Table of Contents

List of Figures

List of Tables

Acknowledgement

All the praises and deepest sense of gratefulness belongs to the Almighty ALLAH, the Merciful, the omnipotent, and the Beneficent but the supreme Ruler of the Universe Who enabled me to complete my work successfully for the Internship program.

It is my great privilege to express deep sense of gratitude and heartfelt indebtedness, sincere appreciation and profound regards to my honorable supervisor Associate Professor **DR. Mohammad Mahmudul Hassan** for the enormous support, supervision, advice, guidance and for offering numerous valuable ideas and suggestions, who has devoted hours and hours of time and patience, motivation, and immense knowledge for me. His guidance helped me during every steps of this study from its inception to completion.

The author deems it a proud privilege to acknowledge his gratefulness, heartfelt gratitude and best regards to **DR. Mahbub Alam,** Lecturer, Department of Animal science and Nutrition, Chittagong Veterinary and Animal Sciences University for his continuous inspiration and constant valuable suggestions.

The author expresses his profound gratitude, gratefulness and heartfelt thanks to his friend **Tofazzal Md. Rakib** for his factual advice, kind cooperation and continuous help during the study.

The author is immensely grateful to his all other friends and well-wishers, although it is not possible to mention every one by name.

The Author

List of Abbreviations

%	Percentage
±	plus-minus
AMR	Antimicrobial Resistance
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
cm	Centimeter
CS	Culture Sensitivity
DA	Clindamycin
E	Erythromycin
E. coli	*Escherichia coli*
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
I	Intermediate sensitive
K	Kanamycin
KF	Cephalothin
MDR	Multiple Drug Resistance
mg	Milligram
ml	Milliliter
mm	Millimeter
NIAID	National Institute of Allergy and Infectious Diseases
NO.	Number
OX	Oxacillin
P	Penicillin
PCR	Polymerase chain reaction
PRTC	Poultry Research and Training Center
R	Resistance
S	Sensitive
S	*Salmonella*
SS	*Salmonella-Shigella*
TSI	Triple Sugar Iron
UK	United Kingdom
US	United States
WHO	World Health Organization
XLD agar	Xylose lysine Deoxycholate agar
β-lactam	Beta-lactam

Abstract

Salmonellosis is one of the most common, zoonotic diseases and presence of antimicrobial resistant *Salmonella* in wild birds is a global public health threats. Throughout the last decade, multi-drug resistance of *Salmonella* spp. has increased, particularly in developing countries. Therefore, a cross-sectional study was conducted to investigate prevalence of *Salmonella* spp. and antimicrobial resistance pattern against *Salmonella* spp. from two species of wild birds such as Crow and Asian pied starling. Samples were collected from cloacal swabs of Crows (*Corvus splendens*) and Asian pied starling (*Gracupica contra*) for isolating *Salmonella* spp. (bacteriological culture methods) followed by antimicrobial susceptibility testing (disk diffusion method) against *Salmonella* spp. isolates during the period March to December, 2014. The prevalence of *Salmonella* in Asian pied starling and Crows were (67%) and (65%), respectively. Within the category of samples from different species, the variation in prevalence were not varied significantly ($p>0.05$). Isolated *Salmonella* spp. was tested for resistance to six different antimicrobial agents. Among six antimicrobial tested 100% resistance were found to Penicillin, Oxacillin and Clindamycin followed by Erythromycin (50-93%), Kanamycin (7-20%) and Cephalothin (30-67%) from both species of birds. Kanamycin remained sensitive in (70-73%), Cephalothin (26-70%) and Erythromycin appeared to be (0-30%) sensitive against *Salmonella* spp. isolates. *Salmonella* isolates was multidrug resistant up to three of the six antimicrobials tested. The high resistance of Penicillin, Oxacillin and Clindamycin to *salmonella* spp. of crow and Asian pied starling of present study might be due to indiscriminate use of these antibiotics to human as well as in livestock and poultry. In addition, sometimes farmers select drugs by their own or rely on neighbor experienced farmers or non-veterinarians and these mal-practices may not always ensure proper drugs doses, frequency of drug administration and complete course of drug treatment. In conclusion, it can be said that the rational use of antibiotics need to be adopt in treatment of disease for livestock, poultry and human of Bangladesh to prevent the emergence of drug resistance to *Salmonella* spp.

Key Words: Antimicrobial, Prevalence, Resistance, *Salmonella*, wild birds

Chapter-1: Introduction

Infectious microbial diseases involve a principal cause of death in many parts of the world, chiefly in developing countries. *Salmonella* has been renowned as an significant food and water-borne pathogen that can contaminate human and animals consequential in major morbidity and mortality (Akkina et al., 1999). The emerging dominance of poultry in food Animal production worldwide has borne witness to the emergency of the diagnosis of avian diseases from a comparative medical perspective to becoming a necessity for the well-being of poultry health, the global agricultural economy, and consumer safety. Poultry sector plays an important role in our national economy. Several constraints among which occurrence of diseases seriously affect the optimal performance of poultry industry in Bangladesh (Haque et al., 1991). Immediate challenge is necessary to find a definitive primary diagnosis and appropriate treatment for the presenting problem. Avian *Salmonella* infections are important as both a cause of clinical disease in poultry and as a source of food borne transmission discuss to humans. *Salmonella* is a natural harbor in common wild bird which is responsible for spreading of the disease salmonellosis both in livestock and public health issue. *Salmonella* is one of the most important zoonotic pathogens and one of its major sources is poultry. *Salmonella* is one of the major bacterial agents that cause foodborne infections in humans worldwide (Herikstad et al., 2002). Most of the infections are zoonotic in origin but some serotypes like *Salmonella typhi* and *Salmonella paratyphi* infect only humans (Steve Yan et al., 2004). Salmonellosis has been found to be major infectious diseases of all ages of birds. These diseases occur sporadically or enzootically in most of the countries of the world including Bangladesh. The majority of economic loss results from the mortality and reducing productivity for the affected birds (Otaki, 1995). Salmonellosis is a major problem in poultry industries of Bangladesh and its prevalence ranged from 28-53.3% (Bhattacharjee et al., 1996; Rahman et al., 2004b; Mahmud et al., 2011). This disease declines egg production (AHMNA et al., 1998) and also causes huge mortality in birds (Kamal and Hossain, 1989; Islam et al., 2006; Ahmed et al., 2008). The probabable cause for spreading of this disease can be *Salmonella* carrying wild birds, from them *Salmonella* spread into livestock and human. Among food-borne bacterial zoonotic diseases Salmonellosis causes huge economic losses in terms of massive mortality and morbidity (Hafez, 2011). The members of the genus *Salmonella* are being isolated, identified and characterized by using various cultural, biochemical, serological and molecular techniques. The reliable Methods for isolation require

the use of media, which encourage the growth of *Salmonella* and inhibit that of other enteric organisms (Deignan et al., 2000). *Salmonella typhimurium* and *Salmonella enteritidis* are prevalent both in poultry and human and categorized as zoonotic hazards (Mahmud et al., 2011). Contamination of *Salmonella* may be prevalent in farm environment. One possible cause of *Salmonella* contamination in developing countries is presence of wild birds with the domestic one. The disease is endemic in many developing countries particularly the asian subcontinent, south and central america (Mandell et al., 2009). Infections with bacteria of the genus *Salmonella* are responsible for a variety of acute and chronic disease of poultry has been reported in Bangladesh (Kamal and Hossain, 1989). In recent years problems related to *Salmonella* have increased significantly, both in terms of the incidence and severity of cases of human salmonellosis.

Antimicrobial resistance, the ability of microorganisms to withstand antimicrobial agents such as antibiotics, is an important and growing public health issue. The emergence of bacteria resistant to antibiotics is common in areas where antibiotics are used (Schwartz et al., 2003). Antibiotics are used extensively to prevent or to treat microbial infections in human and veterinary medicine. Apart from their use in aquaculture, they are also employed to promote more rapid growth of livestock (Kümmerer and Henninger, 2003). In the United States, it is estimated that the amount of antimicrobials used in food animal production is greater than the amount used in humans. The FDA has communicated that about 28.8 million pounds of antibiotics were sold and distributed for use in food animals in 2009 (Food and Administration, 2010). Heavy use of antibiotics for medical and veterinary purposes (White et al., 2000; Balagué and Véscovi, 2001) as well as the domestic and agricultural use of pesticides and related compounds (Balagué and Véscovi, 2001) caused significant antibiotic contamination of the natural environment and consequent development of resistance in communities. However, over the years bacteria that were once controlled by these drugs have developed resistance so that common infections in humans can cause significant harm and even death. The emergence of antimicrobial-resistant *Salmonella* strains is of great concern world wide (Rowe et al., 1990). Since the beginning of the 1990s, strains of *Salmonella* which are resistant to a range of antimicrobials including the first choice agents for treatment of humans have emerged and are threatening to become a serious public health problem. Drug resistant *Salmonella* emerge in response to antimicrobial usage in humans and in food animals and selective pressure from the use of antimicrobials is a major driving force behind the emergence of resistance. Multi-drug resistance to critically important antimicrobials is compounding the problem (Organization, 2005). There are reports of high prevalence of

resistance in *Salmonella* isolates from countries such as Taiwan (Lauderdale et al., 2006), India (Mandal et al., 2004), The Netherlands (Van Duijkeren et al., 2003), resistant isolates from France (Weill et al., 2006), Canada (Poppe et al., 2006), and Ethiopia (Molla et al., 2004). Similarly, there are various reports of multi-drug resistant *Salmonella* organisms isolated in Bangladesh (Ahmed et al., 2010; Begum et al., 2010).

Antimicrobial resistance is an increasingly global problem, and emerging antimicrobial resistance has become a public health issue worldwide (Kaye et al., 2004). A variety of foods and environmental sources like world birds harbor bacteria that are resistant to one or more antimicrobial drugs used in human or veterinary medicine and in food-animal production (Bager and Helmuth, 2001; Anderson et al., 2003; Schroeder et al., 2004). One of the ways multi-resistant bacteria may be introduced into the biocoenosis and into humans via environment (Bohm et al., 2004). One of the studies indicated a rise in the antibiotic resistance found in *Salmonella typhi* (Gautam et al., 2002). In recent years, antibiotic resistance in *Salmonella* has assumed alarming proportions (Murugkar et al., 2005) and the isolates were resistant to at least one of the 15 antibiotics tested. Moreover, antibiotic treatment is considered the most important issue that promotes the emergence, selection and spreading of antibiotic-resistant microorganisms in both veterinary and human medicine (Neu, 1992; Witte, 1998). Antibiotic used as a prophylactic and growth promoter in many developing countries including Bangladesh to treat and prevent bacterial and protozoal infections and diseases (Schwarz et al., 2001; Molla et al., 2004; Enabulele et al., 2010). In veterinary practice, antibiotics are used in livestock production, disease prevention and as growth-promoting feed additives. Due to indiscriminate use of antimicrobials, they are randomly become resistant against different infectious diseases. The use of antibiotics in animals disrupts normal flora of intestine, resulting into emergence of antibiotic-resistant *Salmonella* and their prolonged fecal shedding into the environment. Such practice has led to misuse of antibiotics with the associated high prevalence of antibiotic resistance among isolates from animal and food sources (Enabulele et al., 2010). The fatality rate in people infected with antibiotic-resistant *Salmonella* is 21 times greater than that infected with non-antibiotic resistant *Salmonella* strains. The resistant bacteria of production animal and human often transmitted to the environmental organism though there is a little chance of exposure in nature. A growing body of evidence implicates environmental organisms as reservoirs of these resistance genes; Antibiotic resistance, evolving and spreading among bacterial pathogens, poses a serious threat to human health. However, resistance has also been found in the absence of antibiotic exposure, such as in bacteria from wildlife (Tacão et al., 2012;

Wellington et al., 2013). The exceptional capability of bacteria to build up resistance mechanisms to antimicrobial agents has, from a human health point of view, unspecified catastrophic proportions, rendering more and more infections that are complicated or not possible to treat (Levy and Marshall, 2004). Antibiotics are used to treat or check infections in animals and humans. In the agriculture setting they are also used as growth promoters for food-producing animals, to check infection in fruit trees and in aquaculture. Most reports propose that the main force behind emergence of drug resistance is the use, misuse, and abuse of antimicrobial agents throughout the past decades, but there is also confirmation that epidemic spread of drug-resistant bacteria could be a contributing factor (Livermore, 2003). However, antibiotic-resistant bacteria have been found in hosts and environments apparently free from any antibiotic pressure imposed by man (Gilliver et al., 1999). Most research on the epidemiology of antibiotic resistance spreading has paying attention on human and veterinary medicine, but there is a rising interest to recognize how bacterial resistance is transferred within reservoirs in natural environments (Allen et al., 2010). Due to their diversity in migratory patterns and ecological niches, and their ease in picking up human/environmental bacteria, they act as mirrors of human activities. In addition, bird migration provides a possible mechanism for the establishment of new endemic foci of disease at great distances from where an infection was first acquired (Reed et al., 2003). Besides, due to large range of migration and flying of wild birds close to the human settlement may cause drug resistance. Some oppurtunistic and non-structured studies had been conducted for *Salmonella* in commercial poultry in Bangladesh. Very few studies was conducted isolation and drug resistance in *salmonella* spp. throught the world from wild birds but none in Bangladesh. This study therefore aimed to investigate the prevalence of salmonella spp. and antimicrobial resistance pattern against *Salmonella* isolates from wild birds.

1.1 Aims and objectives

The main objective of this study was to investigate the occurrence of *Salmonella* spp. in two species of wild birds, and antimicrobial sensitivity testing of those isolates to determine resistance pattern. The following specific objectives were fulfilled by the present research:

- To estimate prevalence of *Salmonella* spp. in two species of wild birds (Crow and Asian pied Starling)
- To determine antimicrobial resistance pattern of isolated *Salmonella* spp. from those wild birds.

1.2 Significance of the study

Salmonella spp. was recognized as a major cause of food-borne illnesses in many countries that are closely associated with the presence of wild birds with domestic one where wild birds was a major carrier for this *Salmonella* spp. No avilable studies had been conducted for *Salmonella* of wild birds in Bangladesh and very few in worlds. Some sporadic studies had been attempted to determine *Salmonella* spp., its antimicrobeal resistence from poultry in our country. Lack of data on the prevalence of *Salmonella* spp. in wild birds was increases and the incidence rate of those diseases also increases. The study will help to know the prevalence of *Salmonella* spp. in some common wild birds. On the otherhand, an attempt has been taken to analysis of antibiotic resistant in *Salmonella* spp. isolated and identified from wild birds sample. The identification of antimicrobial resistant *Salmonella* will help for appropriate therapy and provide information on the auxiliary emergence of drug resistance.

Chapter-2: Review of Literatures

2.1 Characteristics, taxonomy and nomenclature of *Salmonella*

Salmonella like other *Enterobacteriaceae* are motile, non-spore former. *Salmonella* reduce nitrates to nitrites and negative in oxidase. *Salmonella* have been known to cause illnesses for more than 100 years when it was discovered by Dr. Daniel Salmon (Steve Yan et al., 2004)**.** *Salmonella* is a facultative anaerobe, Gram-, rod shaped, 2-3 x 0.4-0.6µm in size and motile by peritrichous flagella excluding for *S.* Gallinarum and *S.* Pullorum which are immotile. They are urease and Voges-Proskauer negative and citrate utilizing (Montville and Matthews, 2008). *Salmonella* are typically non-lactose, non-sucrose fermenting but are able to ferment glucose, maltose and mannitol with the manufacture of acid only as in the case of *S.* Typhi and acid with H_2S in the case of *S.* Paratyphi and for most other *Salmonella* serovars (Cruickshank et al., 1975). Optimum temperature for development is in the range of 35 – 37ºC but a few can rise at temperatures as high as 54ºC and as low as 2ºC. *Salmonella* rise in a pH range of 4-9 with the best being 6.5-7.5. They need high water action for growth (> 0.94) but are able to survive at a_w of < 0.2 for example in dried foods. Reserve of growth occurs at temperatures < 7ºC, pH < 3.8 or a_w < 0.94 (El Hussein et al., 2012). Based on differences in 16S rRNA sequence data, the genus *Salmonella* is separated into two species: *S. enterica* and *S. bongori*. *S. enterica* is further divided into six subspecies: subspecies *enterica, salamae, arizonae, diarizonae, houtenae* and subspecies (Popoff and Le Minor, 2001). Kauffmann-White scheme classifies members of *Salmonella* species according to three major antigenic determinants composed of somatic (O-antigens), flagellar (H-) and virulence (K-) antigens. Agglutination by antibodies definite for the diverse O-antigens, groups the *Salmonellae* into six sero-groups: A, B, C_1, C_2, D and E. Hardly ever cross reactivity between O-antigens of *Salmonella* and other genera of *Enterobacteriaceae* do happen. Consequently more categorization of serotypes is based on the very specific H-antigens (Scherer and Miller, 2001). All *Salmonella* strains are serologically classified using Kauffmann-White scheme, and at the present the genus contains more than 2,500 serotypes (Popoff et al., 2003; Tindall et al., 2005). The majority of the *Salmonella* serotypes belong to *Salmonella enterica* subsp, *enterica* (about 60%), followed by subspecies *salamae* (20%), *diarizonae* (13 %), *arizonae* (3.8 %), *houtenae* (2.8%) and *indica* (0.45%). Only (0.8%) belong to the second species *Salmonella bongori* (Pignato et al., 1998). Strains that belong to *Salmonella enterica subsp*.I (*Salmonella enterica* subsp. *entericae*), are frequently pathogenic to humans and mammals while those belonging to subspecies II, IIIa, IIIb, IV, VI and

Salmonella bongori are usually isolated from reptiles and other cold blooded animals (Brenner et al., 2000).

2.2 Epidemiology

In many countries the incidence of human *Salmonella* infection has increased markedly over the year. In the United States, *Salmonella* serotypes affect approximately 1.4 million persons and cause 500 to 2,000 deaths each year (Mead et al., 1999). In 2005, Centers for Disease Control and Prevention documented 36,184 *Salmonella* cases in the U.S. and found that the two most common serotypes isolated from human sources were *Salmonella enteritidis* and *Salmonella typhimurium* (Control and Prevention, 2007). In the last decade a dramatic increase in infections caused by *Salmonella* has been registered in several countries in Europe as well as in North and South America. During the period from 2000 to 2002, a total of 376,856 human and 65,789 nonhuman *Salmonella* isolations were reported into the World Health Organization (WHO) from many countries in the world including Africa, Asia, Latin America and the Caribbean, Europe, North America, and Oceania (Galanis et al., 2006). North America and Europe accounted for 87.9% (389,134) of all reported isolates. The most common *Salmonella* serotypes isolated from human sources were *Salmonella enteritidis* was by far the most common serotype reported from human isolates globally. It accounted for 65% of all isolates, followed by *Salmonella typhimurium* at 12% and *Salmonella newport* at 4%. Among nonhuman isolates, *Salmonella typhimurium* was the most commonly reported serotype accounting for 17% of isolates followed by *Salmonella heidelberg* (11%) and *Salmonella enteritidis* (9%) (Galanis et al., 2006). A total of 565,042 human and 102,113 non-human *Salmonella* isolates were reported into the (WHO) in many countries in the word and found that the most common *Salmonella* serotypes were *Salmonella enteritidis* and *Salmonella typhimurium* (Salm-Surv and Organization, 2006). In developing countries, including Bangladesh there are no sufficient data available on *Salmonella* infection and the likely sources of Salmonellosis due to limited epidemiological studies. Further, where incidence data are available these are frequently out dated. In addition, under-reporting of cases and the presence of other infectious diseases considered to be of high priority may have also overshadowed the problem of Salmonellosis.

2.3 Salmonellosis

Salmonellosis is primarily a food-poisoning syndrome, which occurs when ingesting pathogenic *Salmonella* serotypes. The cause of food-borne Salmonellosis is the penetration and passage of *Salmonella* organism from the gut lumen into the epithelium of the small intestine where inflammation occurs. There is also evidence that the pathogenesis may involve two toxins; an enterotoxin and a cytotoxin (Jay et al., 2005)**.** Salmonellosis is an infectious disease in both humans and animals. Human infections are usually associated with animal contact and the consumption of contaminated food products such as poultry, meat and other dairy products (Uyttendaele et al., 1998). Salmonellosis is usually considered as self-limiting illness, but it can also become invasive and fatal, especially for patients who are young or immune-compromised (Wilson et al., 2003)**.** Non-typhoidal *Salmonella* strains are important causes of infections in both humans and animals. This disease is caused by *Salmonella* serotypes other than *Salmonella typhi* and *Salmonella paratyphi*. It is a major food-borne infection with worldwide distribution. The majority of cases are self-limiting gastroenteritis (Kariuki et al., 2002). The clinical symptoms usually appear 8 to 72h after contact with the pathogen. The typical symptoms are usually nausea, vomiting, abdominal pain and diarrhea with or without fever. Few (<5%) of the patients develop invasive *Salmonella* infections or bacteremia and about 10% of those with invasive disease develop localized infections (Steve Yan et al., 2004). During the past decade, there had been a significant world-wide increase of non-typhoidal Salmonellosis especially in industrialized countries including, the United Kingdom, Germany, France, Austria, Denmark, and the United States of America. In the US, 1.3 million illnesses and 400 to 600 deaths were occurred in each year (Mead et al., 1999). The most common serotypes responsible for the disease are *Salmonella enteritidis* and *Salmonella typhimurium* (Herikstad et al., 2002).

2.4 *Salmonella* Status in developing countries

Poultry and its products have always topped the incidence of Salmonellosis in many developing countries including India, Egypt, Brazil and Zimbabwe (Dahal et al., 2007) and is the most seriously perceived food risks in chicken meat, even in the developed countries (Yeung and Morris, 2001). The reported prevalence of *Salmonella* in chicken carcasses in South Asian countries varies from country to country. Studies in northern Thailand revealed 57% prevalence in chicken meat at the market during 2002-2003 (Padungtod and Kaneene, 2006), and 14.5% prevalence in Kathmandu, Nepal (Maharjan et al., 2006). Sero-prevalence

of poultry *Salmonella* in Bangladesh has been reported to be 23.46% (Sikder et al., 2005). Not much literature has been available on the prevalence of *Salmonella* in chicken carcasses from India, few researches reports negligible to as low as 5% (Rahman et al., 2004a), to a prevalence of 69% (Bajaj et al., 2003). However, the overall annual incidence of food borne Salmonellosis in India is nearly 6 per 1000 inhabitants (Dahal et al., 2007).

2.5 Salmonellosis outbreaks throughout the world

Salmonella enteritidis emerged as a pathogen of poultry in the mid-1970s, but later became an important human pathogen. A human *Salmonella enteritidis* infection showed a dramatic increase since the 1980s, and has become the most commonly isolated serotypes in many countries (Rabsch et al., 2001). *Salmonella enteritidis* came to prominence as a major food-borne pathogen in Europe and America during the 1980 (Velge et al., 2005). In 1997, it was implicated in over 70% of cases of human Salmonellosis in England and Wales (Cogan and Humphrey, 2003) and, despite a recent decline in incidence, it is the serotype most commonly isolated from gastrointestinal infections in the United Kingdom and remains among the most significant *Salmonella* serotypes in public health elsewhere, including North America (Control and Prevention, 2006). In the United States food-borne *Salmonella* are estimated to cause approximately 1.3million illnesses, 15,000 hospitalizations, and 500 deaths each year. About 300,000 of these illnesses may be attributable to *Salmonella enteritidis* (Mead et al., 1999)**.** During the 1980 and 1990, *Salmonella enteritidis* emerged as an important cause of human illness in the United States, and the rate of *Salmonella enteritidis* isolates reported to CDC increased from 0.6 per 100,000 populations in 1976 to 3.6 per 100,000 in 1996. There were a total of 997 reported outbreaks of *Salmonella enteritidis* infection in the United States from 1985-2003, which resulted in 33,687 illnesses, 3,281 hospitalizations, and 82 deaths. The number of reported outbreaks of *Salmonella enteritidis* infection in the United States increased from 26 in 1985 to a high of 85 in 1990, with a gradual decrease thereafter to 34 outbreaks in 2003. In addition, the number of cases in outbreaks each year has decreased, from a high of 2,656 in 1990 to a low of 578 cases in 2003. A food vehicle was confirmed in 44% of outbreaks of *Salmonella enteritidis* infection in the United States. *Salmonella enteritidis* was recognized as a public health problem in Northeastern states during the 1980s and has since spread throughout the United States During 1985-1998, state and territorial health departments reported 796 *Salmonella enteritidis* outbreaks that accounted for 28,689 illnesses, 2839 hospitalizations, and 79 deaths. In United States, during the period 1990-2001,

state and territorial health departments reported 677 *Salmonella enteritidis* outbreaks, which accounted for 23,366 illnesses, 1,988 hospitalizations, and 33 deaths (Control and Prevention, 2004). The incidence of *Salmonella enteritidis* increased several years later in Denmark than in the United States, the United Kingdom and central Europe and found that the incidence of *Salmonella enteritidis* infection among humans increased during the 1990s, and the epidemic peaked in 1997 with an incidence of 70 % reported cases per 100,000 population. On the basis of systemic phage typing of isolates collected from humans and from food chain (Mølbak and Neimann, 2002). Recent empirical evidence suggests that small mammals and birds may also be a significant source of *S. enterica* contamination in animal feed, which by itself is capable of accounting for the prevalence of clinical salmonellosis seen in cattle herds (Carlson et al., 2011).

2.6 Detection techniques for *Salmonella* spp. in wild birds

Conventional culture methods used for the isolation of *Salmonella* spp. from Cloacal swab of birds include, nonselective pre-enrichment followed by selective enrichment and plating on selective and differential agars. Suspect colonies are then confirmed biochemically and serologically. These methods are time consuming and take approximately 4-7 days (Hammack et al., 1999). Since *Salmonella* are closely related to both public and animal health, more rapid and sensitive methods for the identification of this bacterium are required**.** More recently, a number of alternative methods for the detection of *Salmonella* in foods have been developed including, immunoassays, nucleic acid hybridization and polymerase chain reaction (PCR) techniques (Axelsson and Sorin, 1997). The primary advantages of PCR tests are increased sensitivity and less time required to process samples in the laboratory when compared to standard culture methods (Wang and Yeh, 2002).

2.7 Salmonellosis in wild birds

Salmonella bacteria, especially *S. enterica* serotype *typhimurium* are commonly found in the intestine of wild bird (Tizard, 2004). These organisms are maintained within bird populations by several mechanisms. The simplest of these mechanisms occurs in birds since that eat other animals risk eating *Salmonella* infected prey. Both wild and captive birds may be temporary or permanent *Salmonella* carriers or even suffer from clinical salmonellosis as a result of eating infected prey. A similar infection pathway affects scavenging or carrion eating birds such as vultures, crows and most importantly Asian pied starling. For example Crows and Asian pied starlings are opportunistic scavengers who feed at sites where raw sewage is

released. They appear to be relatively resistant to disease but may serve as effective carriers of *Salmonella* and thus a source of infections for other animals. The infected or carrier bird of salmonellosis may transmit infection to human either directly as a result of handling or more commonly as a result of exposure to domestic cat infected by preying or sick and morbid birds. In wild birds *Salmonella* may cause disease and death or even spread from their avian hosts to domestic mammals and man. Although well recognized as an avian disease for over an hundred years, salmonellosis qualifies as an emerging disease because its prevalence in wild bird populations appears to have increased greatly over the past 40 years as a result of artificial feeding by humans. Salmonellosis spread from wild birds to other carnivore, herbivore or omnivore by contaminating feed and water. As enteric bacteria *Salmonella* are found within the intestine. They spread to other individuals either because the intestine is eaten, for example by a predator or alternatively they are shed in feces. Both types of spread by carnivorism or by fecal contamination are common among wild birds. *Salmonella* may present in wild birds for two reasons. In one case, the organism is adapted to the host and established itself as a part of the intestinal flora on a permanent basis. In second situation the *Salmonella* may be present in feces for a short time as a result of environmental contamination. It is to be probable that carrion feeding birds such as crows will also from time to time eat *Salmonella* infected carcass (Tizard, 2004).

2.8 Importance of birds and their movements as Vectors for disease

Birds are well known vectors for pathogenic microorganisms that could cause disease in humans. The importance of birds in the spread and lifecycle of e.g. influenza A virus, West Nile virus and Lyme's disease are well established (Horimoto and Kawaoka, 2001). Birds are also known to be able to harbor enteropathogens as *Salmonella* spp. and *Campylobacter* spp. Many bird species undergo considerable migrations, often involving the crossing of continents. The phenomenon of bird migration creates the potential for the establishment of new endemic foci of disease along the migration routes. The emergence of the West Nile Virus in the USA is a striking example of how quickly a new zoonotic disease can become widely dispersed (Reed et al., 2003). The most commonly reported enteropathogen reported from wild birds are *Salmonella* spp. and several routes of (re-)transmission to humans has been suggested, e.g., sparrows feeding on bird feeders or in close relation to farms, or gulls feeding on grazing fields (Hudson et al., 2000). These and a number of similar routes of transmission are plausible transfer mechanisms of antibiotic-resistant enterobacteria from wild bird to humans. To study birds and their movements as possible potential mechanisms

for dispersal of e.g. antibiotic-resistant bacteria it is important to understand the dynamics and complexity of bird movements. For convenience different patterns of movements has been divided into six main types (Del Hoyo et al., 1992). Apart from the studies in this, bird migration and movements has been suggested in the dispersal of antibiotic-resistant *Salmonella* spp. (Palmgren et al., 1997), vancomycin resistant enterococci (Drobni et al., 2009), and antibiotic-resistant *E. coli* (Literak et al., 2007).

2.9 Prevalence of *Salmonella* in wild birds

Asagi and co-workers isolated serotypes *typhimurium Var Copenhagen* at the year of 1967 from 2/30 healthy crow in japan. This Scavenges may also transmit the infections to other animals, For example watts and wall investigated an outbreak of salmonellosis in sheep in Australia where affected sheep had been isolated within one pasture for a long time and no new animals had been introduced. Serovar *typhimurium* was however isolated from crows in the affected pasture and they surmised that the birds were the source of infection. The weather had been hot and dry, both birds and sheep had congregated at the limited sources of the water. They suggested that the water supply had been fouled by bird feces. In a study it was shown that the birds readily picked up infection by feeding on infected sheep carcass. Once infected, crows shed the *salmonella* in their feces for upto 27 days (Tizard, 2004). In a recent Spanish study 13/310 (4.19%) wild free living raptors were positive for *salmonella*. These birds were infected with many different types of *salmonella* strains. The pattern of multiple serotypes indicates that the infections were acquired from a wide variety of sources. *Salmonella* serotype *Aagar* has been isolated from peregrine falcons (*Falco peregrines*) in Sweden. In North America, kirk patricks and trexler myren examined raptors and found 2 positive out of 150 fecal samples. Nocturnal raptors such as owls may also acquire infection in this way. Thus kirk Patrick and colvin[7] in new jersy found 8/94 (8.5%) in barn owls (*Tyto alba*) *salmonella* positive. In a large Spanish survey 21/286 (7.36%) of captive raptors were *salmonella* positive. Quessy and messier tested ring-billed gulls (*L. delawarensis*) in Qubec and found 8.7% of cloacal swabs were salmonella positive. Snoeyenbos and coworkers examined cloacal swabs from herring gulls (*L. argentatus*) in Massachusetts and found 10/405 positive to *salmonella*. Selbitz in Germany found 42/852 (4.9%) were positive for *salmonella* at the species of black headed gull (*L. ridibundus*). Literak and coworkers found 20%-36% *Salmonella* positive in juvenile gulls. Hubalek and coworkers found 24.7% *Salmonella* positive in panama isolated from gulls. British investigators have also found 22.2% positive to *Salmonella* from fecal sample of gull. Butterfield and coworkers in herring

gull of North east of England found that the prevalence of *Salmonella* increased 2.1% in 1975-76 to 8.4% in 1979. Muller in hamburg found 16% of wild duck feces and 30% of pigeon feces were positive to *Salmonella*. Maeda and coworkers found 15% of fecal sample from healthy sparrow were *Salmonella* positive. Morishita and coworkers collected cloacal swab from 1709 healthy passerines in ohio, they isolated *Salmonella* 4/373(1.07%) from house sparrow and 62/868 (7.1%) from European starling (*Sturnus vulgaris*) at the year of 1999. Sharma and coworkers examined 799 wild birds in india and isolated 20 birds positive to *Salmonella*. Snoeyenbos and coworkers examined several black bird roosts in Massachusetts in healthy birds and found 11/299 cowbirds, 2/108 common grackles and 13/148 from European starling were positive to *Salmonella* at the year of 1967. In United States 892 birds were captured and *Salmonella* spp. was isolated from 22 birds. The prevalence by dairy ranged from 0.7 to 16.7%, whereas the prevalence by bird species ranged from 1.2-3.2%. Cowbirds and English sparrows had the highest prevalence of *Salmonella* organisms. Five serotypes of *Salmonella* organisms were isolated, including *Meleagridis, Montevideo, Muenster, Typhimurium*, and an untyped serotype (Kirk et al., 2002). Gastrointestinal tract samples tested positive for S. enterica 2.5% prevalence in European starling (Carlson et al., 2011).

2.10 Antimicrobial resistance

Antibiotic resistance is a relatively new term. A bacterial strain can be defined resistant if it survives in the presence of higher antibiotic concentrations in comparison with phylo-genetically related strains (Guardabassi et al., 1998). Antibiotic resistance in microorganisms is either genetically inherent or the result of the microorganism being exposed to antibiotic. Most of the antibiotic resistance has emerged as a result of mutation or through transfer of genetic material between microorganisms. Antibiotic resistance is not a bacterial property that can be determined by studying a single strain, but only by comparison under identical conditions of two or more strains belonging to the same species. The above mentioned definition of antibiotic resistance refers to *in vitro* conditions. Under *in vivo* conditions, antibiotic resistance is a context dependent term as it depends on the location of the bacterium and the bioavailability of the drug. Bacteria are less susceptible to antibiotics when assembled in compared with the same organisms living separately (Guardabassi et al., 2000). In aquatic environments, binding of the antibiotic molecule with ions or substances present in sediment strongly reduces both the activity of the drug and its absorption in the intestine (Guardabassi and Dalsgaard, 2002). A broad variety of biochemical and physiological

mechanisms are responsible for the development of resistance. Recent studies of almost 400 different bacteria have demonstrated about 20,000 possible resistance genes (Davies and Davies, 2010). Drug resistance in food borne bacterial enteric pathogens is an almost inevitable consequence of the use of antimicrobial drugs in food-producing animals, and specifically in the developing countries by use of medicines in humans (Threlfall et al., 2000; van den Bogaard and Stobberingh, 2000). A major concern is that the high levels of antibiotic resistance are a result of the use of antibiotics in food animals. A recent estimate in the United States suggests that 24.6 million pounds of antibiotics are given to animals each year as growth promoters at sub-therapeutic amounts in their feed compared to 3 million pounds consumed by humans (Oldfield III, 2003).

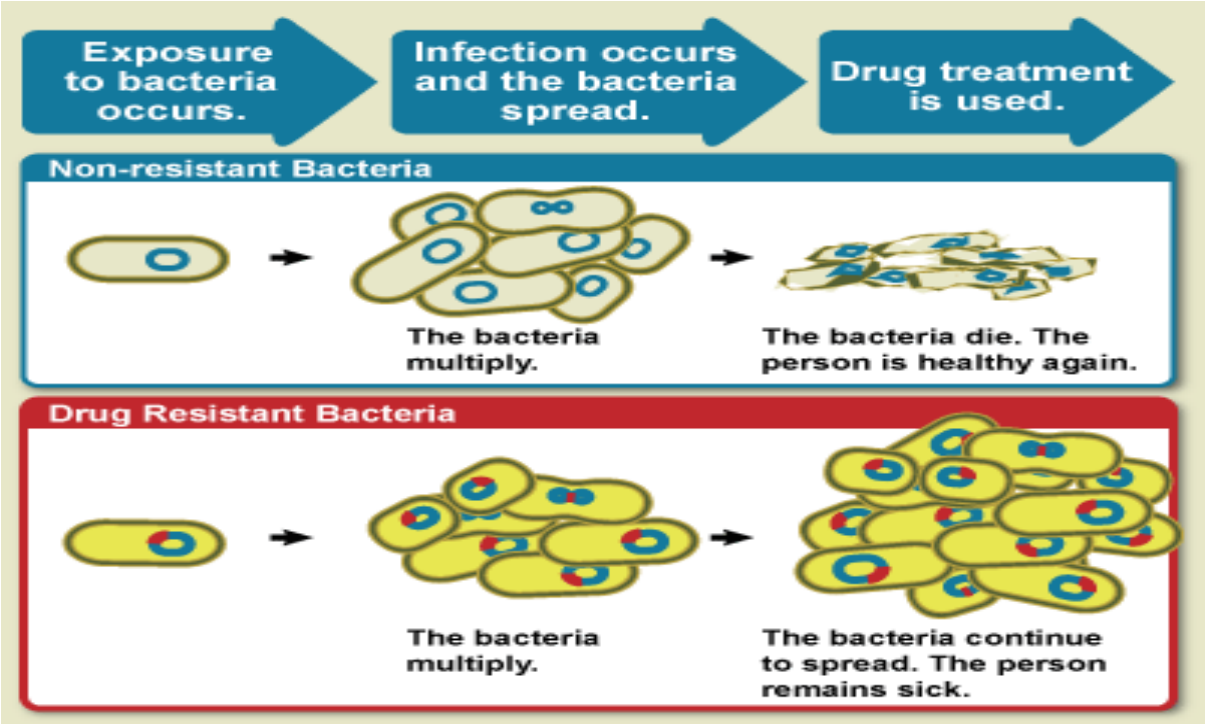


Figure 1: Diagram showing the difference between non-resistant bacteria and drug resistant bacteria. Non-resistant bacteria multiply, and upon drug treatment, the bacteria die. Drug resistant bacteria multiply as well, but upon drug treatment, the bacteria continue to spread (NIAID, 2009)

In recent years the emergence and global dissemination of multi-drug resistant typhoidal strains has posed major public health problems in the developing countries, and over the past decade it has assumed epidemic proportions in South Asia (Okeke et al., 2005).

Antimicrobial resistance among non-typhoid *Salmonella* serotypes has been a serious problem worldwide. The identification of antimicrobial-resistant *Salmonella* in food has raised concerns on treatment of food borne salmonellosis especially the development of

Ceftriaxone and Ciprofloxacin-resistant *Salmonella,* as these are important in treating *Salmonella* infections. The extent of global food trade and the intercontinental transmission of resistant *Salmonella* via foods underscore the potential impact that local geographical agricultural antimicrobial use may have on consumer health worldwide (Butaye et al., 2006). Conventional antimicrobial agents, such as Ampicillin, Chloramphenicol, and Trimethoprim-Sulfamethoxazole had been the drug of choice in the treatment of salmonellosis before the 1980s. However, multi-drug resistance, with rates of resistance to these antimicrobial agents of more than 50% has been reported in many areas of the world. Extended-spectrum Cephalosporin and Fluoroquinolones are increasingly reported after 1991 (Chiu et al., 2004). The possible emergence and spread of *Salmonella* strains resistant to antibiotics commonly used as treatment are concerns, because these infections can be invasive and difficult to treat by the drugs of choice for invasive *Salmonella* disease (Paterson, 2006). In developing countries, household subsistence farming is common, which means that a large proportion of the population has close contact with food animals; therefore, if resistant organisms are common in animals, the chance that they will be transmitted to human beings is more likely (Okeke et al., 2005). Some research studies indicate that the costs associated with antimicrobial resistance are higher by several times (Howard and Scott, 2005).

2.11 Mechanisms of Resistance

Bacterial resistance to antibiotics can be caused by different molecular mechanisms (Guardabassi and Dalsgaard, 2002). The most common mechanisms include: reduced drug uptake; active drug efflux; drug deactivation, modification of the drug target; increased concentration of the drug target, or alternative pathways to elude the drug **Fig 2**.

2.12 Natural and acquired resistance

Intrinsic resistance is the survival genes in bacterial genomes that could create a resistance phenotype. Differences in environmental factors could be responsible in existence of numerous number and sorts of resistance (Davies and Davies, 2010). Antibiotic usage by human and releasing to the environment can promote antibiotic resistance in every place. Antibiotics are used as growth support for animal, therapeutic agent for humans, aquacultures, pets and pest control in agriculture, biocides in toiletries, hand care, culture sterility, cloning and industry. Genetic studies showed that wastewater and plants are reservoirs of antibiotic resistance (Davies and Davies, 2010). An important distinction should

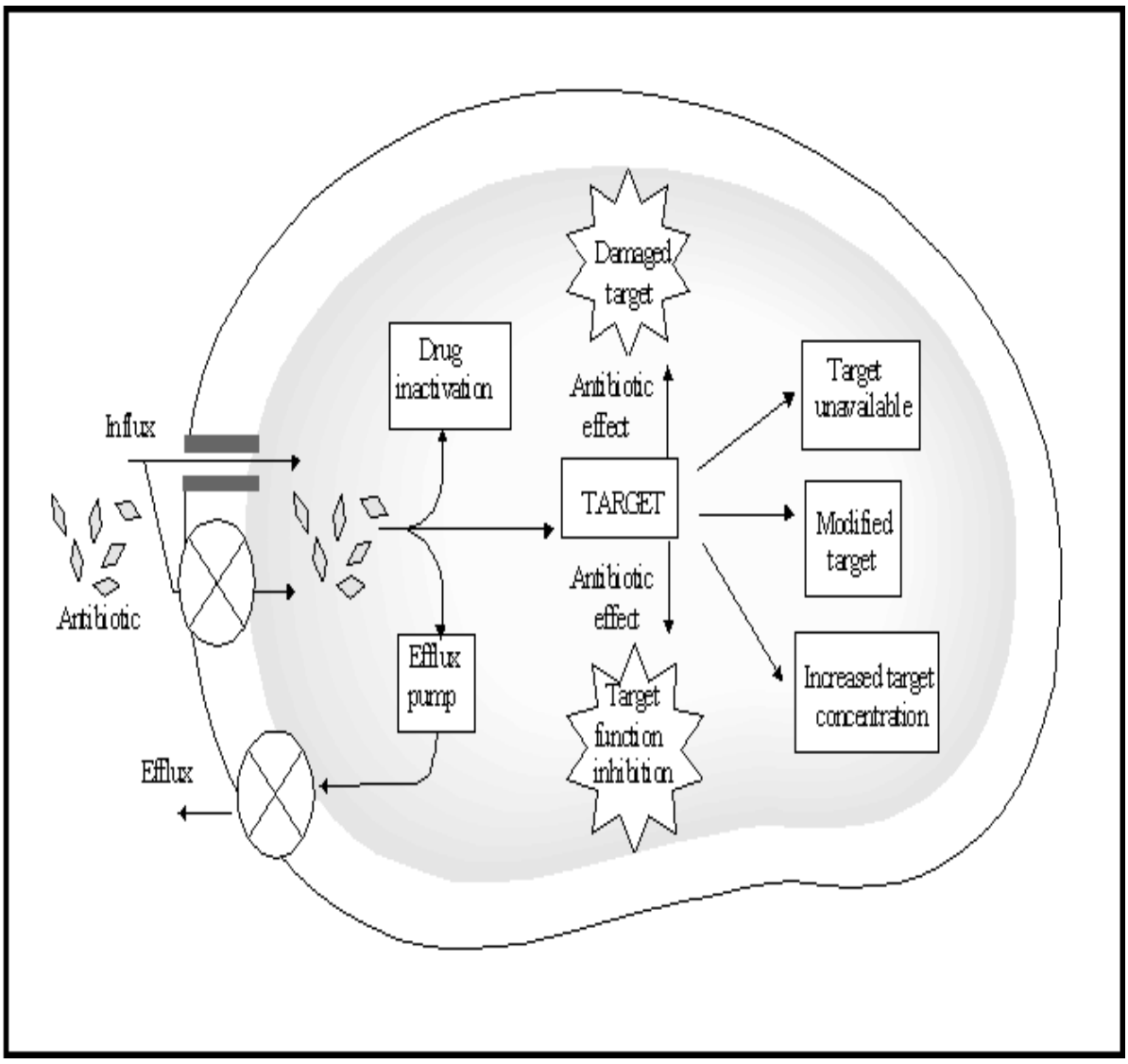


Figure 2: Molecular mechanisms of antibiotic resistance (Hayes and Wolf, 1990).

be made between natural and acquired resistance. Bacteria are termed naturally, intrinsically or constitutively resistant when resistance is due to characteristic features typical of the species. For example, *Pseudomonas aeruginosa* is naturally resistant to penicillins, due partly to the inability of the drug to diffuse through the outer membrane (Chopra and Ball, 1982) and partly to the deactivation of the drug by chromosomally encoded enzymes (Ohmori et al., 1977). In contrast, acquired resistance emerges in a bacterial population that was previously susceptible, because of modifications of the bacterial DNA caused by either chromosomal mutation or horizontal gene transfer. Natural resistance results from a long process of genetic evolution, whereas, acquired resistance can arise within a short time (Hayes and Wolf, 1990).

2.13 Mutation

Resistance in bacteria could be inherent. This can happen by mutation or acquirement of new DNA. Mutation is spontaneous and transfer by plasmids or bacteriophages (Hawkey, 1998). Genetic mutation is the primary cause of antibiotic resistance. Antibiotic resistant is created from different ways, such as antibiotic usage both in medical and veterinary medicine that lead to distribution of bacterial resistance genes through other bacteria (Mathew et al., 2007). Examples- Some of bacteria have the ability to produce specific antibodies that are resistant to an extended Spectrum β-lactamase (ESBLs). The common place for these bacteria is gut. These bacteria are expanded among patients and in hospitals. These groups of bacteria can then be transferred to other people by unwashed hands specially after using toilet (Davies and Davies, 2010). The genes of β-lactamase enzymes are mainly global in circulation; random mutation of the genes encoding and the enzymes have increase to customized catalysts with ever more complete spectra of resistance. Another general family of DNA- binding proteins gyrase inhibitors is answerable for low levels of Quinolone resistance (Davies and Davies, 2010).

2.14 Intracellular migration of resistance genes

Genes can be transferred between bacteria in a horizontal way through conjugation, transduction and transformation. Transfer of genes can happen in the intestinal tracts of animals and human. Thus genes of antibiotic resistance can be shared. Most of antibiotic resistance genes reside on plasmids with self-replicating circular pieces of DNA that make the transfer easier. If a bacteria have several resistance genes, it is called multi-resistant or a superbug or super bacterium. The name superbug refers to microbes with high levels of resistance to antibiotic. Some super resistant strains have high virulence and enhanced transmissibility. Certain antibiotic are associated with higher levels of development of superbugs (Davies and Davies, 2010). It is proposed that soil microbes are reservoirs for antibiotic resistance genes that can transfer to other microbes (Mathew et al., 2007). Stress increases the prevalence of resistance and the skills of bacteria to obtain these genes that may be absorb by the similar genetic elements in the bacterium that receive the resistant gene (Mathew et al., 2007). The efflux systems can force offending compounds, like as antibiotics out of the cell and can produce a wide range of resistance (Mathew et al., 2007). In some cases, the resistance genes can also import unknown advantages beyond those associated with the advantages selected under antibiotic use (McDermott et al., 2002).

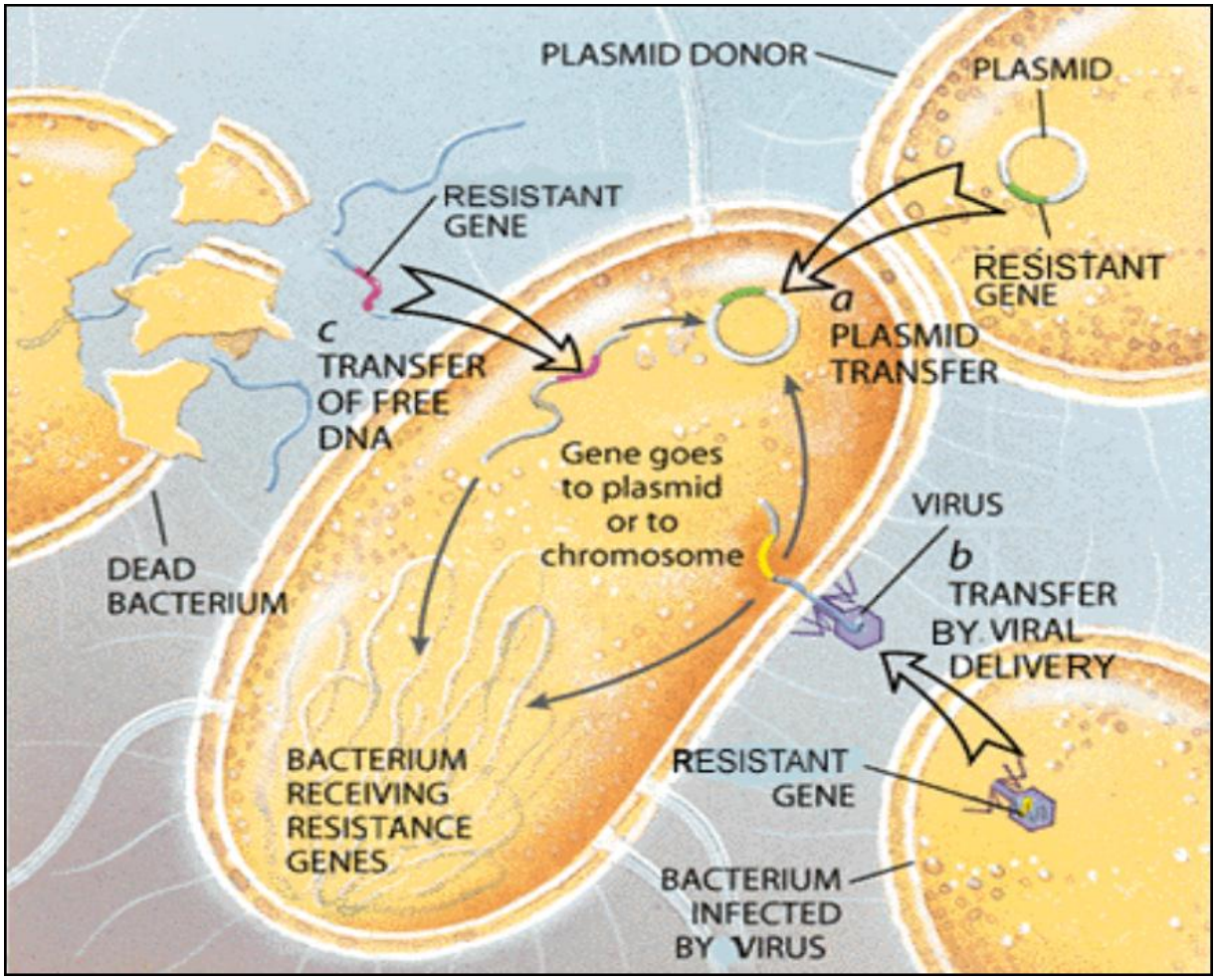


Figure 3: Mechanism of bacterial genetic transfer (Levy, 1998).

Antibiotic resistance genes can migrate from one site to another on the bacterial genome using small vectors called transposons (Mahillon, 1998) and integrons (SUNDSTRÖM, 1998). These genetic elements containing antibiotic resistance genes are able to move between different sites of the bacterial genome without any requirement of DNA homology. This process is known as non-homologous recombination and differs from the normal process of genetic recombination, which requires a high degree of DNA homology (Madigan et al., 1997). Both transposons and integrons make it possible for new antibiotic resistance genes to be acquired by plasmids and subsequently spread in the bacterial population by mechanisms of horizontal gene transfer, as suggested by the frequent recovery of these genetic elements as part of broad host plasmids (Bennett, 1999).

2.15 Multidrug resistance efflux pumps in bacteria

Efflux is the pumping of a solute out of a cell. Efflux pump genes and proteins are present in both antibiotic-susceptible and antibiotic-resistant bacteria. Some systems can be induced by their substrates so that an apparently susceptible strain can overproduce a pump and become resistant. Antimicrobial resistance in an efflux mutant is due to one of two mechanisms: either (i) expression of the efflux pump protein is increased or (ii) the protein contains an amino acid substitution(s) that makes the protein more efficient at export. In either case, the intracellular concentration of the substrate antimicrobial is lowered and the organism becomes less susceptible to that agent. Efflux pumps may be specific for one substrate or may transport a range of structurally dissimilar compounds; such pumps can be associated with multiple drug resistance (Piddock, 2006).

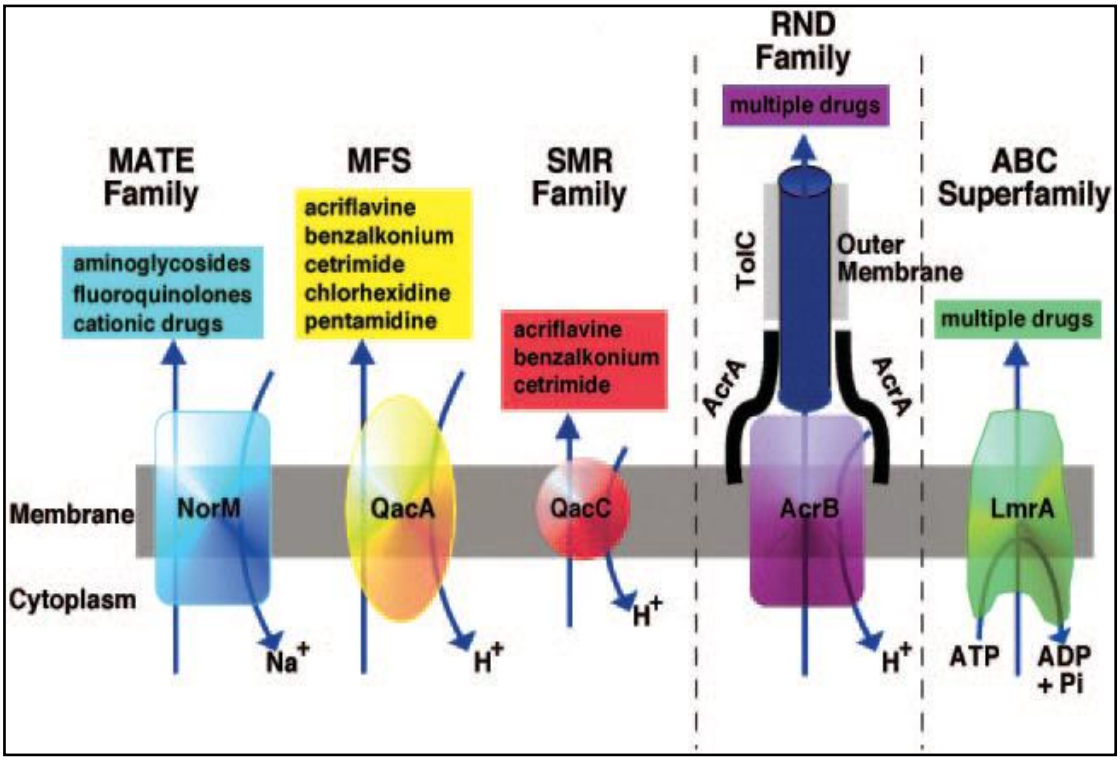


Figure 4: Diagrammatic comparison of the five families of efflux pumps. (Courtesy of Melissa Brown; reproduced by kind permission).

It was reported that resistance of *Salmonella typhi* to chloramphenicol has not been reported so far except in strains made resistant in the laboratory. While examining 52 smooth strains of *S. typhi* and three smooth strains of *S. paratyphi* A 10 strains of *S. typhi* were found to be

resistant to 50 to 500µg chloramphenicol. Of these 10 strains, eight appeared to be tolerant of the antibiotic, but the remaining two strains appeared to produce a substance that antagonizes or destroys chloramphenicol (Murti et al., 1962). Again, it was reported that each year from 1981 through to 1988 the most common serotypes isolated from man in England and Wales and identified at the Division of Enteric Pathogens were *S. typhimurium, S. enteritidis*, and *S. virchow*. In 1981 these three serotypes accounted for 45%, 12%, and 7% of isolations. The remaining 35% comprised strains belonging to a further 188 different serotypes, none of which accounted for more than 1% of the total. In 1988 *S. typhimurium* accounted for 24% of isolations, *S. enteritidis* 57%, and *S. virchow* 4%. The remaining 15% comprised strains of a further 184 serotypes. The resistances to the common antimicrobial drugs in non-typhoidal *Salmonellas* isolated in England and Wales in 1981 and 1988 were reported with particular reference to resistance to four or more antimicrobial drugs (multiple resistances). For *S. typhimurium* the overall percentage of resistant strains varied little, but multiple resistances more than doubled from 5% to 12%; in *S. enteritidis* the incidence remained the same. In *S. virchow* the percentages of strains resistant to all the antimicrobial drugs and in particular, to chloramphenicol, streptomycin, trimethoprim and furazolidone, rose from 0.2% to 10.4% (Ward et al., 1990). Another person reported that in 1996, 6% of *Escherichia coli* from extra intestinal infections were resistant to ciprofloxacin with minimum inhibitory concentrations (MICs) > or =2mg/l (high level resistance). Low level resistance (MIC 0.125-1mg/l) was also (Threlfall et al., 1997).

A total of 1715 *Salmonella* strains, including 600 *S. enteritidis*, 290 *S. derby*, 257 *S. weltevreden*, 235 *S. anatum*, and 211 *S. typhimurium*, originating from 1308 human beings and 407 frozen chicken meat specimens collected in 1993 and 1994 were tested for antibiotic resistance. The disk diffusion method was used with nine disks of chloramphenicol, ceftriaxone, amikacin, kanamycin, ampicillin, sulfamethoxazole plus trimethoprim, nalidixic acid, gentamicin and ofloxacin. It was shown that the resistance rates in 1994 were significantly higher than those in 1993. All isolates were susceptible to ofloxacin with the exception of one isolate (Boonmar et al., 1998). Hakanen *et al.*, stated that during 1995 to 1999, we collected 1,210 *Salmonella* isolates; 629 were from Finnish travelers returning from abroad. These isolates were tested for susceptibility by determining MICs to ciprofloxacin, nalidixic acid, and seven additional antimicrobial agents. The annual proportion of reduced ciprofloxacin susceptibility (MIC > 0.125µg/ml) among all travelers isolates increased from 3.9% to 23.5% ($p<0.01$) during the period of 1995 to 1999. The increasing trend was outstanding among the isolates from Southeast Asia; isolates from Thailand alone increased

from 5.6% to 50.0% ($p<0.01$). The reduced fluoroquinolone susceptibility was non-clonal in character and significantly associated with multidrug resistance. A point mutation in the quinolone resistance-determining region of *gyrA* was present in all isolates with reduced susceptibility. These data provide further evidence for the rapid spread of multidrug-resistant pathogens from one continent to another (Hakanen et al., 2001). It was reported that fifty-one (63.7%) of the 80 *Salmonella* strains were resistant to one or more antimicrobials of which 42 (52.5%) displayed multiple-drug resistance. Among the strains, 51.2% were resistant to sulfisoxazole, 46.2% to spectinomycin, 45% to amoxicillin-clavulanic acid and ampicillin, 41.2% to tetracycline and 30% to chloramphenicol. Less than 27.5% of the strains showed resistance to florfenicol, streptomycin, cotrimoxazole and to trimethoprim. *S. typhimurium* var. Copenhagen (100%), *S. anatum* (62.5%), *S. typhimurium* (33.3%) and *S. braenderup* (34.3%) showed multiple antimicrobial resistance to up to eight antimicrobials. None of the strains were resistant to amikacin, apramycin, gentamicin, kanamycin, neomycin, tobramycin, quinolones, cephalosporins and nitrofurantoin. They also indicated the potential importance of chickens as source of multiple antimicrobial-resistant *Salmonella* for human infections (Molla et al., 2004). Shakespeare *et al.,* stated that *Salmonella enterica* serotype Typhi presenting as a primary psoas abscess. The isolate tested susceptible to ciprofloxacin but resistant to nalidixic acid *in vitro*, a pattern associated with fluoroquinolone therapeutic failures (Shakespeare et al., 2005).

Vo *et al.,* investigated that antimicrobial resistance patterns, integron characteristics and gene cassettes as well as the presence of *Salmonella* genomic island 1 (SGI1) in non-typhoidal *Salmonella* (NTS) isolates from human and animal origin. Epidemiologically unrelated Dutch NTS strains (n=237) originating from food-producing animals and human cases of salmonellosis were tested for their susceptibility to 15 antimicrobial agents. Resistance to 14 of these antimicrobials, including the third-generation cephalosporins was detected. Resistance to sulphonamides, ampicillin, tetracycline, streptomycin, trimethoprim and nalidixic acid was common (>/=10% of the strains were resistant). Resistance against three or more antimicrobials was observed in 57 isolates (Vo et al., 2006). Perron, stated that multidrug-resistance in *Salmonella typhimurium isolated* from swine shown resistance to ampicillin (96%), chloramphenicol (88%), neomycin (72%), tetracycline (90%). No resistance to enrofloxacin or gentamicin was found. The fluoroquinolone susceptibility of 499 *Salmonella enterica* isolates collected from travelers returning to Finland during 2003–2007. Among isolates from travelers to Thailand and Malaysia, reduced fluoroquinolone

susceptibility decreased from 65% to 22% (p = 0.002). All isolates showing nonclassical quinolone resistance were from travelers to these 2 countries (Lindgren et al., 2009).

2.16 Development of Resistance

Antibiotic resistance is a global problem in public health and is growing around the world. Antibiotics have been used for 70 years but during the last decade some treatments have become ineffective and this may lead to spread of some infections in the future. Antimicrobial resistance (AMR) is created by use of antibiotics in a wrong way and develops when a microorganism have mutated or acquired inappropriate use of antibiotics in human and veterinary medicine leads to higher frequencies of AMR (McDermott et al., 2002). Antibiotics are often used in animals. Transfer to human's food of these antibiotics can affect the safety of the meat, milk, and eggs produced and can be the source of superbugs. The resistant bacteria in animals can transfer to humans by three pathways, consumption of meat or other food, direct contact with animals or through the environment. The figure-2.3 shows the transfer ways of antibiotic resistance between human, animals and environment.

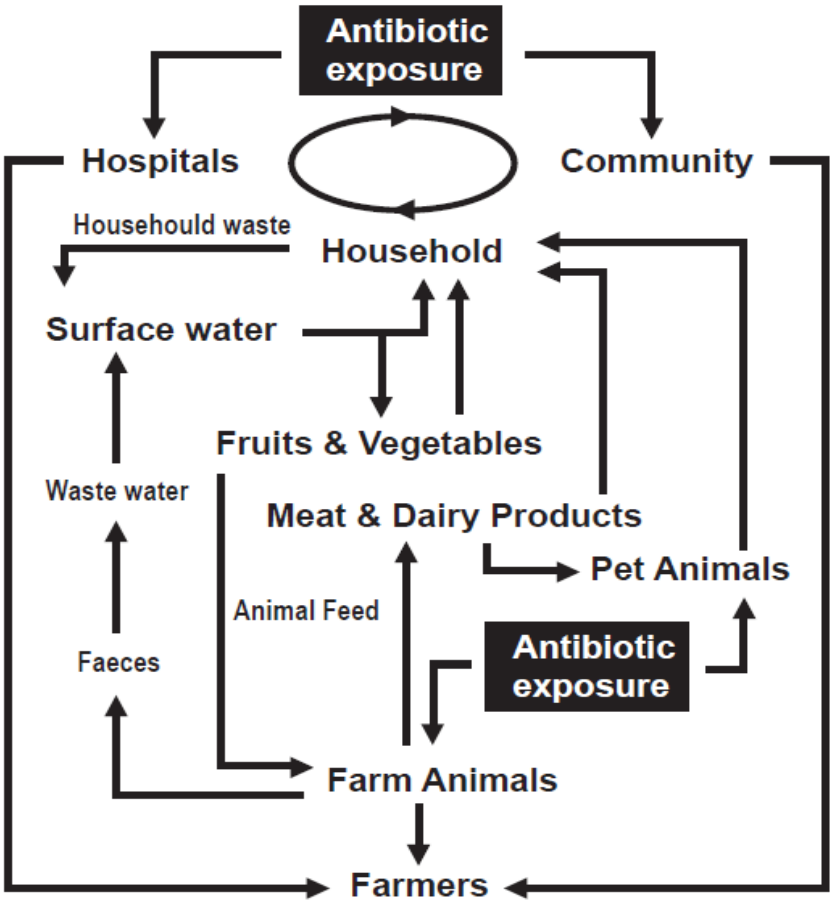


Figure 5: Antibiotic exposure in Human and Animals

2.17 Global trends in resistance pattern

Antimicrobial resistance is one of the biggest challenges facing global public health. Although antimicrobial drugs have saved many lives and eased the suffering of many millions, poverty, ignorance, poor sanitation, hunger and malnutrition, inadequate access to drugs, poor and inadequate health care systems, civil conflicts and bad governance (Byarugaba, 2004), misdiagnosis, counterfeit drugs and lack of education in developing countries have tremendously limited the benefits of these drugs in controlling infectious diseases (Walia, 2003). In Indonesia, *Salmonella paratyphi* isolates recovered between 1995 and 2001 were universally susceptible to commonly used antimicrobials. *Salmonella enteritidis* isolates were resistant to most of the antimicrobials tested, with the exceptions of Fluoroquinolones. A similar study in Zimbabwe reported much lower rates of resistance among *Salmonella enteritidis*, and more than 50% of non-typhoidal *Salmonella* isolates from children in Kenya were multi-drug resistant (Okeke et al., 2005). One of the studies in Spain reported high percentages of resistance of *Salmonella* isolates to Sulfadiazine, Neomycin, Tetracycline and Streptomycin, which might be the result of use of antibiotics as a prophylaxis, growth promoter or treatment (Carramiñana et al., 2004). A similar study in Alberta, Canada indicated high resistance of *Salmonella* isolates from food and food animals to Ampicillin, Streptomycin, Sulfamethoxazole and Tetracycline (Johnson et al., 2005).

In Ethiopia, resistance pattern of *Salmonella* isolates from chickens indicated large proportions of strains resistant to a variety of drugs (Molla et al., 2004). Over the past decade in Nepal, increasing antibiotic resistance in *Salmonella enterica* has lead to a shift in the antibiotics used against this organism from Chloramphenicol and Ampicillin to Trimethoprim-Sulfamethoxazole, Fluoroquinolones and Ceftriaxone, where only a 16-40% positive response to treatment has been achieved (Pokharel et al., 2006). In a study in the United States *Salmonella* isolated from pre-harvest turkey production sources were resistant to multiple antibiotics (Nayak and Stewart-King, 2008). In another study, of 380 *Salmonella* isolates from animal origin in the US, 82% of the isolates were resistant to at least one antimicrobial, and 70% to three or more antimicrobials. Resistance was most often observed to Tetracycline, followed by Streptomycin, Sulfamethoxazole, Ampicillin, Chloramphenicol, Kanamycin, Amoxicillin or Clavulanic acid, and Ceftiofur (Zhao et al., 2007). From 1999 to 2003, 34411 *Salmonella* were isolated from animals in the USA, of which 10.9% were found to be resistant to Ceftiofur, a third generation cephalosporin used in animals, whilst only

0.3% were resistant to Ceftriaxone, a third generation cephalosporin used in human medicine. There was an increase in Ceftiofur resistance (Frye and Fedorka-Cray, 2007). Increased antibiotic resistance among *Salmonella* is not only in the percentage isolates resistant to a particular antibiotic, but also the development of resistance against newer antibiotics (Fluit, 2005). In a study in Nepal, 35 multi-drug-resistant strains out of 132 strains of *Salmonella typhi* were observed showing simultaneous resistance to Ampicillin, Chloramphenicol, and Cotrimoxazole. Although there were no isolates resistant to Ciprofloxacin, 69.23% of 52 isolates tested for minimum inhibitory concentration of Ciprofloxacin showed reduced susceptibility and 76% of 112 strains tested for Nalidixic acid were resistant (Khanal et al., 2007). There are reports of *Salmonella* resistant strains isolated from The Netherlands (Van Duijkeren et al., 2003), France (Weill et al., 2006), Portugal (Antunes et al., 2003) and many other countries. Between the year 1999 and 2004, the number of publications reporting *Salmonella* resistant to β-lactam antibiotics has increased drastically. In 2004, *Salmonella* resistant to extended spectrum cephalosporin were identified in 43 countries (Arlet et al., 2006).

In a retrospective study in Korea, the resistance rate against Chloramphenicol showed mild increase, but the Ampicillin, Trimethoprim or Sulfamethoxazole, Kanamycin or Nalidixic acid remained at a similar level over 9 years (Yoo et al., 2004). Because the majority of human cases of non-typhoidal Salmonellosis are acquired through the consumption of contaminated food and water, data on the proportions of serotypes and their resistance patterns in different countries are important for global public health management, as food consumption practices vary in different countries and increasing global travel and food trade increase the likelihood of acquiring infections from non-domestic sources (Lauderdale et al., 2006). In Pakistan, Bacitracin, Erythromycin and Novobiocin also grew 100% resistance against *Salmonella enteritidis* isolated both from fecal and egg samples (Akhtar et al., 2010). In India Approximately, 78% of *Salmonella typhi* isolates collected from infected patients between 1990 and 1991 demonstrated resistance to Chloramphenicol, Ampicillin and Trimethoprim or Sulfamethoxazole. Approximately 81% of the *Salmonella enterica* serotype Typhi isolates from northern India was resistant to Chloramphenicol (Sharma et al., 2005). A study in Calcutta in India revealed all *Salmonella enterica* sero-groups were uniformly resistant to commonly used drugs with an exception to Norfloxacin and Ciprofloxacin (Saha et al., 2001). In a few of the studies, a changing pattern of the multi-drug resistant *Salmonella* isolates was noted (Madhulika et al., 2004; Das and Bhattacharya, 2006). In a recent study in

England, Scotland and Wales, it was found that 70% of typhoid cases in returning travelers originated from India or Pakistan, with the highest level of antimicrobial resistance from the Indian subcontinent (Cooke et al., 2007). Although resistance patterns in *Salmonella* have been increasingly observed, re-emergence of Chloramphenicol sensitivity has been noted in few of the studies (Tankhiwale et al., 2003; Achla et al., 2005; Mohanty et al., 2006).

2.18 Antibiotic Resistance pattern and antibiotic sensitivity in Bangladesh

In developing countries like Bangladesh, easy availability of a wide range of drugs coupled with inadequate veterinary services result in increased proportions of drugs used as self-medication compared to prescribed drugs resulting in impending health problems and antimicrobials resistance. The common resistant antimicrobials were Streptomycin (41.3%), Tetracycline (31.9%), Gentamycin (28.2%), Ampicillin (25.4%) and Nalidixic acid were observed in many earlier studies in poultry in different countries including Bangladesh (Akter et al., 2007; Mahmud et al., 2011). The prevalence of antimicrobial resistance against *Salmonella* spp isolates at layer poultry farm was recorded to be 62.5% in Dinajpur (Akter et al., 2007) and 28% in Savar, Bangladesh (Mahmud et al., 2011). The recorded resistant antimicrobials were Erythromycin (100%), Amoxicillin (50%), Penicillin (50%) and Tetracycline (5%) in Dinajpur, Bangladesh and Penicillin-G (100%), Ampicillin (99%), Amoxicillin (98%), Tetracycline (93%), Nitrofurantoin (78%), Sulfamethoxazole (60%) and Ciprofloxacin (40%) in Savar, Bangladesh. Many studies on antimicrobial sensitivity against *Salmonella* spp. at poultry farms have indentified the following sensitive antimicrobials in Bangladesh: Ciprofloxacin remained sensitive at 80-100% (Rahman et al., 2004b; Akter et al., 2007), Nitrofurantoin 100% (Akter et al., 2007; Mahmud et al., 2011), Neomycin 75-100%, Sulfamethoxazole/Trimethoprim 50-100%, Tetracycline and Erythromycin 40-60%, Amoxicillin 30-50% and Doxyclycline 13% (Rahman et al., 2004b; Akter et al., 2007).

2.19 Antibiotic resistance in wild birds

Wild birds have been postulated as sentinels, reservoirs, and potential spreaders of antibiotic resistance. Antibiotic-resistant bacteria have been isolated from a multitude of wild bird species. Several studies strongly indicate broadcast of resistant bacteria from human rest products to wild birds. There is proof suggesting that wild birds can extend resistant bacteria through migration and that resistant bacteria can be transmitted from birds to humans and vice versa. Through further studies of the spatial and temporal distribution of resistant bacteria in wild birds, we can better assess their role and thereby help to mitigate the

increasing global problem of antibiotic resistance (Bonnedahl and Järhult, 2014). Wild animals have been found to harbour antibiotic-resistant bacteria, sometimes at surprisingly high levels (Gilliver et al., 1999). The level of resistant bacteria in wild animals seems to correlate well with the degree of association with human activity (Skurnik et al., 2006). Studies of the occurrence of antibiotic resistance among wild birds have increased during the last years and a number of different bird families have now been found to harbour antibiotic-resistant bacteria. There are quite a few studies of entero-pathogenic *Salmonella* spp. and *Campylobacter* spp. but also of *E. coli*. Antibiotic-resistant *E. coli* have been isolated from ducks and geese (Fallacara et al., 2001; Cole et al., 2005; Middleton and Ambrose, 2005), cormorants (Rose et al., 2009), birds of prey (Costa et al., 2008), gulls (Poeta et al., 2008; Rose et al., 2009), doves (Radimersky et al., 2010) and passerines (Dolejska et al., 2008; Blanco et al., 2009; Rybaříková et al., 2010). Most of the studies are from bird populations with relatively frequent interactions with habitat influenced by human activities. Examples of contaminated habitats, where the risk for birds acquiring antibiotic-resistant bacteria is greater, include livestock farms managed under intensive regimes, landfills and wastewater treatment facilities (Blanco et al., 2007).

In Spain, livestock carrion is intentionally left for scavengers to consume at sites called muladares. Since antibiotics are used intensely in the rearing of the livestock, their carcasses can harbour both antibiotics and antibiotic- resistant bacteria that can be passed further on into the food chain and to the environment (Blanco et al., 2007). Red-billed choughs (*Pyrrhocorax pyrrhocorax*) feed on soil invertebrates and pick up antibiotic-resistant bacteria from contaminated manure spread on the soil as fertilizer. Choughs from areas where manure land-spreading is a common agricultural practice harbour a high bacterial resistance to multiple antibiotics, resembling the resistance profile found in the waste (pig slurry and sewage sludge) used in the respective area (Blanco et al., 2009). When antibiotic-resistant bacteria colonize birds, the birds can become a new environmental reservoir of antibiotic resistance and also a vector that disperses these bacteria to new localities. For example, in the Czech Republic, antibiotic-resistant *E. coli* and *Salmonella* occur in the faecal bacteria of rooks (*Corvus frugilegus*), probably reflecting the presence of such isolates in their sources of food and/or water in the environment (Literak et al., 2007). Because most of the rooks wintering in the Czech Republic breed in European Russia and winter in the Czech Republic, they have the potential to disseminate resistant bacteria over long distances throughout Europe, for example from Russia to the Czech Republic and *vice versa* (Literak et al., 2007).

Antibiotic-resistant *Salmonella* strains have also been isolated in Black-headed Gulls (*Chroicocephalus ridibundus*) just arriving in southern Sweden from non-breeding areas in West and Southwest Europe (Palmgren et al., 1997). In a study of Sweden on drug resistance, from the 49 Gentoo Penguins sampled, 42 isolates of *Enterobacteriace* were found. 39 isolates belonged to the genus *Edwardsiellae*, one isolate was an *E. coli.* All *Edwardsiella* and the *E. coli* isolate were highly susceptible to the 17 (Ampicillin, Amoxiclav, Piperacillin/Tazobactam, Cephalothin, Cefuroxime, Ceftazidime, Cefotaxime, Aztreonam, Imipenem, Nalidixic acid, Ciprofloxacin, Gentamicin, Streptomycin, Tetracycline, Chloramphenicol, Trimethoprim, Sulphamethoxazole) antibiotics tested. 180 samples also taken from Black-headed gull, (90 in each colony), *E. coli* isolates were obtained from 153 samples. Resistance was tested towards tetracycline, ampicillin, streptomycin, chloramphenicol, nalidixic acid and cefadroxil. Nearly half (47.1%; 72/153 isolates) of the *E. coli* isolates from the gulls were resistant to at least one antibiotic (Bonnedahl, 2011).

2.20 Isolation of antibiotic-resistant bacteria from wild birds

Wild birds are important with regard to antibiotic resistance in several different ways: 1) as sentinels, mirroring human activity and its impact on the environment because of the diverse ecological niches of birds and as they easily pick up human and environmental bacteria. 2) As a reservoir and melting pot of antibiotic-resistant bacteria and resistance genes. 3) As potential spreaders of antibiotic resistance through the ability to migrate long distances in short periods of time. 4) As a possible source of antibiotic resistant bacteria colonizing and/or infecting human beings. The first antibiotic-resistant bacteria noted in wildlife were in fact from wild bird's strains of *Escherichia coli* resistant to multiple antibiotics, e.g. chloramphenicol was isolated in pigeons around 1975. Not until 10 years later were the famous studies with baboons in South Africa performed, where the degree of antibiotic resistance carriage among baboons was correlated with the degree of human interactivity. Many bird species have been found to carry antibiotic-resistant bacteria (Bonnedahl and Järhult, 2014)

2.21 Wild birds as spreaders of antibiotic resistance

Birds can migrate long distances in short periods of time and the abundance of reports of carriage of antibiotic-resistant bacteria discussed above, there is a possibility that wild birds can act as spreaders of antibiotic resistance. For humans, the spread of antibiotic-resistant bacteria through travel has been demonstrated. There are also examples where wild bird

migration has been linked to the spread of pathogens, such as the dissemination of West Nile Virus in the US. A study on chickens has demonstrated carriage of ESBL-producing bacteria for several weeks and that ESBL-producing bacteria can be rapidly transmitted between individuals. In a study on domesticated bird, it is reasonable to believe that also wild birds can carry antibiotic-resistant bacteria long enough during migration with the potential of intercontinental spread of resistance. A recent study in Chile has shown a prevalence of ESBL-producing E. coli among Franklin's gulls (*Leucophaeus pipixcan*) that is more than twice as high as in humans in the same area. Humans and gulls share sequence types indicating transmission, but interestingly gulls also share sequence types with human clinical samples from central Canada, a nesting place for those gulls suggesting that migration could be a mechanism of dissemination. Similarly, the isolation of antibiotic-resistant Salmonella strains from black-headed gulls (*Chroicocephalus ridibundus*) just arriving in southern Sweden from non-breeding areas in West and Southwest Europe suggests spread through migration. Antibiotic resistant strains including ESBL-producing E. coli have also been isolated from birds from remote and/or preservation areas. This could be interpreted as possible footprint of human activity, but at least in some cases it seems more plausible with spread through bird migration. Spread of antibiotic-resistant bacteria to remote areas that are reached mainly by migrating birds could also influence bacterial communities in these fragile ecosystems, as antimicrobial substances are part of the cross-talk of bacteria (Bonnedahl and Järhult, 2014)

2.22 Public Health importance

Salmonellosis is an important global public health problem causing substantial morbidity, and thus also has a significant economic impact. Although most infections cause mild to moderate self-limited disease, serious infections leading to deaths do occur (De Jong and Ekdahl, 2006). In spite of the improvement in hygiene, food processing, education of food handlers and information to the consumers, food borne diseases still dominate as the most important public health problem in most countries (Domınguez et al., 2002). Many foods, particularly those of animal origin, have been identified as vehicles for transmission of these pathogens to human beings and spreading them to the processing and kitchen environment (Uyttendaele et al., 1998). In developed countries food is recognized as the most frequently implicated vehicle of transmission and causes heavy financial burden on health care systems (Jordan et al., 2006). In the United States alone, an estimated 1.4 million non-typhoidal *Salmonella* infections, resulting in 168000 visits to physicians, 15000 hospitalizations and 580 deaths

occur annually and the total cost associated with Salmonella is estimated at US$ 3 billion annually (Varma et al., 2005). Apart from the food borne infections, the other major epidemiological development in Salmonellosis is the emergence of multiple-antibiotic resistant *Salmonella*, particularly in the developing countries (Okeke et al., 2005). Ciprofloxacin, Neomycin and Tetracycline have been reported to be commonly used both in poultry and human (Chowdhury et al., 2009; Bashar et al., 2011). Therefore, extensive uses of these antimicrobials in food animals (particularly in poultry and wild birds) can transmit antimicrobial residues to healthy humans through food chain; consequently these drugs may become resistant against any infectious bacterial pathogens in humans. Antimicrobial residues exceeding the threshold level have been detected in animal and poultry products in Bangladesh (Notter, 2012; MAHMUD, 2013).

Chapter-3: Materials and Methods

3.1 Study Area

Two area (Phartoli and Bakolia) of Chittagong City Corporation (CCC) were selected for collection of fecal sample from wild birds for *Salmonella* isolation. The study areas were selected because large number of wild bird appears everyday on the dustbin for searching foods. Collection of sample was done directly from cloaca by using swab stick. The species of wild birds were Crow (*Corvus splendens*) and Asian pied starling (*Gracupica contra*).

3.2 Study Design

A cross-sectional study was conducted in two area of chittagong, in order to investigate prevalence of *Salmonella* spp. and antimicrobial resistance pattern against *Salmonella* spp. in wild birds.

Figure 6: Study area map

3.3 Study Period

The study was conducted for a period of 10 months from March to December, 2014.

3.4 Sample size

A total 50 samples were collected from the wild bird, 20 samples from crow and 30 from Asian pied starling. The samples were then carried to PRTC laboratory under sterile conditions and processed immediately for the isolation of *Salmonella* spp.

3.5 Collection & preservation

A sterile cotton swab stick was used to collect the fecal sample and then put the stick immediately into a sterile vial containing 6ml Amines transport media (Oxoid). Individual sample in each vial was given unique identification number then immediately transferred to laboratory, Chittagong Veterinary and Animal Sciences University (CVASU) through ice eskie. Samples with transport media were stored temporarily in refrigerator before laboratory evaluation.

3.6 *Salmonella* isolation and identification procedures

The study was conducted utilizing the conventional methods for the detection of *Salmonella* spp. following the standard guidelines. There were four definite sequential steps: (1) Nonselective pre-enrichment, (2) Selective enrichment, (3) Plating out and identification, and (4) Confirmation by using Triple Sugar Iron (TSI) agar.

Buffered peptone water was used as non-selective pre-enrichment broth, which was prepared according to the instruction of the manufacturer (Oxoid). In brief, 13gram of powder was completely dissolved per 1000ml of distilled water. An amount of fecal mass of a sample was placed into 200ml of buffered peptone water (BPW); the mixture was shaken approximately for 2 minutes and incubated at 37°C ± 1°C for 18 ± *2* hours. Xylose Lysine Deoxycholate (XLD) was used as selective enrichment broth and it was prepared according to the instruction of the manufacturer. After incubation a loop full of broth was streaked on Xylose Lysine Deoxycholate (XLD) medium and incubated at 37°C for 24 hours. Colonies with black centers were considered presumptive *Salmonella* spp., for plating out and identification *Salmonella-Shigella* (SS) agar was used. SS agar (Difco) were prepared according to the instruction of the manufacturer and then autoclaved. After autoclaving the media at 121°C for 15 minutes, it was cooled to 50°C and approximately 30 ml to 50 ml was poured into the 15 x 150 mm Petri dishes. The depth of the agar in the petridishes was maintained approximately at 4 mm. The freshly prepared plates were used on the same day. The pH of the medium was regularly tested for its consistency. After incubation for 48 ± 3 hours, a loop-full black colony was transferred from the XLD and streaked onto the surface of SS agar. The plates were incubated at 37°C± 1°C for 24 ± 3 hours. The plates were incubated in an inverted position

and after incubation. The plates were checked for the development of typical *Salmonella* colonies. Typical colonies of *Salmonella* on SS agar were blackish due to the production of hydrogen sulfide. Triple Sugar Iron agar (TSI agar) was used to see the typical colonies and biochemical reactions for the identification of *Salmonella* spp., typical colonies grown on the SS agar plates were transferred and inoculated in triple sugar iron agar (TSI) slant and incubated at 37°C ± 1°C for 24 ± 3 hours. The TSI agar slant surface was streaked and the butt was stabbed and incubated at 37°C ±1°C for 24 ± 3 hours.

Table 1: Probable biochemical changes produced by bacteria in TSI agar slant

Reaction Area	**Result**	**Interpretation**
Butt	**Yellow**	Glucose fermentation positive (Glucose used)
	Red or Unchanged	Glucose fermentation negative (Glucose not used)
	Black	Formation of hydrogen sulfide
	Bubbles or Cracks	Gas formation from glucose
Slant Surface	**Yellow**	Lactose and/or sucrose fermentation positive
	Red or Unchanged	Lactose and/or sucrose fermentation negative

3.7 Selection of antimicrobials for antimicrobial susceptibility testing against *Salmonella* isolates

The most commonly used antimicrobial agents for either chemoprophylaxis or therapy for control of bacterial diseases in livestock, poultry and human in south Asia includes Sulfadiazine, Sulphamethoxy pyridazine, Neomycin, Furazolidone, Ciprofloxacin, Enrofloxacin, Nitrofurantoin, Colistin, Ampicillin and Cloxacillin (Prakash et al., 2005). In many of the studies carried out in India, against *Salmonella* species, the isolates were found resistant to Amoxicillin, Nalidixic acid, Cotrimoxazole, Chloramphenicol, Ciprofloxacin, Sulphadiazine, Sulphamethoxy pyridazine, Erythromycin, Enrofloxacin, Pefloxacin, Neomycin, Furazolidone, Doxycycline, Ampicillin, Tetracycline, Chlortetracycline, Kanamycin, Gentamycin, Amikacin, Ceftizoxime and Ceftriaxone (Gautam et al., 2002; Prakash et al., 2005; Das and Bhattacharya, 2006) and all of these antimicrobials are listed in the Essential Drugs Program (EDP) of the Department of Health (DOH). Therefore, in present research the *Salmonella* isolates are tested with antimicrobial sensitivity testing to see they are resistant to these antimicrobials (Penicillin, Erythromycin, Clindamycin, Oxacillin, Kanamycin, and Cephalothin) used in Bangladesh.

3.8 Antimicrobial sensitivity test

The antimicrobial sensitivity testing was done by the disk diffusion method as described by NCCLS 2000, now known as the Clinical and Laboratory Standards Institute (CLSI). Mueller- Hinton agar was used for this testing. The agar was prepared as per the instructions provided by the manufacturer. After autoclaving the media at 121°C for 15 minutes, it was cooled at 50°C and approximately 30-50ml was poured into the 150 mm petridishes. The depth of the agar in the petridishes was maintained approximately at 4 mm. The freshly prepared plates were used on the same day. The pH of the medium was regularly tested for its consistency. McFarland 0.5 turbidity standards were prepared as per the standard guidelines described by the CLSI. A volume of 0.5 ml of a 1.175% (wt/vol) barium chloride dihydrate ($BaCl_2.2H_20$) solution was added to 99.5ml of 0.18mol/L (1% vol/vol) sulfuric acid with constant stirring to maintain the suspension. The turbidity standard was then aliquoted into test tubes 4 ml each, identical to those used to prepare the inoculum suspension. The McFarland standard tubes were sealed with parafilm to prevent evaporation. McFarland standards then were stored in the dark at room temperature. Before each use, the standards were shaken well, mixing the fine white precipitate of barium sulfate in the tube. The accuracy of the density of a prepared McFarland standard was checked by using a spectrophotometer with a 1-cm light path; for freshly prepared 0.5 McFarland standards, the absorbance at a wavelength of 625nm was 0.88. The McFarland standards were replaced every week with a fresh preparation. Before antimicrobial sensitivity testing, each *Salmonella* isolate was re-suspended in BPW and inoculated onto Mueller-Hinton agar and incubated at 37°C for 18-24 hours. At least 3-5 well isolated colonies of the same morphological type were selected from the agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 4 ml trypticase soy broth. The broth culture was either directly adjusted to the McFarland standards or by incubation at 35°C until it achieved or exceeded the turbidity of the 0.5 McFarland standards (usually 2-6 hours). The turbidity of the actively growing broth culture was adjusted with sterile broth to obtain turbidity optically comparable to the point of the 0.5 McFarland standards. Optimally within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab then was rotated several times pressed firmly on the inside wall of the tube above the fluid level. This removed excess inoculum from the swab. The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times,

rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step the rim of the agar was swabbed. The procedure was done under laminar flow to avoid contamination. The lid was left ajar for 3-5 minutes but no more than 15 minutes, to allow for any access surface moisture to be absorbed before applying the drug impregnated disks. The predetermined battery of antimicrobial disks like Penicillin (10units/disc), Cephalothin (30µg/disc), Oxacillin (1µg/disc), Kanamycin (30µg/disc), Erythromycin (15µg/disc), Clindamycin (2µg/disc) were chosen and placed centrally onto the surface of the inoculated agar plate. The disk placed in the agar surface was not closer than 24 mm from center to center. A total of 6 disks were placed on one 150 mm plate. The plates were inverted and placed in an incubator set to 35°C within 15 minutes after the disks were applied. After 16-18 hours of incubation, each plate was examined.

The resulting zone of inhibition was uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition (judged by the unaided eye) were measured, including the diameter of the disk. Zones were measured to the nearest whole millimeter, using suitable scale, which held on the back of the inverted petri plate. The Petri-plate was held a few inches above a black, nonreflecting background and illuminated with reflected light. The zone margin was taken as the area showing no obvious, visible growth that could be detected with the unaided eye. Faint growth of tiny colonies, which could be detected only with a magnifying lens at the edge of the zone of inhibited growth, was ignored. The sizes of zones of inhibition were interpreted by referring to zone diameter interpretive standards from NCCLS 2000 and the isolates were considered as sensitive, intermediately sensitive or resistant to this agent tested according to this standard.

Table 2: Panel of antibiotics used, their concentrations and zone diameter interpretative standards (CLSI, 2007).

Group of Antimicrobial	Antimicrobial agent	Disk contents	Zone diameter, nearest whole mm		
			R	**I**	**S**
Penicillinase labile penicillins	Penicillin	10 units	≤28	-	≥29
Penicillinase stable penicillins	Oxacillin	1 µg	-	-	≥20
Aminoglycosides	Kanamycin	30 µg	≤13	14-17	≥18
Lincosamides	Clindamycin	2 µg	≤14	15-20	≥21
Macrolides	Erythromycin	15 µg	≤13	14-22	≥23
Cephems	Cephalothin	30 µg	≤14	15-17	≥18

3.9 Data Analysis

Field and Laboratory data were stored and then cleaned in the MS Excel-2007 programme before exporting to STATA/IC-11.0 for analysis. Descriptive analysis was performed to know the frequency and distribution of *Salmonella* and antibiotic resistance pattern.

Chapter-4: Results

Salmonella spp. isolated from cloacal sample on two species of wild birds such as Crow and Asian pied Starling at Pahartoli and Bakolia under Chittagong City Corporation area of Bangladesh to evaluate antimicrobial susceptibility to estimate the prevalence and pattern of antimicrobial resistance and sensitivity among *Salmonella* spp. isolates.

Table 3: Prevalence of *Salmonella* spp. within two species of birds

Species	Positive (%)	Chi2-value	P-value
Crow (N=20)	13 (65%)	0.014	0.9
Asian pied Starling (N=30)	20 (67%)		

Table 3 showed the prevalence of *salmonella* spp. within two species. The prevalence of Asian pied starling and Crow was (67%) and (65%), respectively. Within the category of samples from different species the variation in prevalence were not varied significantly ($p>0.05$). The strength of association between the prevalence of salmonella spp. within Crow and Asian pied starling were 1.4%, where the strength of association was 1.4% higher in Asian pied starling than Crow.

Table 4: Antimicrobial resistance pattern of *Salmonella* spp. isolates from Crows

Antibiotics	No. of positive sample tested	Pattern		
		Resistance	Intermediate	Sensitive
Penicillin	10	R(10)	I(0)	S(0)
Erythromycin	10	R(5)	I(2)	S(3)
Oxacillin	10	R(10)	I(0)	S(0)
Kanamycin	10	R(2)	I(1)	S(7)
Clindamycin	10	R(10)	I(0)	S(0)
Cephalothin	10	R(3)	I(0)	S(7)

Note: R= Resistance; I= Intermediate; S=Sensitive

Table 5: Antimicrobial resistance pattern of *Salmonella* isolates from Crows

Antibiotics	No. of positive sample tested	Pattern		
		Resistance (%)	Intermediate (%)	Sensitive (%)
Penicillin	10	100	0	0
Erythromycin	10	50	20	30
Oxacillin	10	100	0	0
Kanamycin	10	20	10	70
Clindamycin	10	100	0	0
Cephalothin	10	30	0	70

The prevalence and pattern of antimicrobial resistance of *Salmonella* isolates from Crows has been outlined in **Table 4 & 5**

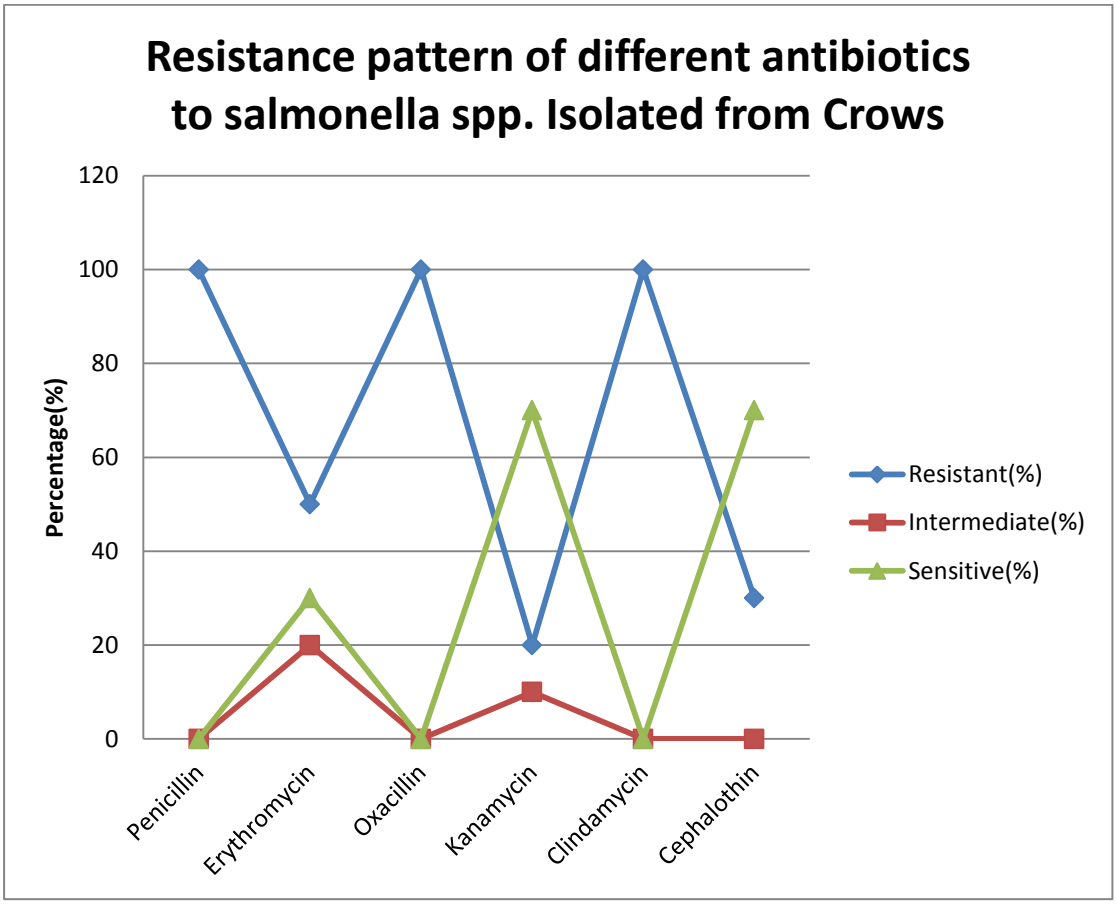


Figure 7: Resistance pattern of *Salmonella* isolates from Crows

From Crow resistance patterns of *Salmonella* were highest in Penicillin, Oxacillin and Clindamycin (100%) followed by Erythromycin (50%), Cephalothin (30%) and Kanamycin (20%). It was revealed that no isolates were found sensitive to Penicillin, Oxacillin and Clindamycin. Kanamycin and Cephalothin showed highest level of sensitivity (70%)

followed by Erythromycin (30%). In current research, all the isolates of *Salmonella* showed multiple antimicrobial resistances. **Fig 7** shows the graphical presentation of resistance pattern of *salmonella* isolates from Crows.

Table 6: Antimicrobial resistance pattern of *Salmonella* isolates from Asian pied Starling

Antibiotics	**No. of positive sample tested**	**Pattern**		
		Resistance	**Intermediate**	**Sensitive**
Penicillin	15	R(15)	I(0)	S(0)
Erythromycin	15	R(14)	I(1)	S(0)
Oxacillin	15	R(15)	I(0)	S(0)
Kanamycin	15	R(1)	I(3)	S(11)
Clindamycin	15	R(15)	I(0)	S(0)
Cephalothin	15	R(10)	I(1)	S(4)

Note: R= Resistance; I= Intermediate; S=Sensitive

Table 7: Antimicrobial resistance pattern of *Salmonella* isolates from Asian pied Starling

Antibiotics	**No. of positive sample tested**	**Pattern**		
		Resistance (%)	**Intermediate (%)**	**Sensitive (%)**
Penicillin	15	100	0	0
Erythromycin	15	93	7	0
Oxacillin	15	100	0	0
Kanamycin	15	7	20	73
Clindamycin	15	100	0	0
Cephalothin	15	67	7	26

The prevalence and pattern of antimicrobial resistance of *Salmonella* isolates from Asian pied Starling has been outlined in **Table 6 & 7**

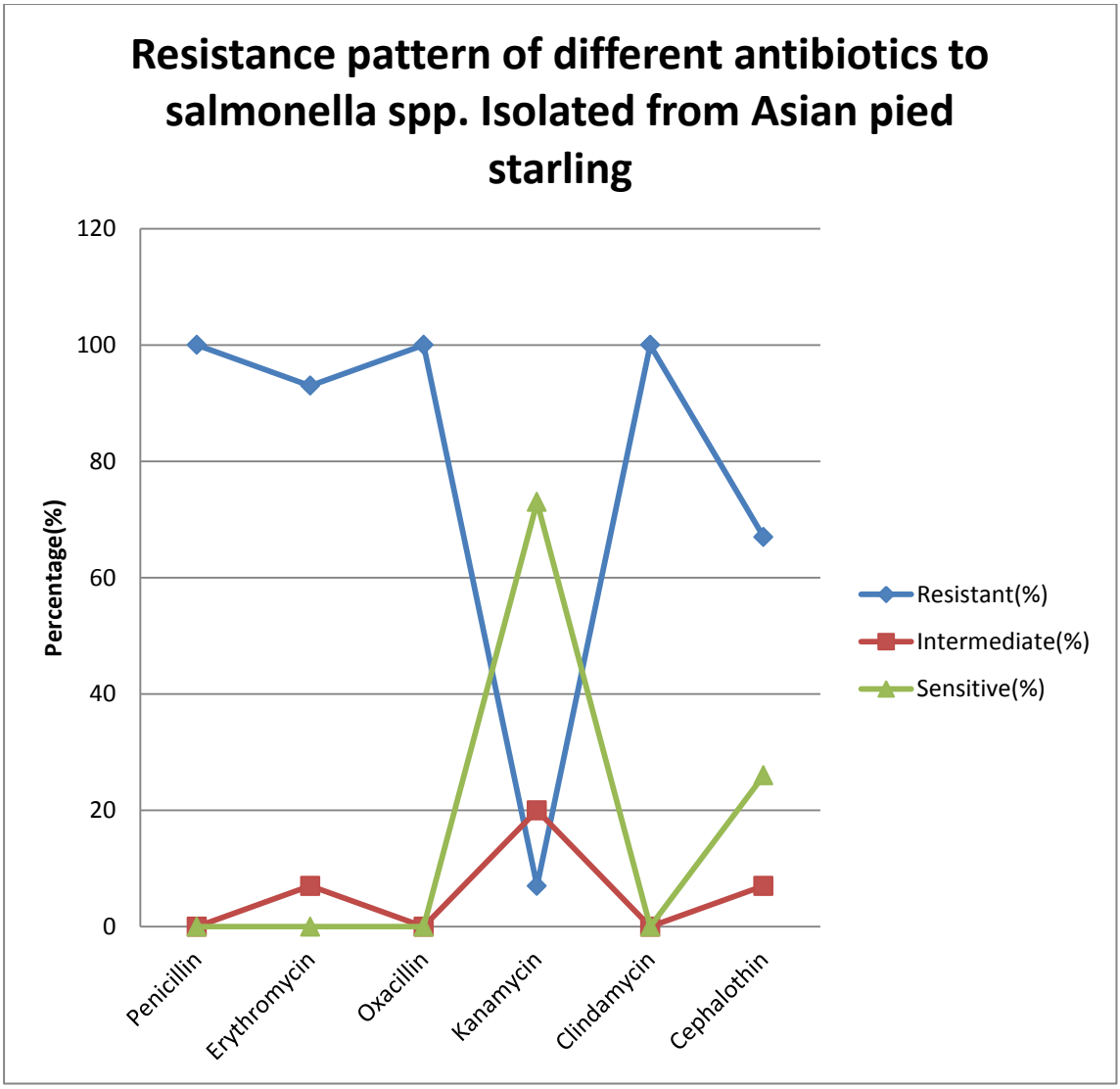


Figure 8: Resistance pattern of *Salmonella* isolates from Asian pied Starling

From Asian pied starling resistance patterns of *Salmonella* were highest in Penicillin, Oxacillin and Clindamycin (100%) followed by Erythromycin (93%), Cephalothin (67%) and Kanamycin (7%). It was revealed that no isolates were found sensitive to Penicillin, Oxacillin and Clindamycin. Kanamycin showed highest level of sensitivity (73%) followed by Cephalothin (26%). In current research, all the isolates of *Salmonella* showed multiple antimicrobial resistances. **Fig 8** shows the graphical presentation of resistance pattern of *salmonella* isolates from Asian pied Starling.

Table 8: Patterns of multidrug resistance in isolates of *Salmonella* from two different species

Antimicrobials	Pattern	Crows (n= 10)	Asian pied starling (n= 15)	Range (%)
Penicillin	R	100%	100%	100
	I	0	0	0
	S	0	0	0
Erythromycin	R	50%	93%	50-93
	I	20%	7%	7-20
	S	30%	0	0-30
Oxacillin	R	100%	100%	100
	I	0	0	0
	S	0	0	0
Kanamycin	R	20%	7%	7-20
	I	10%	20%	10-20
	S	70%	73%	70-73
Clindamycin	R	100%	100%	100
	I	0	0	0
	S	0	0	0
Cephalothin	R	30%	67%	30-67
	I	0	7%	0-7
	S	70%	26%	26-70

Note: R= Resistance; I= Intermediate; S=Sensitive

Among the six tested antimicrobials resistance pattern against *Salmonella* isolates Penicillin, Oxacillin and Clindamycin turned out as the highest level of resistance (100%) followed by Erythromycin (50-93%), Cephalothin (30-67%) and Kanamycin (7-20%) from Crows and Asian pied starling. The rate of sensitivity to individual antibiotics against *Salmonella* isolates from two different species of birds was highest in Kanamycin ranged (70-73%) followed by Cephalothin (26-70%) and erythromycin (0-30%). Penicillin, Oxacillin and Clindamycin showed no variation of resistance level it was (100%) resistance in two species of birds and no isolates were found to sensitive against these three antibiotics. In case of Erythromycin, 93% of *Salmonella* isolates were resistance to Erythromycin in Asian pied starling and resistance of Erythromycin (50%) was in Crows. Antimicrobial resistance of Kanamycin was found in Crows (20%) and (7%) in Asian pied starling. The resistance level

of Cephalothin was higher in Asian pied starling (67%) and lower in Crows (30%). The sensitivity of Kanamycin and Cephalothin to isolated *salmonella* spp. was better within two species of birds among all used antibiotics. Highest Resistance pattern for intermediate type was in Kanamycin ranged (10-20%) followed by Erythromycin (7-20%), Cephalothin (0-7%) and (0%) for other three antibiotics to isolated salmonella for both species of birds. Highest intermediate type resistance was found (20%) to isolated *salmonella* spp. from Asian pied starling in both Kanamycin and Erythromycin. Patterns of multidrug resistance isolates of *Salmonella* within two species of birds presented graphically in **Fig 9.**

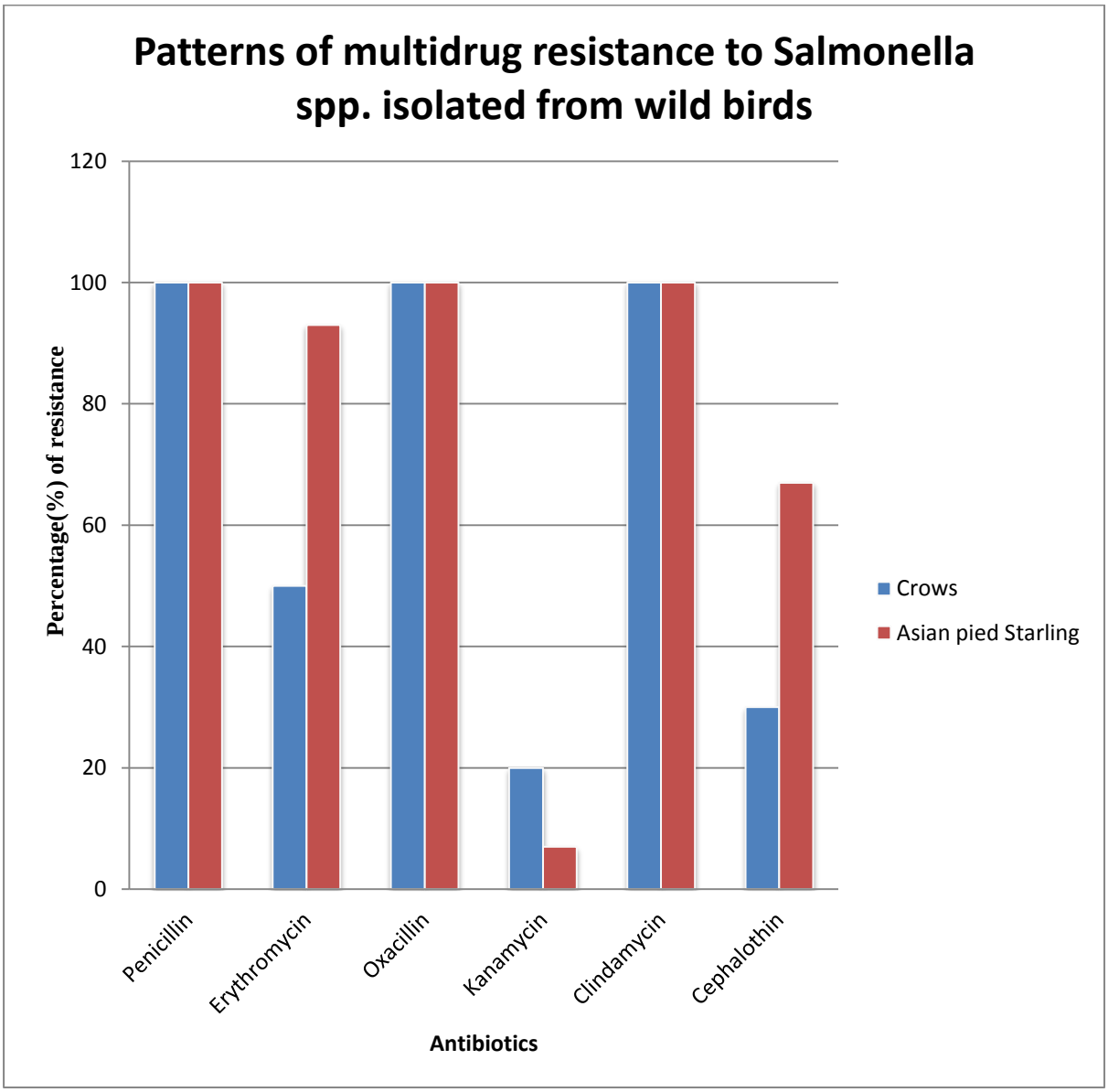


Figure 9: Patterns of multidrug resistance *Salmonella* isolated from wild birds

Chapter-5: Discussion

Crow (*Corvus splendens*) and Asian pied starling (*Gracupica contra*) is very much common in our country, naturally they lives in the forest but they comes out into the populated area for the collection of food. This two species of birds carry lots of pathogenic microorganisms which can easily spread from them to human or other livestock species including poultry by different direct or indirect ways. It is associated with negative health impacts to human and other livestock species. Now a days, for the easy and frequent access of this two species in rural and urban area where different antibiotics were used regularly for human and livestock treatment, Crow and Asian pied starling got some antibiotic residue or antibiotic resistant bacteria from those human and livestock source anyway and they developed antibiotic resistance against some bacterial diseases which have important public health significance, such as salmonellosis. The aim of this study was to determine the occurrence of *Salmonella* spp. in crows and Asian pied starling along with estimating the prevalence of antimicrobial resistance against *Salmonella* spp. in Pahartoli and Bakolia under Chittagong City Corporation of Bangladesh. To the best of my knowledge, it was the first study in Bangladesh to tackle this issue.

5.1 Prevalence of *Salmonella* spp. in Crows and Asian pied starling

20 Cloacal swabs was collected from crow and found 13 samples positive to *salmonella*. Prevalence of *salmonella* in crow was 65% which was very much higher than the result found (Asagi et al., 1976), Asagi and his coworkers found *salmonella* positive 2 out of 30 samples from crows cloacal swabs. 30 samples was also collected from Asian pied starling and found 20 samples were *salmonella* positive. Prevalence of *salmonella* in Asian pied starling was 67% which was also very much higher than the result found (Snoeyenbos et al., 1967; Morishita et al., 1999), Morishita and his co-workers found salmonella positive 62 out of 868 (7.1%) samples from Europian starlings cloacal swabs. Snoeyenbos and coworkers also found 13/148 (12%) samples positive to *salmonella*. Gastrointestinal tract samples tested positive for S. enterica 2.5% prevalence in European starling (Carlson et al., 2011) which also very much lower than the Studied prevalence of *salmonella*. The prevalence level in the present investigation is much higher than other observations. It may be noted that Crow and Asian pied starling is very much common in our country, they are found everywhere all over the Bangladesh. This two species of birds can move easily from forest to the human touched area, that's why they get the contamination of microorganism easily. For the collection of their feed they usually stay near the dirty site like: Dustbin, Sewage, Dead carcass, Waste

food, human stool, Cow dung etc. Those can be identified as a store house for microorganisms. This may be the main cause for the higher prevalence of *salmonella* from those two species. Crows and Asian pied starling's are lives in forest most of the time; they visit to the populated area only during collection of feed. When they stay in the forest they can get contamination of *salmonella* from other birds like Gulls which were act as a carrier for *Salmonella* spp. (Bonnedahl, 2011). On the other hand, higher rate of isolation of *Salmonella* spp. from Cloacal swabs of those two species of birds in developing countries like Bangladesh might be due to favorable climatic condition, sub-standard hygienic condition and lack of proper control measures.

5.2 Antimicrobial resistance

Approaches to prevent and control Salmonellosis in the food animal industry by various means such as improved bio-security, vaccination, use of competitive exclusion products, and the introduction of novel immuno-potentiators with limited success has necessitated the use of antimicrobial chemotherapy in the treatment and control of Salmonellosis (Zhao et al., 2007). To prevent and control Salmonellosis by extensive use of antibiotics in particular has lead to emergence of resistant bacteria. The use of antimicrobials in food animals has resulted in the development of antimicrobial resistance (Oldfield III, 2003), through mutation and acquisition of resistance encoding genes (Fluit, 2005). The situation in developing countries like Bangladesh may be exaggerated by easy accessibility of antimicrobials at a cheaper price and their extensive use in poultry production system (Prakash et al., 2005). Another major setback might be the quality and potency of locally produced antimicrobial drugs; for example, there are over 50 different brands of the Penicillin group in Bangladesh. Thus there is widespread availability and uncontrolled use of antibiotics poses the antimicrobial resistance in food animals and their products which is the actual threat of public health. The current study recorded multiple antimicrobial resistances against *Salmonella* spp. (up to six) and in most cases, estimated 100% resistance for category 2-4 antimicrobials across the study sites. These threatening correspond to the many non-epidemiological and opportunistic earlier studies in Bangladesh (Ahmed et al., 2010; Begum et al., 2010), India (Suresh et al., 2006), Nepal (Pokharel et al., 2006; Dahal et al., 2007), Bhutan (Lan et al., 2005; Dahal et al., 2007).

5.3 Level of antimicrobial resistance *Salmonella* from cloacal swabs of crow and Asian pied Starling

5.3.1 Penicillin

The results of antimicrobial resistance of *Salmonella* isolated revealed that all the isolates from Cloacal swab of crows and Asian pied starling were resistance to Penicillin. Earlier studies conducted in different countries and detected Penicillin was resistant to *salmonella* spp. (Mchugh et al., 1975; Roantree et al., 1977; Fu and Neu, 1978). Penicillin commonly use in livestock and Human treatment also using regularly in poultry. Therefore, residues of these antibiotics could have been passed through different environmental source to the birds or they found penicillin resistant *salmonella* from those sources during their migration. The high resistance of Penicillin to *salmonella* spp. from Cloacal swabs of crow and Asian pied starling of present study might be due to indiscriminate use of these antibiotics to human as well as in livestock. That's why they got the penicillin resistant *salmonella* spp. or residues of that antibiotic from them by taking their dung or stool or other wastage. In addition, sometimes farmers select drugs by their own or rely on neighbor experienced farmers or non-veterinarians and these mal-practices may not always ensure proper drugs doses, frequency of drug administration and complete course of drug treatment. *Salmonella* spp. was G^- bacteria and penicillin was β-lactum antibiotic, we know that β-lactum antibiotic acts mainly on G^+ bacteria which can be another cause of *salmonella* resistance to penicillin. The above factors might have influenced on Penicillin to be resistance against *Salmonella* spp.

5.3.2 Oxacillin

Resistance of Oxacillin in this study was higher and there was no variation in resistance levels among *Salmonella* Crows or Asian pied starlings. 100% resistance of Oxacillin to *salmonella* spp. was found from that study which found the similarity with the result of Oxacillin resistance (Roantree et al., 1977; Guzmán-Blanco et al., 2000; Nishino et al., 2007) against salmonella. Oxacillin is also commonly use in livestock, poultry and Human treatment regularly. Therefore, residues of these antibiotics could have been passed through different environmental source to the birds or they found Oxacillin resistant *salmonella* spp. from those sources during their migration. The high resistance of Oxacillin to *salmonella* spp. from Cloacal swabs of crow and Asian pied starling of present study might be due to indiscriminate use of these antibiotics to human as well as in livestock. That's why they got the Oxacillin resistant *salmonella* spp. or residues of that antibiotic from them by taking their dung or stool or other wastage. In addition, sometimes farmers select drugs by their own or

rely on neighbor experienced farmers or non-veterinarians and these mal-practices may not always ensure proper drugs doses, frequency of drug administration and complete course of drug treatment. *Salmonella* spp. was G^- bacteria and Oxacillin was β-lactum antibiotic, we know that β-lactum antibiotic acts mainly on G^+ bacteria which can be another cause of *salmonella* resistance to Oxacillin. The above factors might have influenced on Oxacillin to be resistance against *Salmonella* spp.

5.3.3 Erythromycin

In this study Erythromycin was resistance more against *Salmonella* spp. isolated from Asian pied starling (93%) than the resistance against *Salmonella* spp. isolated from Crows (50%). In case of isolated *salmonella* from cloacal swab of crows there was found 3 totally sensitive spp. of *salmonella* to erythromycin where in Asian pied starling it was absent. This result was various with many other previous studies where they said erythromycin were totally resistant to *salmonella* spp. (Roantree et al., 1977; Jacobs-Reitsma et al., 1994; Cui et al., 2005). It could be happened due to heavily use of Erythromycin against different infectious diseases including Salmonellosis without proper diagnosis of diseases. Resistance of Erythromycin was evidenced as a serious problem in the study sites at it might be due to Subnormal doses and incomplete treatment course of Erythromycin. The variation between the erythromycin resistance to *salmonella* spp. isolated from crow and Asian pied starling was due to, Asian pied starling got more erythromycin residue or got more erythromycin resistance bacteria than crow.

5.3.4 Kanamycin

Kanamycin showed lower resistance in present study that are good news for the People of Bangladesh cause it can say from the result kanamycin is usable until now in different treatment. From the result we have found that this drug was resistant to *salmonella* spp. isolated from crow was 20% and from Asian pied starling 7%. The Result is more or less similar with the result of different scientist where kanamycin resistance to *salmonella* spp. was 18%-60% (Groisman et al., 1992; Davis et al., 1999; Besser et al., 2000; Carattoli, 2003). The pattern of Kanamycin resistance against *Salmonella* spp. in Bangladesh is one of the commonest antimicrobials. Kanamycin was less widely used in treatment of livestock and human in Bangladesh that's why kanamycin resistant salmonella spp. and residues of that antibiotic was not available in the birds. *Salmonella* spp. was G^- bacteria and Kanamycin was Aminoglycoside antibiotic, we know that Aminoglycoside antibiotic acts mainly on G^-

bacteria which can be another cause of *salmonella* spp. sensitive to Kanamycin. The above factors might have influenced on Kanamycin to be sensitive against *Salmonella* spp.

5.3.5 Clindamycin

Resistance of Clindamycin in this study was higher and there was no variation in resistance levels among *Salmonella* spp. from Crows or Asian pied starlings. 100% resistance of Clindamycin to *salmonella* spp. was found from that study which found the similarity with the result of Clindamycin resistance (Helmuth, 2000; Ang et al., 2004; Maragkoudakis et al., 2009) against *salmonella*. Clindamycin is also commonly use in livestock, poultry and Human treatment regularly in Bangladesh. Therefore, residues of these antibiotics could have been passed through different environmental source to the birds or they found Clindamycin resistant *salmonella* spp. from those sources during their migration. The high resistance of Clindamycin to *salmonella* spp. from Cloacal swabs of crow and Asian pied starling of present study might be due to indiscriminate use of these antibiotics to human as well as in livestock. That's why they got the Clindamycin resistant *salmonella* spp. or residues of that antibiotic from them by taking their dung or stool or other wastage. In addition, sometimes farmers select drugs by their own or rely on neighbor experienced farmers or non-veterinarians and these mal-practices may not always ensure proper drugs doses, frequency of drug administration and complete course of drug treatment. The above factors might have influenced on Clindamycin to be resistance against *Salmonella* spp.

5.3.6 Cephalothin

In this study Cephalothin was resistance more against *Salmonella* spp. isolated from Asian pied starling (67%) than the resistance against *Salmonella* spp. isolated from Crows (30%). In case of isolated *salmonella* spp. from cloacal swab of crows there was found 7/13 totally sensitive spp. of *salmonella* to Cephalothin where in Asian pied starling it was 4/20. This result was more or less similar with many other previous studies where they said Cephalothin was slightly resistant to *salmonella* spp. (Gupta et al., 2003; Archambault et al., 2006; Gebreyes et al., 2006). It could be happened due to less heavily use of Cephalothin against different infectious diseases including Salmonellosis without proper diagnosis of diseases. Resistance of Cephalothin was evidenced as a serious problem in the study sites at it might be due to Subnormal doses and incomplete treatment course of Cephalothin. The variation between the Cephalothin resistance to *salmonella* spp. isolated from crow and Asian pied

starling was due to, Asian pied starling got more Cephalothin residue or got more Cephalothin resistance bacteria than crow.

5.4 Resistance pattern

Overall, three antimicrobial resistance were observed among *Salmonella* spp. isolated from Cloacal swab of crows and Asian pied starling. *Salmonella* spp. was resistant to three of the six antimicrobials tested with simultaneous multi-drug resistance to antimicrobials. Several similar studies were showed higher multi-drug resistance in *Salmonella* spp. isolates from different wild birds (Poppe et al., 1998; Abulreesh et al., 2007). The increasing rates of resistance to Penicillin, Oxacillin and Clindamycin among the isolates might be attributed to the emergence of multi resistance *Salmonella* spp.

5.5 Level of antimicrobial sensitivity

Erythromycin is extensively used against *Salmonella* infection in Bangladesh. However, this drug still remained sensitive against *Salmonella* spp. in studied sites. The results of sensitivity for Erythromycin in this study are more or less similar with other investigations conducted in different places in world (Roantree et al., 1977; Jacobs-Reitsma et al., 1994; Cui et al., 2005). This may be due to the reason of fact that Erythromycin is naturally less resistance drug against *Salmonella* spp. Kanamycin and Cephalothin appeared to be sensitive against *Salmonella* spp. isolated from Cloacal swab of crows and Asian pied starling. This is because these drugs are newly introduced and not commonly used against livestock, poultry and human diseases in Bangladesh as well as in the study areas that's why these drugs may remain sensitive against *Salmonella* spp. *Salmonella* spp. was G^- bacteria and Kanamycin was Aminoglycoside antibiotic, we know that Aminoglycoside antibiotic acts mainly on G^- bacteria which can be another cause of *salmonella* spp. sensitive to Kanamycin. The above factors might have influenced on Kanamycin to be sensitive against *Salmonella* spp. The *Salmonella* strains having similar level of resistance and resistance pattern indicates their origin from a common source. The logical interpretation of the results of the multiple antibiotic resistances (MAR) index is that all *Salmonella* isolated in the study showed that the strains might have originated from environments where antimicrobials are often used. Wild birds are one of the major reservoirs of *Salmonella* spp. are considered to be a high-risk source. Isolates resistant to three or more antimicrobials were classified as multi-drug-resistant (MDR). Multidrug-resistant (MDR) strains of *Salmonella* are now encountered frequently worldwide and the rates of multidrug-resistance have increased considerably in recent years. Even worse, some variants of *Salmonella* have developed multidrug-resistance

as an integral part of the genetic material of the organism, and are therefore likely to retain their drug-resistant genes even when antimicrobial drugs are no longer used, a situation where other resistant strains would typically lose their resistance (Organization, 2005). *Salmonella* spp. isolate from different wild birds showed resistance to several antimicrobials in the Sweden (Bonnedahl, 2011), the isolates of *Salmonella* in this study were found resistant to Tetracycline, Ampicillin, Streptomycin, Chloramphenicol, Nalidixic acid, Cefadroxil. Multi-drug resistant *Salmonella typhimurium* was reported in the past few decades and is frequently reported from the Indian subcontinent (Rahman et al., 2004b). A higher proportion of antibiotic resistance in *Salmonella enteritidis* has been reported from southern Brazil (Dias de Oliveira et al., 2005). Ongoing infection with *Salmonella* organism and frequent migration of crow and Asian pied starling from forest to urban area could considerably increase the prevalence of multiple resistant *Salmonella* spp. in Crows and Asian pied starling in Bangladesh. Therefore, present study demonstrated that the *Salmonella* organism were present in cloacal swab of crows and Asian pied starling and showed different antibiotic resistance pattern which may cause serious health problem in our country.

5.6 The impact on human health

The force on human health of the reservoir in natural environments of these newly emerged resistance genes is clear. Once the genes have mobilized to human pathogenic or commensal bacteria, the genes will increase even more by horizontal transfer to receptive bacterial species and strains, some apparently very fruitfully. This kind of extend is further enhanced by the influence of antibiotic therapy in a hospital setting or under animal husbandry. These new victorious antibiotic-resistant bacterial clones are then unconfined again in the environment through waste water and different dung and stool where the recombination and distribution events may be enhanced even more through the action from pollutants, abnormal microbial communities such as water environments greatly contaminated with waste and sludge from sewage treatment plants and farms. Once there in the aquatic environment the resistant bacteria could easily be picked up by birds such as Crows and Asian pied starling and then reintroduced to humans, e.g. through the animals in food production even in areas far from where the resistance originally emerged, for instance via Crow and Starling feces on fields where cattle are kept. In some areas cattle are held outside in feedlots with hundreds of thousands animals prior to slaughter. The risk of an uncontrolled community spread among humans if the antibiotic-resistant bacteria go into such a food chain is clear. Human, animal and plant pathogens and other bacteria share a common pool of resistance determinants that

simply can be exchanged (Schlüter et al., 2007). The transfer of resistance genes, as well as new combinations of resistance genes and bacterial strains is most likely to occur in environmental compartments with high bacterial density. With the finding of unexpectedly high level of antibiotic resistance determinants in certain bird populations and the heterogeneity in the *Salmonella* strains that harbor them, the gastrointestinal tract of certain bird species, particularly in dense bird colonies and bird roosts could be such a section in the environment. The phenomenon of bird migration could further distribute antibiotic-resistant bacteria to new areas and the fact that birds with their migration are involved in the dissemination of other pathogens such as West Nile Virus, influenza A virus etc. (Reed et al., 2003), indicate that birds are able of introducing antibiotic resistance to new geographical areas.

Chapter-6: Conclusion

Salmonellosis is a leading, zoonotic and widely distributed disease throughout the world. A wide range of cause present to spread such disease but wild birds can be a major spreader for that disease for their migration nature. This multiple antimicrobial-resistant nature of the organism adds to the gravity of the problem. The level of resistance of *Salmonella* to antibiotics should be alarming to the human and livestock. Therefore, it is necessary to inform people about the future alarming condition of the antibiotic resistance. The present situation underlines the increased public and governmental interest in eliminating sub-therapeutic use of antibiotics in livestock, poultry and particularly those that are also used to treat humans. There is need for more rational use of antibiotics in animal production and more careful use in humans. It is important to take concerted action to improve antibiotic resistance surveillance capacity worldwide with a view to monitoring the emerging resistance genes and their transfer in both animal and human. In addition, alternatives to antibiotics should be explored such as the application of probiotics in poultry for production of safe edible products.

Chapter-7: Recommendation

The present study exposes prevalence of *Salmonella* spp. in wild birds collected from two areas of Chittagong. Isolation of antimicrobial resistant *Salmonella* spp. including multi-drug resistance poses a concern to public health threats. The following recommendations should be useful to prevent the contamination of environment.

To prevent the multidrug resistant *Salmonella* spp. the practicing veterinarians should follow the following recommendation

1. Multi-drug resistant *Salmonella* may not be treated by conventional therapeutic agents

2. Sensitive drugs should be identified and choice to treat Salmonella infection

3. Caution should be taken to use the sensitive drugs carefully in terms of dose, frequency of administration and course of drug treatment

To prevent incidence in *Salmonella* spp. in wild bird droppings and its environment

1. In order to control *Salmonella* infection of wild birds in Bangladesh detailed epidemiological investigation is needed

2. Single investigation are not sufficient for formulating a standards by the regulatory agencies, so, large-scale studies are required to explore prevalence of *Salmonella* in wild birds and their environment

3. Step should be taken to prevent wild bird's migration with Salmonella infection

4. Further studies to identify *Salmonella* serotypes and specific antimicrobial resistant gene of *Salmonella* isolates should be needed

5. Proper disposal of dustbin content, sewage water, dead carcass, waste food and human stool should be needed to prevent transmission of *Salmonella* infection

References

Abulreesh, H.H., Goulder, R., Scott, G.W., 2007. Wild birds and human pathogens in the context of ringing and migration. Ringing & Migration 23, 193-200.

Achla, P., Grover, S., Bhatia, R., Khare, S., 2005. Sensitivity index of antimicrobial agents as a simple solution for multidrug resistance in Salmonella Typhi. Ind. J. Med. Res 121, 185-193.

Ahmed, A., Islam, M., Haider, M., Hossain, M., 2008. Seroprevalence and pathology of naturally infected Salmonellosis in poultry with isolation and identification of causal agents. Journal of the Bangladesh Agricultural University 6, 327-334.

Ahmed, M., Rahman, M., Mahbub, K., Wahiduzzaman, M., 2010. Characterization of antibiotic resistant Salmonella spp isolated from chicken eggs of Dhaka city. Journal of Scientific Research 3, 191.

AHMNA, K., Bari, A., Islam, M., Das, P., Ali, M., 1998. Pullorum disease in semi mature chickens and its experimental pathology. Bangladesh Veterinary Journal 32, 124-128.

Akhtar, F., Hussain, I., Khan, A., Rahman, S., 2010. Prevalence and antibiogram studies of Salmonella enteritidis isolated from human and poultry sources. Pakistan Veterinary Journal 30, 25-28.

Akkina, J., Hogue, A., Angulo, F., Johnson, R., Petersen, K., Saini, P., Fedorka-Cray, P., Schlosser, W., 1999. Epidemiologic aspects, control, and importance of multiple-drug resistant Salmonella Typhimurium DT104 in the United States. Journal of the American Veterinary Medical Association 214, 790.

Akter, M., Choudhury, K., Rahman, M., Islam, M., 2007. Seroprevalence of salmonellosis in layer chickens with isolation, identification and antibiogram study of their causal agents. Bangladesh Journal of Veterinary Medicine 5, 39-42.

Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K.A., Davies, J., Handelsman, J., 2010. Call of the wild: antibiotic resistance genes in natural environments. Nature Reviews Microbiology 8, 251-259.

Anderson, A.D., Nelson, J.M., Rossiter, S., Angulo, F.J., 2003. Public health consequences of use of antimicrobial agents in food animals in the United States. Microbial Drug Resistance 9, 373-379.

Ang, J.Y., Ezike, E., Asmar, B.I., 2004. Antibacterial resistance. The Indian Journal of Pediatrics 71, 229-239.

Antunes, P.c., Réu, C., Sousa, J.C., Peixe, L.s., Pestana, N., 2003. Incidence of< i> Salmonella</i> from poultry products and their susceptibility to antimicrobial agents. International journal of food microbiology 82, 97-103.

Archambault, M., Petrov, P., Hendriksen, R.S., Asseva, G., Bangtrakulnonth, A., Hasman, H., Aarestrup, F.M., 2006. Molecular characterization and occurrence of extended-spectrum β-lactamase resistance genes among Salmonella enterica serovar Corvallis from Thailand, Bulgaria, and Denmark. Microbial Drug Resistance 12, 192-198.

Arlet, G., Barrett, T.J., Butaye, P., Cloeckaert, A., Mulvey, M.R., White, D.G., 2006. < i> Salmonella</i> resistant to extended-spectrum cephalosporins: prevalence and epidemiology. Microbes and Infection 8, 1945-1954.

Asagi, M., Oka, C., Sato, G., 1976. Isolation of Salmonella typhimurium var. copenhagen from crows in the city of Otaru. Japanese Journal of Veterinary Science 38, 521-522.

Axelsson, F., Sorin, M., 1997. Transia Salmonella. Diffcham AB, Sweden.

Bager, F., Helmuth, R., 2001. Epidemiology of resistance to quinolones in Salmonella. Veterinary research 32, 285-290.

Bajaj, B., Sharma, V., Koul, S., Thakur, R., 2003. Incidence of Salmonella in poultry and meats and growth inhibition of Salmonella enteritidis by organic acids. Journal of food science and technology 40, 556-558.

Balagué, C., Véscovi, E.G.a., 2001. Activation of multiple antibiotic resistance in uropathogenic Escherichia coli strains by aryloxoalcanoic acid compounds. Antimicrobial agents and chemotherapy 45, 1815-1822.

Bashar, T., Rahman, M., Rabbi, F.A., Noor, R., Rahman, M.M., 2011. Enterotoxin Profiling and Antibiogram of Escherichia coli Isolated from Poultry Feces in Dhaka District of Bangladesh. Stamford Journal of Microbiology 1, 51-57.

Begum, K., Reza, T.A., Haque, M., Hossain, A., Hassan, F.K., Hasan, S.N., Akhter, N., Ahmed, A., Ваша, U., 2010. Isolation, identification and antibiotic resistance pattern of Salmonella spp. from chicken eggs, intestines and environmental samples. Bangladesh Pharmaceutical Journal 13, 23-27.

Bennett, P.M., 1999. Integrons and gene cassettes: a genetic construction kit for bacteria. Journal of Antimicrobial Chemotherapy 43, 1-4.

Besser, T., Goldoft, M., Pritchett, L., Khakhria, R., Hancock, D., Rice, D., Gay, J., Johnson, W., Gay, C., 2000. Multiresistant Salmonella Typhimurium DT104 infections of humans and domestic animals in the Pacific Northwest of the United States. Epidemiology and infection 124, 193-200.

Bhattacharjee, P., Kundu, R., Biswas, R., Mazumder, J., Hossain, E., Miah, A., 1996. A retrospective analysis of chicken diseases diagnosed at the Central Disease Investigation Laboratory, Dhaka. Bangladesh Veterinary Journal 30, 105-113.

Blanco, G., Lemus, J.A., Grande, J., 2009. RETRACTED: Microbial pollution in wildlife: Linking agricultural manuring and bacterial antibiotic resistance in red-billed choughs. Environmental research 109, 405-412.

Blanco, G., Lemus, J.A., Grande, J., Gangoso, L., Grande, J.M., Donázar, J.A., Arroyo, B., Frías, O., Hiraldo, F., 2007. Retracted Geographical variation in cloacal microflora and bacterial antibiotic resistance in a threatened avian scavenger in relation to diet and livestock farming practices. Environmental Microbiology 9, 1738-1749.

Bohm, R., Gozalan, F., Philipp, W., 2004. Comparative study on antibiotic resistance in selected bacterial species isolated from wastewater originating from slaughterhouses and of municipal sources. Proceedings of International Society for Animal Hygiene. Saint-Malo 277.

Bonnedahl, J., 2011. Antibiotic resistance in Enterobacteriaceae isolated from wild birds. Acta Universitatis Upsaliensis.

Bonnedahl, J., Järhult, J.D., 2014. Antibiotic resistance in wild birds. Upsala journal of medical sciences 119, 113-116.

Boonmar, S., Bangtrakulnonth, A., Pornruangwong, S., Samosornsuk, S., Kaneko, K.-i., Ogawa, M., 1998. Significant increase in antibiotic resistance of< i> Salmonella</i> isolates from human beings and chicken meat in Thailand. Veterinary Microbiology 62, 73-80.

Brenner, F., Villar, R., Angulo, F., Tauxe, R., Swaminathan, B., 2000. Salmonella nomenclature. Journal of clinical microbiology 38, 2465-2467.

Butaye, P., Michael, G.B., Schwarz, S., Barrett, T.J., Brisabois, A., White, D.G., 2006. The clonal spread of multidrug-resistant non-typhi< i> Salmonella</i> serotypes. Microbes and infection 8, 1891-1897.

Byarugaba, D., 2004. Antimicrobial resistance in developing countries and responsible risk factors. International journal of antimicrobial agents 24, 105-110.

Carattoli, A., 2003. Plasmid-mediated antimicrobial resistance in Salmonella enterica. Current issues in molecular biology 5, 113-122.

Carlson, J.C., Franklin, A.B., Hyatt, D.R., Pettit, S.E., Linz, G.M., 2011. The role of starlings in the spread of Salmonella within concentrated animal feeding operations. Journal of applied ecology 48, 479-486.

Carramiñana, J.J., Rota, C., Agustin, I., Herrera, A., 2004. High prevalence of multiple resistance to antibiotics in< i> Salmonella</i> serovars isolated from a poultry slaughterhouse in Spain. Veterinary microbiology 104, 133-139.

Chiu, C.-H., Su, L.-H., Chu, C., 2004. Salmonella enterica serotype Choleraesuis: epidemiology, pathogenesis, clinical disease, and treatment. Clinical microbiology reviews 17, 311-322.

Chopra, I., Ball, P., 1982. Transport of antibiotics into bacteria. Advances in microbial physiology 23, 183-240.

Chowdhury, R., Haque, M., Islam, K., Khaleduzzaman, A., 2009. A review on antibiotics in an animal feed. Bangladesh Journal of Animal Science 38, 22-32.

CLSI, 2007. Performance Standards for Antimicrobial Susceptibility testing; Seventeenth informational Suppliment. 26& 27.

Cogan, T., Humphrey, T., 2003. The rise and fall of Salmonella Enteritidis in the UK. Journal of applied microbiology 94, 114-119.

Cole, D., Drum, D.J., Stallknecht, D.E., White, D.G., Lee, M.D., Ayers, S., Sobsey, M., Maurer, J.J., 2005. Free-living Canada geese and antimicrobial resistance. Emerg. Infect. Dis 11, 935-938.

Control, C.f.D., Prevention, 2004. Outbreak of Salmonella serotype Enteritidis infections associated with raw almonds--United States and Canada, 2003-2004. MMWR. Morbidity and mortality weekly report 53, 484.

Control, C.f.D., Prevention, 2006. Multistate outbreak of Salmonella typhimurium infections associated with eating ground beef--United States, 2004. MMWR. Morbidity and mortality weekly report 55, 180.

Control, C.f.D., Prevention, 2007. Multistate outbreaks of Salmonella infections associated with raw tomatoes eaten in restaurants--United States, 2005-2006. MMWR. Morbidity and mortality weekly report 56, 909.

Cooke, F.J., Day, M., Wain, J., Ward, L.R., Threlfall, E.J., 2007. Cases of typhoid fever imported into England, Scotland and Wales (2000–2003). Transactions of the Royal Society of Tropical Medicine and Hygiene 101, 398-404.

Costa, D., Poeta, P., Sáenz, Y., Vinué, L., Coelho, A.C., Matos, M., Rojo-Bezares, B., Rodrigues, J., Torres, C., 2008. Mechanisms of antibiotic resistance in Escherichia coli isolates recovered from wild animals. Microbial Drug Resistance 14, 71-77.

Cruickshank, R., Duguid, J., Marmion, B., Swain, R., Churchill, L., 1975. Medical microbiology:(The practice of medical microbiology), vol. II. Edinburgh and New York.

Cui, S., Ge, B., Zheng, J., Meng, J., 2005. Prevalence and antimicrobial resistance of Campylobacter spp. and Salmonella serovars in organic chickens from Maryland retail stores. Applied and environmental microbiology 71, 4108-4111.

Dahal, N., Ellerbroek, L., Poosaran, N., 2007. Prevalence and antimicrobial resistance of Salmonella in imported chicken carcasses in Bhutan. Unpublished MSc Thesis, Chiang mai university and Freie universität, Berlin.

Das, U., Bhattacharya, S., 2006. Antibiogram, phage typing and biotyping of Salmonella Typhi and Salmonella paratyphi A from Rourkela, Orissa. The Indian journal of medical research 124, 109.

Davies, J., Davies, D., 2010. Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews 74, 417-433.

Davis, M.A., Hancock, D.D., Besser, T.E., Rice, D.H., Gay, J.M., Gay, C., Gearhart, L., DiGiacomo, R., 1999. Changes in antimicrobial resistance among Salmonella enterica Serovar typhimurium isolates from humans and cattle in the Northwestern United States, 1982-1997. Emerging infectious diseases 5, 802.

De Jong, B., Ekdahl, K., 2006. The comparative burden of salmonellosis in the European Union member states, associated and candidate countries. BMC Public Health 6, 4.

Deignan, T., Alwan, A., Kelly, J., McNair, J., Warren, T., O'farrelly, C., 2000. Serum haptoglobin: an objective indicator of experimentally-induced< i> Salmonella</i> infection in calves. Research in Veterinary Science 69, 153-158.

Del Hoyo, J., Elliot, A., Sargatal, J., 1992. Handbook of the Birds of the World. Barcelona: Lynx Editions.

Dias de Oliveira, S.l., Siqueira Flores, F., dos Santos, L.R., Brandelli, A., 2005. Antimicrobial resistance in< i> Salmonella enteritidis</i> strains isolated from broiler carcasses, food, human and poultry-related samples. International journal of food microbiology 97, 297-305.

Dolejska, M., Šenk, D., Čížek, A., Rybaříková, J., Sychra, O., Literak, I., 2008. Antimicrobial resistant< i> Escherichia coli</i> isolates in cattle and house sparrows on two Czech dairy farms. Research in veterinary science 85, 491-494.

Domınguez, C., Gomez, I., Zumalacarregui, J., 2002. Prevalence of< i> Salmonella</i> and< i> Campylobacter</i> in retail chicken meat in Spain. International Journal of Food Microbiology 72, 165-168.

Drobni, M., Bonnedahl, J., Hernandez, J., Haemig, P., Olsen, B., 2009. Vancomycin-resistant Enterococci, point barrow, Alaska, USA. Emerg Infect Dis 15, 838-839.

El Hussein, A.A., Mohy-Eldin, H.S., Elmadiena, M.M.N., El Siddig, M.A., 2012. Prevalence, Detection and Antimicrobial Resistance Pattern of Salmonella in Sudan.

Enabulele, S.A., Amune, P.O., Aborisade, W.T., 2010. Antibiograms of salmonella isolates from poultry farms in Ovia North East local government area, Edo State, Nigeria. Agric. Biol. J. North Am 1, 1287-1290.

Fallacara, D., Monahan, C., Morishita, T., Wack, R., 2001. Fecal shedding and antimicrobial susceptibility of selected bacterial pathogens and a survey of intestinal parasites in free-living waterfowl. Avian diseases, 128-135.

Fluit, A.C., 2005. Towards more virulent and antibiotic-resistant Salmonella? FEMS Immunology & Medical Microbiology 43, 1-11.

Food, Administration, D., 2010. Summary report on antimicrobials sold or distributed for use in food-producing animals. Center for Veterinary Medicine, Accessed September 5, 2012.

Frye, J.G., Fedorka-Cray, P.J., 2007. Prevalence, distribution and characterisation of ceftiofur resistance in< i> Salmonella enterica</i> isolated from animals in the USA from 1999 to 2003. International journal of antimicrobial agents 30, 134-142.

Fu, K.P., Neu, H.C., 1978. Piperacillin, a new penicillin active against many bacteria resistant to other penicillins. Antimicrobial agents and chemotherapy 13, 358-367.

Galanis, E., Wong, D.M.L.F., Patrick, M.E., Binsztein, N., Cieslik, A., Chalermchaikit, T., Aidara-Kane, A., Ellis, A., Angulo, F.J., Wegener, H.C., 2006. Web-based surveillance and global Salmonella distribution, 2000–2002. Emerging infectious diseases 12, 381.

Gautam, V., Gupta, N.K., Chaudhary, U., Arora, D., 2002. Sensitivity pattern of Salmonella serotypes in Northern India. Brazilian journal of infectious diseases 6, 281-287.

Gebreyes, W.A., Thakur, S., Morrow, W., 2006. Comparison of prevalence, antimicrobial resistance, and occurrence of multidrug-resistant Salmonella in antimicrobial-free and conventional pig production. Journal of Food Protection® 69, 743-748.

Gilliver, M.A., Bennett, M., Begon, M., Hazel, S.M., Hart, C.A., 1999. Enterobacteria: antibiotic resistance found in wild rodents. Nature 401, 233-234.

Groisman, E.A., Parra-Lopez, C., Salcedo, M., Lipps, C.J., Heffron, F., 1992. Resistance to host antimicrobial peptides is necessary for Salmonella virulence. Proceedings of the National Academy of Sciences 89, 11939-11943.

Guardabassi, L., Dalsgaard, A., 2002. Occurrence and fate of antibiotic resistant bacteria in sewage. Danish Environmental Protection Agency.

Guardabassi, L., Dijkshoorn, L., Collard, J.-M., Olsen, J., Dalsgaard, A., 2000. Distribution and in-vitro transfer of tetracycline resistance determinants in clinical and aquatic Acinetobacter strains. Journal of medical microbiology 49, 929-936.

Guardabassi, L., Petersen, A., Olsen, J.E., Dalsgaard, A., 1998. Antibiotic Resistance in Acinetobacterspp. Isolated from Sewers Receiving Waste Effluent from a Hospital and a Pharmaceutical Plant. Applied and Environmental Microbiology 64, 3499-3502.

Gupta, A., Fontana, J., Crowe, C., Bolstorff, B., Stout, A., Van Duyne, S., Hoekstra, M.P., Whichard, J.M., Barrett, T.J., 2003. Emergence of multidrug-resistant Salmonella enterica serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. Journal of Infectious Diseases 188, 1707-1716.

Guzmán-Blanco, M., Casellas, J.M., Silva Sader, H., 2000. Bacterial resistance to antimicrobial agents in Latin America: The giant is awakening. Infectious disease clinics of North America 14, 67-81.

Hafez, H.M., 2011. Enteric diseases of poultry with special attention to Clostridium perfringens. Pak Vet J 31, 175-184.

Hakanen, A., Kotilainen, P., Huovinen, P., Helenius, H., Siitonen, A., 2001. Reduced fluoroquinolone susceptibility in Salmonella enterica serotypes in travelers returning from Southeast Asia. Emerging infectious diseases 7, 996.

Hammack, T.S., Amaguaña, R.M., June, G.A., Sherrod, P.S., Andrews, W.H., 1999. Relative effectiveness of selenite cystine broth, tetrathionate broth, and Rappaport-Vassiliadis medium for the recovery of Salmonella spp. from foods with a low microbial load. Journal of Food Protection® 62, 16-21.

Haque, M., Hamid, M., Howlider, M., Huque, Q., 1991. Performance of native chicks and hens reared together or separately under rural conditions of Bangladesh. The Bangladesh Veterinarian 8, 11-13.

Hawkey, P.M., 1998. The origins and molecular basis of antibiotic resistance. Bmj 317, 657-660.

Hayes, J.D., Wolf, C.R., 1990. Molecular mechanisms of drug resistance. Biochemical journal 272, 281.

Helmuth, R., 2000. Antibiotic resistance in Salmonella. Salmonella in domestic animals, 89-106.

Herikstad, H., Motarjemi, Y., Tauxe, R., 2002. Salmonella surveillance: a global survey of public health serotyping. Epidemiology and Infection 129, 1-8.

Horimoto, T., Kawaoka, Y., 2001. Pandemic threat posed by avian influenza A viruses. Clinical microbiology reviews 14, 129-149.

Howard, D.H., Scott, R.D., 2005. The economic burden of drug resistance. Clinical infectious diseases 41, S283-S286.

Hudson, C.R., Quist, C., Lee, M.D., Keyes, K., Dodson, S.V., Morales, C., Sanchez, S., White, D.G., Maurer, J.J., 2000. Genetic Relatedness of SalmonellaIsolates from Nondomestic Birds in Southeastern United States. Journal of clinical microbiology 38, 1860-1865.

Islam, M., Haider, M., Chowdhury, E., Kamruzzaman, M., Hossain, M., 2006. Seroprevalence and pathological study of Salmonella infections in layer chickens and isolation and identification of causal agents. Bangladesh Journal of Veterinary Medicine 4, 79-85.

Jacobs-Reitsma, W., Koenraad, P., Bolder, N., Mulder, R., 1994. In vitro susceptibility of Campylobacter and Salmonella isolates from broilers to quinolones, ampicillin, tetracycline, and erythromycin. Veterinary quarterly 16, 206-208.

Jay, J.M., Loessner, M.J., Golden, D.A., 2005. Modern food microbiology. Springer.

Johnson, J.M., Rajic, A., McMullen, L.M., 2005. Antimicrobial resistance of selected Salmonella isolates from food animals and food in Alberta. The Canadian Veterinary Journal 46, 141.

Jordan, E., Egan, J., Dullea, C., Ward, J., McGillicuddy, K., Murray, G., Murphy, A., Bradshaw, B., Leonard, N., Rafter, P., 2006. < i> Salmonella</i> surveillance in raw and cooked meat and meat products in the Republic of Ireland from 2002 to 2004. International journal of food microbiology 112, 66-70.

Kamal, A., Hossain, M., 1989. Pathological investigation on the mortality of chickens in Bangladesh Agricultural University Poultry Farm. M. Sc.(Vet. Science) Thesis to Bangladesh Agricultural University, Mymensingh.

Kariuki, S., Revathi, G., Gakuya, F., Yamo, V., Muyodi, J., Hart, C.A., 2002. Lack of clonal relationship between non-typhi Salmonella strain types from humans and those isolated from animals living in close contact. FEMS Immunology & Medical Microbiology 33, 165-171.

Kaye, K.S., Engemann, J.J., Fraimow, H.S., Abrutyn, E., 2004. Pathogens resistant to antimicrobial agents: epidemiology, molecular mechanisms, and clinical management. Infectious disease clinics of North America 18, 467-511.

Khanal, B., Sharma, S.K., Bhattacharya, S.K., Bhattarai, N.R., Deb, M., Kanungo, R., 2007. Antimicrobial susceptibility patterns of Salmonella enterica serotype typhi in eastern Nepal. Journal of health, population, and nutrition 25, 82.

Kirk, J.H., Holmberg, C.A., Jeffrey, J.S., 2002. Prevalence of Salmonella spp in selected birds captured on California dairies. Journal of the American Veterinary Medical Association 220, 359-362.

Kümmerer, K., Henninger, A., 2003. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. Clinical Microbiology and Infection 9, 1203-1214.

Lan, Y., Verstegen, M., Tamminga, S., Williams, B., 2005. The role of the commensal gut microbial community in broiler chickens. World's Poultry Science Journal 61, 95-104.

Lauderdale, T.-L., Aarestrup, F.M., Chen, P.-C., Lai, J.-F., Wang, H.-Y., Shiau, Y.-R., Huang, I.-W., Hung, C.-L., 2006. Multidrug resistance among different serotypes of clinical< i> Salmonella</i> isolates in Taiwan. Diagnostic microbiology and infectious disease 55, 149-155.

Levy, S.B., 1998. The challenge of antibiotic resistance. Scientific American 278, 32-39.

Levy, S.B., Marshall, B., 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nature medicine 10, S122-S129.

Lindgren, M.M., Kotilainen, P., Huovinen, P., Hurme, S., Lukinmaa, S., Webber, M.A., Piddock, L.J., Siitonen, A., Hakanen, A.J., 2009. Reduced fluoroquinolone susceptibility in Salmonella enterica isolates from travelers, Finland. Emerging infectious diseases 15, 809.

Literak, I., Vanko, R., Dolejska, M., Čížek, A., Karpíšková, R., 2007. Antibiotic resistant Escherichia coli and Salmonella in Russian rooks (Corvus frugilegus) wintering in the Czech Republic. Letters in applied microbiology 45, 616-621.

Livermore, D.M., 2003. Bacterial resistance: origins, epidemiology, and impact. Clinical infectious diseases 36, S11-S23.

Madhulika, U., Harish, B., Parija, S., 2004. Current pattern in antimicrobial susceptibility of Salmonella Typhi isolates in Pondicherry. The Indian journal of medical research 120, 111-114.

Madigan, M.T., Martinko, J.M., Parker, J., Brock, T.D., 1997. Biology of microorganisms. prentice hall Upper Saddle River, NJ.

Maharjan, M., Joshi, V., Joshi, D.D., Manandhar, P., 2006. Prevalence of Salmonella species in various raw meat samples of a local market in Kathmandu. Annals of the New York Academy of Sciences 1081, 249-256.

Mahillon, J., 1998. Transposons as gene haulers. Apmis 106, 29-36.

MAHMUD, K.K., 2013. Determination of Antibiotic Residue Level in Commercial Poultry Meat and Assess the Heat Tolerance Level of That Residues at Cooking and Autoclaving. CHITTAGONG VETERINARY AND ANIMAL SCIENCES UNIVERSITY, CHITTAGONG.

Mahmud, M.S., Bari, M.L., Hossain, M.A., 2011. Prevalence of Salmonella serovars and antimicrobial resistance profiles in poultry of Savar area, Bangladesh. Foodborne pathogens and disease 8, 1111-1118.

Mandal, S., Mandal, M., Pal, N., 2004. Reduced minimum inhibitory concentration of chloramphenicol for Salmonella enterica serovar typhi. Indian journal of medical sciences 58, 16.

Mandell, G., Dolin, R., Bennett, J., Mandell, G.L., Bennett, J., 2009. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Elsevier.

Maragkoudakis, P.A., Mountzouris, K.C., Psyrras, D., Cremonese, S., Fischer, J., Cantor, M.D., Tsakalidou, E., 2009. Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against< i> Listeria monocytogenes</i> and< i> Salmonella enteritidis</i>. International journal of food microbiology 130, 219-226.

Mathew, A.G., Cissell, R., Liamthong, S., 2007. Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. Foodborne pathogens and disease 4, 115-133.

McDermott, P., Zhao, S., Wagner, D., Simjee, S., Walker, R., White, D., 2002. The food safety perspective of antibiotic resistance. Animal biotechnology 13, 71-84.

Mchugh, G.L., Moellering, R., Hopkins, C., Swartz, M., 1975. Salmonella typhimurium resistant to silver nitrate, chloramphenicol, and ampicillin. Lancet 1, 235-240.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., Tauxe, R.V., 1999. Food-related illness and death in the United States. Emerging infectious diseases 5, 607.

Middleton, J., Ambrose, A., 2005. Enumeration and antibiotic resistance patterns of fecal indicator organisms isolated from migratory Canada geese (Branta canadensis). Journal of wildlife diseases 41, 334-341.

Mohanty, S., Renuka, K., Sood, S., Das, B., Kapil, A., 2006. Antibiogram pattern and seasonality of Salmonella serotypes in a North Indian tertiary care hospital. Epidemiology and infection 134, 961-966.

Mølbak, K., Neimann, J., 2002. Risk factors for sporadic infection with Salmonella Enteritidis, Denmark, 1997–1999. American Journal of Epidemiology 156, 654-661.

Molla, B., Mesfin, A., Alemayehu, D., 2004. Multiple antimicrobial-resistant Salmonella serotypes isolated from chicken carcass and giblets in Debre Zeit and Addis Ababa, Ethiopia. Ethiopian Journal of Health Development 17, 131-139.

Montville, T., Matthews, K., 2008. Food microbiology: An introduction . 428 p. United States of America: ASM Press, Washington, DC. ISBN.

Morishita, T.Y., Ley, E.C., Harr, B.S., 1999. Survey of pathogens and blood parasites in free-living passerines. Avian diseases, 549-552.

Murti, B.R., Rajyalakshmi, K., Bhaskaran, C., 1962. Resistance of Salmonella typhi to chloramphenicol Part IA preliminary report. Journal of clinical pathology 15, 544-548.

Murugkar, H., Rahman, H., Kumar, A., Bhattacharyya, D., 2005. Isolation, phage typing & antibiogram of Salmonella from man & animals in northeastern India. Indian Journal of Medical Research 122, 237.

Nayak, R., Stewart-King, T., 2008. Molecular epidemiological analysis and microbial source tracking of Salmonella enterica serovars in a preharvest turkey production environment. Foodborne pathogens and disease 5, 115-126.

Neu, H.C., 1992. The crisis in antibiotic resistance. Science 257, 1064-1073.

NIAID, 2009. http://www.niaid.nih.gov/topics/antimicrobialResistance/Understanding/Pages/geneTransfer.aspx.

Nishino, K., Nikaido, E., Yamaguchi, A., 2007. Regulation of multidrug efflux systems involved in multidrug and metal resistance of Salmonella enterica serovar Typhimurium. Journal of bacteriology 189, 9066-9075.

Notter, D., 2012. Bangladesh Journals Online. Bangladesh Journal of Animal Science; Vol 41, 35-40.

Ohmori, H., Azuma, A., Suzuki, Y., Hashimoto, Y., 1977. Factors involved in beta-lactam antibiotic resistance in Pseudomonas aeruginosa. Antimicrobial agents and chemotherapy 12, 537-539.

Okeke, I.N., Laxminarayan, R., Bhutta, Z.A., Duse, A.G., Jenkins, P., O'Brien, T.F., Pablos-Mendez, A., Klugman, K.P., 2005. Antimicrobial resistance in developing countries. Part I: recent trends and current status. The Lancet infectious diseases 5, 481-493.

Oldfield III, E.C., 2003. The road to resistance: antibiotics as growth promoters for animals: White DG, Zhao S, Sudler R, et al. The Isolation of Antibiotic Resistant Salmonella From Retail Ground Meats, N Engl J Med 2001; 345: 1147–54. The American journal of gastroenterology 98, 499.

Organization, W.H., 2005. Drug-resistant salmonella. Fact sheet 139.

Otaki, Y., 1995. Poultry disease control programme in Japan. Asian Livestock (FAO).

Padungtod, P., Kaneene, J.B., 2006. < i> Salmonella</i> in food animals and humans in northern Thailand. International journal of food microbiology 108, 346-354.

Palmgren, H., Sellin, M., Bergström, S., Olsen, B., 1997. Enteropathogenic bacteria in migrating birds arriving in Sweden. Scandinavian Journal of Infectious Diseases 29, 565-568.

Paterson, D.L., 2006. Resistance in gram-negative bacteria: Enterobacteriaceae. The American journal of medicine 119, S20-S28.

Piddock, L.J., 2006. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clinical microbiology reviews 19, 382-402.

Pignato, S., Giammanco, G., Santangelo, C., Giammanco, G., 1998. Endemic presence of< i> Salmonella bongori</i> 48: z< sub> 35</sub>:-causing enteritis in children in Sicily. Research in microbiology 149, 429-431.

Poeta, P., Radhouani, H., Igrejas, G., Gonçalves, A., Carvalho, C., Rodrigues, J., Vinué, L., Somalo, S., Torres, C., 2008. Seagulls of the Berlengas natural reserve of Portugal as carriers of fecal Escherichia coli harboring CTX-M and TEM extended-spectrum beta-lactamases. Applied and environmental microbiology 74, 7439-7441.

Pokharel, B.M., Koirala, J., Dahal, R.K., Mishra, S.K., Khadga, P.K., Tuladhar, N., 2006. Multidrug-resistant and extended-spectrum beta-lactamase (ESBL)-producing< i> Salmonella enterica</i>(serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. International journal of infectious diseases 10, 434-438.

Popoff, M., Le Minor, L., 2001. Antigenic formulas of the Salmonella serovars, WHO Collaborating Centre for Reference and Research on Salmonella. World Health Organ.

Popoff, M.Y., Bockemühl, J., Gheesling, L.L., 2003. Supplement 2001 (no. 45) to the Kauffmann–White scheme. Research in microbiology 154, 173-174.

Poppe, C., Martin, L., Muckle, A., Archambault, M., McEwen, S., Weir, E., 2006. Characterization of antimicrobial resistance of Salmonella Newport isolated from animals, the environment, and animal food products in Canada. Canadian journal of veterinary research 70, 105.

Poppe, C., Smart, N., Khakhria, R., Johnson, W., Spika, J., Prescott, J., 1998. Salmonella typhimurium DT104: a virulent and drug-resistant pathogen. The Canadian veterinary journal 39, 559.

Prakash, B., Krishnappa, G., Muniyappa, L., Kumar, B.S., 2005. Epidemiological characterization of avian Salmonella enterica serovar infections in India. International Journal of Poultry Science 4, 388-395.

Rabsch, W., Tschäpe, H., Bäumler, A.J., 2001. Non-typhoidal salmonellosis: emerging problems. Microbes and infection 3, 237-247.

Radimersky, T., Frolkova, P., Janoszowska, D., Dolejska, M., Svec, P., Roubalova, E., Cikova, P., Cizek, A., Literak, I., 2010. Antibiotic resistance in faecal bacteria (Escherichia coli, Enterococcus spp.) in feral pigeons. Journal of applied microbiology 109, 1687-1695.

Rahman, H., Bhattacharya, D., Murugkar, H., 2004a. Prevalence of Salmonella in poultry in Northeastern India. India. J. Vety Res 13, 1-7.

Rahman, M., Samad, M., Rahman, M., Kabir, S., 2004b. Bacterio-pathological studies on salmonellosis, colibacillosis and pasteurellosis in natural and experimental infections in chickens. Bangladesh Journal of Veterinary Medicine 2, 1-8.

Reed, K.D., Meece, J.K., Henkel, J.S., Shukla, S.K., 2003. Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, influenza A and enteropathogens. Clinical medicine & research 1, 5-12.

Roantree, R., Kuo, T.-T., MacPhee, D., 1977. The effect of defined lipopolysaccharide core defects upon antibiotic resistances of Salmonella typhimurium. Journal of general microbiology 103, 223-234.

Rose, J.M., Gast, R.J., Bogomolni, A., Ellis, J.C., Lentell, B.J., Touhey, K., Moore, M., 2009. Occurrence and patterns of antibiotic resistance in vertebrates off the Northeastern United States coast. FEMS microbiology ecology 67, 421-431.

Rowe, B., Ward, L., Threlfall, E., Wallace, M., Yousif, A., 1990. Spread of multiresistant Salmonella typhl. The Lancet 336, 1065-1066.

Rybaříková, J., Dolejská, M., Materna, D., Literák, I., Čížek, A., 2010. Phenotypic and genotypic characteristics of antimicrobial resistant< i> Escherichia coli</i> isolated from symbovine flies, cattle and sympatric insectivorous house martins from a farm in the Czech Republic (2006–2007). Research in veterinary science 89, 179-183.

Saha, M., Saha, D., Dutta, P., Mitra, U., Bhattacharya, S., 2001. Isolation of Salmonella enterica serotypes from children with diarrhoea in Calcutta, India. Journal of Health, Population and Nutrition, 301-305.

Salm-Surv, W.G., Organization, W.H., 2006. Progress report (2000-2005): building capacity for laboratory-based foodborne disease surveillance and outbreak detection and response.

Scherer, C.A., Miller, S.I., 2001. Molecular pathogenesis of Salmonellae. Principles of bacterial pathogenesis, 265-333.

Schlüter, A., Szczepanowski, R., Pühler, A., Top, E.M., 2007. Genomics of IncP-1 antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool. FEMS microbiology reviews 31, 449-477.

Schroeder, C.M., White, D.G., Meng, J., 2004. Retail meat and poultry as a reservoir of antimicrobial-resistant< i> Escherichia coli</i>. Food microbiology 21, 249-255.

Schwartz, T., Kohnen, W., Jansen, B., Obst, U., 2003. Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiology Ecology 43, 325-335.

Schwarz, S., Kehrenberg, C., Walsh, T., 2001. Use of antimicrobial agents in veterinary medicine and food animal production. International journal of antimicrobial agents 17, 431-437.

Shakespeare, W.A., Davie, D., Tonnerre, C., Rubin, M.A., Strong, M., Petti, C.A., 2005. Nalidixic acid-resistant Salmonella enterica serotype Typhi presenting as a primary psoas abscess: case report and review of the literature. Journal of clinical microbiology 43, 996-998.

Sharma, C., Gill, J., Aulakh, R., Bedi, S., Bedi, J., Sharma, J., 2005. Prevalence of enterotoxigenic Escherichia coli and Salmonella typhimurium in meat and meat products. Journal of Food Science and Technology(Mysore) 42, 56-58.

Sikder, A., Islam, M., Rahman, M., Rahman, M., 2005. Seroprevalence of Salmonella and Mycoplasma gallisepticum infection in the six model breeder poultry farms at Patuakhali district in Bangladesh. International Journal of Poultry Science 4, 905-910.

Skurnik, D., Ruimy, R., Andremont, A., Amorin, C., Rouquet, P., Picard, B., Denamur, E., 2006. Effect of human vicinity on antimicrobial resistance and integrons in animal faecal Escherichia coli. Journal of Antimicrobial chemotherapy 57, 1215-1219.

Snoeyenbos, G., Morin, E., Wetherbee, D., 1967. Naturally occurring salmonella in" blackbirds" and gulls. Avian diseases, 642-646.

Steve Yan, S., Pendrak, M.L., Abela-Ridder, B., Punderson, J.W., Fedorko, D.P., Foley, S.L., 2004. An overview of< i> Salmonella</i> typing: Public health perspectives. Clinical and Applied Immunology Reviews 4, 189-204.

SUNDSTRÖM, L., 1998. The potential of integrons and connected programmed rearrangements for mediating horizontal gene transfer. Apmis 106, 37-42.

Suresh, T., Hatha, A., Sreenivasan, D., Sangeetha, N., Lashmanaperumalsamy, P., 2006. Prevalence and antimicrobial resistance of< i> Salmonella</i> enteritidis and other salmonellas in the eggs and egg-storing trays from retails markets of Coimbatore, South India. Food microbiology 23, 294-299.

Tacão, M., Correia, A., Henriques, I., 2012. Resistance to broad-spectrum antibiotics in aquatic systems: anthropogenic activities modulate the dissemination of blaCTX-M-like genes. Applied and environmental microbiology 78, 4134-4140.

Tankhiwale, S., Agrawal, G., Jalgaonkar, S., 2003. A preliminary report on current antibiogram of Salmonella enterica serotype Typhi in Nagpur. Indian journal of medical microbiology 21, 292.

Threlfall, E., Graham, A., Cheasty, T., Ward, L., Rowe, B., 1997. Resistance to ciprofloxacin in pathogenic Enterobacteriaceae in England and Wales in 1996. Journal of clinical pathology 50, 1027-1028.

Threlfall, E.J., Ward, L.R., Frost, J.A., Willshaw, G.A., 2000. The emergence and spread of antibiotic resistance in food-borne bacteria. International journal of food microbiology 62, 1-5.

Tindall, B., Grimont, P., Garrity, G., Euzeby, J., 2005. Nomenclature and taxonomy of the genus Salmonella. International journal of systematic and evolutionary microbiology 55, 521-524.

Tizard, I., 2004. Salmonellosis in wild birds. In, Seminars in avian and exotic pet medicine, 50-66.

Uyttendaele, M., Debevere, J., Lips, R., Neyts, K., 1998. Prevalence of< i> Salmonella</i> in poultry carcasses and their products in Belgium. International Journal of Food Microbiology 40, 1-8.

van den Bogaard, A.E., Stobberingh, E.E., 2000. Epidemiology of resistance to antibiotics: links between animals and humans. International journal of antimicrobial agents 14, 327-335.

Van Duijkeren, E., Wannet, W., Houwers, D., Van Pelt, W., 2003. Antimicrobial susceptibilities of Salmonella strains isolated from humans, cattle, pigs, and chickens in the Netherlands from 1984 to 2001. Journal of clinical microbiology 41, 3574-3578.

Varma, J.K., Mølbak, K., Barrett, T.J., Beebe, J.L., Jones, T.F., Rabatsky-Ehr, T., Smith, K.E., Vugia, D.J., Chang, H.-G.H., Angulo, F.J., 2005. Antimicrobial-resistant nontyphoidal Salmonella is associated with excess bloodstream infections and hospitalizations. Journal of Infectious Diseases 191, 554-561.

Velge, P., Cloeckaert, A., Barrow, P., 2005. Emergence of Salmonella epidemics: The problems related to Salmonella enterica serotyp Enteritidis and multiple antibiotic resistance in other major serotypes. Veterinary research 36, 267-288.

Vo, A.T., Van Duijkeren, E., Fluit, A.C., Wannet, W.J., Verbruggen, A.J., Maas, H.M., Gaastra, W., 2006. Antibiotic resistance, integrons and< i> Salmonella</i> genomic island 1 among non-typhoidal< i> Salmonella</i> serovars in The Netherlands. International journal of antimicrobial agents 28, 172-179.

Walia, K., 2003. Emerging problem of antimicrobial resistance in developing countries: Intertwining socioeconomic issues. In, ISSN 1020 4237© World Health Organization 2002 Publications of the World Health Organization enjoy copyright protection in accordance with the provisions of Protocol 2 of the Universal Copyright Convention. For rights of reproduction or translation, in part or in toto, of publications issued by the WHO Regional Office for South, 1.

Wang, S.J., Yeh, D.B., 2002. Designing of polymerase chain reaction primers for the detection of Salmonella enteritidis in foods and faecal samples. Letters in applied microbiology 34, 422-427.

Ward, L., Threlfall, E., Rowe, B., 1990. Multiple drug resistance in salmonellae in England and Wales: a comparison between 1981 and 1988. Journal of clinical pathology 43, 563-566.

Weill, F.-X., Guesnier, F., Guibert, V., Timinouni, M., Demartin, M., Polomack, L., Grimont, P.A., 2006. Multidrug resistance in Salmonella enterica serotype Typhimurium from humans in France (1993 to 2003). Journal of clinical microbiology 44, 700-708.

Wellington, E.M., Boxall, A., Cross, P., Feil, E.J., Gaze, W.H., Hawkey, P.M., Johnson-Rollings, A.S., Jones, D.L., Lee, N.M., Otten, W., 2013. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. The Lancet infectious diseases 13, 155-165.

White, D.G., Piddock, L.J., Maurer, J.J., Zhao, S., Ricci, V., Thayer, S.G., 2000. Characterization of fluoroquinolone resistance among veterinary isolates of avian Escherichia coli. Antimicrobial agents and chemotherapy 44, 2897-2899.

Wilson, J.S., Hazel, S.M., Williams, N.J., Phiri, A., French, N.P., Hart, C.A., 2003. Nontyphoidal salmonellae in United Kingdom badgers: prevalence and spatial distribution. Applied and environmental microbiology 69, 4312-4315.

Witte, W., 1998. Medical consequences of antibiotic use in agriculture. Science 279, 996-997.

Yeung, R.M., Morris, J., 2001. Consumer perception of food risk in chicken meat. Nutrition & Food Science 31, 270-279.

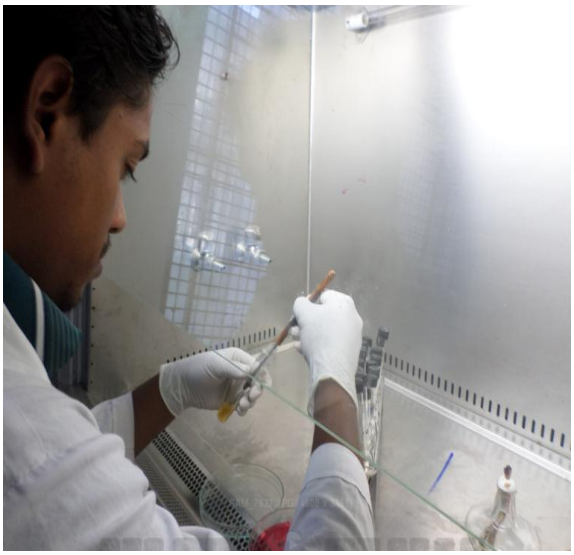
Yoo, S., Pai, H., Byeon, J.-h., Kang, Y.H., Kim, S., Lee, B.K., 2004. Epidemiology of Salmonella enterica serotype typhi infections in Korea for recent 9 years: trends of antimicrobial resistance. Journal of Korean medical science 19, 15-20.

Zhao, S., McDermott, P., White, D., Qaiyumi, S., Friedman, S., Abbott, J., Glenn, A., Ayers, S., Post, K., Fales, W., 2007. Characterization of multidrug resistant< i> Salmonella</i> recovered from diseased animals. Veterinary microbiology 123, 122-132.

Annex-I: Picture gallery of the present study

Trapping birds by net

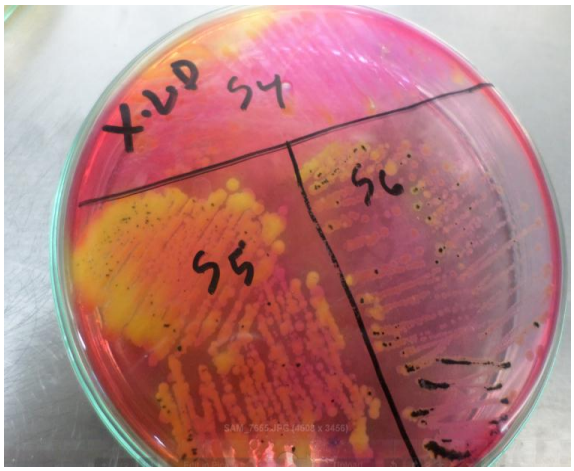
Collection of cloacal swab



Taking inoculation

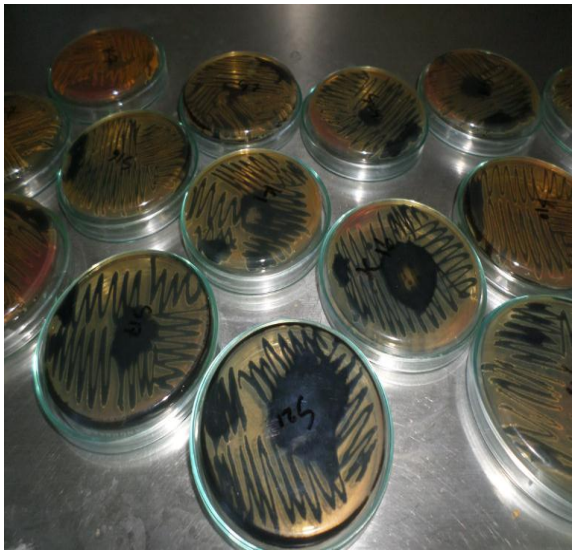
Streaking on XLD agar plate

Incubation of agar plate

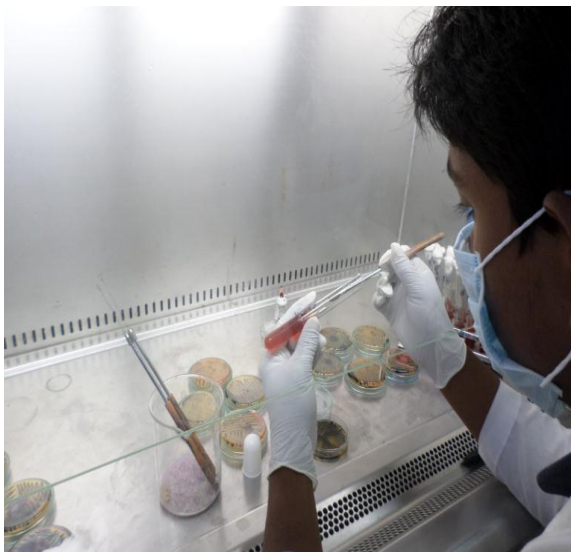


Growth of *Salmonella* on XLD agar

Streaking on SS agar plate

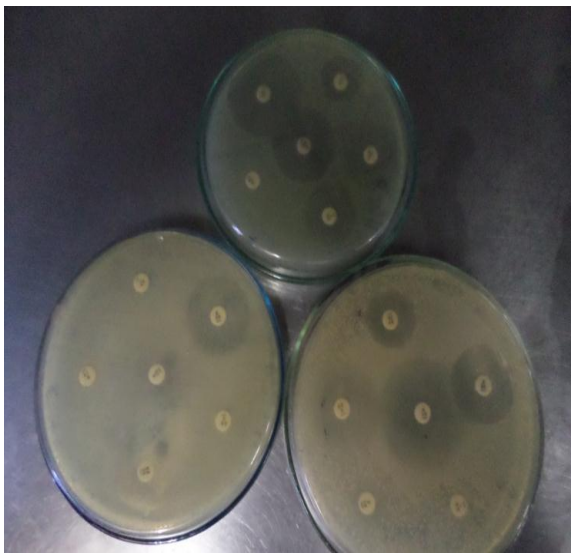


Growth of *Salmonella* on SS agar

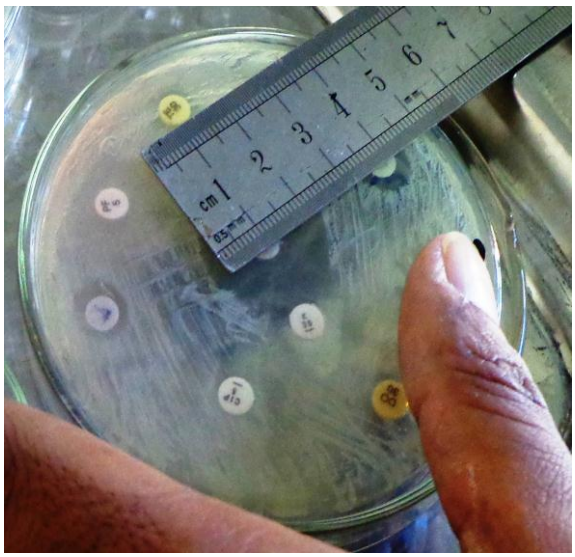


Stab on TSI Slant

Growth of *Salmonella* on TSI Slant



Inhibition on Mueller Hinton agar



Measuring diameter of zone of Inhibition

Annex-II: Preparation of Media and reagents for laboratory evaluation

- **Aimes transport medium (Oxoid):** An amount of 10gm Aimes transport medium powder was dissolved in 1000ml sterile distilled water and mixed it properly before heating to boil for one minute with intermittent gentle swirling for the complete dissolving of the medium. Then the medium was dispensed in vials at 6ml per vial and sterilized by autoclaving at 15 psi (121^0C) for 15 minutes. The vials were placed to cool in upright position and cream color, opalescent gel was appeared afterwards the vials were then stored in refrigerator until being used.

- **Nutrient broth:** A total of 28gm nutrient agar medium powder was suspended in 1000ml of distilled water and heated to boiling to dissolve completely. The media was then sterilized by autoclaving at 121^0C for 15 minutes before poured into test tubes. Tubes containing broths were kept to cool at room temperature and then store in refrigerator for further use.

- **Xylose Lysine Deoxycholate medium:** A total of 53gm XLD medium was suspended in 1000ml of distilled water. The suspension was then heated with frequent agitation until the medium had boiled. After boiling the dissolved medium immediately was transferred to a water bath at 50^0C. Afterwards the medium was poured into petri dishes. The petri dishes containing medium were allowed to dry before storing in a refrigerator for the future use.

- **Mueller-Hinton agar:** An amount of 38gm Mueller-Hinton agar medium powder was suspended in 1000ml of purified water and heated with frequent agitation and boiled for one minute to dissolve the medium completely. The medium was then sterilized by autoclaving at 121^0C for 15 minutes and cooled at room temperature before poured into petri dishes on a level, horizontal surface to give uniform depth and then left to cool at room temperature and stored in refrigerator for future use.

Annex-III: Raw data from *Salmonella* positive sample with results and zone of inhibition diameter of selected antibiotic disc

Sample No.	K	DA	KF	E	OX	P
C16	26mm	R	22mm	32mm	R	10mm
C7	24mm	R	23mm	10mm	R	R
S21	26mm	R	R	R	R	R
S7	20mm	R	R	R	R	R
S5	24mm	R	R	R	R	R
S3	10mm	R	R	R	R	R
S24	25mm	R	R	R	R	R
S25	15mm	R	R	R	R	R
S6	22mm	R	R	R	R	R
C10	20mm	R	R	15mm	R	R
C17	R	R	22mm	R	R	R
S17	20mm	R	22mm	R	R	R
C1	R	R	R	R	R	R
C8	15mm	R	R	R	R	R
S28	16mm	R	R	R	R	R
S27	22mm	R	15mm	12mm	R	R
C12	25mm	R	20mm	8mm	R	R
S20	23mm	R	R	18mm	R	R
S10	18mm	R	12mm	10mm	R	R
C15	20mm	R	22mm	27mm	R	R
C3	25mm	R	18mm	24mm	R	8mm
S8	18mm	R	20mm	R	R	R
S13	22mm	R	24mm	R	R	R
S19	16mm	R	21mm	R	R	R
C6	26mm	R	25mm	18mm	R	R

Note: K= Kanamycin; DA=Clindamycin; KF=Cephalothin; E=Erythromycin; OX=Oxacillin; P=Penicillin; C= Sample positive from Crow; S= Sample positive from Asian pied starling; R= Resistance.