### ACKNOWLEDGEMENT

All sorts of praises go to the **Almighty Allah**, whose blessing enabled the author to complete thesis successfully for the degree of Masters of Science under the Dept. of Pathology and Parasitology, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. The author wishes heartfelt gratitude to the supervisor Prof. **Dr. Sharmin Chowdhury**, Department of Pathology and Parasitology (DPP), CVASU for her valuable supervision and guidance. The author sincerely thanks to co-supervisor Prof. **Dr. Md Shafiqul Islam**, Associate Professor at the Department of Pathology and Parasitology, CVASU for his suggestions and guidance.

The author also gratefully acknowledges Prof. **Dr. Mohammad Alamgir Hossain**, Dean of the Faculty of Veterinary Medicine (FVM), Prof. **Dr. Mohammad Mahbubur Rahman**, Head of the Department of Pathology and Parasitology, Prof. **Dr. Md. Masuduzzaman**, Prof. **Dr. AMAM Zonaed Siddiki**, Prof. **Dr Md Abdul Alim**, Prof. **DR. Towhida Kamal** of Department of Pathology and Parasitology (DPP), CVASU for their valuable provision of information during the research period. I am also indebted to all the staff of the DPP, and One Health Institute, CVASU for their cordial assistance. It's the author's immense pleasure to thank **DR. Md. Sirazul Islam**, for providing support to extend the spectrum of this thesis work. The author humbly thanks to **DR. Jahan Ara**, DR. Md. Masud Parves Munna, DR. Abid Hasan, for their suggestions, encouragement and support during the research work.

The author would like to express his deep sense of gratitude to the Director of Advance Study and Research, CVASU; Bangladesh Agricultural Research Council (BARC) and Ministry of Science and Technology, People's Republic of Bangladesh for providing necessary research funds for the research.

Finally, the author expresses his thankfulness to his parents, seniors, juniors and wellwishers.

THE AUTHOR

Md. Hafizar Rahman

December, 2022

# Table of content

| Acknowledgement   | i    |
|---|------|
| List of content   | ii   |
| List of acronyms and symbols used                                   | v    |
| List of tables  | vii  |
| List of figures   | vii  |
| Summary   | viii |
| Chapter 1: Introduction   | 12   |
| Chapter 2: Review of literature                                     | 14   |
| 2.1. An overview of Salmonella                                      | 14   |
| 2.1.1. Classification and nomenclature                              | 14   |
| 2.1.2. Morphology   | 14   |
| 2.1.3. Growth requirements  | 15   |
| 2.1.4. Antigenic structure  | 15   |
| 2.1.5. Biochemical properties                                       | 15   |
| 2.1.8. Transmission of Salmonella                                   | 16   |
| 2.1.7. The Factors Affecting Salmonella Colonization in Chickens    | 16   |
| 2.2. Salmonella infections in layer chicken                         | 20   |
| 2.3. Salmonella infections in broiler chicken                       | 20   |
| 2.4. The Overall Prevalence of Salmonella in poultry                | 21   |
| 2.5.1. The Antimicrobial Resistance Pattern of Salmonella Infection | 22   |
| 2.5.2. The Resistance to Penicillins                                | 25   |
| 2.5.3. The Resistance to Cephalosporins                             | 26   |
| 2.5.4. The Resistance to Carbapenems                                | 26   |
| 2.5.5. The Resistance to Fluroquinolones                            | 27   |
| 2.5.6. The Resistance to Aminoglycosides                            | 28   |
| 2.5.7. The Resistance to Macrolides                                 | 28   |
| 2.5.8. The Resistance to Lincosamides                               | 29   |
| 2.5.9. The Resistance to Tetracyclines                              | 29   |
| 2.5.10. The Resistance to Phenicols                                 | 30   |
| 2.5.11. The Resistance to Rifampicin                                | 30   |
| 2.5.12. The Resistance to Glycopeptides                             | 31   |
| 2.5.13. The Resistance to Sulphur Drugs                             | 31   |
| 2.5.14. The Resistance to Polymyxins                                | 32   |
| 2.3. Antimicrobial resistant gene in Salmonella                     | 32   |
| 2.4. Diagnosis of Salmonella  | 33   |

| 2.4.1. Serological diagnosis   | 33 |
|--|----|
| 2.4.2. Molecular diagnosis   | 33 |
| 2.5. Pathological Findings   | 33 |
| 2.6. The Status of non-typhoidal Salmonella in Bangladesh  | 35 |
| 2.7. The Pathomicrobial studies on <i>Salmonella</i> sp infection in broiler chickens  | 35 |
| Chapter 3: Materials and methods   | 37 |
| 3.1 Study area   | 37 |
| 3.2 Sample collection duration   | 37 |
| 3.3. Biological sample collection  | 37 |
| 3.4. Data collection   | 37 |
| 3.5 Bacteriological Investigation  | 38 |
| 3.5.1 Isolation of <i>Salmonella</i> sp  | 38 |
| 3.5.2 Sub-culturing on blood agar  | 38 |
| 3.5.3. Preservation of isolates  |    |
|  | 38 |
| 3.6. Molecular detection of Salmonella   | 39 |
| 3.6.1 DNA extraction from the isolates   | 39 |
| 3.6.2 Identification of Salmonella by polymerase chain reaction (PCR)  | 39 |
| 3.7 Antimicrobial susceptibility testing (AST)   | 40 |
| 3.7.1 Detection of antimicrobial resistance genes  | 40 |
| 3.8. Histopathological study of <i>Salmonella</i> sp   | 41 |
| 3.8.1. Equipment and appliances for histopathology   | 41 |
| 3.8.2. Collection of samples and processing  | 41 |
| 3.8.3. Routine hematoxylin and eosin staining procedure  | 42 |
| 3.8 Statistical analysis   | 43 |
| Chapter 4: Results   | 44 |
| 4.1. Results of postmortem findings of <i>Salmonella sp</i> infection in broiler chicken                                     | 44 |
| 4.2. The histopathological examination of <i>Salmonella</i> sp infection in broiler chicken                                  | 44 |
| 4.3. Prevalence of <i>Salmonella</i> sp infection in farm level  | 45 |
| 4.4. Analysis of risk factors  | 45 |
| 4.4.1. Prevalence of <i>Salmonella</i> sp according to potential explanatory variables in different upazila in Chattogram    | 45 |
| 4.4.2. Univariable association of risk factors with the occurrence of <i>Salmonella</i> sp in broiler chickens at farm level | 47 |
| 4.4.3. The Antimicrobial resistance profile and percentage of multidrug resistance to <i>Salmonella</i> sp. isolates         | 49 |
| 4.4.4. The distribution of antimicrobial resistance genes  | 50 |
| 4.4.5. The results of growth characteristics of Salmonella sp in XLD   | 52 |

| and Blood agar   |    |
|--|----|
| 4.4.6. The result of DNA extraction, PCR and culture sensitivity test of | 53 |
| Salmonella sp  |    |
| Chapter 5: Discussion  | 54 |
| Chapter 6: Conclusion  | 60 |
| Chapter 7: Limitation: recommendations and future perspectives           | 61 |
| Appendix   | 62 |
| References   | 64 |
| Biography  | 78 |

# Frequently used abbreviation

| Abbreviation and | Elaboration  |
|------------------|--|
| symbols          |  |
| AMR              | antimicrobial resistance                             |
| MDR              | multidrug resistant                                  |
| %                | percent  |
| >                | greater than   |
| <                | less than  |
| 2                | greater than equal                                   |
| <u> </u>         | less than equal                                      |
| =                | equal to   |
| °C               | degree celsius                                       |
| BHI              | brain heart infusion                                 |
| bp               | base pair  |
| BPW              | buffered peptone water                               |
| CDC              | center for disease control and prevention            |
| CI               | confidence interval                                  |
| CLSI             | clinical and laboratory standards institute          |
| CRE              | carbapenem resistant enterobacteriaceae              |
| CSE              | centre for science and environment                   |
| CS               | culture sensitivity                                  |
| CVASU            | Chattogram veterinary and animal sciences university |
| DNA              | de-oxy ribonucleic acid                              |

| μL   | microliter                                  |
|------|---|
| mA   | milli ampere                                |
| MCR  | plasmid-mediated colistin resistance        |
| MFS  | major facilitator superfamily               |
| mL   | milliliter                                  |
| Mm   | millimeter                                  |
| MRSA | methicillin resistant staphylococcus aureus |
| OR   | odds ratio                                  |
| PCR  | polymerase chain reaction                   |
| Rpm  | rotation per minute                         |
| ST   | heat stable toxin                           |
| Stx  | shiga toxin                                 |
| TAE  | tris acetate edta                           |
| VTEC | verotoxigenic E. coli                       |
| WHO  | world health organization                   |
| w/v  | weight/volume                               |
| CIP  | ciprofloxacin                               |
| TE   | tetracycline                                |
| CRT  | ceftriaxone                                 |
| SXT  | sulfamethoxazole & trimethoprim             |
| CN   | gentamycin                                  |

## LIST OF TABLES

| Table 2.1 | Taxonomic names of subspecies   | 14 |
|-----------|---|----|
| Table 3.1 | The primer sequences for the polymerase chain reaction (PCR) used to identify genes for antibiotic resistance | 40 |
| Table 4.1 | Prevalence of Salmonella infection according to different factors at farm level                               | 46 |
| Table 4.2 | Univariable logistic regression of Salmonella infection in farm level   | 48 |
| Table 4.3 | The percentage of multidrug resistance to <i>Salmonella</i> spp. isolates (Broiler chicken, N=8)              | 49 |
| Table 4.4 | The occurrence of antimicrobial resistance genes among Salmonella isolates $[n = 8]$ from broiler chicken     | 51 |

### LIST OF FIGURES

| Figure 2.1 | Postmortem findings of Salmonella affected broiler chicken.<br>(A), (B)               | 44 |
|------------|---|----|
| Figure 2.2 | Diffuse necrosis in liver (A) and congestion and infiltration of lymphocyte (B, C, D) | 45 |
| Figure 4.1 | The antimicrobial resistance pattern of Salmonella isolates [n = 8]                   | 50 |
| Figure 4.2 | Salmonella sp on Xylose Lysine Deoxycholate   | 52 |
| Figure 4.3 | Salmonella sp on blood agar plates (A), (B)   | 52 |
| Figure 4.4 | Gram's staining properties of Salmonella sp   | 52 |
| Figure 4.5 | DNA extraction for the detection of Salmonella sp                                     | 53 |
| Figure 4.6 | PCR assay for the detection of Salmonella sp.   | 53 |
| Figure 4.7 | Bacterial zone of inhibition.   | 53 |
| Figure 4.8 | Bacterial zone of inhibition (A), (B)   | 53 |
| Figure 4.9 | Comparing inoculum with McFarland Standard  | 53 |

#### SUMMARY

Globally, antimicrobial resistance (AMR) is a public health concern, since antibiotics are among the most prescribed classes of drugs in humans and animals. Random use of antimicrobials in the poultry industry is considered as a contributing factor for AMR that can jeopardize human health through the potential dissemination of AMR pathogens. It is noteworthy that Salmonella is one of the bacterial groups considered to be of high priority in surveillance programs in the food chain and infectious diseases in poultry. Information on the circulation of Salmonella strains at the commercial poultry farm level is limited in many parts of the world. The present study aimed to determine the prevalence and stereotyping of Salmonella strains circulating in the broiler farm environment with their detailed AMR profiling. Pooled cloacal samples were collected randomly from commercial broiler farms in Chattogram district, Bangladesh. Then the standard bacteriological procedure was followed to isolate Salmonella sp, and identification was confirmed by the basis of morphology, cultural characters, and genus-specific polymerase chain reaction (PCR). After phenotypic characterization of the resistance profile against commonly used antibiotics by disc diffusion technique, all strains were screened by PCR for some selected resistance genes. Out of the 105 samples, Salmonella sp was isolated and identified from 8 samples. In antimicrobial sensitivity testing, 100% isolates showed resistance to ampicillin and amoxicillin, and 87.5% to gentamycin followed by tetracycline, and ciprofloxacin (75%), doxycycline (50%), Trimethoprim/Sulfamethoxazole, and Ceftriaxone (25%). The results of PCR assays revealed that all the eight isolates were carrying the *tetA* gene, the *tetB* and 16.67% the *tetC* gene. The prevalence of the isolates bearing the *Sul-I* gene, *blaTEM*, *blaCTX-M* were 100%, 87.5%, and 50%, respectively. The present study was conducted to find out the prevalence of poultry Salmonella in broiler chickens and to find out that there is a great risk to securing healthy poultry products due to the circulation of the multi drug resistant (MDR) Salmonella sp.

Keywords: Prevalence, Antimicrobial resistance, tetA, tetB, tetC, sul-1, blaTEM, blaCTX-M gene,