonso-Calleja, C. (2013) Antibiotic-resistant bacteria: A challenge for the food industry. *Crit. Rev. Food Sci. Nutr.*, 53(1): 11-48.

****