UNDERSTANDING THEECOLOGY OF ANTIMICROBIAL RESISTANCE OF PET BIRDS IN BANGLADESH

Md.Saddam Hossain

Roll no: 0116/08

Registration no.: 335

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Department of Medicine and Surgery
Faculty of Veterinary Medicine
Chittagong Veterinary and Animal Sciences University
Chittagong-4225, Bangladesh

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Md. Saddam Hossain

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

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Prof. Dr. Mohammad Mahmudul Hassan	**Prof. Dr. Kohei Makita**
Supervisor	**Co-supervisor**

Prof. Dr. Md. Mizanur Rahman
Chairman of the Examination Committee

Department of Medicine and Surgery
Faculty of Veterinary Medicine
Chittagong Veterinary and Animal Sciences University
Chittagong-4225, Bangladesh

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List of Abbreviations

Abbreviation	Elaboration
AMR	Antimicrobial Resistance
AST	Antimicrobial susceptibility test
BGA	Brilliant green agar
BCS	Body Condition Score
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
EDTA	Ethylene diamine tetraacetic acid
ESBL	extended-spectrum beta-lactamase
GDP	Gross domestic product
I	Intermediate
ICDDR,B	International Centre for Diarrhoeal Disease Research, Bangladesh
IUCN	International Union for Conservation of Nature
KAA	Kanamycin aesculinazide
MRSA	Methicillin-resistant Staphylococcus aureus
NHP	Non-human primate
OR	Odds ratio
PCR	Polymerase chain reaction
R	Resistant
ROC	Receiver operating curve
S	Sensitive
S.	*Staphylococcus*
TB	Tuberculosis
USDA	United States Department of Agriculture
VISA	Vancomycin-intermediate *Staphylococcus aureus*
VRE	Vancomycin-resistant *Enterococcus*
VRSA	Vancomycin-resistant *Staphylococcus aureus*
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

Symbols

Symbols	Stands for
&	And
>	Greater than
<	Less than
˚C	Degree centigrade
≥	Greater than equal
%	Percentage
Mg	Microgram
χ2	Chi square
w/v	Weight/volume
≤	Less than equal

Abstract

Pet birds are recreational bird species that reared as pets with the exception of poultry. In Bangladesh more than twenty species of pet birds are reared by pet lovers. Budgerigar is the most common among all pet birds. However, antimicrobial resistances (AMR) became a threatened issue for pet birds as well as pet lovers. Thus, this health related sustainable development aims are considerably less attainable with the growing threat of antimicrobial resistance. As the bird keepers may have close contact with cage pet birds, they can be exposed to resistant organisms carried by them. Therefore this study was aimed to understand antimicrobial resistance patterns of *Escherichia coli* and *Staphylococcus* sp. isolated from Budgerigar in Chattagram. From December, 2016 to June, 2017, 220 cloacal swab samples were collected from budgerigars. A structured questionnaire survey was conducted on farm owners to know the management and disease ecology of Budgerigar at the farm level. The standard microbiological procedures were followed for isolation of zoonotic bacteria's. Budgerigar was found to be 24.74% among all pet species. The prevalence of *E. coli* and *Staphylococcus* sp. were recorded as 22.27% (n=49) and 18.18% (n=40), respectively. Poor Body Condition Score ($p \leq 0.04$) and tap water ($p \leq 0.03$) showed significant influences on AMR of *E. coli* in budgerigar. In case AMR of *Staphylococcus* spp. young bird ($p \leq 0.003$) and diseased bird ($p \leq 0.001$) were found as significant variables. Antibiotic Susceptibility tests against *E. coli* and *Staphylococcus* spp. were conducted using disc diffusion method for nine antibiotics. All (100%, n=49) *E. coli* isolates were resistant against amoxicillin, sulfomethoxazol, trimethoprim, and cefixime but lowest resistant was found in ciprofloxacin (6.12%). Moreover, we found 100% (n=40) multidrug resistance in *Staphylococcus* spp. for enrofloxacin and gentamycin followed by others and lowest for ciprofloxacin and azithromycin (5%). In conclusion, this study presented multidrug resistant *E. coli* and *Staphylococcus* sp. isolated from the pet birds. The indiscriminate use of antibiotics on pet birds should be reduced to lessen the risk of public health importance multi-drug resistance bacteria. By placing antimicrobial resistance in pet birds within the sustainable development agenda, we seek to intensify the national and international commitments to finding a solution to this emerging threat in pet birds sector before it turns into a global crisis.

Key words: Antimicrobial, Sustainable development, Ecology, Resistance, *E. coli*, *Staphylococcus* sp.

Chapter-1: Introduction

The term 'pet bird' defines as those birds having capability of living, breeding and surviving in captive condition and purposes of rearing are hobby and sometimes commercially. This birds having two categories includes Psittaciformes (parrots, parakeets, budgerigars, love birds, lorry, macaw etc.) and Passeriformes (e.g. canaries, finches, sparrows etc.) (Boseret et al., 2013). Parrots are found in Bangladesh like all over the world from a long time. They are popular as pets due to their sociable and affectionate nature, intelligence, bright colors, and ability to imitate with human voices. Economically they can be beneficial to communities as sources of income from the pet trade. However budgerigar (*Melopsittacus undulates*) is one of the most common captive pet parrots from Psittaciformes group. It is small pet birds with big personality, smart looking and can be very loving. Budgerigars are predominant naturally in Australia. They have different color but mainly found as green-yellow faced bird. Pet birds are the source of recreation for human especially children. Pet birds are kept in a cage or aviary; though generally, tame parrots should be allowed to be taken out regularly. Species of parrot vary in their temperament, noise level, talking ability, cuddliness with people. Budgerigars are becoming increasingly more popular as household pets (Bangert et al., 1988). The Budgerigar are normally found as small flocks, but can form very large flocks under favorable conditions. The nomadic movement of the flocks is tied to the availability of food and water. Drought can drive flocks into more wooded habitat or coastal areas. Naturalized feral budgerigars have been recorded since the 1940s in the St. Petersburg, Florida, area of the United States, but are much less common now than they were in the early 1980s. Increased competition from European starlings and house sparrows is thought to be the primary cause of the population decline (Parr and Juniper, 2010). Now Budgerigar farming is available in Bangladesh. Budgerigar population in Chattagram is higher than from other parts of Bangladesh. Parrots are excellent companion birds, and can form close, affectionate bonds with their owners. However they habitually required a massive amount of attention, care and intellectual prompt to blood. Depending on locality, Budgerigar may be either wild immovable or be enslaved bred. Among a larger number of species of parrots, Alexandrine Parakeet, Cockatiel, Rose-ringed Parakeet, Red breast parakeet, Blossom Headed parakeet,

Macaw, Lovebird etc. are available in Bangladesh and widely reared in cage as decorative birds (Akhter et al., 2010a). Though the have pet bird socio-economic impacts, they are also probable carriers and/or transmitters of zoonotic diseases (Reaser et al., 2008). Budgerigar is often suffered from many bacterial diseases with the association of normal flora or environmental pathogens due to stress and immune-suppression. For example bacterial enteritis is a natural stress associated disease caused mainly by *E. coli, Klebsiella, Salmonella, Pasteurella, Pseudomonas, Aeromonas*and *Citrobacter* (Altman and Robert, 1997). Besides bacterial enteritis, bacterial respiratory diseases are also often a stress associated phenomenon where *Klebsiella, Escherichia coli, Enterobacter, Pseudomonas, Pasteurella and Mycoplasma* are commonly involved (Friend and Franson, 1999).

It is important to discuss the risks encountered by bird handlers (including children, students), professional workers (e.g. veterinarians, traders or shop owners) and the general people with the propose of preventing the transmission of disease from pet birds to humans. Birds of the Passeriformes and Psittaciformes order usually do not reservoir a large quantity of microbes in their intestinal tract; however, birds are susceptible to a variety of bacterial infections (Benskin et al., 2009). Several genera of these family have been reported having different diseases like *Escherichia coli, Salmonella* spp., *Citrobacter*spp., *Staphylococcus spp., pseudo tuberculosis* and *Klebsiella* spp (Martin and Ritchie, 1994).

The Enterobacteriaceae is a large family of gram-negative bacteria (Quinn, 1994) also called enterobacteria, are not belong to commonsel of digestive microbiota of pet birds and their presence in clinically healthy birds are the indication of direct contact with human(Asterino, 1996). Birds are susceptible and can also transmit entero-pathogens to humans and surprisingly, there are few comprehensive surveys done for wild and most domesticated birds (Reed et al., 2003). There are reports of human infections caused by *E. coli* and *S.* Typhimurium transmitted indirectly from migra-tory birds belonging to the passeriformes order (Tsiodras et al., 2008) which suggest that the transmission of these pathogens by passerines can occur when reared in home environment . *E. coli* in particular, is not belong to the intestinal flora of pet birds indicating by the fact that, the feces of only 9% of healthy budgerigars and 17% of finches were found positive for enterobacteria (Glünder, 2002).

The recurrently identified bacterial organisms belonged to the genus *Staphylococcus* spp in budgerigar (Lamb et al., 2014). *Staphylococcus aureus* was isolated from 6 cases infected Budgerigar, *Staphylococcus hyicus* from 3 and *Staphylococcus intermedius* from one case. *S. aureus* was most often involved in outbreaks of septicemia, with or without 'Megabacterium' proventriculitis (Devriese et al., 1994). Various causes of disease, such as polyomavirosis, Pacheco's disease, chlamydophilosis, *Enterococcus hirae* septicaemia, aspergillosis, helminth infections and vitamin A deficiency were diagnosed. *S. aureus* was isolated from 13 birds in Belgium and the prevalence was 6%. (Hermans et al., 2000). Enterotoxin-producing strains were found in clinically healthy poultry, indicates that precautions should be taken during the handling and cooking of poultry products. Methicillin-resistant *S. aureus* (MRSA) has been isolated from poultry meat in a number of countries, but the prevalence and significance for human health are incompletely understood (Rao, 2013).

Antimicrobial resistance (AMR) is a global community health threat. Nowadays important bacteria's are not only single drug resistant but also multiple drugs resistant. Human and animal health are now in great danger (Levy and Marshall, 2004).Today it became a global crisis rather than regional, as AMR can spread one country to another and continent to continent. The pattern of antimicrobial resistance may vary from country to country but it is clear that Asia is an epicenter of AMR, especially Bangladesh, India, Pakistan due to their high-density population and abuse of antibiotic (Kang and Song, 2013). Due to globalization and massive travelling, the spread of resistant pathogens may increase. Moreover pet bird's owners are not conscious about using antibiotics with the suggestions of authorized veterinary doctors. They used to treat their birds by themselves or by local doctors or quack. Besides therapeutic and prophylactic applications antibiotics are using as growth promoter thus increase the chance of getting resistance (Roess et al., 2013). Statistics showed that per annum about 50000 people are dying in Europe and USA and about 700000 worldwide due to antibiotic resistance(O'Neill, 2014). In 2013, about 214000 child deaths recorded due to resistant sepsis infections all over the world out of which 11523 were contributed by India, Pakistan, Nigeria, Democratic Republic of Congo and China (Robinson et al., 2016). AMR is a One Health issue; it has clear links to people, animals and environment. The contribution of animal production, terrestrial

livestock, agriculture and aquaculture to the global AMR crises is questioned by some on the grounds that animal-associated infections in humans increases due to abuse of antibiotics in animal production. Multidrug-resistance genes now highly prevalent in many important and common pathogens like *E. coli*, *Klebsiella pneumoniae*, *Salmonella*, *Enterococcus* and *Staphylococcus* spp. (Robinson et al., 2016). Indeed, the rate of antibiotic resistance emergence is related to the total consumption of antibiotics, regardless whether adequately used or not. However, antibiotic-resistant bacteria have been found in hosts and environments apparently free from any antibiotic pressure imposed by man (Caprioli et al., 1991; Gilliver et al., 1999; Souza et al., 1999). Most research on the epidemiology of antibiotic resistance dissemination has focused on human and veterinary medicine, but there is an increasing interest to understand how bacterial resistance is transferred within reservoirs in natural environments (Alley et al., 2002). The first antibiotic-resistant bacteria noted in wildlife were in fact from wild bird strains of *E. coli* resistant to multiple antibiotics, e.g. chloramphenicol were isolated in pigeons in around 1975. Many bird species have been found to carry antibiotic-resistant bacteria. Resistant *E. coli* have been isolated from ducks and geese, cormorants, birds of prey, gulls, doves, and passerines. Extended spectrum beta-lactamase (ESBL)-producing *E. coli* were first isolated from wild birds in 2006. In recent years, many reports have followed, mostly from Europe. ESBL-producing *E. coli* have now been isolated from wild birds from all continents of the world except Australia and Antarctica. The high level of antibiotic resistance in avian pathogens from Bangladesh is worrisome and indicates that widespread use of antibiotics as feed additives for growth promotion and disease prevention could have negative implications for human and animal health and the environment. For therapeutic and preventive management of salmonellosis and colibacillosis farmers do always not seek veterinary advice for drug choice and farm management. They also rely on feed and drug sellers, bio-medical suppliers and experienced and educated neighbor farmers for choosing drugs against different pet bird diseases. Sometimes, they take their own judgments to select therapeutic management against these diseases. Moreover, diagnosis of diseases in pet bird is broadly based on less sensitive clinical signs and symptoms along with postmortem lesions and therefore misdiagnoses with wrong selection of antimicrobials are frequently occurred. The above facts therefore suggest antimicrobials are being used indiscriminately with non-

specific drugs and doses as well as incomplete course of drug treatment against colibacillosis in pet birds, consequently antimicrobials randomly become resistant. These resistant microbes may function as a potential source in the transportation of antimicrobial resistance to human pathogens (van den Bogaard and Stobberingh, 2000; Schwarz et al., 2001). Antimicrobial susceptibility testing is not a common and routine practice to identify sensitive drugs against different bacterial pathogens in pet birds sector in Bangladesh. Hence, there is no strategy in place to deal with the emergence of antimicrobial resistance against bacterial pathogen in pet birds. Also no specific study has been conducted yet to know the epidemiology of antimicrobial usage in pet bird's farms in Bangladesh. The aforementioned facts and factors therefore encouraged to conduct an epidemiological research to explore the current status of antimicrobial usage as well as to assess antibiogram against *E. coli* and *Staphylococcus* spp.in budgerigar bird farms in Bangladesh.

Objectives

1) .To estimate the prevalence and patterns of antimicrobial resistance against *E. coli* and *Staphylococcus* spp. in Budgerigar
2) To identify the possible risk factors for AMR
3) To assess the socio-economic status of pet birds farm owners in Bangladesh

Chapter-2: Literature review

Significant literatures on pet bird's ecology, budgerigar condition in world, antimicrobials, antimicrobial resistance, prevalence, consequences and diagnostic techniques have systematically been reviewed in this chapter. This chapter is to provide scientific information based on related past studies and accordingly identify gaps and rationalize the present epidemiological MS research on antimicrobial resistance in pet birds. The review findings of relevant published articles have been presented under the following headings as below.

2.1. Ecology of pet birds

Pet birds are the source of recreation for human especially children. Pet parrots are kept in a cage or aviary; though generally, tame parrots should be allowed to be taken out regularly. Parrots are found all over the world from a long time. They are popular as pets due to their sociable and affectionate nature, intelligence, bright colors, and ability to imitate with human voices. Economically they can be beneficial to communities as sources of income from the pet trade. The domesticated budgerigar, a small parrot, is the most popular of all pet bird species. Species of parrot vary in their temperament, noise level, talking ability, cuddliness with people. Parrots are excellent companion animals, and can form close, affectionate bonds with their owners. However they invariably require an enormous amount of attention, care and intellectual stimulation to thrive. Depending on locality, parrots may be either wild caught or be captive bred, though in most areas without native parrots, pet parrots are captive bred. Among a larger number of species of parrots, Alexandrine Parakeet, Cockatiel, Rose-ringed Parakeet, Red breast parakeet, Blossom Headed parakeet, Macaw, Lovebird, Conur, Lory, Amazon Parrot, Rozella, Grey Parrot, Cockatoos and other species are available in Bangladesh (Akhter et al., 2010b). Budgerigars (*Melopsittacus undulatus*) are popular exhibit animals in public display facilities and were often managed as flocks in open aviaries. Management of budgerigars in these situations provides many challenges, including the potential for disease outbreaks. Many families own their "kitchen pet bird", which represents a lucrative business for pet shops or local breeders, since a single male canary is sold around 30 euros in Belgium and a female around 20 euros. Prices are about the same for zebra finches or budgerigars, and 50% to 100% higher for "special" finches like Gould diamonds. Bird

fairs and live bird markets also gather many people. In addition, some species are bred for their very high value; for example, in the case of canaries, male and female breeding stock reproducers with recognized genetic potential are presented in national and international contests for their posture (the bossu belge), their color (red mosaic) or for their song (harzer). Therefore, their offspring could be sold at high for rising prices. Finally, exotic birds like greater psittaciforms (parrots, e.g. ara or cockatoo), legally or illegally traded from for example Asia or South America, remain high in the ranking of popular pets and are also profusely represented in zoos and parks. Notwithstanding these socio-economic facts, these animals are potential carriers and/or transmitters of zoonotic diseases. Some of these pathologies could have an important impact on human health, like chlamydophilosis, salmonellosis or even highly pathogenic avian influenza A H5N1, but also have an economic impact if some of these pathogens were spread via carriers or vectors like wild birds, human beings, insects or mites to poultry breeding units or cattle facilities, then entering the food chain. The aim of this review is to enlighten and discuss the risks encountered by bird handlers (including children), professional workers (e.g. veterinarians, traders or shop owners) in particular and the human population in general, and to assess the eventual health and economic consequences, and propose some guidelines to prevent transmission from such birds to humans.

2.2. Disease impact of budgerigar

Budgerigars are susceptible to a wide range of bacterial, viral, fungal, and parasitic diseases that pose threats in a flock situation. Escherichia coli are gram-negative, rod-shaped bacteria in the family Enterobacteriaceae. There are hundreds of serotypes of E. coli that are classified using a numbering system based on the type of outer membrane lipopolysaccharide (O-antigen), the flagella that exist in some motile strains (H-antigen), and the polysaccharides that form either a discrete capsule or amorphous layer (K-antigen). 26 Several serotypes of *E. coli* are nonpathogenic commensal organisms of the gastrointestinal tract in many species. 13 Pathogenic strains of *E. coli* are determined by specific virulence factors and their effect in susceptible species. One virulence factor found in pathogenic strains of E. coli is the attaching and effacing (*eae*) gene that leads to intimate bacterial adherence to the host epithelium, creating characteristic attaching and effacing lesions. The *eae* gene encoded on a pathogenicity island called the locus of enterocyte effacement (LEE).

The *eae* gene produces the protein Intiman that, in coordination with additional receptors encoded on the LEE Pathogenocity Island, forms a pedestal that allows for intimate attachment of the bacteria to the epithelium and destruction of the underlying host tissue. Attaching and effacing *E. coli* (AEEC) have a tropism for the small intestine. Unlike cattle, birds were not usually considered significant reservoirs of *eae*-positive *E. coli*. 18 This report describes enteritis in a population of captive budgerigars at Zoo New England's Franklin Park Zoo (Boston, Massachusetts 02121, USA), in which the lesions resembled those of AEEC in other species.

Enterobacteriaceae, *E. coli* in particular, do not belong to the intestinal flora of granivorous pet birds. This is indicated by the fact that the feces of only 9% of healthy budgerigars and 17% of finches tested were positive for Enterobacteria. Stressful situations such as overcrowding in small cages coincident with increased noise and low light levels can enhance the colonization of the gut with *E. coli*. On the other hand it seems nearly impossible to colonize the intestine of budgerigars with *E. coli* or *Klebsiella* spp. even under favorable conditions (Priya et al., 2008) The frequent findings of enterobacteria in deceased granivorous birds suggest that *E. coli* and other enterobacteria are involved in the course of diseases with predisposing factors. Nutritional experiments with young chickens suggest that a diet consisting exclusively of seeds has an inhibitory effect on intestinal colonization with *E. coli*. Determination of *Aeromonashydrophila* in nearly 3500 wild and pet birds provides statistically significant evidence that the composition of the intestinal flora may depend on dietary habits: infection was found in 1.9% of the granivorous and herbivorous species, in 7.1% of the omnivorous and in 12.4% of the carnivorous and insectivorous birds. The occurrence of enterobacteria and *Aeromonas hydrophila* in the digestive tract is obviously influenced by the composition of the nutrients (Glünder, 2002).

2.3. History of antibiotic development and scope of Antimicrobial resistance

In order to consider the problem of antimicrobial resistance as it occurs today, it is very important the history and advanced of both antimicrobials and antimicrobial resistance. There are generally two categories of antimicrobials one includes the synthetic drugs, such as the sulfonamides and the quinolones, and the second is antibiotics, synthesized by microorganisms. In current years, growing numbers of chemical derivatives of antibiotics have been developed which are semi-synthetic

drugs, thereby modifying the difference between synthetic and natural antibiotics. As microorganisms were the main reason for infectious disease, grown the interest in antimicrobial therapy. At the beginning of an era, various types of plant products and derivatives were applied as treatment of many diseases but doctors or patient didn't know the actions of those medicinal agents. Various drugs were used previously to treat the diseases caused by protozoan than that of bacterial diseases. History told that in 1619 malaria was treated by the juice of cinchona bark (quinine) and dysentery caused by amoebas treated by ipecacuanha root (emetine). In the period of when chemotherapy started, only a few numbers of antibacterial were used eg. Mercury was the drug to treat syphilis. Dyes, as possessing differential affinities for various issues was conjectured to be used as antimicrobial drugs by Paul Ehrlich in the early 1900's. Later in 1904, Ehrlich and Shiga detected that trypanrot (red dye) was effective against trypanosomes (Mitsuhashi, 1993). Around this time Ehrlich drew attention on arsenicals and started working with Sahachiro Hata. In 1909, they got a result that arsphenamine (Salvarsan) is effective against spirochetes and an effective treatment for syphilis. Gerhard Domagk first discovered truly the active class of antimicrobial drugs named sulfonamides (Domagk, 1935). In Bayer Company, two scientists named Klarer and Mietzsch produced Prontosil red in 1932, which is a red dye constrained to a sulfonamide group.But it was very unfortunate for bayer; there was no antibacterial activity by Prontosil red in vitro. Prontosil red divided its component dye and sulfanilamide in vivo, where sulfanilamide is an effective antibacterial agent that was previously described that interpreted by (Trefouél, 1935). After that, many companies started to produce sulfanilamide and improved the molecule to increase the performance with the addition to reduce the side effects. Companies also tried to increase the action of a broad spectrum. Though the penicillin was the first natural antibiotic that was discovered by Alexander Fleming the way of using of microorganisms as treatment was not first. First invented antibiotic, Penicillin was discovered by Alexander Fleming in 1928 from fungus (*Penicilliumnotatum*) (Fleming, 1929), but he was unable to show the therapeutic value of penicillin. In 1941 Norman Heatley, Ernst Chain and Howard Florey had manifest the therapeutic value of penicillin (Chain et al., 1940). In 1943, Robert Coghill and Andrew Moyer jointly tried to produce penicillin at the USDA's Northern Regional Research Laboratory in Illinois and got successes. After that worldwide research was started for founding *Penicillium*strains which could produce extra penicillin, Raper and Fennel

(Raper and Fennell, 1946) discovered *Penicillium chrysogenum* that has the ability of yield more amount of penicillin (Demain and Elander, 1999). Discovery of penicillin made a new channel and after that,severalnumbers of antibiotics were swiftly invented and started to use. Selman Waksman started finding for antibiotics that originated by soil microorganisms in 1940. In 1943, one student of Selman Waksman invented streptomycin (Schatz et al., 1944) and in the same time period, Rene Dubos invented gramicidin which was the first active antibiotic against gram-positive bacteria (Hotchkiss and Dubos, 1941). Within few years, some other antibiotics such as chlortetracycline and chloramphenicol were discovered. Many discoveries happened of drugs, some of them were too toxic for human use. In spite of that, within 10 years many new drugs developed and mainly antibiotics drugs were in this group. By the side of soil, many drugs were discovered from many unusual sources. As for example, from different sources such as wound, sewage, chicken throat, wet wall of Paris etc. bacteria isolated those could produce antibiotics (Garrod and O'GRADY, 1971). The first synthetic drug was discovered in 1962, a nalidixic acid which one is the first quinolones that were described. Though it is not significant by itself but the improvement of nalidixic acid guide to the discoveries of more effective fluoroquinolones. With the times, antibiotics of this class such as ciprofloxacin, norfloxacin, enrofloxacin have become more popular for the treatment in both human medicine and veterinary medicine (Mitsuhashi, 1993). After 1960's, there have some development and modification of existing drugs that led few inventions of new antibiotics. In case of treating infectious diseases, those new antibiotics were highly effective. Nevertheless, those new antibiotics have been very useful such as the increased killing of microorganisms, the increasingly wide range of action, limited toxicity, and reduced side effects. It is very unfortunate that, after the 1970's, only one antibiotic has been established (Lipsitch et al., 2002). Now a day, the effectiveness of different antibiotics has been decreased and to increase effectiveness and defeat problem of resistance, applied different combination of drugs with different mechanisms of action.

Microbial resistance to antibiotics is a worldwide problem in human and veterinary medicine. Commonly, it is usual that the principal risk factor for an increase in this situation is the extensive use of antibiotics leading to the dissemination of resistant bacteria and resistance genes in animals and humans (van den Bogaard&Stobberingh,

2000). The appearance of multi-resistant bacteria of human and veterinary origin was probably accompanied by contamination of the environment often leading to serious health concerns (Grobbel et al., 2007). Bacteria may present resistance to antibiotics under selective pressure, but they may also acquire antibiotic resistance determinants without direct exposure to an antibiotic through horizontally mobile elements including conjugative plasmids, integrons and transposons (Middleton & Ambrose, 2005). These mobile elements can simply transfer antibiotic resistance genes from one bacterium to another (Coque et al., 2008). The bacteria of the normal flora of the gut, such as *Escherichia coli* and enterococci, can easily acquire and transfer resistance genes. These commensal bacteria, which constitute a reservoir of resistance genes for pathogenic bacteria, can thus be used as indicators of changes in antimicrobial resistance (Caprioli et al., 2000). Antibiotic resistance in faecal indicator bacteria could have a number of consequences. For example, *E. coli* and enterococci have become more efficient human nosocomial pathogens (Jett et al., 1994) as they have developed increased antibiotic resistance. The common buzzard is a medium to large bird of prey, with a geographical distribution that covers most of Europe and also extends into Asia. As a great opportunist, it is well adapted to a varied diet of pheasants, rabbits, other small mammals, snakes and lizards, and can often be seen walking over recently ploughed fields looking for worms and insects (IUCN, 2010). In addition to the currently common detection of multi-resistant bacteria in areas with high human density (Cole et al., 2005), the emergence of such bacteria in more remote areas such as high mountain regions is even more alarming (Dolejska et al., 2007). Although wild birds have only rare contact with antimicrobial agents, in disagreement with the existence of direct selective pressure, they can be contaminated or colonized by resistant bacteria. Water contact and acquisition via food seem to be the major routes of transmission of resistant bacteria of human or domestic animal origin to wild animals (Cole et al., 2005). Wild birds in general may therefore represent reservoirs of resistant bacteria and genetic determinants of antimicrobial resistance (Dolejska et al., 2007). Monitoring the prevalence of resistance in indicator bacteria such as faecal *E. coli* and enterococci in different populations such as animals, patients and healthy humans makes it feasible to compare the prevalence of resistance and to detect the transfer of resistant bacteria or resistance genes from animals to humans and vice versa (Martel et al., 2001). However, few reports of the level of antimicrobial resistance in *E. coli* and enterococci of wild animals have been

published (Nulsen et al., 2008; Poeta et al., 2005b, 2007b; Radhouani et al., 2009; Silva et al., 2010). The aim of the present study was to analyse the prevalence of antimicrobial resistance and the mechanisms implicated in faecal *E. coli* isolates and Enterococcus species of common buzzards in Portugal.

After the invention of new antibiotics got resistance immediately within few years due to improper use of those antibiotics. There isa lot of evidence of the presence of resistant microorganisms in nature but those microorganisms are not present in human (Hughes and Datta, 1983). However, in the recent years, the microorganisms which are resistant to antibiotics are alarmingly high in human as well as animals. By the side of the discovery of new antibiotics, researcher began to find out microorganisms that are resistant to new drugs. Surprisingly by the year 1909, Ehrlich discovered resistant trypanosomes when he started to work with dyes and arsenicals. After the invention of penicillin became much popular to use as treatment and a research showed *S. aureus* resistance in hospitals was 14% in 1946 to 38% in 1947 and today about 90% resistance in hospital cases. All over the world, penicillin and ampicillin together found resistance to *S. aureus* is about 80%. After the end of the World War II, sulfonamides were used very commonly for the treatment of *Shigella* infections in Japan but it was resistant to about 80% by the year of 1952. After that Japanese started to shift to streptomycin, tetracycline and chloramphenicol as a results *Shigella* became multi-drugs resistance quickly (Falkow, 1975). Sulfonamides were a successful drug for the treatment of meningococcal disease within 30 years of discovery but recently it became resistance to almost all antibiotics. Some researcher and clinicians already predict a crisis stage of antibiotics and we may go to face some destructive diseases which will not be cure with our antimicrobials (Baquero and Blázquez, 1997; Lipsitch et al., 2002). We found that resistance has been observed in microorganisms commonly but some microorganisms are remarkably concern. The resistance organisms are becoming increase significantly due to tremendously frequency of travel worldwide, highly increase of population in both developed and developing countries.

2.4 *E. coli*: zoonotic significance and resistance pattern

Drug resistance in *Escherichia coli* strains isolated from pet birds (mynahs, macaws, finches, common bengals, parrots, and flamingos) imported into Japan from 10

foreign countries in 1977 and 1978 was investigated. Of the 309 strains isolated from 127 pet birds in the Animal Quarantine Service, 232 (75.1 %) were drug resistant. Furthermore, strains resistant to oxytetracycline hydrochloride, dihydrostreptomycin, and sulfadimethoxine were relatively common. Resistance patterns varied from single to sextuple resistance, and 148 (63.8%) of the resistant strains had conjugative R plasmids. These results suggest that the high incidence of drug resistance and R plasmids in *E. coli* strains isolated from these pet birds may be a reflection of the prophylactic use of antibiotics for the prevention of diseases which increasingly occur with importation of the birds. Furthermore, the results suggest that the birds may be potential reservoirs of drug-resistant *E. coli* for families who raise and have intimate contact with such birds (Nakamura et al., 1980). Many zoonotic diseases are transferred from cage or pet birds to human through direct or indirect contact of the diseased or carrier birds. Visitors are more susceptible to acquire zoonotic diseases from cage birds in zoo. Bacteria are one of the most common causes of zoonotic diseases. For this, proper isolation, identification and characterization of the bacteria are essential to control zoonotic diseases. Outbreaks of zoonoses have been traced to human interaction with and exposure to animals at fairs, petting zoos, and in other settings. In 2005, the Center for Disease Control and Prevention (CDC) issued an updated list of recommendations for preventing zoonoses transmission in public settings. The CDC recommendations, which were developed in conjunction with the National Association of State Public Health Veterinarians, include sections on the educational responsibilities of venue operators, managing public and animal contact, and animal care and management (CDC, 2005). In 2002, seven people became ill with *E. coli*: 0157117 infections after visiting a large agricultural fair in Ontario, Canada. Investigators of outbreak conducted a case-control study, which indicated that goats and sheep from a petting zoo were the source of the *E. coli* among fair visitors. Other indications were that the fencing and environment surrounding the petting zoo that could have been a source of transmission (Warshawsky et al., 2002). Very few works have been studied on the isolation and identification of bacteria from caged birds in Bangladesh and the present study, therefore, was undertaken to isolate and identify important species of bacteria from apparently healthy caged parrots, and to determine antibiotic sensitivity pattern of the isolated bacteria (Akhter et al., 2010b).

2.5 *Staphylococcus* spp.: zoonotic significance and resistance pattern

*Staphylococci are*gram-positive cocci, normal flora of the body which are frequently found on the nose and respiratory tract and associated with nosocomial infections. At present days, *Staphylococcus* spp. is resistance to several antimicrobials. In the clinical perspective, *Staphylococcus* is classified into two groups: coagulase-negative and coagulase positive. The most common important opportunistic microorganisms on the skin are from coagulase-negative group staphylococci is *Staphylococcus epidermidis.* On the other hand, important coagulase-positive group Staphylococci are *S. aureus* which is commonly found in the nasal cavity of human and animals. *S. aureus* is always opportunistic can cause various complications such as pimples, impetigo, furuncles, folliculitis, abscesses and life-threatening diseases such as pneumonia, osteomyelitis, endocarditis, septicemia and meningitis etc. (Le Loir et al., 2003)**.** There are some virulence factors that indicate the ability to cause diseases of *S. aureus.* It can cause food poisoning and toxic shock syndrome. Antimicrobial resistance in staphylococci is very usual. History told that AMR in staphylococci started at the beginning of the antibiotic era. In 1948, when penicillin started to use, all *Staphylococcus* isolates were resistant to penicillin and other N-lactam antibiotics also including ampicillin in the hospital. That time 59% of *S. aureus* were resistant to penicillin, all were from hospital patients. Around 1950, most of the strain of *Staphylococcus* group were penicillin resistant in most of the hospital all over the world (Garrod and O'GRADY, 1971). Due to nosocomial infection of penicillin-resistant *S.aureus*, pathogens transferred to the community very rapidly. Nowadays, the percentage of resistance of *Staphylococcus* increased many times, now more than 90% *S. aureus* are resistance and coagulase-negative *Staphylococcus* are resistance about 50% to 70% (O'Brien and 2, 1987). A recent study from Portugal with healthy young volunteers revealed that *S. aureus* was highly resistant (94%) to penicillin or penicillin and erythromycin (Sá-Leão et al., 2001). Other antibiotics are in the same situation after introduction became resistance in a short time. *Staphylococcus* resistance to other antibiotics such as streptomycin, tetracycline, chloramphenicol, and novobiocin was reported in 1953. A pandemic emerged by a notorious penicillin-resistant strain of *S. aureus* (phage type 80/81) in 1950's and spread all over the world, but it was under control after the invention of penicillinase-resistant β lactams (Robinson et al., 2005). The resistance was recorded in independent strain but

sometimes together in a single. The *S. aureus* wasresistant to new drugs as for example fluoroquinolones and quinupristin (Srinivasan et al., 2002). In 1960, when *S. aureus* became resistant to penicillin, penicillinase-resistant β lactams such as methicillin began to use the patient in the hospitals (Garrod and O'GRADY, 1971). But, unfortunately, it was not safe, it became started to resistant and methicillin-resistant *S. aureus* (MRSA) distributed all over the world very quickly. At present, MRSA is one of the most important nosocomial organisms. In the UnitedStates from intensive care unit 47% *S. aureus* were isolated that were methicillin resistant (Srinivasan et al., 2002). In 2000, 48% *S. aureus* isolates were methicillin resistant in Portugal. Most of the research presented that, until now MRSA is a hospital-based problem, on the other hand, some publications reported some community-based problem in some countries. MRSA in day-care centers, among the children with some cases of death has been reported (Sá-Leão et al., 2001). Along with the hospital, community-based MRSA is very low (1-2)% that are increasing gradually. *Staphylococcus* spp. was resistant not only to methicillin but also other antibiotics except glycopeptides. At present best treatment for MRSA infections is vancomycin, but some countries showed the resistance to vancomycin. In 1997, vancomycin-resistant *S. aureus* (VRSA) was first identified in Japan (McCormick, 1998). After that VRSA was isolated in the USA including other countries. After research, the background of VRSA come to us, the vancomycin resistance gene *vanA* from *Enterococcus faecalis* could be transferred to *S. aureus* by in vitro (Noble et al., 1992). After 1997, no record was found of connection to VRE but in 2004, from patients of U.S., VRSA containing *van A* gene been isolated (Ruef, 2004; Witte, 2004). In case of identification of VSRA, a large number of strains isolation didn't follow CLSI standards so they know as vancomycin-intermediate *S. aureus* (VISA). If VISA strains failed to meet the principle of resistance in vitro, patients will not respond to vancomycin properly (Sakoulas et al., 2004). Data from 26 European countries from 1999 to 2002 examined and there was <1% MRSA prevalence found in northern Europe and >40% in southern and western Europe and MRSA significantly increased in Belgium, Germany, Ireland, the Netherlands, and the United Kingdom, and decreased in Slovenia (Tiemersma et al., 2004). In the Asianregion, 74 MRSA strains were identified from 12 countries and all MRSA strains were resistant to penicillin and gentamycin. Other antibiotics were resistance in very high level, amoxicillin-clavulanic acid (96%), cefuroxime (85%), clarithromycin (85%),

ciprofloxacin (84%), trimethoprim-sulfamethoxazole (50%) (Ko et al., 2005). A study of antibiotic susceptibility testing in 2006 to 2007 in Mymensingh, Bangladesh presented that *S. aureus* of MRSA strains were 100% resistant to penicillin, oxacillin, cloxacillin and amoxicillin. Wild animal research presented diversified microorganisms, 61% *Staphylococcus* spp. isolated from non-human primates in Africa (Schaumburg et al., 2012). Rhesus macaque was infected 39% with *Staphylococcus aureus* in Netherland. Wild animals were also susceptible to Methicillin-Resistant *Staphylococcus aureus*, A study of cynomolgus macaques (*Macacafascicularis*) showed that, 22% of monkey were positive to MRSA (Kim et al., 2017).

2.6 Mechanisms of antimicrobial resistance

Based on the mode of action of different antimicrobials, antibiotics can be classified as several major groups. Antimicrobial resistance in different microorganisms can be caused by variety of mechanisms (i) the presence of an enzyme that inactivates the antimicrobial agent; (ii) the presence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent; (iii) a mutation in the antimicrobial agent's target, which reduces the binding of the antimicrobial agent; (iv)posttranscriptional or post- translational modification of the antimicrobial agent's target, which reduces binding of the antimicrobial agent; (v) reduced uptake of the antimicrobial agent; (vi) active efflux of the antimicrobial agent; and (vii) overproduction of the target of the antimicrobial agent. In addition, resistance may be caused bya previously unrecognized mechanism. On the other hand, a gene which is not expressed in vitro may be expressed in vivo (Fluit et al., 2001).

Genetically encoded resistances can vary from mutations in endogenous genes to horizontally acquired foreign resistance genes carried by mobile genetic elements like plasmids (Frye and Jackson, 2013). Point mutations in a promoter or operator can result in the over expression of endogenous genes such as an antimicrobial inactivation enzyme like the Amp C *β-lactamase* gene, or an efflux system like the mar locus. Point mutations in genes encoding antimicrobial targets can result in a resistant target, such as mutations to the *gyrase* gene leading to the expression of a fluoroquinolone-resistant gyrase enzyme (Hopkins et al., 2005). Exogenous resistance genes encoded on plasmids, integrons, phage, and transposons can be horizontally

transmitted by transformation, conjugation, or transduction and these foreign genes can encode all three mechanisms of resistance. This includes genes encoding enzymes that inactivate the antimicrobial, such as *β-lactamases* that cleave the four-membered ring in *β-lactams*, genes which encode efflux systems like tet(A), genes encoding a modified version of the enzyme that is the target of the antimicrobial, such as *dfrA*, or genes encoding an enzyme that modifies the antimicrobial target like a ribosomal RNA methylase, such as *erm*(B) (Ajiboye et al., 2009). Analysis of these resistance mechanisms can then be used to determine the genetic relationship between resistance found in isolates from animals and humans. Because of the diversity of genetic elements that lead to an antimicrobial resistance, it may be possible to determine if resistances seen in bacterial isolates from human infections are closely related to those found in animal isolates, thus identifying animal sources of resistant bacteria in human infections that can be targeted in order to reduce human disease (Frye and Jackson, 2013).

2.7Sources of resistance in the environment

Concern over resistance was originally confined to the acquisition of resistance by microorganisms which cause epidemic disease and was an issue only with respect to clinically isolated strains. However, in recent years, antibiotic-resistant bacteria have been isolated from virtually every environment on earth. This came as a surprise to many clinicians because resistance was found in regions never exposed to human impacts. Even as awareness of environmental resistance has increased, many investigators have continued to restrict their concern to only those pathogens that survive in the environment. It was believed that they posed a danger to humans only if the disease they caused involved resistance to antibiotics. For many years, the focus of research on resistance in the environment reflected this viewpoint. However, we now know that resistance genes can be spread far wider than once believed and a pool of resistance is developing in non-pathogenic organisms found in humans, animals, and the environment. These non-pathogenic organisms serve as a source from which pathogens can acquire genes conferring resistance, and in turn, they can become resistant by acquiring genes from pathogens discharged into the environment, e.g. via sewage or agricultural runoff. Thus, dissemination of resistant bacteria is not only a problem of the resistant pathogens themselves but also the availability of resistance genes to pathogens via gene transfer. Although resistant organisms can be found

naturally in the environment, most resistance is associated with man-made impacts of some type, either agricultural or direct human impact. Antibiotic use in humans can lead to resistance in the environment via discharge of domestic sewage, hospital wastewater, and/or industrial pollution. In addition, to using in humans, antibiotics are added to animal feed to treat infections, as prophylactics, and in sub-therapeutic doses as growth promoters. Although no definitive numbers are available, some authors have published estimates and, by 1980, almost half of the antimicrobial agents used in the United States were used in animal feed (DuPont and Steele, 1987). In Denmark in 1994, a total of only 24 kg of vancomycin was used to treat infections in humans versus 24,000 kg for animals (Witte, 1998a). According to (Levy, 2001), in 1998 in the U.S., half of the 50 million pounds of antibiotics produced were used for agricultural applications. There are a variety of positive effects from using antibiotics in animal feed, namely, inhibition of harmful gut flora which leads to increased growth rates and decreased mortality. This has allowed more concentrated farming and an estimated $3.5 billion savings in production costs per year in the United States alone (DuPont and Steele, 1987). However, the practice has resulted in the selection of antibiotic-resistant organisms in the guts of food animals. From there, these organisms enter the human food chain via contamination during slaughtering or the environment via waste discharge. Resistance has been found to follow closely the use of any given antibiotic (Aarestrup, 1999). Although some investigators dispute any danger being posed by selection of resistant flora within the guts of animals, there is no doubt that such antibiotic use leads to higher concentrations of resistant pathogens and non-pathogens, as well as resistance genes, throughout the farm environment and nearby environments affected via runoff from farms. As will be discussed later, once resistant organisms are spread into the environment, they pose a health risk if they colonize or spread resistance genes to bacteria that colonize humans.

2.8 Problems associated with antimicrobial resistance

It is an alarming issue and major concern about the problem of antimicrobial resistance in the whole world. World Health Organization (WHO) is much concern about the AMR and increased anxiety about the role of antimicrobials used in animal husbandry. Many meetings and conferences occurred to prevent and control the emergence and spread of antimicrobial resistant micro-organisms. Now it is

impossible to return the pre-antibiotic era so we have to concern about the antimicrobial resistance.

AMR is a global threat to both human and animals and day by day it is increasingly growing and poses a huge health risk to the human, animals and environment. Antimicrobial resistance has the direct and indirect effects on the health. When the levels of antimicrobials are high, then it can be toxic to the human or animals. Most of the antibiotics have the direct effect as for example Penicillin causes hypersensitivity reactions and produces allergy. In USA, self-reported penicillin allergy was reported about 80% to 90% of the individuals. The report also suggested that unnecessarily exposed to broader-spectrum antibiotics leads to developing of antimicrobial resistance microorganisms (Pongdee and Li, 2018). Some antimicrobials cause endocrine disruption such as oxytetracycline, tetracycline and sulfamethoxazole and some causes nervous effects (cefuroxime, neomycin) (Lee et al., 2001).

The main problem of AMR is growing the resistance to the specific antibiotics that wouldn't work further. Improper and inappropriate use of antibiotics leads to develop the resistance. Most antibiotics are used in two disciplines: treatment of humans and growth promotion and prophylaxis in animals. Data shared a book related issues and options of AMR, suggested that about 75% of antibiotic use with questionable therapeutic uses (Lederberg and Harrison, 1998). In recent years increasingly usebroad-spectrum agents to the patients and crowd of the person in the nursing home and hospitals another major cause of transferring resistant microorganisms.

AMR is accompanied with high mortality rates; it provokes hindrance of treatment of the diseases with the spreading of resistant pathogens, resulting in a persistent time of infection to the patient. The cost of the treatment increased due to the resistant pathogens, in most of the cases commercially available drugs didn't work to the patients. So they need to buy uncommon antibiotics with a high price.

2.9 Management and remedies of AMR

Global collaborative efforts are necessary for the management and prevention of AMR and it should be individual, community, regional, national and international level. Strategies should develop the appropriateuse of antibiotics; reduce involuntary interaction between microorganisms and antibiotics. The WHO Global Action Plan

emphasizes on increased awareness and understanding on antimicrobial use and associated AMR; build up knowledge regarding AMR through proper surveillance and research; optimal and rational use of antibiotics; lowering the incidence of infectious diseases; and on organizing resources, research, and development for proper integrated prevention and containment of antibiotic resistance (Organization, 2015). Management of AMR in both human and veterinary sectors needs ideal action plans for the development of newer antimicrobials, possible intervention measures. Drugs should be only prescribed by the professionals and drugs should be taken by proper prescription. Patients should be complete treatment course of antibiotics, stopping of medication in the middle, generate resistant organisms. Self-medication by the patients and livestock should be avoided. Use of leftover drugs and sharing of those drugs should not be done and not to be saved for next time of illness. There is a great role of the scientists and policymakers. The researcher should develop novel drugs for effective treatment. Awareness programs should be a buildup for suitable use of drugs and increase cooperation and information networking among stakeholders. Proper law enforcement should be done to limit the sale of un-prescribed drugs.

The spread resistance to antibiotics among pathogenic microbes has made development of alternatives to antibiotics a pressing public concern. Extensive studies have established bacteriophages (phages) and phage-encoded lytic enzymes (virolysins) as two of the most promising families of alternative antibacterial for the treatment and prophylaxis of bacterial infections. They have shown great potential in veterinary and human medicine for the treatment and prophylaxis of infections. Technologies have also been patented employing phages and virolysins in other pathogen related applications including detection and decontamination (Dorval Courchesne et al., 2009).

Chapter-3: Materials and methods

3.1 Description of study area

Chittagong officially known as Chattagram is a major coastal city and financial centre in southeastern Bangladesh. The total area of the district is 5282.92 sq. km. (2039.74 sq. miles) of which 1700 sq. km. (456.37 sq. miles) belongs to coastal area. Its assessed populace remains at more than 5 million and populace density for every square km is 1527. The district lies between $21^0$54' and $22^0$59' north latitude and between $91^0$17' and $92^0$13' east longitude(Mitra et al., 1994). Chattagram peoples are mostly engaged with business and they habitat to rear pet birds and other animals for their recreation. A study was conducted on<HOW MANY> pet birdfarms in Chattagram Metropolitan city to evaluate the socioeconomic status of pet bird farm owners and the epidemiology of antimicrobial resistance of and *E. coli*. And *staphylococcus* spp.

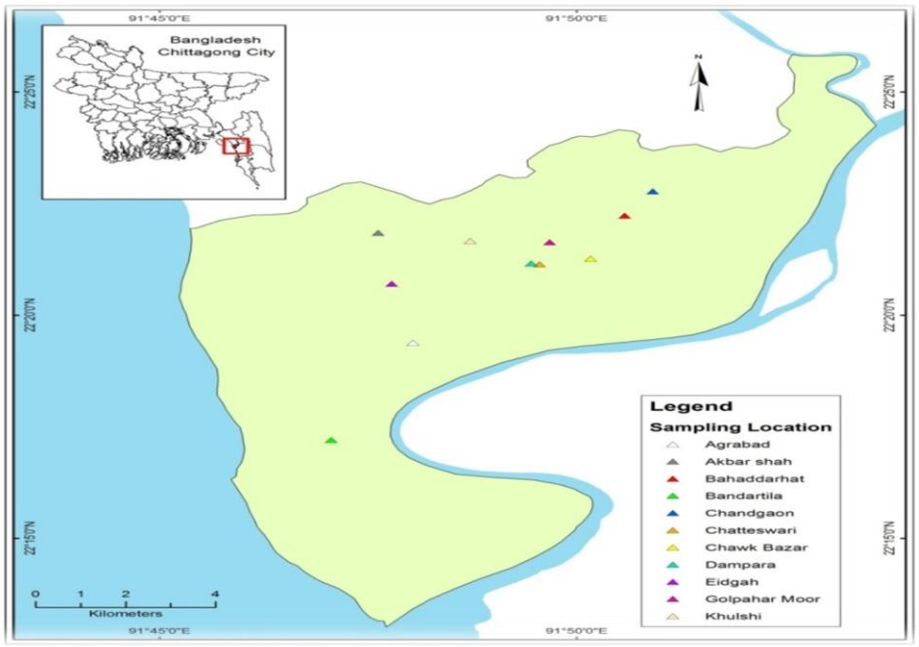


Fig: 1 Study Area

3.2 Ethical approval

Ethical approval was taken from Chittagong Veterinary and Animal Sciences University-Animal Experimentation Ethics Committee (AEEC), Chittagong, Bangladesh (**AEEC approval number: CVASU/Dir (R&E) AEEC/2015/751**) before starting the research. With the help of different protocol, we had assured the

animal ethics and animal safety as well as the safety of working personnel in both field and laboratory throughout the whole study period.

3.3 Study design

Cross-sectional study was done in different location from Chattagram Metropolitan area (CMA). 50 pet bird farms were randomly selected from the list of total pet bird farms of CMA. Those farms having 5-10 birds were included in this study. A total of220 cloacal samples were collected from budgerigar species of selection 50 farms for laboratory testing.

3.4 Sample size calculation

Simple random sampling was used for the sample collection and the sample size was estimated by the

The sample size was calculated by the formula by (Daniel and Cross, 1995)

n= $\frac{Z^2P(1-P)}{d^2}$ Where n=sample size,

Z= Z statistic for a level of confidence,

P = expected prevalence or proportion,

d = precision

3.5 Sample collection

All the samples were collected aseptically maintaining the standard procedure. Samples were collected from cloaca of budgerigar in different pre-selected farm. Cool chain was maintained during sample collection and shipment of samples. For collection of samples wire swab stick was used and submerges in transport media. Samples were transferred to Poultry Research and Training Centre laboratory under sterile conditions and processed immediately for the isolation of bacterial species.

3.6 Study period

The study was conducted between December 2016 and June 2017. Sample collection and laboratory test were done simultaneously.

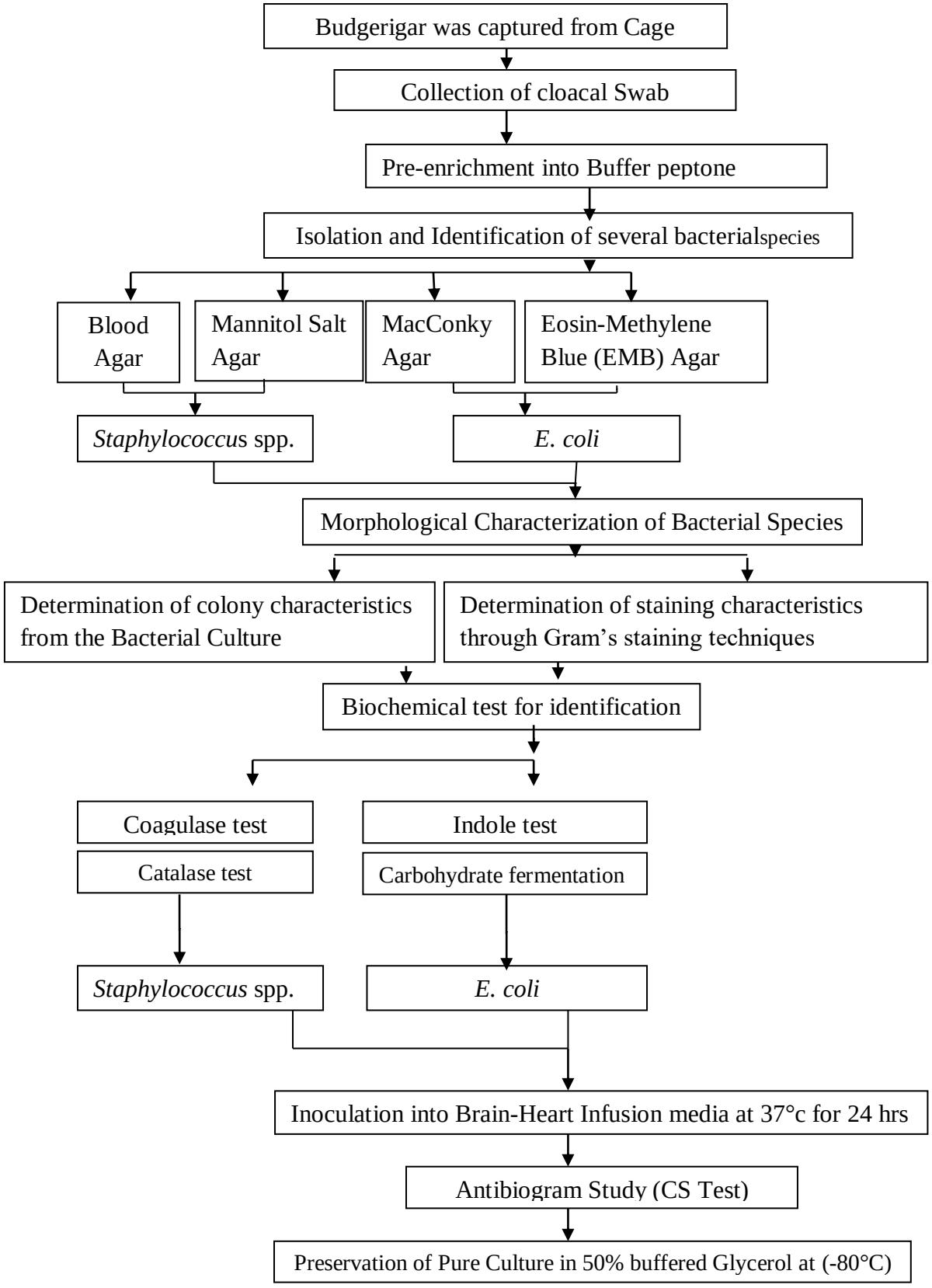
3.7 Data collection

A pre-structured questionnaire was administered to the pet bird farmers at the time of sample collection. The questions were aimed to collect ecological data on the pet birds. Geographical location's data was collected during sample collection.

3.8Laboratory study design

The experimental design is schematically presented in the figure 2.The entire study was divided into 4 major steps: The first step included a collection of samples from different areas, their transportation to the laboratory and inoculation into different culture media. In the second step, isolation and identification of the bacterial pathogens were done based on their cultural characteristics including pigment production, hemolytic activity, Gram's staining character etc. In the third step, characterization of the organism was done using various biochemical tests and other's confirmatory test's. Finally, in last step: their antibiotic sensitivity test was performed.

Figure 2: Experimentaldesign



3.9 Conceptual frame work

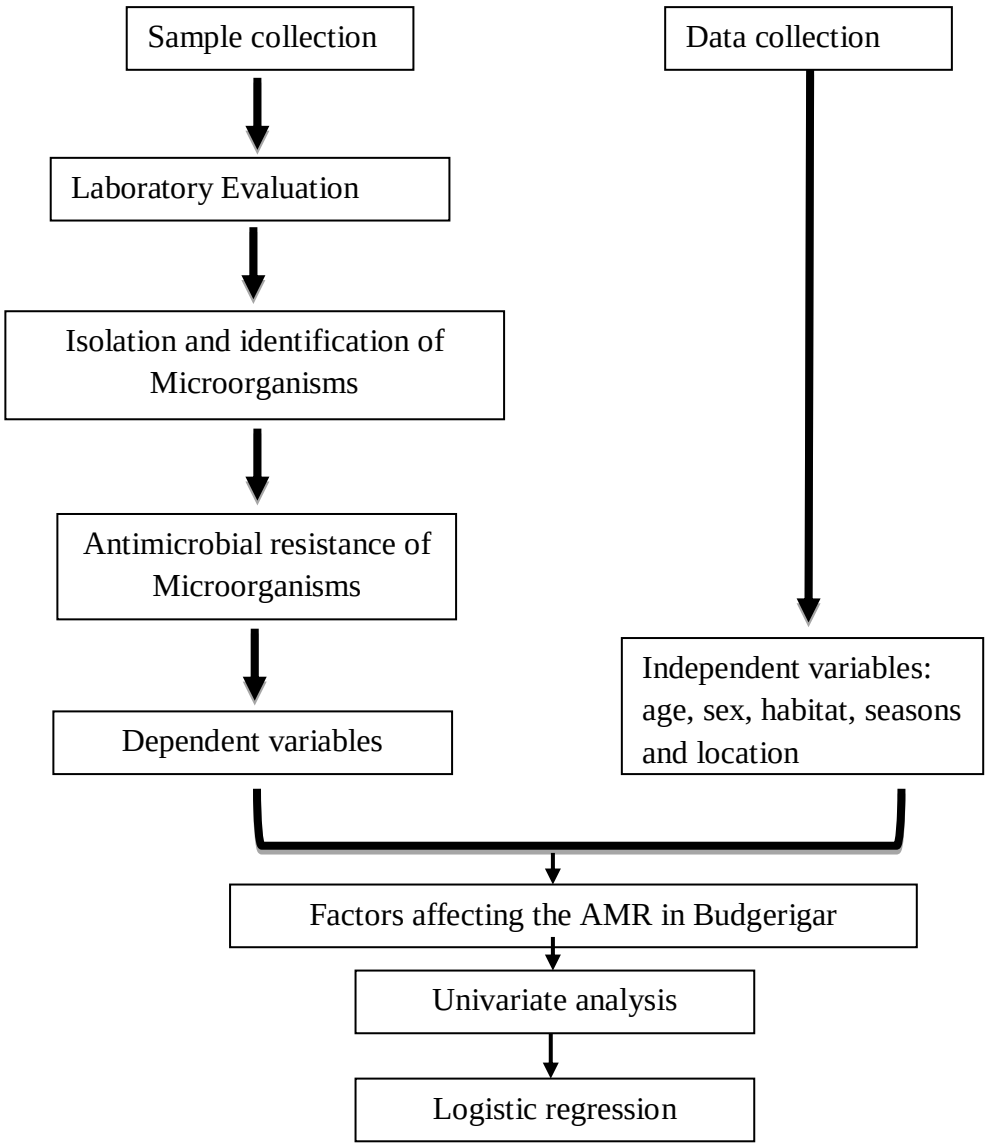


Figure 3: Conceptual frame work

3.10 Laboratory evaluation

3.10.1 Isolation of *Staphylococcus* spp.

Cloacal swabs of the budgerigar (N=220) from transport media were placed into sterile Buffered Peptone Water (BPW, OXOID Ltd, Hampshire, UK) and enriched for 24 hours at 37 ℃ (Parkar et al., 2013). Both Mannitol Salt Agar (MSA,OXOID Ltd, Basingstoke, Hampshire, UK)) medium and Blood agar(OXOID Ltd, Hampshire, UK) base were prepared according to the instructions of the manufacturer (OXOID Ltd, Hampshire, UK). Blood agar was prepared by adding 5% citrated-bovine blood in the blood agar base (Thakkar et al., 2014). A loopful of inoculums from enrichment broth were streaked onto Blood agar and incubated at 37℃ for 24 hours for detection of hemolysis. The growth of yellow colonies on MSA (surrounded by yellow zones as a result of fermentation of mannitol after 24 hours of incubation at 37℃ indicated a positive result (Kateete et al., 2010). The smear was prepared from the isolated colony on clean grease free microscopic glass slide and stained with Gram's Method of staining. All the positive samples were subjected to Coagulase and Catalase tests for biochemical confirmation of *Staphylococcus* spp.(Kateete et al., 2010). After that 5 such cross-sectional colonies were picked up and transferred to a 10 ml test tube containing 5 ml of brain heart infusion broth (BHIB, OXOID Ltd, Basingstoke, Hampshire, UK), were prepared according to the instructions of the manufacturer incubated at 37 °C for 6 hours.

3.10.2 Identification of *Staphylococcus* spp.

A) Staining

Grams staining strategy was done to consider morphology and staining characters of bacteria.For this a single colony from MSA was grabbed with a bacteriological circle, spread on a glass slide and settled by delicate warming. Then it was spread to recolor for two minutes and then washed with running water. Drops of Gram's iodine were then added for a moment and washed with running water. CH_3C_2O liquor was then included for a moment as a decolorizer. In the wake of washing with water, safranin was added as a counterstain and permitted to recolor for 2 minutes. The slides were then washed with water, blotched and dried in air and then analyzed under magnifying lens with high power objective (100X) utilizing submersion oil. Positive

Staphylococcus spp. have demonstrated pink hued round-molded grape-like group under the magnifying lens after gram's staining (Magee et al., 1975).

B) Biochemical test

a. Coagulase test

Entire blood from a stallion was gathered into monetarily accessible sterile tubes containing EDTA to play out the test. At that point, blood was centrifuged at 2600rpm for 10 minutes utilizing a refrigerated axis gadget. The subsequent supernatant, the plasma, was then quickly exchanged to a clean 1.5 ml eppendorf tube utilizing sterile tips and put away at - 20ºC for some time later (Ieven et al., 1995).

i) Tube coagulase test

From each tube developed in BHIB, 50 µL was exchanged to sterile tubes containing 50 µL of stallion plasma. The incubation was done at a temperature of 37ºC for 6 hours. The nearness of coagulates was legitimized, considering vast sorted out coagulation and coagulation of the considerable number of the substance of the tube which does not fall off when the tube was reversed (Alcaráz et al., 2003). A control tube also is placed to validate the result.

ii) Slide coagulase test

Staphylococcus spp. were additionally affirmed by slide coagulase test. One drop of the steed plasma was set on a spotless oil free glass slide. A loopful of suspected culture was blended with plasma independently and checked for agglutination. The presence of agglutination was recorded as positive for coagulase test for *Staphylococcus* spp.(Sperber and Tatini, 1975).

b. Catalase test

i) Tube catalase test

Nutrient agar slant was prepared according to the instructions of the manufacturer (OXOID Ltd, Basingstoke, Hampshire, UK)). Suspected bacterial colonies inoculated onto agar slant and incubated at 37°C for 24 hours. 1 ml of 3%, H_2O_2 was added and rapid ebullition of gas considered as the positive reaction for *Staphylococcus* spp.(Qian et al., 2007).

ii) Slide catalase test

A small amount of colony was placed on a fresh, clean and grease free slide. One drop of 3% H_2O_2 poured onto the colony, a coverslip was placed and bubble formation was indicated as a positive result (Lairscey and Buck, 1987).

C) Preservation of the culture

Biochemical test positive isolates were inoculated into (BHIB, OXOID Ltd, Basingstoke, Hampshire, UK), incubated overnight at 37^{o}C and then preserved at -80^{o}C with 50% glycerol in 1.5 ml eppendorf tubes for further analysis(Gherna, 1994).

3.10.2 Isolation of *Escherichia coli*

Pre-enrichment of *E. coli* was done in BPW broth (OXOID Ltd, Basingstoke, Hampshire, UK) using cloacal (N=220) swab samples (Thaker et al., 2013). A loopful of culture inoculates on MacConky (OXOID Ltd, Basingstoke, Hampshire, UK) agar. Pink colonies obtained from MacConky agar were taken and inoculated on Eosin methylene blue (EMB) (OXOID Ltd, Basingstoke, Hampshire, UK) agar to verify whether the bacterial population was *E. coli*, or not. EMB dyes react with products released by *E. coli* from lactose or sucrose as carbon and energy source and form metallic green sheen.(Virpari et al., 2013). Formation of metallic green sheen color regarded as positive isolates of *E. coli.*

3.10.2Identification of*Escherichia coli*

A) Staining

Grams staining method was done to study morphology and staining characteristics from a suspected colony of EMB agar. For this a single colony was picked up with a bacteriological loop, smeared on a glass slide and fixed by gentle heating. Crystal violate solution was then applied to smear to stain for 2 minutes and then washed with running water. Few drops of Gram's iodine were then added to act as mordant for 1 minute and then again wash with running water. Acetone alcohol was added for few seconds who act as a decolorizer. After washing with water, safranin was added as a counterstain and allowed to stain for 2 minutes. The slides were then washed with water, blotted and dried in air and then examined under a microscope with high power

objective (100X) using immersion oil. Positive *E. coli* have shown red coloredrod-shaped bacteria under the microscope after gram's staining(Krieg and Manual, 1984).

B) Biochemical test

a) Indole test

The unadulterated bacterial culture was developed in sterile peptone broth for 24 hours. Following incubation, 05 drops of Kovac's reagent was added to the way of culture stock. A positive outcome was reflected by the nearness of a red or red-violet shading in the surface layer of the stock. A negative outcome seems yellow. A variable outcome can likewise happen, demonstrating an orange shading subsequently. This is because of the nearness of skatole otherwise called methyl indole or methylated indole, another conceivable result of tryptophan corruption(Virpari et al., 2013).

b) Carbohydrate fermentation test

The test was performed by inoculating 0.2 ml of supplement nutrient broth culture of the isolated organism into the tubes containing five fundamental sugars, for example, dextrose, lactose, sucrose, maltose and mannitol and hatched for 24 hrs at 37ºC. Acid generation was shown by the shading change from red to yellow and gas creation was noted by the aggregation of gas rises in the rearranged Durham's tube (Hugh and Leifson, 1953).

C) Preservation of the culture

Biochemical test positive isolates were inoculated into BHIB (OXOID Ltd, England), incubated overnight at 37ºC and then preserved at -80ºC with 50% glycerol in 1.5 ml eppendorf tubes for future investigation (Morrison, 1977).

3.12Antibiogram Study

The antibiotic susceptibility tests were performed on Muller-Hinton Agar (Liofilchem, Italy) by Kerby-Bauer micro-disc diffusion techniques (Bauer et al., 1966). Measurement of the growth inhibition zone permitted the classification of each

isolate as susceptible, intermediate and resistant according to data provided by guideline for CS test(Guideline, 2007).

The most commonly used antimicrobial agents for either chemoprophylaxis or therapy for control of bacterial diseases in poultry and livestock in South Asia including Bangladesharesulfadiazine, sulphamethoxazole, tetracyclin, neomycin, ciprofloxacin, enrofloxacin, eitrofurantoin, colistin, ampicillin, amoxycillin, cloxacillin, erythromycin, metronidazole and pefloxacin (Prakash and Gupta, 2005; Mahmud et al., 2013).

A recent study in Bangladesh showed that in human penicillin, oxacillin, cloxacillin, amoxycillin, vancomycin, ciprofloxacin, erythromycin, fusidic acid, and rifampicin, ceftriaxone, cefixime, amoxycillin, ampicillin, erythromycin, metronidazole etc due to various infection (Islam et al., 2008).

Above the circumstances, our study derived to test the antimicrobial resistance against *Staphylococcus* spp. and *E. coli* in some antibiotics, which are commonly used in both human, and animal in Bangladesh, thereby we selected 9 common antibiotics from several genera of antibiotics. We selected sulphamethoxazole+trimethoprim, azithromycin, ciprofloxacin, oxytetracycline, cefixim, amoxycillin, gentamycin, norfloxacin and enrofloxacinin our study.

Antimicrobial susceptibility of the isolates were determined by using the micro disc diffusion method, and the method was used according to guidelines established by Clinical and Laboratory Standards Institute (Wayne, 2010). For the Culture Sensitivity test, a bacterial turbidity equivalent to 0.5 McFarland standards was used for each isolate. Mueller-Hinton agar was prepared in Petri dishes as per the instructions of the manufacturer. Pure colonies of the *Staphylococcus* spp. and *E. coli* isolates were inoculated in nutrient broth and incubated at 37°C for overnight. The isolates were streaked thoroughly on the Mueller Hinton agar using sterile glass rod (60° cone shaped) and the antimicrobialdisc wasplaced centrally using antimicrobial disc dispenser. The Petri-dish and its contents were incubated in an incubator at 37°C for 24 hrs. The plates were observed for antimicrobial susceptibility pattern by measuring the zone of inhibition developed against the *Staphylococcus* spp. and *E. coli* isolates on the plate. After the predefined period of incubation, the size of the zone of inhibition around a micro-disk was measured in millimeter with digital slide

calipers and the result was taken on a paper. The susceptible and resistance breakpoint levels of the antimicrobials were based mainly on those specified by guideline (CLSI, 2007) and the isolates were considered as Sensitive (S), Intermediately sensitive (I) or Resistance (R) to tested antimicrobials according to the manufacturer‘s (OXOID, UK) standard protocol and interpretation criteria described against specific antimicrobial in case of *Staphylococcus* spp. and *E. coli* isolates. The results of antibiotic sensitivity test were then recorded, analyzed and discussed.

Table 1. A panel of antibiotics used their concentrations and zone diameter interpretative standards for *Staphylococcus* spp. and *E. coli.*

Agent name	Disc Code	Potency (µg)	*Staphylococcus* spp.			*E. coli*		
			Resistant ≤	**Intermediate**	**Susceptible ≥**	**Resistant ≤**	**Intermediate**	**Susceptible ≥**
SXT	**Sulphamethoxazole & Trimethoprim**	25	10	11 to 15	16	10	11 to 15	16
CRO	**Cefixim**	30	13	14 to 20	21	13	14 to 20	21
ENR	**Enrofloxacin**	1	16	17 to 18	19	11	12 to 28	29
AZM	**Azithromycin**	15	13	14 to 17	18	14	15 to 18	19
CIP	**Ciprofloxacin**	5	15	16 to 20	21	20	21 to 30	31
NOR	**Norfloxacin**	5	15	16 to 18	19	15	16 to 18	19
AML	**Amoxycillin**	10	18	19 to 20	21	13	14 to 17	18
CN	**Gentamycin**	10	12	13 to 14	15	12	13 to 14	15
OT	**Oxytetracycline**	30	14	15 to 18	19	11	12 to 14	15

3.13 Collection of data and statistical analysis

Ecological data were collected using questionnaire and GIS techniques simultaneously were imported to the Epi Data v3.1 and Microsoft Office Excel-2007 and then imported to STATA/IC-13 software for analysis. Descriptive statistics were done using the STATA software(Rabe-Hesketh and Everitt, 2003) to express individual results of each category as a percentage of antibiogram pattern of *Staphylococcus* spp. And *E. coli i*solated from cloacal swabs of the budgerigar. The results were also expressed in range and 95% confidence interval (CI).The associated factor was correlated with the high frequency of resistance transmission within environment and animal level were analyzed through descriptive statistics.

3.13. a Univariable analysis

Univariable and multivariable statistical analyses were performed to identify the potential risk factors associated with the prevalence of antimicrobial resistance. The following set of selected risk factors were tested to identify their association with animal of prevalence of study sites such as age of bird, sex of bird, Body Condition Score (BCS), disease condition and water sources.

Univariate chi-square test was performed to assess the association between the categorized response variable of antimicrobial resistance and selected independent variable. Age, sex and species of the positive samples were significantly ($p<0.05$) associated with the prevalence of antimicrobial resistance. Therefore, these variables were forwarded to develop the final logistic model to study the prevalence of antimicrobial resistance.

3.13. b Logistic regression model analysis

In this part of data analysis, interaction was assessed between factors by constructing two interaction products terms for the significant main effect factors in the model, forcing them into the model and examining changes in the odds ratio (OR) and *p* values of the main effects. Evidence of confounding was checked by dropping one of the variables and assessing the changes of odds ratio (≥10% change meant confounding). The model was then assessed for goodness-of-fit while predictive ability was determined using the

receiver operating characteristics (ROC) curve (Dohoo et al., 2003). The results were presented for each adjusted selected variable as an OR, *p* value and 95% CI.

Chapter-4: Results

4.1 Preliminary ecological and management study on pet birds in Bangladesh

A cross-sectional study was conducted to know the general concept of the pet bird's farm with a view to know more about the ecological and biological information of different species. The study has an aim to correlate the ecological factors of different bird species with the antimicrobial resistance and its transmission. Overall prevalence of *E. coli* and *Staphylococcus* spp. was found as 22.27% and 18.18 % respectively.

Table: 2: Pet birds rearing farmer's information

Criteria		N (%)
Sex	Male	45(91.84)
	Female	4(8.16)
Occupation	Business	20(40.82)
	Service holder	11(22.45)
	Student	14(28.57)
	Others	4(8.16)
Education	SSC	4(8.16)
	HSC	9(18.37)
	Bachelors	24(48.98)
	Masters	12(24.49)
Role in developing new Pet bird Farming	Motivate to establish new farm	39(79.59)
	Provide technical support	1(2.04)
	Provide financial support	4(8.16)
	Consultancy	5(10.20)
Purpose of farming	Hobby	34(69.39)
	Business	16(30.61)
Farm size	Small(10-30)	24(48)
	Medium(31-100)	16(32)
	Large(101-500)	10(20)

Table 2 showed that maximum (91.84%) per bird farmers were male and most of them were business man (40.82%) and followed by student (28.57%). They having a role to motivate new people for new pet bird farming (79.59%) because they are mostly educated (approximately 49% having Bachelor's degree). At the beginning they started

their farm as like hobby (69.39%) and afterward its goes to business purpose (30.61%). Most of the farms are small having 10-30 birds (48%) followed by medium (32%) and lowest percent are large having 101-500 Birds (10%).Table 3 stated that people having different species in his/her farm among them budgerigar (24.74%), cockatiel (15.79%), finch (16.32%) and those bird having high price like macaw (0.53%) and cockatoos (2.11%).

Table: 3: Different species information, their management and diseases

Criteria		N (%)
Species	Budgerigar	47(24.74)
	Cockatiel	30(15.79)
	Dove	14(7.36)
	Finch	31(16.32)
	Java	9(4.74)
	Lorry	5(2.63)
	Love bird	13(6.84)
	Ring neck parrot	16(8.43)
	Sunconure	5(2.63)
	Cockatoo	4(2.11)
	Rosella	4(2.11)
	Macaw	1(0.53)
Source of bird	Farm	126(66.32)
	Market	64(33.66)
Housing	Inside the flat	28(56.82)
	Verandah	21(42.86)
Feeder	Plastic	166(87.37)
	Tin	24(12.63)
Waterer	Plastic	170(89.47)
	Tin	20(10.53)
Source of water	Tape	105(56.15)
	Filter	82(43.85)
Frequency of disease	Frequent	26(53.06)
	Less frequent	23(46.94)
Season of occurring disease	Summer	14(32.56)
	Rainy	4(9.3)
	Winter	23(53.49)
	Spring	2(4.65)
Experience of Endemic diseases within 1 year	CRD	38(26.02)
	Fowl Cholera	24(16.43)
	New castle	29(19.86)
	Salmonellosis	40(27.39)
	Colibacillosis	17(11.64)
	Mite infestation	8(5.47)

Farmers collect their birds from another farm (66.32%), from market (33.68%) and reared these birds inside their flat (56.82%). They supply tap water (56.15%) for their bird mostly. For supplied food and water most of the farmers use plastic feeder (87.37%) and waterier (89.47%). Most frequent (53.06%) diseases are occurred in winter season (53.49%). Most common diseases are salmonellosis, Chronic Respiratory disease (CRD), Fowl Cholera, New Castle disease, colibacillosis and mite infestation.

4.2 Univariable and multivariable associations between AMR of *E. coli* and selected variables

The prevalence of *E. coli* in budgerigar32.1 % was highest in Agrabad area ($p \leq 0.05$) and lowest in Akbar shah and the percentage is 15.6% (Table 4).

The significant variables (BCS and Water source, $p \leq 0.3$) identified through univariable chi-square analyses were forwarded to the logistic regression model. After adjustment of the factors each other through the model, the odds of antimicrobial resistance of *E. coli* was significantly higher in poor BCS birds (OR=1.98; CI: 20.2-38.2, $p=0.04$) than that good. On the other hand, the odds of AMR was higher in tap water sources (OR=2.6; CI: 20.6-34.8, $p=0.03$) than that of filtered water source (Table 4).

Table: 4 Frequency distribution of AMR of *E. coli* in budgerigar in Chittagong

Variables	Categories	AMR of *E. coli*			Logistic regression Model		
		n (%)	95% CI	*P* (χ^2-test)	OR	95% CI	*P*
Location N=200	Agrabad (n=28)	9 (32.1)	15.8-52.3	0.83			
	Akbarshah (n=32)	5 (15.6)	5.3-32.8				
	Bohoddarhat (n=41)	8 (19.5)	8.8-34.8				
	Chakhbazar (n=16)	3 (18.7)	4.1-45.6				
	Halishohor (n=48)	11 (22.9)	12.1-37.3				
	Khulshi (n=30)	7 (23.3)	9.9-42.3				
	Potenga (n=25)	6 (24)	9.4-45.1				
Age N=220	Young (n=88)	23 (26.1)	17.3-36.6	0.26	1		
	Adult (n=132)	26 (19.7)	13.3-27.5		0.7	0.3-1.4	0.27
Sex N=220	Female (n=96)	18 (18.7)	11.5-28	0.26	0.7	0.4-1.4	0.36
	Male (n=124)	31 (25)	17.6-33.6		1		
BCS N=220	Poor (n=105)	30 (28.6)	20.2-38.2	0.03	1.98	1.1-3.9	**0.04**
	Good (n=115)	19 (16.5)	10.2-24.6		1		
Disease condition N=220	Non diseased (n=101)	19 (18.8)	11.7-27.8	0.25	1		
	Diseased (n=119)	32 (25.2)	19.2-35.8		1.5	0.7-3	0.23
Water source N=220	Filtered (n=65)	7 (10.8)	4.4-20.9	0.01	1		
	Tap water (n=155)	42 (27.1)	20.3-34.8		2.6	1.1-6.3	**0.03**

4.3 Antimicrobial resistance pattern of *E. coli*.

Ciprofloxacin (89.79%) and Gentamycin (73.49%) were highest sensitive among all drugs whereas Enrofloxacin was lowest sensitive (46.93%) against *E. coli*. Sulphamethoxazol and Trimethoprim, Cefixim and Amoxicillin showed (100%) among all drugs whereas Ciprofloxacin showed the least resistance (6.12%, Figure 3).

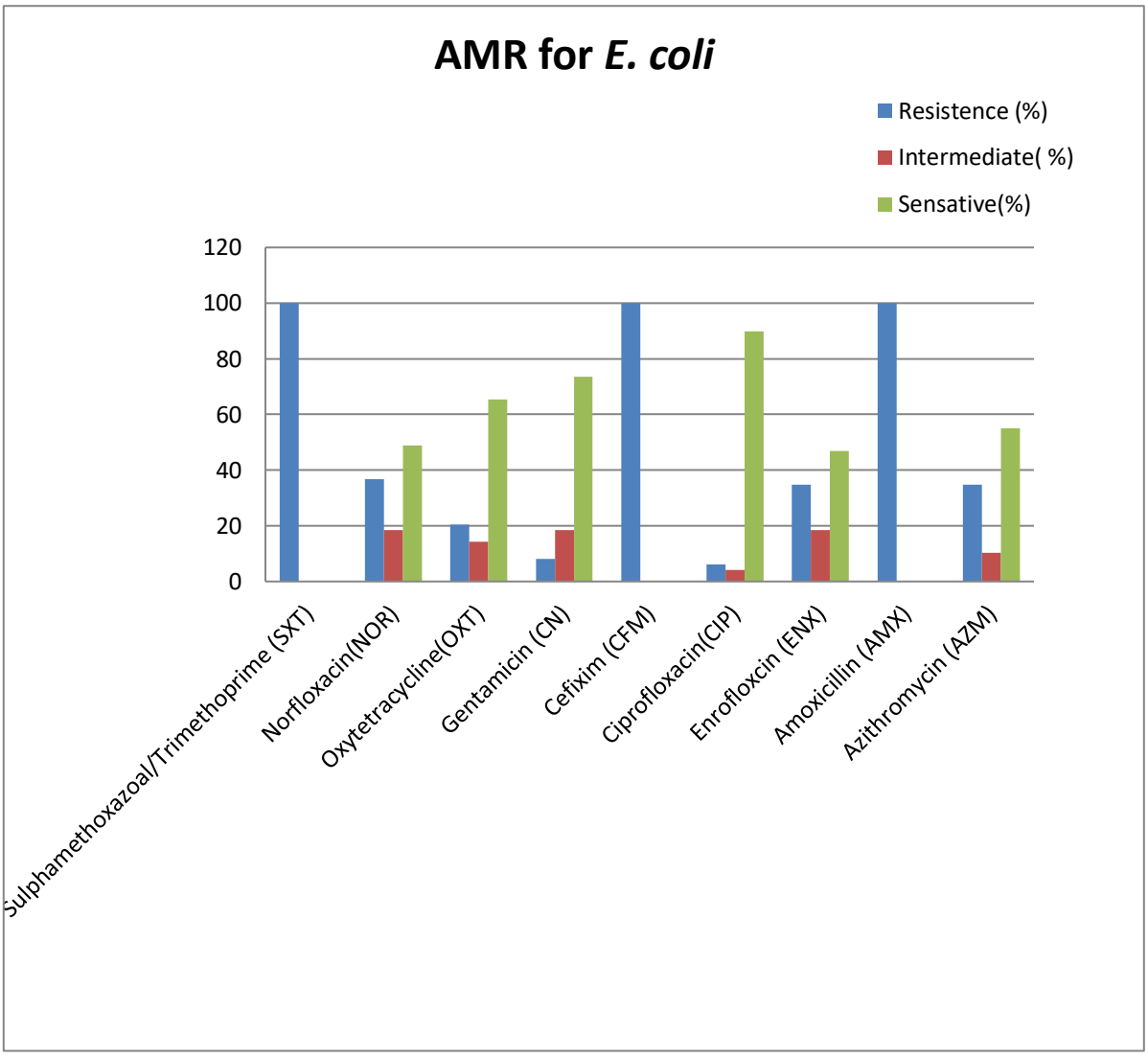


Figure 3: Antimicrobial resistance pattern of *E. coli*

4.4 Univariable and multivariable associations between AMR of *Staphylococcus* spp. and selected variables

The prevalence of *Staphylococcus* spp was 25%, which is highest in Akbarshah area ($p \leq 0.05$) whereas lowest prevalence found among the budgerigar of Halishohor and Chakhbazar, and the rate was 12.5% (Table 5).

The significant variables (Age and Disease condition, $p \leq 0.3$) identified through Univariate chi-square analysis were forwarded to the logistic regression model. After adjustment of the factors each other through the model, the odds of antimicrobial resistance of *Staphylococcus* spp was significantly higher in young bird (OR=1; CI: 0.1-0.6, $p=0.003$) than the adult bird. On the other hand, the odds of AMR was higher in diseases bird (OR=4.5; CI: 1.9-10.5, $p=0.001$) than that of non-diseased bird (Table 5).

Table: 5 Frequency distribution of AMR of *Staphylococcus* spp. in budgerigar of Chittagong

Variables	Categories	AMR of *Staphylococcus* spp			Logistic regression Model		
		n (%)	95% CI	*P* (χ^2-test)	OR	95% CI	*P*
Location N=220	Agrabad (n=28)	5 (17.8)	6.1-36.8	0.80			
	Akbarshah (n=32)	8 (25)	11.5-43.4				
	Bohoddarhat (n=41)	8 (19.5)	8.8-34.8				
	Chakhbazar (n=16)	2 (12.5)	1.5-38.3				
	Halishohor (n=48)	6 (12.5)	4.7-25.3				
	Khulshi (n=30)	7 (23.3)	9.9-42.3				
	Potenga (n=25)	4 (16)	4.5-36.1				
Age N=220	Young (n=88)	23 (26.1)	17.3-36.6	0.01	1		
	Adult (n=132)	17 (12.8)	7.7-19.8		0.3	0.1-0.6	0.003
Sex N=220	Female (n=96)	18 (18.7)	11.5-28	0.84			
	Male (n=124)	22 (17.7)	11.4-25.6				
BCS	Poor (n=105)	22 (20.9)	13.6-	0.30	1.5	0.7-3.1	0.27

N=220			29.9				
	Good (n=115)	18 (15.6)	9.5-23.6		1		
Disease condition N=220	Non diseased (n=101)	9 (8.9)	4.2-16.2	0.001	1		
	Diseased (n=119)	31 (26.1)	18.4-34.9		4.5	1.9-10.5	0.001
Water source N=220	Filtered (n=65)	6 (9.2)	3.5-19.1	0.02	1		
	Supplied (n=155)	34 (21.9)	15.7-29.3		2.2	0.8-5.7	0.10

4.3 Antimicrobial resistance pattern of *Staphylococcus* spp

Ciprofloxacin (85%) and Azithromycin (82.5%) were highest sensitive among all drugs whereas Cefixim was lowest sensitive (2%) against *Staphylococcus* spp.Gentamycin and Enrofloxacinshowed the highest resistance (100%) amongst all drugs whereas Ciprofloxacin and Azithromycin showed the lowest resistance (5%)(Figure4).

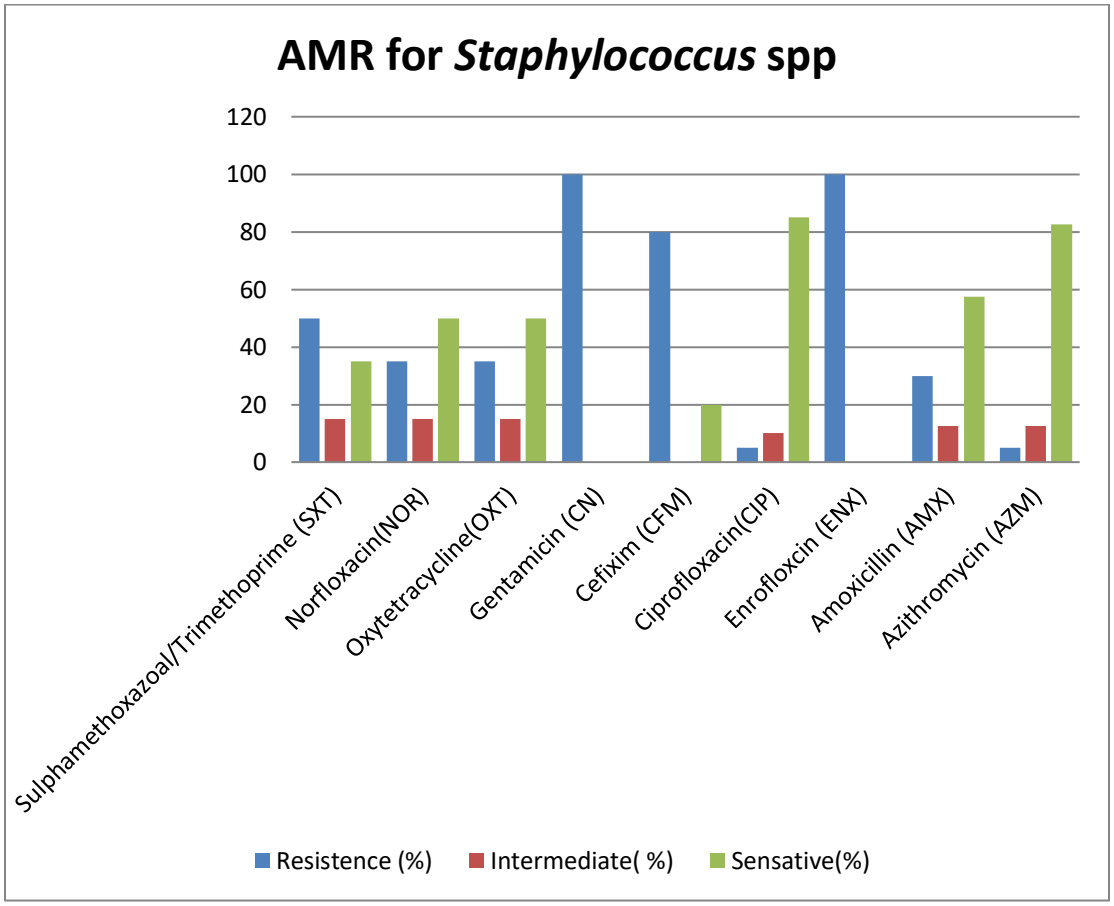


Figure 4: Antimicrobial resistance pattern of *Staphylococcus* spp

Chapter-5: Discussion

Antimicrobial resistance (AMR) in livestock and wildlife is an emerging public health threat whole over the world and also in Bangladesh. Pet bird's usually lives inside the owner house and got chance to transmit the resistance microorganisms easily. Although it is a serious health concern for budgerigar as well as other pet birds but unfortunately there are no studies so far conducted related to AMR in Budgerigar. Few studies have been performed on antimicrobial resistance in parrot in Bangladesh. Antimicrobials are widely used in livestock and poultry production for treatment and growth promotion and ultimately got the resistance which may spread to other animals. Therefore, the present study was conducted to estimate the status of eco-epidemiology of antimicrobial resistance of budgerigar. The important findings, their implications, limitation, conclusion and recommendation have been discussed in this section.

This study found that 45% pet bird farmers were male but female farmers are also growing day by day in Bangladesh. Establish farmers 39% found the role to motivate new people for new pet bird farming and they are mostly educated (around 49% Bachelor's degree). Due to recreation purpose most of the farmers found to have connection with new friends or younger to establish or rearing pet birds. At the beginning they establish their farm as hobby (34%) and transformed into its business (16%). Mostly they rear budgerigar (47%) due to low price and beauty of the bird. Other like cockatiel (30%), finch (31%) and those bird having high priced bird like macaw (0.53%) and cockatoos (2.11%) are also found. Among them macaw and cockatoos are most expensive bird in Bangladesh as well as in the world (Forshaw, 2010).

This hobby with business build up a good relationship and farmers can collect pet birds from other farms (66.32) or market (33.68) and most of the farmers reared pet birds inside their house (56.82%). Study found that supply tap water (56.15%) for their bird which may not be healthy mostly for pet bird as well as other animal(Juranek, 1995). For supplying food and water (87.37%) farmers use plastic feeder and 89.47% use plastic waterer. Most frequent diseases are occurred in winter season (53.49%). This study found that common diseases of pet bird are Salmonellosis, Chronic Respiratory disease (CRD),

Fowl Cholera, New Castle disease, colibacillosis and mite infestation which is similar with information found in poultry farming book (Sonaiya and Swan, 2007).

In our study overall prevalence of *E.coli* in budgerigar is 22.27 %. Prospective study was in brazil showed that overall frequency of healthy birds hosting *E. coli* was 8.47% in budgerigars (5/59) (Gioia-Di Chiacchio et al., 2016). *Escherichia coli* O157:H7 strains transmitted from wild passerines (European 428 starlings mostly) to cattle and then introduced into the food chain has been reported 429 in several studies (Gaukler et al., 2009).

The prevalence of *E. coli* was 32.1 % which was highest in Agrabad area ($p \leq 0.05$) and lowest in Akbar shah (15.6%). It could be due to high density of pet bird population in this area. We know that water supply system is not good in that area of Chittagong so that organism also can transmit via tap water (Rahman et al., 2011). In Bangladesh zoo state that the bacteria isolated in different types of caged parrots were *E. coli* (64.44%) (Akhter et al., 2010b).The isolated Gram-negative bacteria considered as normal inhabitant in the gut of healthy budgerigars, however, under certain conditions became pathogenic so, consideration of them as pathogens would lead to unnecessary antibiotic treatment. This result came in accordance with that reported by (Flammer and Drewes, 1988)and completely disagreed with that reported by (Harrison and Harrison, 1986) who stated that, E. coli and other gram negative bacteria are abnormal inhabitants of the psittacine gut and should be considered pathogen. Antimicrobial resistance of *E. coli* was significantly higher in poor BCS birds (OR=1.98; CI: 20.2-38.2, p=0.04). Poor body condition score birds not fit for good heath that's why their immunity would be down and affection of organism so high. On the other hand, the odds of AMR was higher in tap water sources (OR=2.6; CI: 20.6-34.8, p=0.03) than that of filtered water source. It's clearly said that tap water is very contaminated source of water and in contact with different organism. Since (Moore et al., 1946) reported that a small amount of streptomycin added to the diet caused chickens to grow more rapidly, many other growth-promoting agents, i.e., antibiotics and synthetic chemotherapeutics, have been used as feed additives to increase animal protein production in livestock and poultry. The prolonged use of these drugs for growth promotion, however, led to a high incidence of

enteric bacteria exhibiting drug resistance. The effect of the use of antimicrobial agents at a nutritional level on the emergence of resistant *E. coli* strains in the alimentary tract of livestock and poultry has been investigated (Sokol et al., 1969) and resistance frequencies reaching 70-90% have been found in *E. coli* strains isolated in recent years (12, 22). Ciprofloxacin (89.79%) and Gentamycin (73.49%) showed highest sensitivity among all drugs whereas Enrofloxacin showed lowest sensitive (46.93%) against *E. coli*. Sulphamethoxazol and Trimethoprim, Cefixim and Amoxicillin showed the highest resistance (100%) among all drugs whereas Ciprofloxacin showed the lowest resistance (6.12%). There have been few reports concerning the antibiogram of *E. coli* strains isolated from wild animals. Sato et al. reported that crows and feral pigeons which live close to humans exhibited a high frequency of drug-resistant against *E. coli* strains.

As pet birds have little or no contact with human before them caught. The probability of having drug-resistant *E. coli* strains is very less before caught. Therefore, the high incidence of drug resistance and R plasmids in *E. coli* strains isolated from pet birds may reflect the unethical use of antibiotics for the prevention and treatment of diseases. There have been reports in the last decade of the presence of drug-resistant and R plasmid-carrying *E. coli* strains in normal healthy humans (Grabow et al., 1974). One of the sources of these drug-resistant bacteria in humans is thought to be livestock and poultry that carry a large number of drug-resistant bacteria in the intestinal tract.

Horn et al. (2015) state that the antimicrobial to *E. coli* of canary which the strains presented most resistance was sulfonamides with 55.7%, followed by ampicillin with 54.1% and tetracycline with 39.3%. The total of multidrug-resistant bacteria (MDR) was 34 (55.7%) (Horn et al., 2015).

The overall prevalence of *Staphylococcus* spp. is 18.18%. The prevalence of *Staphylococcus* spp. was 25 % which highest in Akbarshah area ($p \leq 0.05$) whereas lowest prevalence found among the budgerigar of Halishohor and chakhbazar, the percentage is 12.5%. *Staphylococcus* (22.69%) were predominate in the intestinal tract of clinically healthy budgerigars, similar findings were obtained by (Bangert et al., 1988) and this may attributed to environmental conditions, crowded cages as those recorded by (Glünder, 2002).

Antimicrobial resistance of *Staphylococcus* spp was significantly higher in young bird (OR=1; CI: 0.1-0.6, *p*=0.003) than the adult bird. Some study shows that young animal is more susceptible against any disease or bacteria due to immunity development and body thermal condition. Body thermal condition is higher in adult bird than the young bird. On the other hand, the odds of AMR was higher in diseases bird (OR=4.5; CI: 1.9-10.5, *p*=0.001) than that of non-diseased bird due to use of antibiotic in diseased bird not in healthy bird. Ciprofloxacin (85%) and Azithromycin (82.5%) were highest sensitive among all drugs whereas Cefixim was lowest sensitive (2%) against *Staphylococcus* spp. Gentamycin and Enrofloxacin showed the highest resistance (100%) amongst all drugs whereas Ciprofloxacin and Azithromycin showed the lowest resistance (5%). On the other hand, the antibiotics of fluoroquinolone group such as ciprofloxacin, norfloxacin and enrofloxacin showed moderate to high sensitivity against almost all the bacterial isolate. Of these, ciprofloxacin was found to be consistently highly sensitive to all the bacterial isolates which is consistent with the findings of previous study(Morishita et al., 1996).Thus, the results of this study may help pet clinicians to interpret microbiological culture and sensitivity results in budgerigar and other psittacine pet birds as well.

Chapter-6: Conclusion

Antimicrobial resistance is a most concern and the public health hazard due to inappropriate use of antimicrobials. By the side of pet bird, human, livestock, wildlife is most prevalent in resistant organisms. In Budgerigar, prevalence of *E.coli* and *Staphylococcus* spp. were found 22.27% and 18.18% respectively. Among many species of pet birds the most common species found was Budgerigar (24.74%).The standard microbiological procedure were followed for the isolation of these zoonotic bacteria. Susceptibility of *E. coli* and *Staphylococcus* spp.to antibiotics was conducted using disc diffusion method with nine commercially available antibiotics. All (100%, n=49) *E. coli* isolates were resistant against amoxicillin, sulfomethoxazol, trimethoprim, and cefixime but lowest resistant was found in ciprofloxacin (6.12%). Moreover, we found higher (100%, n=40) multidrug resistance in *Staphylococcus* spp. All of these two bacteria have an evidence of antimicrobial resistance ata higher level, especially it is found as MultidrugResistance, which is a threat to us. However, for detecting virulence factor affecting genes of *Staphylococcus* spp.and *E. coli* further work is needed for establishing a molecular diagnostic method. In conclusion, this study presented multidrug resistant in *E. coli* and *Staphylococcus* sp. isolated from the pet birds. The indiscriminate use of antibiotics on pet birds should be reduced to lessen the risk of public health importance multi-drug resistance bacteria. By placing antimicrobial resistance in pet birds within the sustainable development agenda, we seek to intensify the national and international commitment for finding a solution to this emerging threat in pet birds sector before it turns into a global crisis.In this circumstances of resistance pattern of antimicrobial agents, we should take necessary steps to avoid or control the antimicrobial resistance and improve the public health.

Chapter-7: Recommendations and limitation

Pet birds especially budgerigar is most common in Bangladesh as well as in whole world. So now we need to concern about this bird health issue and also public health concern due to very close contact with people. Human and pet bird interaction with the budgerigar to increase the resistance of different antimicrobials, so necessary steps should be taken to reduce the effect of antimicrobial resistance through awareness build up program. Water and food in the environment are contaminating with various resistant microorganisms due to improper management of waste disposal as well dust in pet bird local food. So, proper waste management is needed to solve this antimicrobial resistance problem in Budgerigar. We need you build up some awareness to farmers that don't use antibiotic in non-diseased animal. Need to develop educational based campaign for awareness. Improper utilization of antimicrobials should be stop. In terms of treatment of Budgerigar and other pet birds antimicrobial susceptibility testing should be done to select proper drugs. Everyone should follow proper withdrawal period and drugs prescribed by veterinarian. Further research should be done to know source of resistance pattern and identify the risk factors. Public awareness should be increase regarding antimicrobial resistance.

Chapter: 8 References

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Chapter: 9 Appendix

Understanding of Ecology of Antimicrobial Resistance of Pet Birds in Bangladesh

Section-A: Farmers Data:

01. *Name:*

02. *Address:*

03. *Sex:* ☐ Male ☐ Female

04. Main Occupation: ☐ Business ☐ Service Holder ☐ Student ☐ Others

05. Family Members: .....

06. Age Group:☐<20 yrs ( ) ☐ 20–29 yrs ( ) ☐30–39 yrs ( ) ☐40–49 yrs ( ) ☐ 50 yrs & above ( )

07. Educational Background:☐Below SSC ☐SSC ☐HSC ☐ Bachelors' ☐ Masters' ☐PhD

08. Marital Status:☐Married ☐Unmarried

09. How you become interested in pet bird raising/faming?

10. Role in spreading pet bird farming:☐ Provide training ☐ Motivate to establish new farm ☐Provide technical support ☐Provide financial support ☐Consultancy ☐None ☐Others

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Section-B: Farm Related Data:

01. Name of the Farm/Company: ……..

02. Date of Establishment:

03. Registration from forest ministry:☐Yes ☐No

04. Purpose of Farm:☐Hobby ☐ Business ☐ Conservation ☐Recreation

05. Farm Size Total Bird: ……number

06. Species Specific Data:

No.	Species Name	Variety	Number of birds	Age of sexual Maturity (month)	Age of first laying (month)	No. of eggs per month per pair	No. of Egg/ Pair/Year	No. of baby/ Pair/ month	Adult Price (Pair) (BDT)	Baby Price (Pair) (BDT) (1 to 5 month of age)	Hatchability rate (%)	Fertility rate (%)
01.												
02.												
03.												
04.												
05.												

06.												
07.												
08.												
09.												
10.												

08. Biomorphometric Data:

No.	Species	Variety	Egg Weight (g)			Length (mm)	Width (mm)
			1–3 days	8–10 days	16–18 days		
01.							
02.							
03.							
04.							

05.							
06.							
07.							
08.							
09.							
10.							

08. Types of hatching box: ☐Using wooden box ☐Using soil box ☐ Both

09. Future Plan:

Section-C: Management Related Data:

01. Type of housing:☐Farm House ☐ Inside Own Flat ☐Berandah ☐ Others

02. Rearing system: ☐Single Cage ☐ Colony cage ☐Open in house

03. Pairing Different Varity:☐Yes☐No

05. Pairing For:☐Life☐ ( ) Year ☐ ( ) Month

Section-D: Feeds & Feeding:

Species	Name of feed ingredients	Feed brand name	Amount of feed/bird/day	Price of feed/kg	Feed cost/year	Amount of feed/bird/year	Feeder (plastic feeder=1, earthenware=2, Tin feeder=3	Waterer (plastic waterer=1, earthenware=2, Tin waterer=3	Source water	Time of feeding	Changing time of water

Section-E: Disease & Disease Management Data:

01. Have you experienced any disease problem? ☐Yes ☐No

02. Frequency of Disease: ☐Frequent ☐Less Frequent ☐Rare

03. Occurrence of Disease: ☐ Summer ☐ Rainy Season ☐Winter ☐ Spring

04. No. of Shell Death/ Year: .....number

05. No. of baby Died/ Year:.....number

06. Which disease or symptoms affect most?.....

07. Pigeon of which age affect most: ☐Baby ☐ Adult

08. Drugs/Additives used with feed & water regularly: ☐Yes ☐No

If yes, then name of the drug–……..

09. Vet/Consultant's Advice: ☐Regularly ☐At Intervals ☐ When Needed ☐Never

If not, how get advised?.....

10. Any Endemic Disease:.....

11. Use Any Vaccines: ☐Yes ☐No

If yes, then which vaccine-…….

12. Bio-security measures: ☐Strictly followed ☐normally followed ☐Never followed

13. Use footbath? ☐Yes ☐No

14. Breed Specific Diseases:

No.	Breed/ Variety	Name of Disease(s)	No. of Baby		No. of Adult		Total No.	
			Affected	Died	Affected	Died	Affected	Died
01.								
02.								
03.								
04.								
05.								
06.								
07.								
08.								
09.								
10.								

Section: F: Treatment related data

Species	Disease name	Name of Antibiotic	Dose	Giving time	How long time given drug	Another supportive therapy	Recovery time	Time between new antibiotic use

Note: Amoxicillin=1, Ampicilin=2, Oxytetracycline=3, Sulpher drug=4, Pefloxacin=5, Ciprofloxacin=6, Erythromycin=7, Trimethoprim=8, Doxycycline=9, Colistin sulphate=10, Enrofloxacin=11, Gentamycin=12, Metranidazole=13, Ceftriaxon=14

1. Entry of any other wild birds in the farm? ☐Yes ☐No
2. **Entry of other poultry species in the farm?** ☐Yes☐No **if yes name of species.....**
3. **Any isolation cage or shed?** ☐Yes ☐No
4. **Use Antibiotics with feed?** ☐Yes ☐No

Section-F: Economics:

Cost-Profit Analysis From Pigeon Farming

Initial capital (BDT)	Current Capital (BDT)	Permanent Cost (Building & Materials, Instruments) (BDT)	Feed Costs (BDT)	Worker Cost (BDT)	Medicine Cost (BDT)	Other Costs (BDT)	Income from Sale (BDT)		Total Income (BDT)	Net Profit (BDT)
							Baby	Adult		

Thank you very much for your kind co-operation.

Signature of observer