EPIDEMIOLOGY OF ANTIMICROBIAL RESISTANCE OF *SALMONELLA* SPP., *STAPHYLOCOCCUS SPP*. AND *ESCHERICHIA COLI* IN FREE RANGING BATS

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Roll No: 0116/05
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A thesis submitted in the partial fulfillment of the requirements for the degree of Masters of Science in Epidemiology

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JUNE 2018

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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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JUNE 2018

DEDICATED
TO
MY BELOVED FAMILY
MEMBERS
WHO
ALWAYS VALUED EDUCATION
ABOVE
EVERYTHING ELSE

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List of Abbreviations

Abbreviation	Elaboration
AMR	Antimicrobial Resistance
β	Beta
BGA	Brilliant green agar
BHIB	Brain heart infusion broth
BPA	Baird parker agar
C.	Cynopterus
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
CS	Culture sensitivity
DNA	Deoxyribonucleic acid
E.	Escherichia
ESBL	Extended-spectrum beta-lactamase
ExPEC	Extra intestinal pathogenic E. coli
I	Intermediate
ICDDR,B	International Centre for Diarrhoeal Disease Research, Bangladesh
IUCN	International Union for Conservation of Nature
MDR	Multidrug resistance
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant Staphylococcus aureus
NTS	Non typhoidal salmonella
OR	Odds ratio
P.	Pteropus
PBP	Penicillin binding protein
PCR	Polymerase chain reaction
PRSP	penicillin-resistant S. pneumoniae
R	Resistant
ROC	Receiver operating curve
S	Sensitive
S.	Staphylococcus
TB	Tuberculosis
TCM	Traditional Chinese medicine
TS	Typhoidal salmonella
VGA	Virulent gene analysis
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

List of Symbols

Symbols	Stand for symbols
$>$	Greater than
$<$	Lesser than
$^{\circ}C$	Degree Centigrade
$\geq$	Greater than equal
$\leq$	Less than equal
μg	Microgram
%	Percentage
$\chi 2$	Chi-square
W/v	Weight/volume

Summary

Antimicrobial resistance (AMR) is currently one of the greatest challenges to the global health. The prevalence of resistant microbes in wildlife are poorly understood. Bats are a diverse and highly mobile taxon that often lives in close proximity to people and livestock. Bats use of shared environments may contribute to their exposure to AMR enteric pathogens. The identification of zoonotic and anthropozoonotic agents carried by bats is critical for understanding the ecology of zoonotic pathogens. A cross-sectional study was conducted to determine the epidemiology of AMR in *Salmonella* spp., *Staphylococcus spp.* and *Escherichia coli* (*E. coli*) isolated from bats and test the hypothesis that drinking behavior of bats may result in their infection with AMR bacteria. We collected fecal samples (N=369) from free-ranging bats and 21 water samples from different water bodies in Dhaka city between December 2016 and June 2017. Pre-enrichment was done in buffered peptone water for *Salmonella* spp. & *E. coli* and in Mueller Hinton broth for *Staphylococcus spp*. Samples were cultured on selective media; biochemical test and PCR were done to confirm bacterial isolates. Culture sensitivity test was determined using Kirby-Bauer disc diffusion technique of 12 antibiotics for each organism on selective media. Overall prevalence of *Salmonella* spp., *Staphylococcus* spp and *E. coli* in *Pteropus medius* & *Rousettus leschenaulti* bats was found 10.04-2.86%, 19.65-40.71% & 31.85-25.71%, respectively. Analysis showed 7.32% (27/369; 95% CI: 5-11) of fecal samples of bats positive for resistant *Salmonella* spp., 26.29% (97/369; 95% CI: 22-31) for resistant *Staphylococcus* spp. and *E. coli* resistant to 28.18% (104/369; 95% CI: 24-33). Around 4% (n=21, 95 % CI: 18-62) of water samples were positive for *Salmonella* spp. The final multivariate model revealed that odds of having resistant *Staphylococcus* spp. and *E. coli* were significantly higher in Faridpur (OR=3.1; CI: 2-6, P=0.00) and Dhaka (OR=2.3; CI: 1-5, *P*=0.03), compared than other locations. In case of *Salmonella* spp., prevalence was significantly higher in summer season (OR=8.9; CI: 4-24, P=0.00). Results of the present study exposed that all isolates had developed resistance to multiple antibiotics. *Salmonella* spp. were resistant to Tetracycline (93%), Sulphamethoxazoal-Trimethoprime (80%), Azithromycin (76%), Chloramphenicol (62%), Amoxicillin-Clavulanic acid (42%) & Cefotaxime (41%). *Staphylococcus sp.* showed high resistance to Ampicillin (55%) and Methicillin, Oxacillin, Streptomycin & Tigecycline (by 12% on average). *E. coli* showed resistance against Cefepime (16%) followed by Ampicillin (13%).

Furthermore, water samples showed remarkable resistance to Tetracycline (86%), Amoxicillin-Clavulanic acid (75%), Ceftriaxone (72%), Ciprofloxacin (71%) followed by Chloramphenicol (57%) & Sulphamethoxazoal-Trimethoprime (58%). The study results indicate presence of AMR *Salmonella* spp., *Staphylococcus spp.* and *E.coli* in bats. It is unclear how bats were infected with AMR bacteria, however water contaminated by people and/or livestock may be a source of infection. Screening people and livestock for similar resistant bacterial species will improve our understanding of pathogen transmission among wildlife, livestock and people interface.

Keywords: Antibiotics, Bat, *Escherichia coli*, Prevalence, Resistance, *Salmonella* spp., *Staphylococcus* spp, Water

Chapter-1: Introduction

1.1 Antimicrobial resistance

For many decades, antimicrobial resistance (AMR) has been regarded as a serious clinical and public health problem. Antibiotics used for clinical, veterinary and agricultural practices acting as the driving force for the emergence and persistence of acquired resistance (Thaller et al., 2010). AMR has been considered by world health organization (WHO) as one of the top health challenges facing the 21st century (Marshall and Levy, 2011; Fair and Tor, 2014). Now a days, multi drug resistant bacteria is very common and is a great danger for livestock and human health (Mølbak, 2004; Spellberg et al., 2016). Currently, AMR is a not a native problem rather than global concern, as resistant microbiota are spreading either locally and/or globally through shared environment. South-east Asian countries are considered as the epicenters of AMR, particularly regions like Bangladesh due to their high population density (Okeke et al., 2005; Kang and Song, 2013). Moreover, in developing countries, most of the time animal owners treat their animals by themselves and/or local doctor or quack and are not aware about using various antimicrobial drugs through registered veterinarian or doctor. Unfortunately, in developing countries, antibiotics are widely used in livestock, growing fish farming and poultry farming for growth promotion and disease prevention (Mellon et al., 2001; Roess et al., 2013). In general, antimicrobials are used widely for the treatment of infectious diseases which has got the potential of transferring resistant bacteria and its factors from animals to humans (Stanton, 2013). World Health Organization (WHO) has reported a very high rate of multidrug-resistance (MDR) bacterial genes in many important and common bacteria such as Non typhoidal *Salmonella*, *Neisseria gonorrhoeae*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Enterococcus spp* and *Escherichia coli* (WHO, 2014; Chang et al., 2015). Almost all capable infectious agents have already attained high levels of drug resistance with higher morbidity and mortality and thus, are referred as “super bugs.” (Tanwar et al., 2014).

Several studies were conducted on AMR in agricultural settings and animal agriculture is hypothesized to be a dominant source of AMR. One study reported that application of compost fertilizer to agricultural soil led to an outgrowth in AMR, even though the animals that produced the manure had not been treated with antibiotics (Udikovic-Kolic

et al., 2014). The manure fertilization allowed for enrichment of inhabitant soil bacteria that harbored AMR elements and representing AMR source. The dispersal of resistant genes in natural environments can challenge the population dynamics and natural microbial populations. The effect of antibiotic resistant gene and resistant bacteria that are released from anthropogenic sources, along with the disproportionate use of antibiotics in both human and veterinary settings, is currently considered to be a serious ecological and environmental hazard. Antibiotics from both urban and agricultural sources persist in soil and aquatic environments and the selective pressure imposed by these compounds may affect the human diseases as well as wildlife (Allen et al., 2010; D'Costa et al., 2011).

Bats (family *Pteropodidae*) are a highly diverse and mobile taxon that often live in close proximity to water bodies and are observed roosting in botanical gardens, cities, villages human residences; agricultural land and habitat selection is highly dependent on availability of food resources (synanthropic) (Chakravarthy et al., 2009; Krystufek, 2009). As tropical landscapes become increasingly human-dominated, conflicts between people and wildlife, threaten ecological processes (urban, rural) and play as an important bio-indicator of ecosystem health. According to Hahn et al. (2014), 2–17% of Bangladesh's land area is suitable roosting habitat for bats. The bat species *Pteropus medius* and *Rousettus leschenaulti* are grouped under the Megachiroptera (suborder) and widely distributed in Bangladesh. The bat is found partial in the forest of Sundarban as well as near the human habitation having green space (Srinivasulu et al., 2010). The prime habitat of bat in urban area is formation of colony in old temple, remote house, thatched building or in banana of *Fiscus* tree (Khan, 2001; Cleveland, 2009). The bat often eat fresh fruits like banana, manago, guyava, papaya, Monkey Jack (*Artocarpus lacucha*), Cotton tree (*Bombax ceiba*), Indian rose chestnut (*Mesua ferrea*), Indian Fig (*Ficus racemosa*) which also are recognized as human food. Although the IUCN deems it as a species of 'Least Concern', the population trend is decreasing (Thapa, 2014). A total of 138 species of mammals were evaluated during the existing IUCN Red List process of which 127 species are found in the country and 11 species were extinct.

1.2 Justification of the study

Bat populations are in constant threat in many parts of the country due to habitat destruction caused by urbanization or deforestation, roost disturbance, bush meat

hunting, competition for food and medicinal use, biomedical research and over hunting (Streicker and Allgeier, 2016).

Bats can be unwillingly dangerous to humans. They are natural reservoir of wide range of pathogens including virus and bacteria and others (Wood et al., 2012) (Calisher et al., 2006). Several of zoonotic pathogens have been detected in bat faeces, therefore might be a likely source of zoonosis (Mühldorfer, 2013). However, they are close with anthropogenic inhabitance which has increased the chance of emerging and reemerging infections (Wong et al., 2007).The relationship between bats and humans is closer in over populated country such as Bangladesh (Epstein et al., 2010). In recent periods special attention has been given on these airborne mammals as vector of zoonotic pathogens such as Rabies virus, Ebola virus, Nipah virus, Marburg virus, or coronavirus (Luis et al., 2013; Plowright et al., 2015; Allocati et al., 2016). In recent survey on flying foxes, antibodies to leptospirosis, and bacteria were found 11% in renal tissue and 39% in urine of individuals and Bartonella found 35% in faeces (Cox et al., 2005; Bai et al., 2017). Bats are prevalent in urban areas and come in close interaction with both native animals and humans, contaminating houses with urine and faeces and also, humans sporadically invade into bat habitats. Because bats are important contributors to ecological processes and transmission of microorganisms within the colony and directly or indirectly to humans (Hayman et al., 2013), it is important to screen and recognize bats and their related microbiota.

High inter and intraspecific interaction rates can facilitate rapid transmission of resistant microorganisms through direct and/or indirect contact of several species and large population sizes could rapidly disseminate resistant bacteria or resistant gene. Though bats are highly mobile and ecologically diverse with a varieties of feeding habitats, peri domestic behaviors, roosting in houses and other buildings, as well as trees in dense rural areas, lead to frequent human contact with bat excreta (O'Shea et al., 2011; Plowright et al., 2011). Microbial transmission within bat colonies is promoted by the behavior of several species of these mammals aggregating in crowded roosts. The close association of bat colonies with highly urbanized environments represents a possibly significant health risk, with faecal deposits between human and bat populations. Direct and/or indirect disease transmission, through consumption and contact with contaminated water supplies, fruit, crops, forage or soil with their feces, and/or areas

irrigated by these supplies can also transmit resistant bacteria and gene (Henry et al., 2018).

In Bangladesh, bats live nearby human surroundings in several locations of Dhaka, Faridpur and Madaripur. Conflict can arise when bats roost in human residences, raiding of fruit and householders are affected adversely by their presence. It causes many adverse effects, including damage to forage or crops and properties, damages of water quality, destruction of habitats, injuries to people, livestock, and wildlife. A recent study on bats in Bangladeshi villages found 11% of bat roost near their homes, 65% reported seeing bats flying over their households at dusk, 31% seeing bats inside their compounds or courtyard areas, 20% of at least daily exposure to bats, having physical contact with bat urine (1%), faeces (2%) or large-bodied fruit bat placentas (0.3%), disposal of death bats in local bodies of water (Openshaw et al., 2017). Inspite of the threat to human health, human–bat conflicts occur habitually and are driven by complex environmental, ecological, social and economic factors (Wood et al., 2012).

Widespread range of wild animal both from captivity and free range showed evidence of antibiotic resistant bacterial infection by different researcher. Antimicrobial resistance has been detected in fecal bacteria from a variety of wild animals, including birds, reptiles, mammals, and fish, throughout the world (Sayah et al., 2005). Presence of selected genera containing potentially zoonotic species and AMR genes were isolated from bats in different countries (Nowak et al., 2017). The prevalence of *Salmonella* spp; *Campylobacter jejuni* and *Clostridium perfringens* respectively, was 20%, 19.3-44% & 98% in free roaming bats (Hatta et al., 2016; Vengust et al., 2018). A study in Germany reported 17% investigated bat dies due to opportunistic type bacterial diseases *Pasteurella spp*., and *Shigella flexneri* were identified 7% and 3% in bats. Bacterial pathogens like *Salmonella enterica* serovars *Typhimurium, S. Enteritidis* and *Yersinia pseudo tuberculosis* were identified almost 12% of affected bats (Rózalska et al., 1998; Mühldorfer et al., 2011). Leptospira is a vector born pathogenic in both humans and animals and bacterial detection rates was 11% in kidney, 39% urine samples and 18-28% sero prevalence was found in Native flying fox (Smythe et al., 2002; Cox et al., 2005). AMR microorganisms were isolated from bats of Africa and Bangladesh previously (Ngoa et al., 2012; Olalekan et al., 2012; Aiken et al., 2014; Schaumburg et al., 2015; Islam et al., 2016).

With regard to wide range of migration and flying close to the human settlement, the identification of anthropozoonotic and zoonotic agents carried by bats is serious for understanding the ecology of zoonotic pathogens. Though there are some study has been conducted on antibiotic resistance in production animal, no study yet has been conducted in any free ranging wild animal in Bangladesh except Hasan et al. (2012). So, this study aimed to demonstrate the current status of antimicrobial resistance and its pattern in *Salmonella*, *Staphylococcus* and *E. coli* in free ranging bats in human, wildlife and livestock interface in Bangladesh and develop hypothesis that close contact with human, drinking behavior of bats etc. may result in their infection with antimicrobial resistant bacteria.

1.3 Objectives of the study

i. To estimate the prevalence of *Salmonella* spp., *Staphylococcus spp*. and *Escherichia coli* in bats in Bangladesh.
ii. To determining the antimicrobial resistance pattern of *Salmonella*, *Staphylococcus spp* and *E. coli* isolates from free ranging bats in human, wildlife and livestock interface in Bangladesh.
iii. To evaluate the current status of presence of resistant bacteria in water bodies used by humans and bats

Chapter-2: Review Literature

Relevant literature on antimicrobial drug uses, antimicrobial resistance, prevalence, consequences and/or impacts and diagnostic techniques have thoroughly been reviewed in this chapter. The main purpose of this chapter is to provide up-to-date scientific information based on previous studies and to identify knowledge gaps and justify the present Eco-epidemiological Master's research on Antimicrobial resistance in bats. Google Scholar, Research Gate and PubMed were used to search literature. The relevant findings of published and unpublished articles have been presented under the following headings as below.

2.1 Order: Chiroptera

Bats (Chiroptera) are the second most diverse group of small mammals after rodentia. Approximately, 1100 species of living bats make up around 20% of all known living mammalian species (Mickleburgh et al., 2002; Eick et al., 2005). Bats are found throughout the world in tropical and temperate habitats but they are absent in polar region and in some isolated islands (Hutson and Mickleburgh, 2001; Schipper et al., 2008). In some tropical areas, there are more species of bats than of all other kinds of mammals combined. Over 200 species fruit bats are considerable ecological and economic importance. Bats play a pivotal role as pollinations, bio indicator, pest controlling agents, seed dispersal and maintenance of tropical forest tree diversity and regeneration (Calisher et al., 2006; Goveas et al., 2006; Muscarella and Fleming, 2007; Seltzer et al., 2013). The distribution of bats is largely dependent on the spatial and temporal variation of their food resources. Bats of this species feed on both wild and cultivated fruits including guava, lychee, banana, and papaya (Tang et al., 2008). The fruit bats can cover a 50 km of distance in search of food and water at a single night (Wilson and Reeder, 2005; Mahmood-ul-Hassan and Nameer, 2006).

2.1.1 Status of Bats in South-east Asia Subcontinent

A total of 128 species of bats are reported from South Asia, with the microchiropteran being better represented than the megachiroptera (115 vs 13 species). Among the Microchiroptera large number of species are from the family Vespertilionidae, followed by the families Rhinolophidae, Hipposideridae, Molossidae, and Megadermatidae. The Megachiroptera (consisting of a single family, the *Pteropodidae* or Old World fruit bats) Family *Pteropodidae* is comprised of 42 genera are found throughout the Old

World tropics and sub-tropics from Africa through southern Asia to Australia and on islands in the Indian and western Pacific Oceans (Simmons, 2005). Of this diversity, 10 species, namely, *Pteropus medius* (*Pteropodidae*), *Pteropus melanotus* (*Pteropodidae*), *Latidens salimalii* (*Pteropodidae*), *Rhinolophus cognatus* (Rhinolophidae), *Rhinolophus mitratus* (Rhinolophidae), *Hipposideros durgadasi* (*Hipposideridae*), *Hipposideros hypophyllus* (*Hipposideridae*), *Myotis csorbai* (*Vespertilionidae*), *Eptesicus tatei* and *Harpiola grisea* (*Vespertilionidae*) are endemic to the region. Family wise percent endemicity is greatest in *Pteropodidae* (21.4 species) followed by *Hipposideridae* (13.3 species), *Rhinolophidae* (11.7 species) and *Vespertilionidae* (4.8 species).

Apart from India fruit bats is also distributed in Bangladesh, Bhutan, China, Maldives, Myanmar, Nepal, Pakistan and Sri Lanka (Molur et al., 2012). The global recognized number of taxa now exceeds 1100 (Simmons, 2005). Most recent and obsolete review of the bats in the Indian subcontinent, has obtained with 119 species of bats belonging to eight families and 37 genera (Bates and Harrison, 2000). A total of 189 species are listed in Lower Risk categories, 62 are Near Threatened, 29 species are Data Deficient and 128 are Least Concerned (Hutson and Mickleburgh, 2001). In Pakistan, one species of bat is Endangered, four are Vulnerable, nine are Near Threatened, eighteen are Least Concerned, seven are Data Deficient and one is Not Evaluated (Walker and Molur, 2003; Mahmood-ul-Hassan and Nameer, 2006). In Nepal, Bats are the least known groups with 60 species being Data Deficient. In Bangladesh, 125 species of a mammals have been reported from the country (IUCN, 2000) and about 31 species of bats listed from Bangladesh (Khan, 1982).

Among the countries representing South Asia, India has more than 90% of the total bat diversity of this region, while others have less than 50% diversity. Bhutan has 51%, Nepal has 40%, Pakistan has 33%, Bangladesh has 29%, Afghanistan has 28%, Sri Lanka has 23% and Maldives has 2% of the total bat diversity of South Asia (Srinivasulu et al., 2010).

2.2 History of Antimicrobials Development

The discovery of antibiotics more than 70 years ago initiated a period of drug innovation and implementation in human and animal health and agriculture. These discoveries were tempered in all cases by the emergence of resistant microbes (Wright,

2007; Livermore, 2009). History told that in 1619 malaria was treated by the juice of "sacred bark" (quinine), extracts of the quina-quina bark to treat fever and dysentery caused by amoebas treated by emetine root (Achan et al., 2011). Besides this, many drugs were discovered from many uncommon sources such as wound, sewage, chicken throat, wet wall of Paris etc. bacteria isolated those could produce antibiotics (Garrod and O'Grady, 1971). Here they report targeted metagenomics analyses of rigorously genuine ancient DNA from 30,000-year-old Beringian permafrost sediments and the identification of a highly diverse collection of genes encoding resistance to β-lactam, tetracycline and glycopeptide antibiotics. Structure and function studies on the complete vancomycin resistance element VanA confirmed its similarity to modern variations. Antimicrobial activity seems present in a number of other herbs used in traditional Chinese medicine (TCM) (Wong et al., 2010) and the discovery of active components in these ancient remedies may enrich the arsenal of antimicrobials used by the conventional medicine. At the same time, selective pressures obligatory by these antimicrobial activities during the long-term history of TCM may have been one of the factors contributing to the accumulation of antibiotic resistant genes in human populations.

A variety of microorganisms were elucidated to cause infectious diseases in the latter half of the 19th century. Later in 1904, Ehrlich and Shiga detected that trypanrot (red dye) was effective against trypanosomes (Mitsuhashi, 1993). The first antimicrobial agent in the world was salvarsan, a remedy against spirochetes and therefore, was an effective cure for syphilis (Finch et al., 2010) that was synthesized by Ehrlich in 1910. In 1928, Fleming discovered penicillin. He found that the growth of *Staphylococcus aureus* was inhibited in a zone surrounding a contaminated blue mold (a fungus from the *Penicillium* genus) in culture dishes, a microorganism would produce substances that could inhibit the growth of other microorganisms and used as a clinical & therapeutic in the 1940s (Chain et al., 1940). Penicillin, in terms of safety and efficacy, led in the era of antimicrobial chemotherapy by saving the lives of many wounded soldiers during World War II (Finch et al., 2010). In 1935, sulfonamides were developed by Domagk and other researchers. These drugs were made by synthetic compounds and had limitations in terms of safety and efficacy. In Bayer Company, two scientists named Klarer and Mietzsch produced Prontosil red in 1932, which is a red dye constrained to a sulfonamide group. In 1935, Domagk observed that infection with

hemolytic streptococci was relieved by Prontosil red and *Pseudomonas aeruginosa (pyocyanase)* was used as a cure of wound (Garrod and O'Grady, 1971). But it was very unfortunate for Bayer, There was no antibacterial activity by Prontosil red in vitro. Later, many companies started to produce sulfanilamide and developed the molecule to increase the performance with the addition to reduce the side effects and to increase spectrum of action. In 1939, Rene Dubos become the first scientist to discovered Gramicidin, which is still used today to treat skin infection (Zhang, 2007). In 1944, streptomycin, an aminoglycoside antibiotic, was obtained from the soil bacterium *Streptomyces griseous*. Thereafter, Chloramphenicol, tetracycline, Macrolide, and Vancomycin (e.g., glycopeptide) were discovered from soil bacteria. The synthesized antimicrobial agent was obtained in 1962, which is nalidixic acid, a quinolone antimicrobial drug (Saga and Yamaguchi, 2009). Other two classes of synthetic antibiotics successful in clinical use were the quinolones, such as ciprofloxacin, and oxazolidinones, such as linezolid (Walsh, 2003). With the times, antibiotics of this class such as ciprofloxacin, norfloxacin, enrofloxacin, and Ofloxacin have become more popular for the treatment in both human medicine and veterinary medicine (Mitsuhashi, 1993).

Later the 1960's, there have been developed and chemical modifications of existing drugs. Those modifications were very useful in treating infectious diseases, leading to enhanced killing of pathogens, increased spectrum of action, reduced toxicity, and side effects. Inappropriately, since the 1970's, only a new class of antibiotics has been introduced (Lipsitch et al., 2002). A recent trend in antibiotic therapy is to apply drugs with different mechanisms of action with combinations of altered drugs, in order to increase their effectiveness & overcome of resistance.

2.2 History of Antimicrobial resistance

In the recent years, the microorganisms which are resistant to antibiotics are highly alarming in both human and animals. Since, the discovery of new antibiotics, researcher began to find out microorganisms that are resistant to new drugs. There are lots of evidence of presence of resistant microorganisms in nature but those microorganisms are not present in human (Hughes and Datta, 1983). The traces of tetracycline, have been found in human skeletal remnants from ancient Sudanese Nubia dating back to 350–550 CE. The dissemination of tetracycline in bones is only reasonable after

exposure to tetracycline-containing materials in the diet of these ancient people (Bassett et al., 1980; Nelson et al., 2010). In the case of streptomycin, in 1944 it was introduced for the treatment of tuberculosis (TB; "The Great White Plague"), a mutant strains of *Mycobacterium tuberculosis* resistant to therapeutic concentrations of the antibiotic was found during patient treatment (Davies and Davies, 2010). After invention, penicillin became much popular to use as treatment and a research showed that *Staphylococcus 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 resistant to *Staphylococcus aureus* is about 80% (O'Brien, 1987). After the end of 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 (Clewell, 2001). In 1959, Japanese found *Shigella* species were resistance to streptomycin, tetracycline, Sulphonamides, and chloramphenicol due to plasmid, which carried different antibiotics resistance genes (Zhang, 2007). *Staphylococcus aureus* is the resistant bacterium most familiar in the clinical setting. This bacterium rapidly developed resistance to sulfonamides when they were in use. Penicillin was initially effective against this microbe, but resistant strains that produce penicillinase increased in the 1950s. Therefore, penicillinase-stable methicillin was developed in 1960. Then the following year, 1961, methicillin-resistant S. aureus (MRSA) was isolated in the UK (Jevons, 1961). Although penicillin-resistant *S. pneumoniae* (PRSP) strains found in the latter half of the 1970s. In Japan, PRSP was found in the 1980s (Saga and Yamaguchi, 2009). After that Japanese started to shift to streptomycin, tetracycline, and chloramphenicol, as a results *Shigella* became multi-drugs resistance quickly (Falkow, 1975). Ampicillin was initially effective for *Haemophilus influenza*. However, in the 1980s, some of this species were found to produce -lactamase, thereby becoming resistant to ampicillin. In the 1990s, such β-lactamase-producing strains decreased in Japan, however, strains that acquired highly resistance to -lactam through mutations in penicillin-binding protein (PBP) genes. The antibiotic resistance is so complex and difficult, due to increased demand of animal protein in developing countries, irrational use of antibiotics both in the clinical and agriculture settings, highly increase of population in both developed and developing countries, and low socioeconomic status, poor sanitation and hygienic status, as well as

that zoonotic bacterial pathogens are not regularly cultured, and their resistance to commonly used antibiotics are scarcely investigated.

2.3 Enteric Bacteria

Enteric bacteria have consistently threatened human health and have posed significant public health threats. John Snow, who is widely considered to be the father of epidemiology, identified and mitigated the now famous Broad Street Pump cholera epidemic of 1854. Since then, we have made tremendous advancements in molecular epidemiology, with the help of pulse-field gel electrophoresis, restriction fragment length polymorphisms, multi-locus sequence typing, and more. With these advancements, epidemiologists no longer merely identify and mitigate outbreaks caused by enteric bacteria, such as *Salmonella* spp., *Staphylococcus spp* and *Escherichia coli*, but they can also identify asymptomatic individuals and prevent outbreaks from occurring. And yet, enteric disease outbreaks remain central topics in public health.

The term "Enteric Bacteria" is generally used in reference to bacteria of the Family "Enterobacteriaceae", many members of which reside in the intestinal tract flora in a wide variety of animals and also humans, these intestinal bacteria can be easily disseminated in different ecosystems though water, soil, food and other media (Skurnik et al., 2006). Examples of enteric bacteria relevant to this research are reviewed below.

2.4 *Salmonella* spp: zoonotic significance and pattern of resistant

Salmonella is a genus gram-negative, facultative aerobic, rod-shaped, non-spore forming, motile bacteria and peritrichous flagella. *Salmonella* are found worldwide in all warm blooded animals and its surrounding environment. They are natural inhabitant in the gastrointestinal tract of many animals, including birds, livestock, and humans. They are facultative intracellular pathogens, which transfer microorganism's human to human (anthropozoonosis) and human to animal (zooanthroponosis). They have the ability to survive in water, food, and soil for a long time (Angulo et al., 2000). Typhoid fever is a deathly disease, many peoples have died in early ages. A recent report from the Centers for Disease Control and Prevention suggested nearly half (46%) of foodborne illnesses (Painter et al., 2013). Present days 16.6 million cases found a year all over the world, in developed countries the incidence decreased remarkably but in

developing countries the huge number of deaths around 0.6 million (Shanahan et al., 2000). Asia is the main hotspot of *S. typhi,* a research said that about 30-40% blood culture from the hospital is *Salmonella typhi* in the part of Asia (McCormick, 1998). Although actual data on human *Salmonella* in South Asia are limited, a hospital-based surveillance from 1996–2011 shows that 1.3% diarrheal patients in Bangladesh are suffering from non typhoidal salmonella (NTS) and 2.46% patient from typhoidal salmonella (TS) (Leung et al., 2013).

Salmonellosis is a main reason of gastroenteritis in both humans & animals as well as global bacterial disease of public health concern and economic importance in industrial livestock (Brenner et al., 2000; Foley and Lynne, 2008). Wild animal and livestock species are considered as an important reservoir of human-pathogenic *Salmonella* serotypes (Sanchez et al., 2002; Tizard, 2004). However, wild animals and many domestic animals are colonized by *Salmonella*, harbouring the bacteria in their gastrointestinal tracts without apparent clinical signs (Sanchez et al., 2002). A variety of different *Salmonella* serotypes have been isolated from apparently healthy and diseased bats (**Table 2.2**). Almost all of them are serotypes with a broad-host range (Hoelzer et al., 2011). In particular, *Salmonella Enteritidis* and *Salmonella Typhimurium* have been frequently recognized, which belong to a small group of *Salmonella* serotypes mainly related with disease in animals and human (Sanchez et al., 2002; Foley and Lynne, 2008). Both serotypes have been isolated from organ tissues of three individual bats of the family *Vespertilionidae* that were found dead or severely injured near human residence (Mühldorfer et al., 2011). Histo-pathologic investigation revealed inflammatory lesions in several organs of these bats, including interstitial pneumonia and purulent meningitis. Other non typhoidal *Salmonella* serotypes have been isolated only once from the intestine of bats. Among these, *Salmonella* serotypes Anatum, Blockley, Rubislaw, Saintpaul and Sandiego are widespread in companion animals and livestock of medical importance to humans (Hoelzer et al., 2011; CDC, 2013). However, *Salmonella Caracas* and *Salmonella landoff* have rarely been identified from human salmonellosis cases (CDC, 2013). In contrast to broad-host-range *Salmonella* serotypes found in bats, *Salmonella Typhi* is the causative agent of typhoid fever and almost exclusively associated with disease in humans (Uzzau et al., 2000). Interestingly, this serotype was isolated from heart blood, internal organs and bile of 58 *Pteropus rufus* from Madagascar (Brygoo et al., 1971), which again provide

some evidence for systemic bacterial infection possibly was induced by capture and handling of bats inapparently infected with *Salmonella Typhi*.

Antibiotic resistant Enterobacteriaceae is reported in Chimpanzees in Uganda transmitted from human in semi captive condition. Gastrointestinal bacterial transmission with resistant properties also documented in Tanzania and UK (Nizeyi et al., 1999; Lilly et al., 2002; Garcês et al., 2017). The prevalence *Salmonella* on rodents in different landscape was found 6%, 7.3%, 10%, 16.2% & 32% in worldwide (Seguin et al., 1986; Henzler and Opitz, 1992; Hilton et al., 2002). The frequency of isolation of 3.4% for Campylobacter spp. found in the African country, 18% prevalence reported for rats trapped in France and 57.4% for black rats in Portugal (Cabrita et al., 1992; Henzler and Opitz, 1992).The prevalence of Salmonella spp; *Campylobacter jejuni* and *Clostridium perfringens* respectively, was 20%, 19.3-44% & 98% in free roaming bats (Hatta et al., 2016; Vengust et al., 2018). Bacterial pathogens like *Salmonella enterica* serovar Typhimurium, and *S. Enteritidis* were identified almost 12% of affected bats (Rózalska et al., 1998; Mühldorfer et al., 2011). Daffner., (2001) conducted a study in UK to determine the prevalence of *Salmonella* in Vespertilionidae bat and reported 1.36% from blood samples of heart. Antibiotic resistant *Salmonella* spp, reported at overall 8% in captive zoo of South Africa (Mitsuhashi, 1993). The frequency of resistance to the 9 antimicrobial agents tested was 75% for *Salmonella*, 85.7% of Campylobacter spp. (Nkogwe et al., 2011). The prevalence of resistance to antimicrobial agents among *Salmonella* was reported as 57.1% found in fruit-eating bats at the Emperor Valley Zoo in Trinidad (Gopee et al., 2000b). According to (Reyes et al., 2011) conducted a study to detect the *Salmonella* in *Pteropoid* bat in Philippines in both PCR assay and conventional method. The prevalence of *Salmonella* spp was recorded at 4.3% in PCR assay and 9.4% in conventional culture assay. Other study on different species of bats (Souza et al., 1999; Mühldorfer et al., 2011) were recorded 17% and 34% *Salmonella* infection, respectively.

Since 1989 outbreak of resistant *Salmonella typhi* occurred in developing counties of south-east Asia especially in Pakistan and India. Strains of *S. typhi* were mainly resistant to chloramphenicol, ampicillin, and trimethoprim; also other antibiotics such as streptomycin, sulfonamides, and tetracycline (Rowe et al., 1997). In between 1990 to 1992, 236 isolates of *Salmonella typhi* were identified in Bangladesh and resistant to

ampicillin (66.5%), co-trimoxazole (72.9%), chloramphenicol (78.8%), tetracycline (58.5%) and nalidixic acid (14%) (Azad et al., 2013).

2.5 *Staphylococcus* spp.: zoonotic significance and pattern of resistant

Staphylococcus spp is the normal flora associated with skin, glands and mucous membranes of warm-blooded animals (mammals and birds), and recent studies have indicated that animals could be a source of *Staphylococcus spp* infections in humans (Van Cleef et al., 2011; Mediavilla et al., 2012). They are occasionally found in the intestinal, genitourinary, and upper respiratory tracts of these hosts. They have also been isolated from animal products, and other sources such as soil, sand, seawater, fresh water, dust and air. It is an opportunistic, adaptable pathogen with abilities to persist and multiply in a several of environments and has a wide variety of diseases (Cucarella et al., 2004). In human, *Staphylococcus aureus* is a common bacterium found on the skin and nasal passages of healthy people. About 25- 40% of the population is colonized with *Staphylococcus spp*. It is also a common cause of skin and soft tissue infections (Francois et al., 2005) and sometimes causes severe disease such as pneumonia, bacteremia, meningitis, sepsis, and pericarditis and food poisoning (Von Eiff et al., 2001; Gao and Stewart, 2004). In animals, *Staphylococcus spp* bacteria isolated and identified as an important opportunist that can cause superficial to life threatening illness in a variety of animal (White et al., 2003). *Staphylococcus aureus* is a bacterium of significant importance because of its capacity to adapt to diverse environmental forms showing multiple antibacterial resistant patterns. It might be resistant to other β-lactam and methicillin antimicrobials and are referred to as methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA are recognized as a variety of bacteria that is resistant to certain antibiotics including methicillin, penicillin, oxacillin, amoxicillin (Wichelhaus et al., 1997). In developed countries, humans and domestic animals are an important reservoir for methicillin resistant *Staphylococcus aureus* (MRSA) infections in free living wildlife (Graveland et al., 2011). Thus, MRSA remained of great concern in both human and veterinary medicine (Saleha and Zunita, 2010). Free-living small mammals may also be a source of potential pathogenic *staphylococci* in natural environment. Humans and livestock can become infected with pathogens by contact with infected small mammals (Pacha et al., 1987; Ahlm et al., 1997). Numerous studies have been published on the characterization of staphylococci isolated from various

animals but there is little known about the distribution of staphylococci of certain wild small mammals.

In the Asian region, 74 MRSA strains were identified from 12 countries and all MRSA strains were resistant to penicillin and gentamycin. Other antibiotics were resistant in very high level, amoxicillin-clavulanic acid (96%), cefuroxime (85%), clarithromycin (85%), ciprofloxacin (84%), 50% was to trimethoprim-sulfamethoxazole (Ko et al., 2005). A study of antibiotic susceptibility testing in 2006 to 2007 in Mymensingh, Bangladesh presented that *Staphylococcus aureus* of MRSA strains were 100% resistant to penicillin, oxacillin, cloxacillin and amoxicillin. A latest study on hospitalized patient showed that from a total number of 209 *Staphylococcus aureus* were isolated of which 64 (30.6%) isolates were Methicillin-resistant; among which 36 (56.2%) isolates were MDR. The isolates were resistant to Amoxicillin, Tetracycline, Ciprofloxacin, Gentamycin, Erythromycin, and Trimethoprim-sulfamethoxazole (Ghasemian et al., 2014). A recent study on human wound infection showed that, from a total of 40 isolates of *Staphylococcus spp*. were found 100% resistant to Penicillin and approximately 75% of them were found to be resistant to Methicillin, Oxacillin, Azithromycin, Ciprofloxacin, and Tetracycline and almost 45% isolates exhibited resistance to Amikacin, Chloramphenicol, Gentamycin, and Tobramycin (Hassan et al., 2015). About, 26 out of 29 strains of *Staphylococcus aureus* were resistant to penicillin G and 75% of isolates were resistant to azithromycin, ciprofloxacin, methicillin, oxacillin, and tetracycline. Nearly 65% of isolates revealed resistance to erythromycin, and trimethoprim–sulfamethoxazole. Around 65% of isolates exhibited resistance to erythromycin, and trimethoprim/ sulfamethoxazole (Hasan et al., 2016). A previous study on Cockroach (*Periplaneta americana*) sample captured from Bangladesh shows that the overall prevalence of *Staphylococcus spp* was 38%. Higher prevalence of *Staphylococcus spp*. was seen among the cockroaches from the restaurant 49.3% and household 26.7%. The prevalence of resistance *Staphylococcus spp*. was found to 68% to Penicillin followed by 60% to Erythromycin, 46% to Oxacillin and 31% were resistance to Clindamycin (Islam et al., 2016).

The prevalence of resistance rates of *Staphylococcus spp* was found 89.5% to penicillin and 5.3% to Cefoxitin from Côte d'Ivoire. The resistance rates in urban populations was found 95% to penicillin and 3% to methicillin in Gabon, 8% resistance to methicillin, 3.7% to penicillin & clindamycin, while 5.6% and 7.5% isolates were

resistant to ciprofloxacin and erythromycin, respectively in Nigeria, and penicillin was resistance to 69.8% & methicillin was resistance to 7% in Kenya (Akobi et al., 2012; Ateba Ngoa et al., 2012; Aiken et al., 2014; Schaumburg et al., 2014). The antibiotic resistance analysis of selected isolates revealed high prevalence of tetracycline, erythromycin, and chloramphenicol resistance (in about 80 %, 100 %, and 60 % of isolates, resp.) in *Staphylococcus spp*. of European bats (Vandžurová et al., 2013).

2.6 *Escherichia coli*: zoonotic significance and pattern of resistant

Escherichia coli is the most prevalent enteric bacteria, belongs to the large bacterial family, *Enterobacteriaceae*, which are facultative anaerobic Gram-negative bacteria (Sørum and Sunde, 2001). *Escherichia coli* is one of the primary intestinal commensal organisms found in warm-blooded animal (endothermic) and it is widely disseminated in the different ecosystem. There are two broad types of *E. coli*, the *E. coli* strains that are harmless commensals of the intestinal tract and others that are major pathogens of human and animals. The pathogenic *E. coli* strains are divided into those causing disease inside the intestinal tract and others capable of infection at extra-intestinal sites (De Sousa, 2006). Besides commensal strains, the species *E. coli* comprises several zoonotic pathovars causing intra- and extra intestinal diseases in humans and animals, such as diarrhoea, septicemia, urinary tract infections or meningitis (Kaper et al., 2004; Tenaillon et al., 2010). While much is known about the prevalence and pathogenic potential of *E. coli* in humans and food-producing animals, concern has been raised about the potential impact of antibiotic-resistant bacteria on public health (Gilliver et al., 1999; Adesiyun et al., 2009), little is known about *E. coli* in wildlife. Few studies revealed mainly commensal strains in captive wild animals (Adesiyun and Downes, 1999; Baldy-Chudzik et al., 2008), whereas others reported zoonotic and potentially extra intestinal pathogenic *E. coli* (ExPEC) strains originating from wildlife, indicating that wildlife might serve as a source or reservoir of virulent *Escherichia coli* strains (Ewers et al., 2009; Guenther et al., 2012). Attention has also been focused on the fact that antibiotic-resistant *E.coli* has been detected from wild animals that have not been exposed to artificial drugs (Piddock, 1996; Costa et al., 2008). For this reason, fecal *E. coli* is considered to be an important indicator for the selective pressure exerted by the use of antimicrobials on intestinal populations of bacteria (Van den Bogaard and Stobberingh, 2000). However, some strains are identified as pathogenic *E. coli* by their ability to possess specific virulence factors and specific toxin-encoding genes (Nataro

and Kaper, 1998). *E. coli* include the production of one or more shiga toxin (Mead and Griffin, 1998). Zoonotic pathogens Enterohemorrhagic *E. coli* are associated with severe human illness such as hemolytic uremic syndrome, hemorrhagic colitis, and thrombocytopenic thrombotic purpura (Caprioli et al., 2005). *E. coli* was first recognized as a pathogen in 1982 during an outbreak of hemorrhagic colitis caused by the consumption of undercooked meat in Oregon and Michigan (Riley et al., 1983) Ground beef, cattle and other bovine sources have been identified as the main natural reservoir for *E. coli*. Other animals such as swine in the United State also harboured potentially pathogenic *E. coli* (Feder et al., 2003). Birds and rodents have also been reported to harbour *E. coli*. (Wallace et al., 1997) reported the isolation of *E. coli* from wild birds in Morecambe Bay and Lancaster, UK which implicated that wild bird can serve as potential vectors for the dissemination of *E. coli*.

As bats provide a considerable protein source for people in many countries of the World (Mickleburgh et al., 2009; Kamins et al., 2011; Vora et al., 2014) including Bangladesh, it would be highly desirable to get more insight into the prevalence and genetic background of *E. coli* from these animals. Another important aspect is that wild animals have increasingly been recognized as carriers and putative distributors of antimicrobial resistant *E. coli* (Gilliver et al., 1999; Guenther et al., 2011; Radhouani et al., 2014). Indeed, bats have been shown to harbour AMR *E. coli*, demonstrating that they can be considered an important pool for genetic material of bacteria (Souza et al., 1999; Gopee et al., 2000a; Oluduro, 2012). In contrast, little is known about bats as carriers of pathogenic bacteria because only a few authors have investigated enteric bacteria, including *E. coli*, in bats (Chaverri, 2006; Oluduro, 2012). *Escherichia coli* is a commensal bacterium found in the intestine of mammals and birds, with a higher prevalence reported in herbivores and omnivores compared with carnivores, marsupials, and bats (Gordon and Cowling, 2003). Information concerning the prevalence of *E. coli* in bats is scarce. Previous research has shown that only a low proportion of bats are carrying *E. coli*, which could be due to the lack of a cecum and, thus, microbial fermentation in these animals (Gordon and Cowling, 2003). However, *E. coli* has been reported as the cause of urinary tract infection in Vespertilionidae bats (Mühldorfer, 2013).

Prevalence of antimicrobial-resistant *E. coli* has been shown to be widespread among geographic locations and animal species (Gilliver et al., 1999; Osterblad et al., 2001).

In mammalian wildlife that are free-ranging, 58% were positive for *E. coli*, for wildlife kept on private farms and at the zoo, the prevalence of *E. coli* in faecal materials was 88.2% and 88.1%, respectively (Adesiyun, 1999; Gopee et al., 2000a). A study conducted on rodents near the pig farm in the found prevalence of *E. coli* was 24.95-83.8% in Canada & Africa (Nkogwe et al., 2011; Jardine et al., 2012). The prevalence of *E.coli* in bats were as to be found as 12.6% in India (Banskar et al., 2016), 16.3% in Okinawa Island, Japan (Obi et al., 2014), and 9.6% in European free tailed-bats Portugal (Garcês et al., 2017). According to Literak et al., 2010 were conducted a study in Small terrestrial mammals (Rodentia, Insectivora) in a suburban and forest environment and were recorded an average of 57.4% E coli in free ranging wild animal. Hasan et al., 2012 also reported a higher prevalence of 73.3% of *E coli* in wild birds from Bangladesh. Van Dongen et al., 2013 describe isolated *E. coli* from variety of different species at the rate of 9-61%. Isolation of *E coli* in wild rodents and rats ranges from 20-65% (Adesiyun, 1999; Jardine et al., 2012), wild birds ranges from 13%-47% (Cernicchiaro et al., 2012; Veldman et al., 2013). A higher prevalence of 72.4% *E coli* infection was reported in urban free ranging wild animal in Australia (Caprioli et al., 2005), Canada (Cole et al., 2005), New Zealand (Corn et al., 2005) and United States of America (Eggert et al., 2013) by different earlier study. On the other hand, relatively lower prevalence of *E coli* infection in free ranging wild animal of less disturbed landscape of forest and urban area also reported by different authors. An average of 12% *E coli* infection was found in Spain in rural rodents while 47.4% recoded in urban area close to human settlement (Skurnik et al., 2006). The overall occurrence rates of *E. coli* among these hosts were 14%, 17% and 54% for bats, birds and rodents, respectively (Bilung et al., 2014). The frequency of tetracycline resistance *E. coli* in the poultry farm in Bangladesh reported as 70 100% and about 77-100% to amoxicillin. A British study which found that 97% of *E. coli* isolates from wild animal species (bank voles and wood mice) were resistant to amoxicillin-clavulanate (Jardine et al., 2012). Amongst *Escherichia coli* isolates, resistance was 18.1% to tetracycline, 15.8% to ampicillin and chloramphenicol was resistance to 8.2% and lower prevalence were recorded to cephalothin (0.0%), streptomycin (0.0%), and gentamicin (3.5%) in free roaming wild animals (Nkogwe et al., 2011). The prevalence of *E.coli* isolates resistant strains was comparatively high 61% to Erythromycin, 16.3% in tetracycline and 26.5-27% in streptomycin but lower to sulphamethoxazole/trimethoprim and gentamycin in free living bats (Adeshina et al., 2009).

2.7 Mechanisms of antimicrobial resistance

Prior to the 1990s, the problem of antimicrobial resistance was never taken to be such a threat to the management of infectious diseases. But gradually treatment failures were increasingly being seen in health care settings against first-line drugs and second-line drugs or more. Microorganisms were increasingly becoming resistant to ensure their survival against the arsenal of antimicrobial agents to which they were being bombarded. They achieved this through different means but primarily based on the chemical structure of the antimicrobial agent and the mechanisms through which the agents acted. The resistance mechanisms therefore depend on which specific pathways are inhibited by the drugs and the alternative ways available for those pathways that the organisms can modify to get a way around in order to survive (WHO, 2014).

Antimicrobial agents act selectively on vital microbial functions with minimal effects or without affecting host functions. Different antimicrobial agents act in different ways. The understanding of these mechanisms as well as the chemical nature of the antimicrobial agents is crucial in the understanding of the ways how resistance against them develops. 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 by a previously unrecognized mechanism. On the other hand, a gene which is not expressed in vitro may be expressed in vivo (Langton et al., 2005; Toma and Deyno, 2015).

Some common acquired resistance mechanisms can occur through various ways are given below:

1. **Acquisition of chromosomal mutations:** Mutation is a heritable change in the sequence of the DNA occurring due to errors during DNA replication (Snyder

et al., 2013).The frequencies of chromosomal mutations leading to antibiotic resistance depend on the specific antibiotic. For example, mutation frequencies are high for compounds like Nalidixic acid, Rifampicin, and Streptomycin, low for Erythromycin and are not known to occur for Vancomycin and Polymyxin-B. For antibiotics like Streptomycin, a single mutation can determine a 1000-fold increase in the resistance levels (Makovec and Ruegg, 2003). In contrast, for other drugs, the acquisition of resistance is a gradual, step-wise process in which different mutations are involved (Baquero, 2001).

2. **Acquisition by horizontal gene transfer**: Horizontal gene transfer is the relocation of genetic material from one bacterial cell (donor) to another (recipient). Such a transfer may occur directly by physical contact or indirectly, using the surrounding medium or bacteriophage as vectors (Guardabassi and Dalsgaard, 2002). Bacterial transfer of antibiotic resistance has been demonstrated to occur in various natural habitats, including water, sediment, soil, plants and animals (Davison, 1999). The DNA transferred from the donor to the recipient may be contained in mobile genetic elements called plasmids, structures of circular DNA that reproduce independently from the chromosome (Lin and Biyela, 2005). Functions that are of importance under particular conditions, such as antibiotic resistance, heavy metal resistance, metabolic functions, or production of antibiotics, toxins and virulence factors (Snyder et al., 2013).
3. **Intracellular migration of resistance genes:** Antibiotic resistance genes can migrate from one site to another on the bacterial genome using small vectors called “transposons” and “integrons”. These genetic elements containing antibiotic resistance genes are able to move between different sites of the bacterial genome without any requirement of DNA homology. This process is known as non-homologous recombination and differs from the normal process of genetic recombination, which requires a high degree of DNA homology (Cox and Wright, 2013). Both transposons and integrons make it possible for new antibiotic resistance genes to be acquired by plasmids and subsequently spread in the bacterial population by mechanisms of horizontal gene transfer, as suggested by the frequent recovery of these genetic elements as part of broad host plasmids (Frost et al., 2005; Bennett, 2008).

Some common antibiotics which are being used in both human and veterinary treatment and mode of action along with the method of getting resistance are described in **Table 2.1**.

TABLE 2.1: Antimicrobials Agents with Modes of Action and Resistance Mechanisms of commonly used Antibiotics against Microbes:

Antimicrobial Types	Mode of Action	Resistance (s) Mechanism
β-Lactams: Penicillin, Ampicillin, Cephalosporin	Peptidoglycan Biosynthesis	Hydrolysis, efflux, altered target
Aminoglycosides: Gentamicin, Neomycin, Streptomycin, kanamycin	Translation	Phosphorylation, acetylation, nucleotidylation, efflux, altered target
Glycopeptides: Vancomycin, Teicoplanin	Peptidoglycan Biosynthesis	Reprogramming peptidoglycan biosynthesis
Tetracyclines: Tetracyclines, Minocycline, Tigecycline	Translation	Monooxygenation, efflux, altered target
Macrolides: Erythromycin, Azithromycin	Translation	Hydrolysis, glycosylation, phosphorylation, efflux, altered target
Lincosomides: Clindamycin	Translation	Nucleotidylation, efflux, altered target
Streptogramins: Synercid	Translation	C-O lyase, acetylation, efflux, altered
Oxazolidones: Linezolid	Translation	Efflux, altered target
Phenicols: Chloramphenicol	Translation	Acetylation, efflux, altered target
Quinolones: Ciprofloxacin	DNA replication	Acetylation, efflux, altered target
Pyrimidines: Trimethoprim	C1 metabolism	Efflux, altered target
Sulfonamides: Sulfamethoxazole	C1 metabolism	Efflux, altered target
Rifamycins: Rifampin	Transcription	ADP-ribosylation, efflux, altered target
Lipopeptides: Daptomycin	Cell membrane	Altered target
Cationic peptides: Colistin	Cell membrane	Altered target, efflux

2.8 Environment Sources of Resistance

The environment is the melting pot of antimicrobial resistance. In recent years, antibiotic-resistant bacteria have been isolated from virtually every environment on earth. Recent research points to the environment as an important component for the transmission of resistant bacteria and in the emergence of resistant pathogens. Numerous authors have emphasized the need to take on a holistic outlook on antibiotic resistance, including humans, animals and environment through one-health approach (Collignon, 2015; So et al., 2015). 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. Although, transmission of resistant genetic materials to pathogens from environmental bacteria, which occupy other habitats & often less phylogenetically associated and environmental stressors may induce horizontal gene transfer to and from human pathogens in environmental settings. Over the past years, the role of the environment as an important source and dissemination route of resistance has been increasingly recognized (Bondarczuk et al., 2016). Bacteria typically not associated with the human microbiota may have the chance to interact with human-associated species in several settings. Sole possibility is that environmental bacteria can be rapidly present in the human microbiome, after interaction with wild animals, intake of raw food, or drinking contaminated water (De Boeck et al., 2012; Ghaly et al., 2017). Resistance determinants between the wild environments present a critical threat to both human and animal health. Wildlife populations those living in closer vicinity to humans have higher levels of antibiotic resistance than those wild populations with virtually no contact with humans or anthropogenic disturbance (Osterblad et al., 2001). Wild birds and domestic animals living close to humans are also known to port bacteria carrying resistance genes, and may contribute to spreading those genes across huge areas (Bonnedahl et al., 2009; Stedt et al., 2015). In addition, global food trade has been contributing to distribute resistant pathogenic bacteria around the world; for example, the German Shiga-toxin producing *Escherichia coli* (O104:H4) outbreak in 2011 (Buchholz et al., 2011). Environments facilitating dissemination of resistant bacteria also enable spread of non-resistant human pathogens, and generally also opportunistic pathogens. Thus, Environments facilitating dissemination of resistant bacteria also assist spread of non-resistant human pathogens, and also opportunistic pathogens. Allowing human-

associated bacteria to use environmental bacterial populations as reservoirs for resistance genes that can later be engaged into the human-associated resistoms (Baquero et al., 2008; Martínez, 2008). Therefore, wastewater treatment plants, sewage, farm land, water bodies & travel, also air-borne aerosols, dust, and food inhabited by bacteria, are significant vectors allowing bacterial transmission between hosts & environment (Pal et al., 2016; Bengtsson-Palme, 2017). Domestic animals frequently drink such surface water either treated or untreated and may subsequently spread resistant bacteria to humans. There is evidence for resistance genes transfer among diverse organisms such as strains of *Bacteroides*, *Clostridium perfringens*, *Streptococcus pneumoniae*, and *Enterococcus faecalis* (Shoemaker et al., 2001). Opportunistic pathogens frequently flourishing in soil, for example *Ochrobactrum intermedium Stenotrophomonas maltophilia*, and *Burkholderia cepacia*, (Berg et al., 2005; Johnning et al., 2013), may be in between organisms that can act as recipients of resistance genes from human-associated bacteria and which could transfer those genetic factor back at an future or even infected humans themselves. Agricultural use of manure and treated sewage for fertilizer, leakage from waste storage facilities can also be strengthening this issue (Sarmah et al., 2006). In Southeast Asia large amounts of antibiotics are used for human medicine, as a growth promoters in livestock farming, aquaculture and antibiotics are released into the environment. Thus, antibiotic resistance genes are widely disseminated and that resistant genes can persist uniformly in the absence of a positive selection pressure (Martínez, 2012). Natural environments and, in particular, wild animals represent reservoirs of antibiotic resistance genes (Radhouani et al., 2012).

2.8 Spread of resistance bacteria among environmental

In recent years, antibiotic-resistant bacteria have been isolated from virtually every environment on earth. Antibiotic resistance is not only found in pathogenic bacteria but also in environmental organisms occupying terrestrial and aquatic habitats. The occurrence of resistant bacteria in nature may have originated from antibiotic producing organisms, however some cases the mechanisms and genes protecting these organisms from the antibiotics they produce are similar to those responsible for resistance in clinical isolates (Davies and Davies, 2010). 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, antibiotics are added to animal feed to treat infections, as prophylactics, and in sub-therapeutic doses as growth promoters. However, higher numbers of resistant bacteria occur in polluted habitats compared with unpolluted habitats, indicating that humans have contributed substantially to the increased proportion of resistant bacteria occurring in the environment (Pathak et al., 1993). Possible mechanisms by which humans enhance the spread of antibiotic resistance among environmental bacteria include the deliberate or accidental introduction of antibiotics, resistant bacteria and resistance genes into the environment. The main risk for public health is that resistant genes are transferred from environmental bacteria to human pathogens. The ability of resistant bacteria and resistant genes to move from one ecosystem to another is documented by the various cases in which transmission of resistant bacteria has been demonstrated between animals and humans. The inclusion of certain growth promoters in animal feed has been recognized as a cause for the selection of resistant genes in the commensal microflora of animals and their transmission to humans via the food chain (Marshall and Levy, 2011). Similarly, drinking and bathing water could represent a source for the acquisition of resistant bacteria in humans. The ecological consequences associated with the dissemination of resistant bacteria in the environment have been scarcely investigated. However, it appears evident that environmental contamination with antibiotics, resistant bacteria and resistant genes affects the biodiversity of natural ecosystems. Antibiotics are likely to determine a reduction in the levels of microbial diversity by the suppression of susceptible organisms, including bacteria, fungi, protozoa and algae. Resistant bacteria and genetic elements could find favorable conditions to become predominant in habitats contaminated by antibiotics, thereby, altering the original composition of natural microbial communities (Muthaiyan et al., 2011). 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.

2.9 Present status of antimicrobial resistant in Bats

Recently, bats receiving growing consideration in infectious disease studies, because of their well familiar status as reservoir species for many transmissible agents. This is even more important, as bats with their capability of long distance dispersion and complex social structures are unique in the way microbes could be spread by these small fruit

bats species. However, AMR studies in bats are predominantly limited to the identification of specific pathogens presenting a potential health threat to humans. But the effect of AMR patterns on the individual host and their importance is largely unknown and has been neglected in most studies published to date. Antibacterial resistance is now a greatest challenge for the human for the consistent uses of antibiotics inappropriately. It is now a major concern in case of common livestock (cattle, sheep, goat and buffalo). In spite of a number of researches on AMR in humans and livestock are most common but in small fruit bats are rare. In Germany, 42 different bacterial genera with more than 53 bacterial species were identified; predominant bacteria isolated were *Enterococcus faecalis* (14.7%), *Hafnia alvei* (11.2%), *Serratia liquefaciens* (10%), and *Pasteurella multocida* (7.7%). Primary bacterial pathogens like *Salmonella enterica* serovars Typhimurium, *Salmonella Enteritidis* and *Yersinia pseudotuberculosis* were identified almost 12% of bats (Mühldorfer et al., 2011). In Brazil, *Salmonella* spp. in bats, ranging from 2.6% to 9.1% have been previously reported (Cassel-Beraud and Richard, 1988). Leptospira act as a vector born pathogen in both humans and animals and bacterial detection rates was 11% in kidney, 39% urine samples and 18-28% sero prevalence was found in Native flying fox of Australia (Smythe et al., 2002; Cox et al., 2005). *Campylobacter jejuni* and *C. coli* isolate from rectal swab of fruit bats, were found in 17 out of 631 fecal samples, in 14 different bat species from diverse habitats of Netherlands (Hazeleger et al., 2018). A total of 88 Megachiroptera, the identification of 66 families and 103 genera. The predominant families and genera were *Clostridiaceae* (69.5%), *Campylobacteraceae* (24.2%) and *Enterobacteriaceae* (5.1%) and *Clostridium* (65.7%) & *Campylobacter* (24.2%), respectively. The identification of 170 species and more predominant species were *Clostridium butyricum* (21.0%) and *Campylobacter coli* (19.3%) (Hatta et al., 2016). In total, 34 potential pathogens, representing 15 genera, isolate from Victoria, Australia were found *Enterobacteriaceae* (62.8%), *Pasteurellaceae* (19.9%) and *Moraxellaceae* (9.4%) dominate in flying fox faeces (Henry et al., 2018). In Nigeria, A total of 250 fecal samples were collected and 53 isolates were identified as *S. aureus* (*n* = 28), *S. schweitzeri* (*n* = 11) and *S. argenteus* (*n* = 14). Only one *S. aureus* was resistant to penicillin and another isolate was intermediately susceptible to tetracycline (Olatimehin et al., 2018). In routine microbiological examination, a methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated from an infected wound of a bat in Germany (Walther et al., 2006). From Gabon, colonization rates of *S. aureus* (4–6%)

and *S. schweitzeri* (4%) in 2 out of 4 species of fruit bats, namely *Rousettus aegyptiacus* and *Micropteropus pusillus* (Held et al., 2017). From January 2008 to February 2010, about 107 *Staphylococcus aureus* isolates were recovered from 560 faecal samples from different roosting sites of Africa. Molecular characterization of 70 isolates showed that 65 (92.9%) were assigned to coagulase type VI, while gene typing classified 69 isolates into the following: type I (17.1%), type II (4.3%), type III (1.4%) and 75.7% type IV. About 3.7% isolates were resistant to clindamycin & penicillin, while six (5.6%) and eight (7.4%) isolates were resistant to ciprofloxacin and erythromycin, respectively (Akobi et al., 2012). In Portugal, *E. coli* isolates were recovered from 14 of 146 faecal samples (9.6%) and a total of 19 isolates were completely recognize ESBL producing. The beta-lactamase genes detected were *bla*CTX-M-1 (57.9%) and *bla*CTX-M-3 (36.8%), followed by *bla*SHV (31.6%), *bla*TEM (21.1%), *bla*OXA (10.5%) and *bla*CTX-M-9 (10.5%). Among isolates resistance genes studied, tet (A) and tet (B) were predominant and fimA was the main virulence factor detected (Garcês et al., 2017). Enteropathogens in bats from Trinidad was found antimicrobial resistance in *E. coli* against streptomycin (26.5%) and tetracycline (16.3%) with 8% of bacterial strains resistant to both antimicrobial agents and neomycin 15%, ampicillin 46% and streptomycin 100% (Adesiyun et al., 2009).

2.10 Problems associated with antimicrobial resistance

AMR is an alarming issue and major concern worldwide. World Health Organization (WHO) is much concern about 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. AMR is a global threat to both human and animals and day by day it is gradually growing. Antimicrobial resistance has the direct and indirect effects on the health. When the levels of antimicrobials are high, then it can be toxic to 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 antimicrobial resistant microorganisms (Pongdee and Li, 2018). The indirect impact of antimicrobial resistance, however, extends outside increased health risks and has many public health consequences with wide implications. Some antimicrobials cause

endocrine disturbance such as Oxytetracycline, tetracycline & sulfamethoxazole and some causes nervous effects like cefuroxime, neomycin (Lee et al., 2001). AMR is accompanied with high mortality rates, it provokes interruption 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 doesn't work to the patients. So they need to buy occasional antibiotics with a high price. The main problem of AMR is growing the resistance to the specific antibiotics that wouldn't work further.

2.11 Managing and Remedies of AMR

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 (WHO, 2017). Effective antimicrobial drugs are prerequisites for both preventive and curative measures. This action plan underscores the need for an effective "one health" approach involving coordination between several international sectors and actors, including human and veterinary medicine, agriculture, finance, environment, and well informed consumers. Global collaborative efforts are necessary for the management and prevention of AMR and it should be individual, community, regional, national and international level (Uchil et al., 2014). Strategies should develop the appropriate use of antibiotics, reduce involuntary interaction between microorganisms and antibiotics. Drugs should be only prescribed by the professionals and drugs should be taken by proper dosage. Patients should complete treatment course of antibiotics, stopping of medication in the middle generates 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. Management of AMR in both human and veterinary sectors needs ideal action plans for the development of newer antimicrobials, possible intervention measures (Union, 2014). 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.

Table 2.2: Number of *Salmonella* spp., *Staphylococcus spp* and *Escherichia coli* isolates from bat and their frequency in different country in the world

Bats Family	Bacterial	Sources	%	Location	Authors
Vespertilionidae	*Salmonella* spp.	Intestine	2/46	Philippines	(Reyes et al., 2011)
	Salmonella spp.	Heart blood	1/73	UK	(Daffner, 2001)
	Salmonella Enteritidis*, *Typhimurium	Organ sample	3/486	Germany	(Mühldorfer et al., 2011)
Molossidae	*Salmonella* spp.	Faecal	2/37	Trinidad	(Adesiyun et al., 2009)
Nectarivorous	*Salmonella* spp.	Faecal	1/47	Brazil	(De Sousa, 2006)
Noctilionidae	*Salmonella* **Moladeb,** *Salmonella* **Rubislawb**	Gastrointes tinal tract	1/11	Trinidad	(Adesiyun et al., 2009)
Nectarivorous	***Salmonella Typhi, Salmonella Typhimurium***	Heart. blood, organ	58/481	Madagascar	(Brygoo et al., 1971)
Pteropodidae *Cynopterus sphinx, C. brachyotis*	***Escherichia coli***	Rectum	21/27	Indonesia	(Graves et al., 1988)
C. brachyotis brachyotis	***Escherichia coli***	Stomach and intestine	16/34	Peninsular, Malaysia	(Daniel et al., 2013)
Pteropodidae, Vespertilionidae	***Escherichia coli***	Faecal	19/56	Philippines	(Italia et al., 2012)
Nectarivorous, Vespertilionidae	***Escherichia coli***	Faecal	18/82	Brazil	(Cabal et al., 2015)
Unknown species of bats	***E. coli*** 015:H7	Rectum	28/84	Malaysian Borneo	(Apun et al., 2011)
Eidolon helvum	***Staphylococcus aureus***	Faecal	107/560	Nigeria	(Akobi et al., 2012)
Rousettus aegyptiacus	***Staphylococcus aureus***	Faecal	4/55	Gabon	(Held et al., 2017)
Pteropus pumilus	***Staphylococcus spp***	Rectum	25/56	Philippines	(Heard et al., 1997)
Pteropodidae *Pteropus giganteus; P. hypomelanus*	***Staphylococcus aureus***	Rectum	9/133	Indonesia	(Heard et al., 1997)
Pteropodidae *P. giganteus*	***Salmonella*** Virchow	Faecal	3/302	Bangladesh	(Islam et al., 2013)

Chapter 3: Materials and Method

3.1 Description of study area

Bangladesh as a deltaic floodplain of topical source has a wide variety of habitations suitable for a diverse fauna and supports about 160 million people. Dhaka division is a governmental division of Bangladesh, has an area of 147,570 km^2 and lies between 20° 34' to 26° 38' N and 88°91' to 92°41'E (**Figure 3.1**). The city lies between 2 to13 m above sea level, on the eastern bank of the Buriganga River in the heart of the Bengal delta. The capital and largest city Dhaka situated at center of Dhaka division with about 18.89 million of people. Dhaka has a tropical savanna (wet and dry) climate with an annual average temperature of 25°C (77°F), near 80% of the annual average rainfall of 1,854 mm (73.0 in) occurs during the monsoon season and humidity 70.5% to 78.1% (Anon (2016). From the early civilization, all these habitats as well as man- made structures such as abandoned houses, forts, places of worship, sparingly used office blocks, monuments, mosques, castle and shrines, realm and dilapidated buildings, ruins, discussed road and railway bridges and culverts were traditional in and around on it and those areas were particularly occupied by wild small mammal animals like bats.

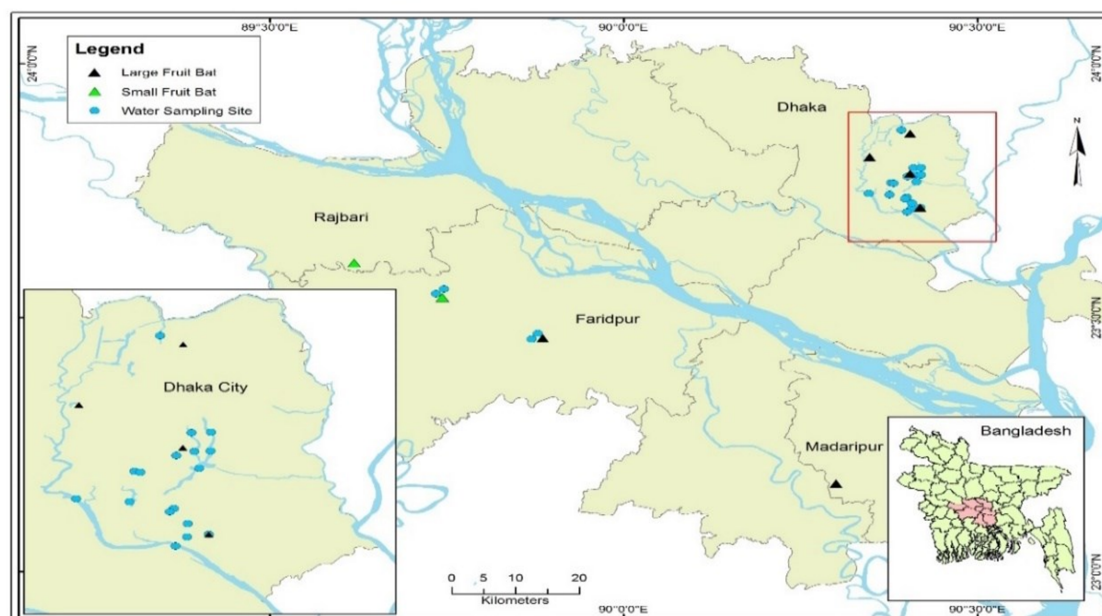


Figure 3.1: Distribution of bat roasting sites and environmental sample (water body) in Dhaka division

3.2 Distribution of Bats in Bangladesh

To achieve the objective of the study three districts were chosen where bats are uniformly ubiquitous. According to their different habitat (rural and urban) within 3 districts eight sites were selected. We choose four sites from Dhaka, three sites from faridpur and a sites from madaripur districts were selected. Two species of bats (*Pteropus medius, Rousettus leschenaulti*) are designated for sample collection **Table 3.1.**

Table 3.1: Distribution of Bats in Bangladesh

District	Location	No. of Roosts	Species	Assumptive population size	sample size
Dhaka	Uttara	1	*Pteropus medius*	500-600	41
	Mirpur botanical Garden	1	*Pteropus medius*	700-800	50
	Mohakhali	1	*Pteropus medius*	800-900	53
	Boldha Garden	2	*Pteropus medius*	600-700	35
Faridpur	Nagarkanda	2	*Rousettus leschenaulti*	2000-2200	40
	Baliakandi, Rajbari	1	*Rousettus leschenaulti*	700-800	50
	Kanaipur	2	*Rousettus leschenaulti*	2200-2300	50
Madaripur	Charmuguria	1	*Pteropus medius*	800-900	50

Boldha Garden, Nagarkanda and Kanaipur have two groups of bats in Dhaka and Faridpur districts. On the other hand, Uttara, Mirpur botanical Garden, Mohakhali, Baliakandi and Charmuguria have one roosts of bats from each location.

The population size in each roosts of bats ranges from 500-900 in Dhaka district. On the other hand, the roosts of Nagarkanda and Kanaipur have varies from 2000-2300 bats on an averages of each group where as Baliakandi and Charmuguria have bats ranges 700-900 bats varies in each group.

In the current study, we collected 41, 50, 53 and 35 samples from Uttara, Mirpur Botanical Garden, Mohakhali and Boldha Garden of Dhaka districts respectively. In

case of Faridpur districts 40, 50 and 50 samples collected from Nagarkanda, Kanaipur and Baliakandi, Rajbari respectively. In case of madaripur, 50 sample were collected from Charmuguria. (**Table 3.1**).

3.3 Ethical approval

Ethical approval to conduct the study 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 initiating the research. With the help of different protocol from various scientific journals, we had assured the animal ethics and animal safety as well as the safety of working personnel in both field and laboratory throughout the study period.

3.4 Study Design

A cross-sectional study was conducted in eight locations of Bangladesh; four locations of Dhaka, three from Faridpur district and one from Madaripur district. The study sites were selected based on expert's knowledge on human-bat interface and bats habitat. Areas like Dhaka (Mirpur national botanical garden, Mohakhali T&T colony, Uttara & Boldha garden), Madaripur (Charmuguria), Rajbari (Baliakandi) and Faridpur (Kanaipur, Nagarkanda) were suggested as common location for bats and therefore were selected as study area. Each study area was visit based on previous survey and/or consult with local peoples and sample was collected at the same time.

3.3 Sample size calculation

The sample size of the study was estimated using standard method described by (Thrusfield and Christley, 2005).

$$N = \frac{Z^2 \text{ X } P(1-P)}{d^2}$$

Where N = Number of samples to be collected (sample size)

Z = Statistic for a level of Confidence at 95% (standard value of 1.96)

d = Precision or Margin of error at 5%

P = expected prevalence

The prevalence of resistant microbes in wildlife is poorly understood. There is no record of prevalence of antimicrobial resistance in bats of Bangladesh, neither in neighboring countries. So maximum sample size was calculated based on assuming 50% prevalence (P= 0.50) as per available literature (Lwanga et al., 1991), taking 5% precision (d=0.05) and 95% confidence interval (i.e. Z=1.96). Calculated sample size was 385 using Epi-Tool software. Sampling and sample collection was done upon judgments and convenience sampling as described by Morrison et al., (2008) for wild life sampling. In this present study, 369 samples were collected that included mainly bat fecal samples and water samples from surrounding water bodies of bats habitat. Due to limitation of proper time, availability of facilities and limited resource target sample collection are not possible.

3.5 Study period

Samples were collected through the period of January to June 2017. Sample collection and laboratory test was done simultaneously. Tests such as PCR and culture and sensitivity test were done up to December 2017.

3.6 Sample collection

3.6.1 Bat fecal sample collection

Freshly voided fecal sample from free-ranging two different species of bats (*Pteropus medius* and *Rousettus leschenaulti* or large /small size) were collected for the present study. Polythene sheets were placed under the bat roosting sites at mid-night to collect fresh fecal samples in the next early morning from free-ranging bats with keeping best concern to the ethical and animal welfare issues. Freshly voided fecal samples were collected (**Appendix III**) by sterilized swab sticks and environmental sample (water sample) were collected. The collected fecal swabs were placed immediately in the sterile falcon tube containing (10ml) buffered peptone water (BPW) for *Salmonella* spp. and *E. coli* (Putturu et al., 2013; Thaker et al., 2013) and Mueller-Hinton broth (MHB) supplemented with 6.5% NaCl (24 hour's at 37°C) for *Staphylococcus spp* (Kateete et al., 2010).Water samples were placed in falcon tubes (1L bottle) for isolation of *salmonella* spp. The sample containing tubes were labeled with a unique identification number corresponding to each species/roosting sites. The collected samples in falcon tube were kept in cool boxes (4°C) and transported to Bangladesh Livestock Research Institute (BLRI), Food safety laboratory, Savar for bacteriological laboratory analysis

as soon as possible. The samples were stored in the refrigerator at -20°C until further laboratory evaluation being performed.

3.6.2 Water

3.6.2.1 Introduction:

Water bodies in third world countries like Bangladesh with a large amount of water sources are used for drinking, bathing, washing and fishing by multiple parties including humans, livestock, domestic animals and wild animals. In addition, the large frugivorous bat, *Pteropus medius* & *Rousettus leschenaulti* have also been seen drinking from shared water sources. The drinking habits of fruits bats *Pteropus medius* & *Rousettus leschenaulti* are not well understood at this time, however. It was reported that they make contact with water bodies and later drink the water off of their chests. *Pteropus* usage of water bodies may be dependent on the water content of the forage they are seasonally eating. *Pteropus* are generalist feeders (Sudhakaran and Doss, 2012) who eat fruits, leaves, and flowers of multiple plant species. It is currently hypothesized that *Pteropus sp*. may be exposed to bacteria from utilizing shared water sources. Hence, the question arose of how water sources were being utilized by *Pteropus* bats in Bangladesh and how these patterns of utilization may relate to *Pteropus* species ecology.

3.6.2.2 Methods:

Water bodies were observed in Dhaka as well as in rural Bangladesh during the day as well as in the evening during the month of March and May. Two water bodies were observed in Dhaka; one in Ramna Park, a large park located near old Dhaka. This park is also home to a bat roost of approximately 160 bats. These bats have a roost approximately 400 m from a large body of water in the park. Another urban area of observation was the Botanical Garden in Dhaka, which is next to the city zoo. A roost of approximately 80-85 bats was located within the Botanical Garden and the water body nearest to this roost (approximately 400 m) was observed.

Three water bodies were observed in rural Bangladesh during the day as well as during early evening in early March 2017 to identify how the water bodies were utilized by humans, livestock, domestic animals, wildlife and bats. All three of these water bodies were located in the vicinity of the city of Faridpur, which is an area that is currently and

historically being used for bat sampling. These locations were recorded with GPS. The first rural water body that was observed (WB1) was the Kataghang River. The dates of observation coincided with the dry period in Bangladesh so this river was broken into many smaller lagoons. This particular water body was located near a village that utilized the water for bathing, washing, and fishing. Livestock also used this water body. The second water body that was observed (WB2) near Faridpur was a privately owned man-made lagoon known as Engineer's Pond. This water body was chosen for observation because of its proximity to a *Pteropus* bat roost approximately 600 m away. Finally, a third water body (WB3) was observed as well. This water body was the Kumar River, which is located within Faridpur itself. This was the largest water body observed.

Before leaving Faridpur, 500 ml water samples were taken from the three rural water bodies. These water samples were frozen for the return trip to Dhaka, where they were then stored at the Bangladesh Livestock Research Institute (BLRI), Food safety laboratory, (BLRI), Savar for testing for the presence of bacteria.

3.7 Data Collection from water bodies

A data collecting sheet was developed and administered at the same time of sample collection from water bodies. A map of each observation site was made, and the visits was marked on the map in order to have a rough estimate of where the bat visits are occurring in comparison to the visits of other animals. Collection of geographical location's data such as type of habituation of the animal (rural and urban), GPS coordinates, tree, topography, bats species, water resources, etc. were recorded.

3.8 Laboratory study design

The microbiological laboratory design is schematically presented in (**Figure 3.2**). The entire study was divided into three steps. In first step, collection of samples from free ranging bats, pre-enrichment of samples and transportation to the laboratory was done. In second step isolation and identification of the bacterial pathogens was done based on their culture characteristics and Gram`s staining character. Finally in third step, characterization of the organism was done using various biochemical tests and PCR. Finally, antibiotics sensitivity test was performed to explore drug resistance profile of bacterial isolates from bats feces.

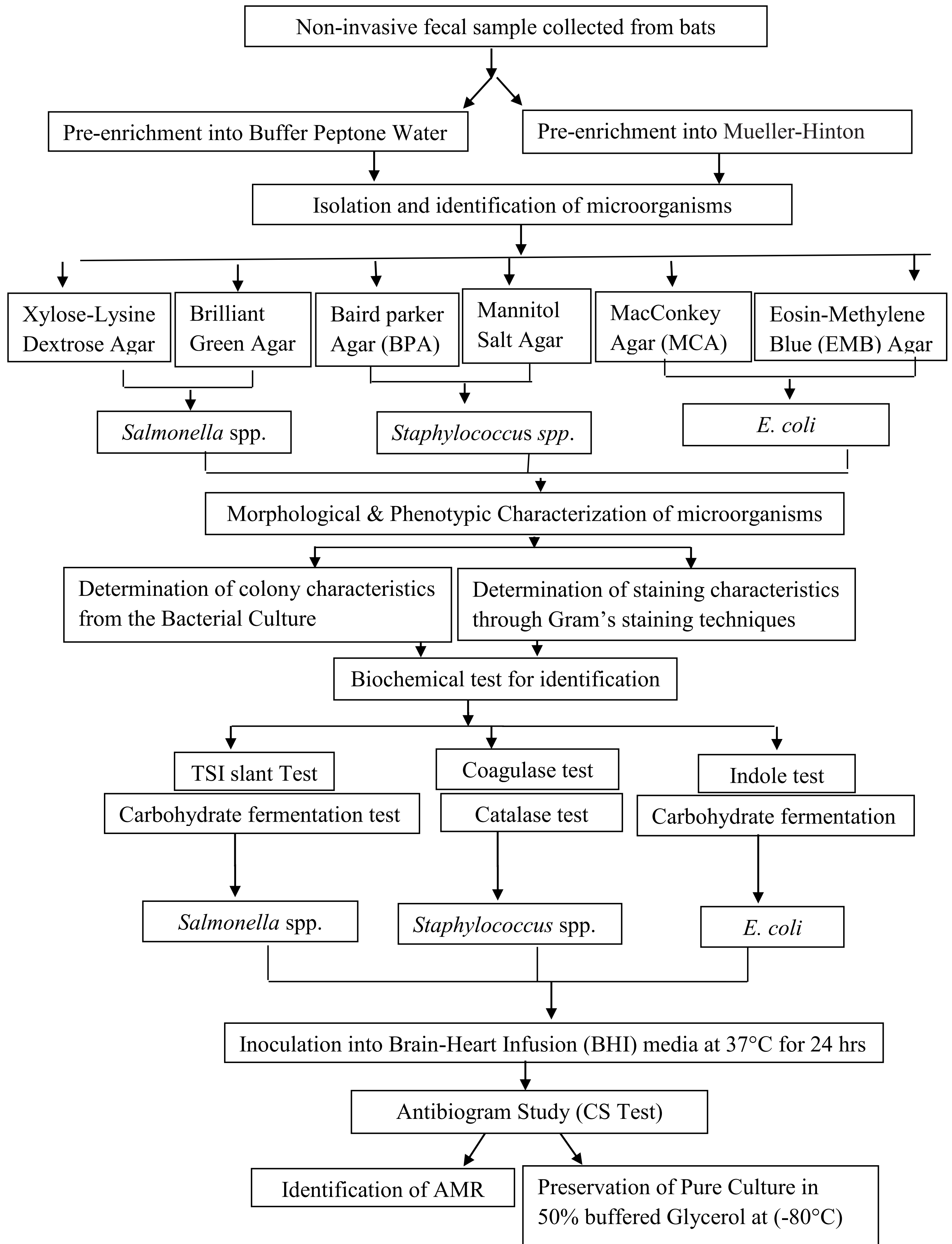


Figure 3.2: Experimental design of laboratory analysis

3.9 Bacteriological investigation

3.9.1 Isolation of *Salmonella* spp

To identify *Salmonella* spp in collected freshly defecated fecal sample, pri-enrichment was done in buffer peptone water (Oxoid Ltd, P^H: 7.2±0.2) following a modified standard method (De Medici et al., 2003; Hendriksen, 2003) and incubated at 37-42°C for 24 hr. Selective enrichment was carried out following standard method (Antunes et al., 2003; Michael et al., 2003; Carrique-Mas and Davies, 2008; Schönenbrücher et al., 2008; Putturu et al., 2013) using Rappaport-Vassiliadis broth medium. After selective enrichment, one lope of inoculums were streaked on Brilliant Green Agar (BGA) (Oxoid Ltd, P^H: 6.9±0.2) and incubated in 37°C for 18-24 hours and colonies with red color in BGA considered as positive for *Salmonella* spp (Holt et al., 1994; Nesa et al., 2011; Hazeleger et al., 2018). These colonies were further streaked on Xylose-Lysine-Deoxycholate (XLD) (Oxoid Ltd, P^H: 6.9±0.2) agar medium and incubated for 37°C for 22-24 hours. Colorless or Red/pink colour colonies with black centre were confirmed as the typical colonies of *Salmonella* spp (Adesiyun et al., 2009; Reyes et al., 2011; Soria and Bueno, 2016).

3.9.2 Identification of *Salmonella* spp.

3.9.2.1 Staining method

Grams staining method was done to study morphology and staining characters. Suspected colonies from EMB (*E. coli*), BGA (*Salmonella* spp) and Mannitol salt agar (*Staphylococcus spp*) was stained as described by Manual of veterinary investigation laboratory technique (Merchant and Packer, 1967; Hunter, 1998; Brown, 2009). Briefly the procedure was as follows:

A small colony was picked up with a loop, smeared on clean grease free glass slide and fixed up by gentle heating. Crystal violate solution was then applied on smear to stain for two minutes and then washed with running water. Few drops of Gram's iodine were then added to act as mordant for one minute and then again washed with running water. Acetone alcohol was then added for few seconds (5-7s) to decolorize. After washing with water, safranin was added as counter stain and allowed to stain for 2 minutes. The slides were then washed with water, blotted and dried in air and then examined under microscope with

high power objective (100X) using immersion oil. The same method was followed to stain other 2 studied bacteria (*Staphylococcus spp* and *E. coli*).

3.9.2.2 Bio-chemical test:

3.9.2.2.1 Reaction in TSI agar slant

To identify sucrose, lactose and dextrose fermentation, the TSI (triple sugar iron) agar (Oxoid Ltd, Basingstoke, Hampshire, UK) slant was used. It also helped to determine the ability of the microorganisms to produce H_2S. Minimum of three black centered or black colonies were inoculated into TSI agar slant and incubated at intervals of 37°C for 24-72 hours. Isolates with positive reaction (pinkish slant and yellow butt or black slant and yellow butt) was considered as positive of *Salmonella* spp (Pao et al., 2005; Hossain et al., 2006) and blackening in butt indicated H_2S reaction (Jones et al., 2000; Dargatz et al., 2016) and bubble of air in the butt indicated gas production in slants (White et al., 1997).

3.9.2.2.2 Carbohydrate (sugar) fermentation test

Tube with different sugar media (dextrose, lactose, sucrose, maltose and mannitol) were inoculated by a loopful of nutrient broth culture of the microorganisms and incubated at 37◦C for 24 hours. The isolates which were unable to ferment lactose and sucrose remain red and gas production was noted by the accumulation of bubble in the inverted Durham's tube and thus *Salmonella* spp was suspected in the broth (Douglas et al., 1998; Hossain et al., 2006).

3.9.2.2.3 Serotyping By Slide Agglutination Test

Slide agglutination test was done mainly for serotyping of Salmonella; agglutinating antiserum poly "O" and poly "H" (S & E reagents Lab, Bangkok, Thailand/ Denka Seiken Co. Ltd., Tokyo, Japan) was used to perform the serotyping. The macroscopic slide agglutination tests were performed by the method describe by Popoff and Le Minor (2001). The cultures to be tested were first checked with Salmonella "poly-O" polyvalent antiserum. A drop of serum and a drop of physiological saline were placed in the slide and a clean grease free slide was taken as control. A single drop of thick bacterial suspension was placed on glass slide and a drop of polyvalent antiserum was added. The slide was gently rotated to mix the fluid thoroughly. Cultures that were agglutinated within one to

two minutes were selected as positive for *Salmonella* spp and subjected to agglutination test with Salmonella agglutinating antiserum (poly "H"). A loopful of colony from nutrient agar was placed on the glass slide and mixed properly by tilting the glass slide and after 1-2 min if agglutination occurred, the sample was considered positive for *salmonella*. Leon-Velarde et al. (2004) described that poly 'O' antiserum gave positive agglutination reactions with any serovars for preliminary screening of *Salmonella* and poly "H" antiserum gives specific agglutination reaction for motile *Salmonella* spp (Santiviago et al., 2001; Vlahović et al., 2004).

3.9.3 Isolation of *Staphylococcus spp*

After primary enrichment, a loop full of inoculum was streaked on Baird-Parker Agar (Oxoid Ltd., P^H: 7.0±0.2) medium supplemented with Egg Yolk emulsion (5%) and saturated potassium Tellurite (0.3%) solution (Merck, Darmstadt, Germany) as selective medium. The plates were then incubated for 24-48 hour's at 37°C as per standard protocol. A shiny black colony surrounded by opaque zone of halo was regarded as a positive *S. aureus* colony (Loncarevic et al., 2005; Pelisser et al., 2009; Rohinishree and Negi, 2011; Karmi, 2013).

The typical colonies from Baird Parker agar were sub-cultured onto mannitol salt agar (Oxide Ltd., P^H: 7.4±0.2) and 5% defibrinated-sheep blood agar (Oxoid Ltd., P^H: 7.3±0.2). t After incubation at 37°C for 24 h, growth of a sticky golden yellowish coloured colonies on MSA was considered positive (Kateete et al., 2010; Grema et al., 2015). Furthermore, blood agar plates with 5% defibrinated sheep blood was used for the detection of hemolysis (Roberson et al., 1992; Boerlin et al., 2003). From the MSA plates, further the colonies were used for other biochemical tests.

3.9.4 Identification of *Staphylococcus spp*

3.9.4.1 Biochemical Test:

Biochemical confirmation of all the positive samples were subjected to coagulase and catalase tests for *Staphylococcus sp.* as described by Cheesbrough (2006). Three to five well separated colonies were picked up and moved to a 10 ml test tube containing Muller

Hinton Broth supplemented with 6.5% Nacl (HiMedia Ltd, Mumbai-400086, India) and incubated at 37°C for 6 hours.

3.9.4.2 Coagulase test (Clumping factor)

Commercially available sterile tubes containing horse whole blood/rabbit plasma with EDTA was used to perform the test. The tubes were centrifuged at 2600 rpm for 10 minutes. The follow-on supernatant, the plasma, was instantly transferred by a sterile tip to a sterile 1.5 ml Eppendorf tube and kept at -20°C for future use.

3.9.4.3 Tube coagulase test

From each tube cultivated in Brain heart infusion broth (BHIB), 50µL was transferred to sterile tubes containing 50µL of horse plasma. The incubation was done at a temperature of 37°C for 6 hours. The presence of coagulates was by large organized coagulation and coagulation of all the contents of the tube which do not come off when inverted (Graham et al., 2006; De Oliveira et al., 2011). A control tube was placed to validate the result (Bannerman, 2007).

3.9.4.4 Slide coagulase test

Slide coagulase test was conducted further to confirm the presence of *Staphylococcus* sp. (which were confirmed by tube coagulase test). A drop of the horse plasma sample was placed on a clean grease free glass slide. Suspected cultures were mixed by a loop to plasma and tested for agglutination. Agglutination of the mixture was recorded as a *Staphylococcus sp*. positive for coagulase test (Jay et al., 2005; David and Daum, 2010).

3.9.4.5 Tube Catalase test

Nutrient agar slant was prepared according to the instructions of manufacturer (Oxoid Ltd, Basingstoke, Hampshire, UK). Suspected bacterial colonies were inoculated into agar slant and incubated at 37°C for 24 hours. After that 1ml of 3% H_2O_2 was added. Rapid ebullition of gas was considered as positive reaction of *Staphylococcus spp* (Winn, 2006; Rushdy et al., 2007).

3.9.4.6 Slide Catalase test

Further a slide catalase test was conducted to confirm the presence of *Staphylococcus* spp. One suspected colony from agar plate was taken on a clean new glass slide using sterile inoculating loop. Then one drop of recently prepared 3% Hydrogen peroxide (3% H_2O_2) solution was taken over the colony. Production of bubbles in 5-10 seconds was an indication of positive test (Forbes et al., 2002; Cheesbrough, 2006).

3.9.5 Preservation of the pure culture

Isolated colonies of positive bacterial sample were inoculated in nutrient broth/brain-heart infusion broth and incubated overnight at 37°C for 24 hours. Then, 700 µl broth, containing bacteria, was added to 300 µl of 50% glycerin in an Eppendorf tube and preserved at -80°C for future investigation (Buxton and Fraser, 1977).

3.9.6 Isolation of *Escherichia Coli*

Escherichia coli were isolated from freshly voided feces following the method described by (Virpari et al., 2013) with slight modification. For the isolation of *E. coli,* sample was inoculated in screw cap test tube containing buffer peptone water (Thaker et al., 2013) for primary enrichment (Oxoid Ltd, P^H:6.2±0.0) and incubated overnight at 37ºC. After primary enrichment, bacterial culture was streaked on MacConkey agar (Oxoid Ltd, P^H: 7.4±0.2) and Blood agar plates (Oxoid Ltd., P^H: 7.3±0.2) and incubated for another 24 hours at 37ºC. After incubation the bacterial growth was observed. Characteristic pink colored colonies on MacConkey agar (Kumar and Prasad, 2010; Park et al., 2012) and hemolytic and mucoid colonies around the growth on blood agar were suspected as *E. coli* (March and Ratnam, 1986; Pedersen et al., 2006). Then sub-culture was done on Eosin-Methylene Blue (EMB) agar (Merck, P^H: 7.1±0.2) and incubated at 37ºC for 24 hours. The colonies with characteristic metallic sheen green appearance were confirmed as *E. coli* (KHMNH et al., 2005; Singh and Prakash, 2008; Adesiyun et al., 2009; Oluduro, 2012; Tanzin et al., 2016).

3.9.7 Identification of *Escherichia Coli*

3.9.7.1 Biochemical test

3.9.7.1.1 Hanging drop Motility test technique

The motility test was performed by hanging drop technique as described by (Cowan, 2004) to differentiate the motile bacteria and non-motile bacteria. Hanging drop slide were prepared by broth culture and examined under 100X power objective. The motile organisms were regarded as *E. coli.*

3.9.7.1.2 TSI (Triple sugar iron) agar slant reaction with organisms

A straight inoculating needle was used to pick up isolated colony from culture on selective agar. The TSI slant was inoculated by stabbing the butt down to the bottom and then streaked over the surface of the slant uniformly without scratching its surface. The TSI slant was then incubated overnight at temperature of 37ºC for 18-24hrs. Presence of gas bubbles, yellow slant (acids accumulate) & yellow butt (acidic reaction) and absence of black precipitate in the butt (due to the H_2S gas production and sugar fermentation) was indicative of *E. coli* (Carter et al., 1995; Krieg and Padgett, 2011).

3.9.7.1.3 Carbohydrate (sugar) fermentation test

The test was performed by inoculating 0.2 ml of nutrient broth culture of the isolated organisms into the tubes containing five basic sugars such as dextrose, maltose, lactose, sucrose and mannitol and incubated for 24 hour at 37ºC. Color change from red to yellow and gas production was noted by the accumulation of gas bubbles in the inverted Durham's tube (Mac Faddin, 1976; Cheesbrough, 2006) was indicative of acid production.

3.9.7.1.4 Catalase test

A volume of 3 ml of 3% H_2O_2 (catalase reagent) was taken in a test tube. Single colony from the pure culture of *E. coli* was taken with a sterile glass rod and merged in the reagent. Formation of bubble within three second (3-5s) was indicative of positive test (Ewing, 1986; Cheesbrough, 2006).

3.9.7.1.5 Methyl Red test

Single colony of the organism from the pure culture was inoculated in 5 ml of sterile MR-VP broth. After 5 days incubation at 37ºC, methyl red solution (5 drops) was added and

color formation was observed. Growth of red and yellow color indicated positive and negative result, respectively (Ewing, 1986; Cheesbrough, 2006).

3.9.7.1.6 Voges -Proskauer (V-P) test

The test organisms were grown at 37ºC for 48 hour in 3 ml of sterile MR-VP broth and then 0.6 ml of 5% alpha-naphthol and 0.2 ml of 40% potassium hydroxide containing 0.3% creatine were added per ml of broth culture. Following well shaking, the broth was allowed to stand for 5-10 minutes to observe the color formation. Development of pink-red color indicated positive result (Ewing, 1986; Douglas et al., 1998; Cheesbrough, 2006).

3.9.7.1.7 Indole test

The test organisms were cultured in 3 ml of peptone water containing tryptophan at 37ºC for 48hrs. One ml of diethyl ether was added, after shaking well it was allowed to stand until the ether rises to the top. Then 0.5 ml Kovac's reagent was gently ran down the side of the test tube to form a ring in between the medium and the ether. Development of brilliant red colored ring indicated positive test (Ewing, 1986; Cheesbrough, 2006).

3.9.1.8 Preservation of the culture

An isolated colony of bacteria those positive in biochemical test were inoculated into Brain-Heart Infusion broth (BHIB)/Nutrient broth (Oxoid Ltd, Basingstoke, Hampshire, UK), incubated overnight at 37ºC for 24 hours. Then, 700 µl broth, containing bacteria, was added to 300 µl of 50% glycerol in an Eppendorf tube (1.5ml)v and preserved at -80°C with for further testing (Buxton and Fraser, 1977; Gherna and Reddy, 2007).

3.10 Confirmation of Bacterial genus

Bacterial genus was identified following the previously described properties of bacteria in terms of growth on selective media and reaction in different biochemical tests.

Table 3.2: Standard to confirm bacterial genera based on colony morphology and biochemical test.

Organisms	Growth on selective agar	Colony properties on selective agar	Staining properties	Biochemical tests	Result of biochemical test
Escherichia coli	MAC	Pink and dry colony	Gram negative Medium sized rod	Indole test	Cherry red color ring formation
	EMB	Metallic sheen		TSI	Slant yellow, Butt yellow, Gas +/-ve, H_2S –ve.
Salmonella spp	BGA	Red colony		TSI	Slant red, Butt yellow, Gas +/- ve, H_2S +/- ve
	XLD	Colorless colony with black centre			
Staphylococcus spp	BPA	Shiny Black, Opaque colony	Gram positive, cluster forming cocci	Catalase	+ve
				Coagulase	+ve or -ve
	MSA	Yellowish, sticky colony	Gram positive, cluster forming cocci	Catalase	+ve
				Coagulase	+ve or -ve

MAC= MacConkey Agar; EMB= Eosin-Methylene Blue Agar; BPA= Baird parker Agar; MSA= Mannitol Salt Agar; XLD= Xylose-Lysine-Deoxycholate Agar; BGA= Brilliant Green Agar; TSI= Triple Sugar Iron Agar; +ve = Positive; -ve= Negative; H_2S= Hydrogen Sulfide Gas.

3.11 Polymerase chain reaction (PCR)

Positive samples after different biochemical test was performed polymerase chain reaction for confirmation of microorganisms. For Salmonella, Staphylococcus and E. coli were confirmed up to genus level.

3.12 Antimicrobial susceptibility testing (*Salmonella* spp, *Staphylococcus spp*, *Escherichia Coli*)

Antimicrobial sensitivity test was carried out by disk diffusion method on Mueller-Hinton agar (Oxoid Ltd, pH 7.2±7.4) according to conventional procedure (Bauer-Kirby disk diffusion procedure) (Bauer et al., 1966) and Clinical and Laboratory Standards Institute (Wikler, 2007; CLSI, 2008; Wayne, 2011).

Antimicrobial agents frequently used for both chemoprophylaxis and therapeutics for control of bacterial diseases in livestock and poultry in South Asia including Bangladesh were tested in this study (Prakash and Gupta, 2005; Mahmud et al., 2013). A bacterial turbidity was matched by 0.5 McFarland standards for each isolate. The 0.5 McFarland standard was prepared by adding 0.5ml of 1.175% (w/v) barium chloride dehydrate ($BaCl_2$.2H2O) solution to 99.5ml of 0.18 mol/L (1% v/v) sulfuric acid (Cockerill, 2010; Carter and Cole Jr, 2012). The tubes of McFarland standard were sealed with parafilm to stop vaporization and stored in the dark room at temperature 22°C to 25°C (Wiegand et al., 2008).

3.12.1 Culture inoculation

3 to 5 well isolated colonies of the microorganisms were selected from the agar plate culture. Each colony was touched with the help of loop and growth was transferred into 4ml tube containing Nutrient broth /Tryptic soy broth (TSB) (Oxoid Ltd, Basingstoke, Hampshire, UK). The broth culture was directly adjusted to the McFarland standards or by incubation at 35ºC until it achieved or exceeded the turbidity of the 0.5 McFarland standards (2-6 hours).

A sterile cotton swab was dipped into the adjusted suspension and rotated the swab several times pressed firmly within the inside wall of the tube above the fluid level. This removed excess inoculum from the swab. The surface of the dried Mueller-Hinton agar (MHA, Basingstoke, Hampshire, UK) plate was inoculated by streaking the swab. This procedure was repeated by streaking two more times and rotated the plate approximately 60°C for even distribution of inoculation. Each disc was pressed down to the agar surface to ensure complete contact. The disc was placed not more than 24mm from each other and a total 6 disc in 150mm plate was placed. The plate was incubated at 35°C for 20-24 hours

(Matuschek et al., 2014) within 5% CO_2 incubator (Binder CB-150 CO_2 Incubator, Chelmsford, UK).

3.12.2 Reading plates and interpreting results

After 24h of incubation, the plate was examined. The resulted zone of inhibition was uniformly circular with a confluent lawn of growth. Zones diameters of complete inhibition were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter using slide calipers/regular scale. The zone margin was taken as the area showing no obvious, visible growth that could be detected with the unaided eyes. The zone of inhibition sizes were interpreted by zone diameter interpretive standards (Tuohy et al., 2000; CLSI., 2011) and resistant pattern of the organism was reported as susceptible (S), intermediate (I) or resistant (R) to the antibiotics that have been tested as recommended by Clinical and Laboratory Standards Institute (CLSI, 2007; Simjee et al., 2007; Chayani et al., 2009; Silley, 2012) and the British Society for Antimicrobial Chemotherapy (BSAC, 2001).

Table 3.3: A panel of antibiotics used, concentrations and zone diameter interpretative standards for *Salmonella* spp. (CLSI, 2007).

Groups of antimicrobial agents	Drug code	Agents name	Potency (µg)	Disk diffusion zone break point (mm)		
				R	I	S
β-lactam	AMC	Amoxicillin-Clavulanic acid	30 µg	≤30	14-17	≥18
Cephems	CFT	Cefotaxime	30 µg	≤30	23-25	≥26
	CFX	Cefixime	5 µg	≤5	16-18	≥19
	CTR	Ceftriaxone	30 µg	≤30	20-22	≥23
Aminoglycosides	CN	Gentamicin	10 µg	≤10	13-14	≥15
Macrolides	AZT	Azithromycin	15 µg	≤12	-	≥13
	C	Chloramphenicol	30 µg	≤12	13-17	≥18
Tetracyclines	TE	Tetracycline	30 µg	≤11	12-14	≥15
Fluoroquinolones	CIP	Ciprofloxacin	5 µg	≤20	21-30	≥31
Carbapenam	IMP	Imipenem	10 µg	≤10	20-22	≥23
Folate pathway inhibitors (Sulphonamides	SXT	Sulphamethoxazole-trimethoprim	23.75/1.25 µg	≤10	11-15	≥16
Quinolone	NA	Nalidixic acid	30 µg	≤13	14-18	≥19

Note: Manufacturer of disc: Oxoid Limited, Basingstoke, Hampshire, England.

Table 3.4: A panel of antibiotics used, Concentrations and Zone diameter interpretative standards for *Escherichia Coli* (CLSI, 2007).

Group/Class of Antimicrobial agents	Disk code	Agents name	Potency (µg)	Zone Diameter, nearest whole (mm)		
				R	I	S
β -lactam	AMX	Amoxicillin	30µg	≤13	14-17	≥18
	AMP	Ampicillin	10µg	≤13	14-16	≥17
Cephems	CFT	Cefotaxime	30µg	≤22	23-25	≥26
	CFX	Cefixime	5µg	≤15	16-18	≥19
	CTR	Ceftriaxone	30µg	≤19	20-22	≥23
	CFP	Cefepime	30µg	≤18	19-24	≥25
Folate pathway inhibitors (Sulphonamides)	CS	Colistin sulfate	10µg	≤10	-	≥11

Note: Manufacturer of disc: Oxoid Limited, Basingstoke, Hampshire, England.

Table 3.5: A panel of antibiotics used, Concentrations and Zone diameter interpretative standards for *Staphylococcus spp*. (CLSI, 2007)

Groups of antimicrobial agents	Drug code	Agents name	Potency (µg)	Disk diffusion zone break point (mm)		
				R	I	S
β-lactam	AMP	Ampicillin	30 µg	≤30	14-16	≥17
	OX	Oxacillin	30 µg	≤30	-	≥18
	MET	Methicillin	5 µg	≤5	10-13	≥14
Aminoglycosides	CN	Gentamicin	30 µg	≤30	13-14	≥15
	S	Streptomycin	10 µg	≤10	12-14	≥15
Tetracyclines	TE	Tetracycline	30 µg	≤11	12-14	≥15
	TGC	Tigecycline	15µg	≤15	-	≥18
Macrolides	C	Chloramphenicol	30 µg	≤12	13-17	≥18
Folate pathway inhibitors (Sulphonamides	SXT	Sulphamethoxazole-trimethoprim	23.75/1.25 µg	≤10	11-15	≥16
Lincosomides	DA	Clindamycin	2 µg	≤14	15-20	≥21
Ansamycin-rifamycins	RD	Rifampicin	5 µg	≤16	17-19	≥20
Oxazolidones	LZD	Linezolid	30 µg	≤20	-	≥21

Note: Manufacturer of disc: Oxoid Limited, Basingstoke, Hampshire, England.

3.13 Statistical analysis

All attributed data on risk factors and results of laboratory analysis and AMR were stored in Microsoft Excel 2013. The data was cleaned, coded and checked for integrity in MS Excel 2013 and exported to STATA-13 (StataCrop, 4905, Lakeway Drive, College station, Texas 77845, USA) to perform the statistical analysis.

3.13.1 Descriptive analysis

Prevalence of three microorganisms (*Salmonella* spp., *Staphylococcus spp*, *Escherichia Coli*) were calculated using positive samples divided by the total number of samples tested and the results were expressed as percentage with 95% confidence interval . In case of positive samples, antimicrobial susceptibility testing and the percentage of susceptibility was done according to the resistance, intermediate and sensitive antimicrobials.

3.13.2 Risk factor analysis

Based on data collection, bats sample were grouped according to the locations they were collected from, land gradients (urban vs rural), species (*Pteropus medius* vs *Rousettus leschenaulti*) and season (winter vs summer).

3.13.3 Random effect model

Chi-square test ($\chi 2$) was performed to screen the risk factors initially. The significant risk factors ($p \leq 0.2$) were transferred to random effect model. If the random effect model was not fitted properly, the data was forwarded to run a logistic regression model to estimate odds ratio.

3.13.4 Logistic regression model

At first univariable logistic regression model was used without adjusting the clustering effects. Significant risk factors ($p \leq 0.2$) were then entered in to multivariable logistic regression model. Confounding effect of a factor was checked by observing the variation in the coefficient with the presence and absence of a factor. If the variation was greater than 10%, then the factor was considered as a confounder. The validity of the model was checked by receiver operating curve (ROC) and goodness of fit test (lfit) describe by Dohoo et al. (2003). The results were expressed as OR, *P* value and 95% CI.

Chapte-4: Result

4.1 General prevalence of *Salmonella spp., Staphylococcus spp., E. coli* in Bats of Bangladesh.

In *Pteropus medius*, prevalence of *E. coli* was highest (31.85%; 95% CI 25.9-38.4) compared to *Staphylococcus* spp. and *Salmonella* spp. (19.65%; 95% CI: 14.8-25.4 and 10.04%; 95% CI: 6.4-14.7, respectively) (**Table 4.1**).

In *Rousettus leschenaulti*, prevalence of *Staphylococcus spp* was 40.71% (95% CI: 32-49.3) which was higher contrast to *E. coli* and *Salmonella* spp. (25.71%; 95% CI: 18.71-33.8 and 2.86%; 95% CI: 1-7.15, respectively) (**Table 4.1**).

Table 4.1: General Prevalence of *Salmonella spp., Staphylococcus spp., E. coli* in Different species of Bats of Bangladesh.

Species	Micro-organisms	Total positive (n), sample (N)	Percentage (%)	95% CI
Pteropus medius	***Salmonella spp.***	23 (229)	10.04	6.4-14.7
	Staphylococcus spp.	45 (229)	19.65	14.8-25.4
	E. coli	73 (229)	31.85	25.9-38.4
Rousettus leschenaulti	***Salmonella spp.***	4 (140)	2.86	1-7.15
	Staphylococcus spp.	57 (140)	40.71	32-49.3
	E. coli	36 (140)	25.71	18.71-33.8

4.2 Prevalence of resistance *Salmonella* spp., *Staphylococcus spp., E. coli* in Bats of Bangladesh.

The prevalence of resistance microorganisms of Bats *Salmonella* spp., *Staphylococcus spp*. and *Escherichia coli* was to be found 7.32%, 26.29% and 28.18% respectively. (**Table 4.2**).

Table 4.2: Prevalence of Resistance microorganisms of Bats of Bangladesh.

Microorganisms	n (N)	%	95% CI
***Salmonella* spp**	27 (369)	7.32	4.88-10.5
Staphylococcus spp	97 (369)	26.29	21.87-31.1
Escherichia coli	104 (369)	28.18	23.65-33.1

4.3 Prevalence of *Salmonella* spp. based on location

Overall prevalence of *Salmonella* spp. in bats was estimated as 7.32% (27/369). Prevalence (34.29%) was remarkably higher in samples from Boldha Garden followed by 13.21% from Mohakhali, Dhaka. Lowest prevalence (2%) was recorded in bats from Mirpur Botanical Garden and Baliakandi. Bats from Uttara and Nagarkanda were found free of *Salmonella* spp. **(Table 4.3).**

Table 4.3: Location wise prevalence of *Salmonella spp.* in bats of Bangladesh.

Location	Species	No. of sample tested (N)	No. of sample positive to *Salmonella* spp. (n)	Percent of sample positive, %	95% CI
Uttara, Dhaka	*Pteropus medius*	41	0	0	0-8.6
Mirpur botanical Garden, Dhaka	*Pteropus medius*	50	1	2%	0.1-10.7
Mohakhali, Dhaka	*Pteropus medius*	53	7	13.21%	5.4-25.3
Boldha Garden, Dhaka	*Pteropus medius*	35	12	34.29%	19.1-52.2
Nagarkanda, Faridpur	*Rousettus leschenaulti*	40	0	0	0-8.9
Baliakandi, Rajbari	*Rousettus leschenaulti*	50	1	2%	0.1-10.7
Kanaipur, Faridpur	*Rousettus leschenaulti*	50	3	6%	1.3-16.6
Madaripur	*Pteropus medius*	50	3	6%	1.3-16.6
Total		**369**	**27**	**7.32%**	

4.3.1 Factors associated with AMR isolates of *Salmonella* spp in bats

Univariable analysis showed a significant association (P≤0.01) between AMR isolates of *Salmonella* spp. and location of bats. Highest AMR isolates was observed in Dhaka (11.2%) and lowest in Faridpur (2.9%). Prevalence of AMR *Salmonella* spp. were significantly (P≤0.01) varied between *Pteropus medius* (10.04%) and *Rousettus leschenaulti* (2.9%) species. AMR isolates of *Salmonella* spp. was significantly (P≤0.00) higher in summer (34.2%) than winter. (**Figure 4.4**)

Significant variables (Location, species, habitats and seasons; p≤0.2) identified through univariate chi-square analysis were forwarded to the logistic regression model. Species and Habitats were omitted due to effect of collinearity. In the final model, only season showed a significant (P<0.001) influence on the prevalence of AMR *Salmonella* spp., in bats of Bangladesh; the odds of having the isolates was 8.9 times higher in summer **(Table 4.4).**

Table 4.4: Univariable and final multivariable logistic regression model of association between different variables and prevalence of AMR isolates of *Salmonella* spp. in Bats of Bangladesh (N=369).

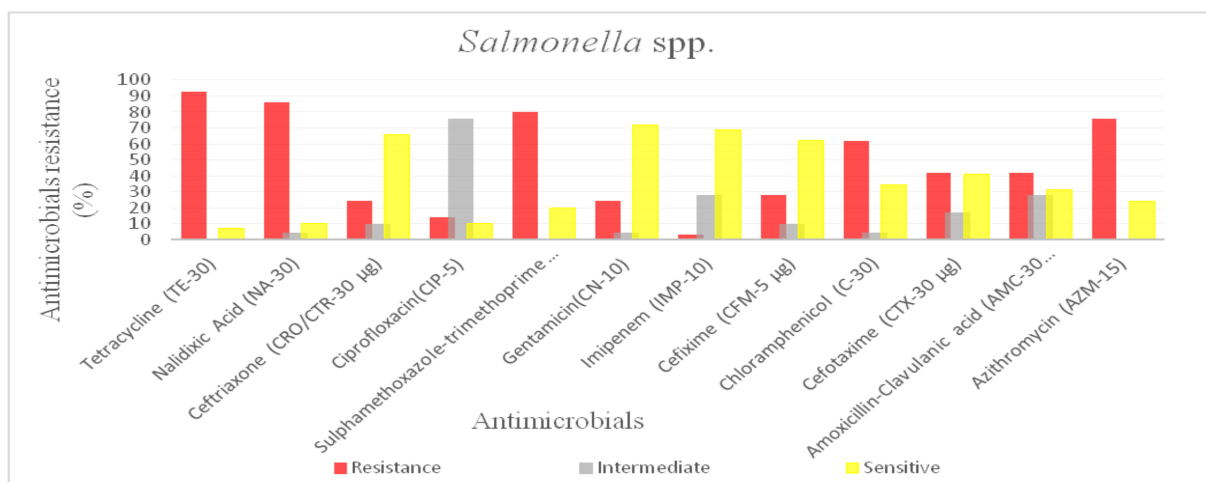
Variables	Catergory (N)	Prevalence of AMR isolates of *Salmonella* spp			Final logistic regression Model		
		n (%)	95% CI	*P* (X^2-test)	OR	95% CI	*P* value
Location	Dhaka (179)	20 (11.2%)	6.9-16.7	0.01	2.0	0.6 - 6.8	0.2
	Madaripur (50)	3 (6.0%)	12.6-16.6		2.2	0.5 - 10.1	0.3
	Faridpur (140)	4 (2.9%)	7.8-7.1		1.0		
Species	*Pteropus medius*(229)	23 (10.1%)	5.5-12.5	0.01			
	Rousettus leschenaulti (140)	4 (2.9%)	1-7.15				
Habitats	Rural (190)	7 (3.7%)	1.4-7.4	0.006			
	Urban (179)	20 (11.2%)	6.9-16.7				
Season	Summer (35)	12 (34.2%)	19.1-52.2	0.00	8.9	3.3 - 24.1	0.00
	Winter (334)	15 (4.4%)	2.5-7.3		1.0		

p: Probability; OR: Odds Ratio; CI: Confidence Interval

4.3.2 Antimicrobial resistance (AMR) pattern of *Salmonella* spp

More than 60% isolates of *Salmonella* spp were resistant against Tetracycline (93%) followed by Nalidixic Acid (86%), Sulphamethoxazoal-trimethoprime (79%), Azithromycin (76%) and Chloramphenicol (62%). Isolates were highly sensitive to Gentamicin (72%), Imipenem (69%) followed by Ceftriaxone (66%) and Cefixime (62%). Resistance pattern for other drugs are shown in **Figure 4.1**.

Fig 4.1: Antimicrobial resistance pattern of *Salmonella* spp.



4.4 Prevalence of *Staphylococcus* spp. based on location

Overall prevalence of *Staphylococcus* spp. was 26.29% (97/369) Prevalence of *Staphylococcus spp* in bats was highest in Baliakandi, Rajbari (44%) and lowest in Mohakhali, Dhaka (7.6%). Prevalence was observed noticeable in Faridpur (Kanaipur, 40% and Nagarkanda, 32.5%) (**Table 4.5**).

Table 4.5: Location Wise Prevalence of *Staphylococcus spp.* in Bats of Bangladesh.

Location	Species	No. of sample tested (N)	No. of sample positive to *Staphylococcus spp.* (n)	Percent of sample positive %	95% CI
Uttara, Dhaka	*Pteropus medius*	41	17	41.5%	26.3-57.9
Mirpur botanical Garden, Dhaka	*Pteropus medius*	50	4	8%	2.2-19.2
Mohakhali, Dhaka	*Pteropus medius*	53	4	7.6%	30.1-18.2
Boldha Garden, Dhaka	*Pteropus medius*	35	4	11.4%	3.2-26.8

Nagarkanda, Faridpur	*Rousettus leschenaulti*	40	13	32.5%	18.6-49.1
Baliakandi, Rajbari	*Rousettus leschenaulti*	50	22	44%	29.9-58.8
Kanaipur, Faridpur	*Rousettus leschenaulti*	50	20	40%	26.4-54.9
Madaripur	*Pteropus medius*	50	13	26%	14.7-40.3
Total		**369**	**97**	**26.29%**	

4.4.1 Factors associated with prevalence of AMR isolates of *Staphylococcus spp.* in bats

All four variables (location, species, rural vs. urban and season) showed significant association with the prevalence of AMR isolates of *Staphylococcus* spp. in univariable analysis. Prevalence found highest in Faridpur; 39.3% isolates were retrieved from this location. *Staphylococcus* spp. was more prevalent in the bat species *Rousettus leschenaultia* (39.3%) in contrast to *Pteropus medius* (16.2%). More bats of the rural areas (35.8%) were affected compared to urban in the present study. Prevalence was higher in winter (27%) than summer (11%) (**Table 4.6**).

Having adjusted different factors (location, species, Habitats and season) using multivariable logistic regression model, only location & season of the bats had a significant association in the final model. Species of bats and Habitats were omitted from the model due to collinearity. The odds of antimicrobial resistance of *Staphylococcus* spp. was significantly higher in Faridpur district (OR=3.1; 95% CI: 1.77-5.4; *P*=0.00) followed by Madaripur district (OR=1.7, CI: 0.78-3.5; *P*=0.1) compared to Dhaka district. However was not significantly (P=0.03) associated, odds of having AMR isolates were higher in winter season (OR=1.7; CI: 0.52-5.1) than summer season (**Table 4.6**).

Table 4.6: Univariable and final multivariable logistic regression model of association between different variables and prevalence of AMR isolates of *Staphylococcus* spp. in Bats of Bangladesh (N=369)

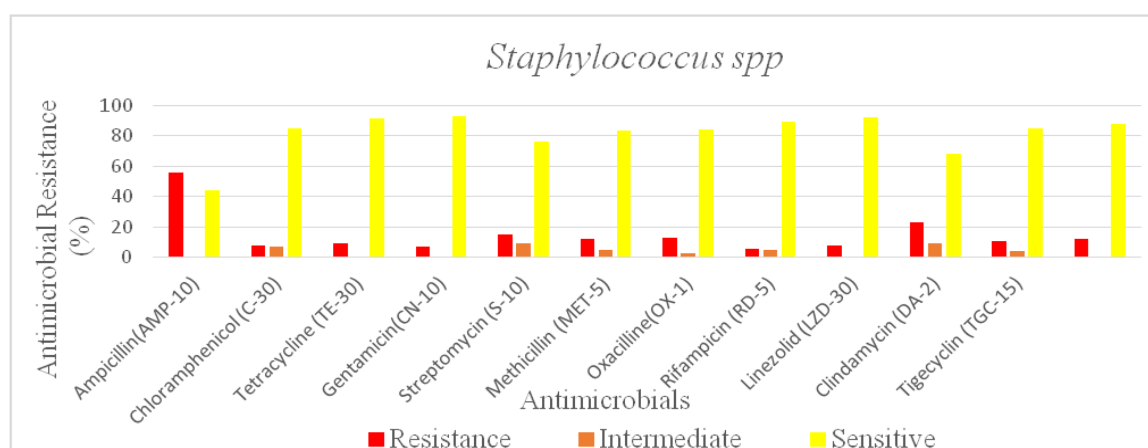
Variables	Catergory (N)	Prevalence of AMR isolates of *Staphylococcus* spp.			Logistic regression Model		
		n (%)	95% CI	*P* (X^2-test)	OR	95% CI	*P* Value
Location	Dhaka (179)	29 (16.2%)	11.13-22.4		1.0		
	Madaripur (50)	13 (26%)	14.63-40.3	0.00	1.7	0.78- 3.5	0.1
	Faridpur (140)	55 (39.3%)	31.15-47.9		3.1	1.77-5.4	0.00
Species of bats	*Pteropus medius* (229)	42 (18.3%)	13.55-23.9	0.00			
	Rousettus leschenaulti (140)	55 (39.3%)	31.15-47.9				
Habitats	Rural (190)	68 (35.8%)	28.98-43.1				
	Urban (179)	29 (16.2%)	11.13-22.4	0.00			
Season	Summer (35)	4 (11.43%)	3.2-26.7	0.03	1		
	Winter (334)	93 (27.9%)	23.1-32.9		1.7	0.52-5.1	0.3

p: Probability; OR: Odds Ratio; CI: Confidence Interval

4.4.2 Antimicrobial resistance (AMR) pattern of *Staphylococcus spp*

Antimicrobial resistance pattern in *Staphylococcus* spp. isolates was observed against 12 commonly used antibiotics. More than 30% resistance was observed only against Ampicillin (56%); second highest was against Clindamycin (23%). A remarkable number of isolates showed sensitivity to most of the antibiotics. Gentamicin (93%) were highly sensitive followed by Linezolid (92%), Tetracycline (91%), Rifampicin (89%), Sulphamethoxazole-trimethoprime (88%), Tigecycline & Chloramphenicol (85%), Oxacillin (84%) and Methicillin (83%) (**Figure 4.2**).

Fig 4.2: Antimicrobial resistance pattern of Staphylococcus *spp*.



4.5 Prevalence of *E. coli* according to location

Overall prevalence of *E. coli* was recorded as 28.18% (104/369) among which highest was observed in bats of Boldha Garden, Dhaka (45.8%). In Baliakandi, Rajbari, prevalence of *E.coli* was estimated lowest (14%; 95% CI: 5.9-26.8). Prevalence in Mirpur Botanical Garden, Dhaka (40%) and Nagarkanda, Faridpur (35%) were noticeable (**Table 4.7**).

Table 4.7: Location Wise Prevalence of *E. coli* of Bats in Bangladesh.

Location	Species	No. of sample tested (N)	No. of sample positive to *E. coli* (n)	Percent of sample positive %	95% CI
Uttara, Dhaka	*Pteropus medius*	41	13	31.8%	18.1-48.1
Mirpur botanical Garden, Dhaka	*Pteropus medius*	50	20	40.0%	26.4-54.9
Mohakhali, Dhaka	*Pteropus medius*	53	16	30.2%	18.3-44.4
Boldha Garden, Dhaka	*Pteropus medius*	35	16	45.8%	28.9-63.3
Nagarkanda, Faridpur	*Rousettus leschenaulti*	40	14	35%	20.7-51.7
Baliakandi, Rajbari	*Rousettus leschenaulti*	50	7	14%	5.9-26.8
Kanaipur, Faridpur	*Rousettus leschenaulti*	50	8	16%	7.1-29.1
Madaripur	*Pteropus medius*	50	10	20.0%	10.1-33.8
Total		**369**	**104**	**28.18%**	

4.5.1 Factors associated with prevalence of AMR isolates of *E. coli* in bats

Chi square test suggested significant association of all explanatory variables with the presence of AMR isolates of *E. coli*. Prevalence of AMR isolates was higher in Dhaka district, *Pteropus medius species, in urban area and summer season.* Two variables, species and Habitats were omitted due to collinearity in the final multivariable model. Only location of the bats showed a significant association in final multivariable model where the

odds of getting AMR isolates was 2.3 times higher in Dhaka and 1.1 times higher in Faridpur compared to Madaripur (baseline) **(Table 4.8).**

Table 4.8: Univariable and final multivariable logistic regression model of association between different variables and prevalence of AMR isolates of *E. coli* in Bats of Bangladesh (N=369)

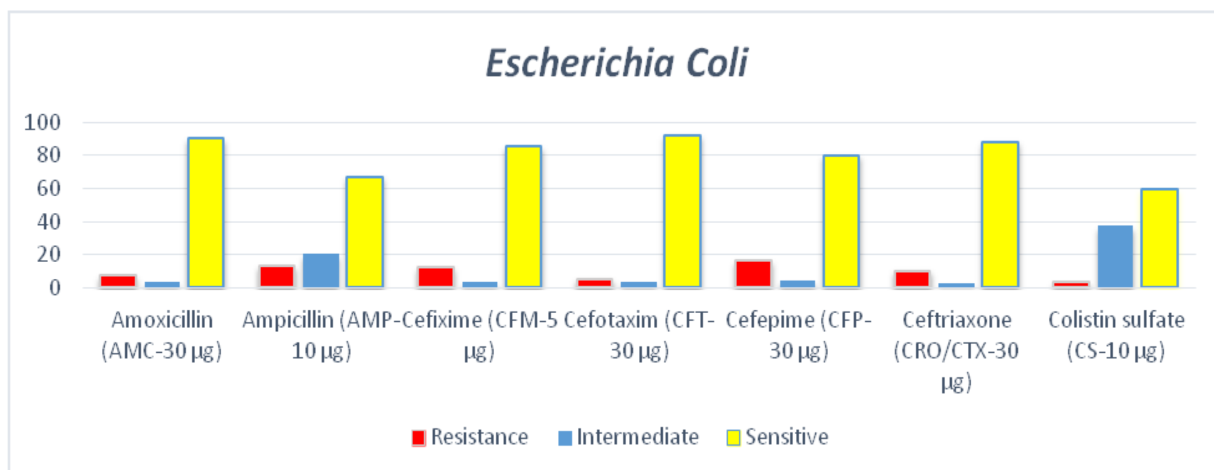
Variables	Catergory (N)	Prevalence of AMR isolates of *E. coli*			Logistic regression Model		
		n (%)	**95% CI**	***P* (X^2-test)**	**OR**	**95% CI**	***P* Value**
Location	Dhaka (179)	65 (36.3%)	29.2-43.8	0.003	2.3	1.1-4.9	0.03
	Madaripur (50)	10 (20%)	10.03-33.7		1.0		
	Faridpur (140)	29 (20.8%)	14.3-28.3		1.1	0.5-2.34	0.9
Species	*Pteropus medius* (229)	75 (32.8%)	26.7-39.2	0.013			
	Rousettus leschenaulti (140)	29 (20.8%)	14.33-28.3				
Habitats	Rural (190)	39 (20.6%)	15.02-26.97	0.001			
	Urban (179)	65 (36.3%)	29.2-43.9				
Season	Summer (35)	16 (45.8%)	28.83-63.3	0.015	1.1	0.5 - 2.2	0.9
	Winter (334)	88 (26.4%)	21.8-31.4		1.0		

p: Probability; OR: Odds Ratio; CI: Confidence Interval

4.5.2 Antimicrobial resistance (AMR) pattern of *E. coli* isolates

Number of isolates of *E. coli* resistant to different antimicrobials was less than 20%; highest resistance observed against Cefepime (16%) followed by Ampicillin (13%). Higher sensitivity was recorded against Cefotaxime (92%) followed by Amoxicillin (90%), Ceftriaxone (88%), Cefixime (85%), Ampicillin (65%) and Colistin sulfate (60%) (**Figure 4.3**).

Fig 4.3: Antimicrobial resistance pattern of *E. coli*



4.6.1 Frequency Distribution of *Salmonella* spp. in water sample (N=22)

The prevalence of AMR isolates of *Salmonella* spp in water was 75% in Faridpur which was significantly higher (*P*=0.04) than Dhaka (22.3%). AMR *Salmonella* spp. isolates of water sample was significantly (*P*=0.04) more prevalent in rural area (75%) than that of urban (**Table 4.9**).

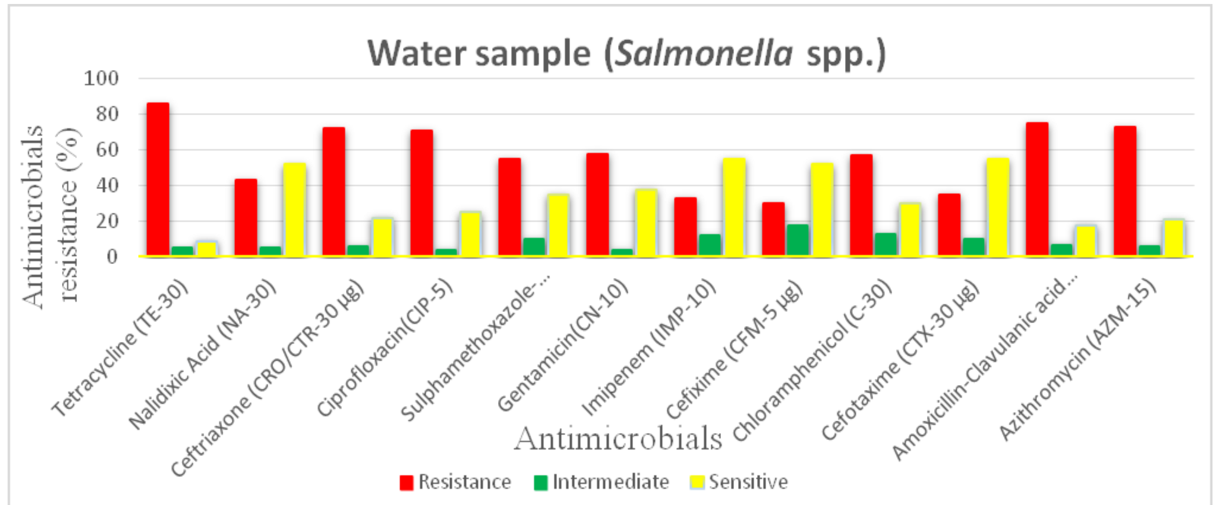
Table 4.9: Frequency Distribution of *Salmonella* spp. in water sample (N=22)

Microorganisms	Variables	Catergory (N)	n (%)	95% CI	*P* (X^2-test)
***Salmonella* spp. (Water sample)**	**Location**	Dhaka (18)	4 (22.3%)	6.41-47.7	0.04
		Faridpur (4)	3 (75%)	19.4-99.3	
	Habitats	Rural (4)	3 (75%)	19.4-99.3	0.04
		Urban (18)	4 (22.2%)	6.41-47.7	

4.6.2 Antimicrobial resistance (AMR) pattern of S*almonella* spp isolates in water samples

Higher resistance to Tetracycline (86%) followed by Amoxicillin-Clavulanic acid (75%), Azithromycin (73%), Ceftriaxone (72%), Ciprofloxacin (71%), Gentamicin (58%), Chloramphenicol (57%) and Sulphamethoxazole-trimethoprime (55%) were recorded in *Salmonella* spp. isolates recovered from water. On the other hand, Cefotaxime & imipenem (55%) had highest sensitivity followed by Nalidixic Acid & Cefixime (52%), Gentamicin (38%), Sulphamethoxazole-trimethoprime (35%) and Chloramphenicol (30%) (**Figure 4.4**).

Fig 4.4: Antimicrobial resistance pattern of water sample (S*almonella* spp)



Chapter-5: Discussion

Antimicrobial resistance (AMR) is becoming a central topic as it's a growing public health threat for humans, livestock and wildlife in developing country like Bangladesh. Bats live in thriving atmosphere with livestock, human and environment, and it is hypothesized that they might harbor and spread resistant microorganisms within and between populations. Although AMR is a serious health concern for humans, animals and wildlife but unfortunately there are no studies on the potential of bats in spreading resistant bacteria in Bangladesh. Few studies have been performed on antimicrobial resistance in wild species in this country. There are many emerging infectious diseases associated with bats species can have major impacts on both ecosystem and public health. Despite their importance in maintaining forest diversity and their role in the zoonotic diseases transmission, research on bats remain neglected in Bangladesh; while limited studies have been conducted on bat bacterial diseases. Antimicrobials are widely used and abused in humans and livestock. Moreover, it is used as growth promoter in animal production. Bats normally live in the vicinity of human and animal habitations both in urban and rural ecologies, ultimately the resistant organisms spread to the free ranging bats. Bat infection dynamics are driven by a complex interaction of ecological, immunological, behavioral, and anthropogenic factors. Therefore, the present study was conducted on bats of urban and rural habitations to assess the status of eco-epidemiology of antimicrobial resistance.

5.1 Prevalence of Bacterial microorganism of Bats

Although some research efforts have been observed on antimicrobial resistance in different wildlife but information on bats AMR was found limited (Mühldorfer, 2013). The present study was aimed to estimate the prevalence of antimicrobial resistance status of *Salmonella* spp., *Staphylococcus* spp. and *Escherichia coli* isolated from free ranging bats of Bangladesh.

In *Pteropus mediums*, the prevalence of *E. coli* was estimated as 31.85% in the present study which is not concordant with an early finding in Costa Rica (Chaverri, 2006) but has resemblance with the studies conducted in Philippines (Italia et al., 2012) and USA (Heard et al., 1997). The prevalence of *Staphylococcus spp*. in bats fecal sample was found 19.65% in this study which is supported by previous study conducted in Poland (Jarzembowski,

2002) but higher prevalence was found in Germany (Kristin et al., 2011) and in USA (Heard et al., 1997). Prevalence of *Salmonella* spp. was found 10.04% in *Pteropus mediums* fruits bats in present study but a previous study in Bangladesh observed a different result (Islam et al., 2013). Presence of *Salmonella* spp. in fruit bats was reported from Pakistan (Gulraiz et al., 2017), Australia (Henry et al., 2018) and Brazil (Cláudio et al., 2018).

In *Rousettus leschenaulti*, prevalence of *Staphylococcus spp* was recorded 40.71% in bats fecal sample in the present study but a lower prevalence was recorded in Gabon (Held et al., 2017). *E coli* was isolated from 20.9% bat fecal samples in the present study; this result is supported by a study in India (Banskar et al., 2016), however dissimilar findings were reported by studies in Philippines (Italia et al., 2012), in Congo (Nowak et al., 2017) and Davao region, Philippines (Hatta et al., 2016). The prevalence of *Salmonella* spp. was found 2.86% in this study which corroborates with the findings of Philippines fruits bats (Reyes et al., 2011).

5.2 Prevalence of Antimicrobial Resistance in *Salmonella* spp. in Bats

The overall prevalence of antimicrobial resistance in *Salmonella* spp. was found 7.32%. The present finding is not supported by the study conducted in Faridpur, Bangladesh (0%) (Islam et al., 2013).The prevalence of present study was higher compared to other overseas studies; 0% in Grenada and Italy (Everard et al., 1979; Di Bella et al., 2014), 1.1% in Trinidad and Tobago (Adesiyun et al., 2009), 1.36% in UK (Daffner, 2001) and 0.2% in Colombian bats (Arata et al., 1968).

5.2.1 Antimicrobial resistance pattern of *Salmonella* spp., in Bat

High occurrence of resistance to different antibiotics, particularly Tetracycline (93%), among *Salmonella* spp. isolated from bats in the present study is comparable to the results of studies conducted in other countries. Tetracycline was 72-93% resistant in another Bangladeshi study (Mahmud et al., 2013), 63.4-65.2% (Musgrove et al., 2006; Sigirci et al., 2018) in Europe (France, Turkey) but 29-39.9% isolates were resistant in Africa (Gopee et al., 2000b; Zhao et al., 2008). This is not surprising since tetracycline is often used as a first-line antimicrobial in disease prevention as well as growth promotion in food animals

and its common use has likely contributed to emerging bacterial resistance and high rates of resistance (Okeke et al., 2005).

Sulfamethoxazole-Trimethoprim is an essential and widely used drug; 79% *Salmonella* spp. isolates were resistant to it in bats in the present study. This result is comparatively higher than the previous study (68%) of AMR in *Salmonella* isolates in Bangladesh (Hafsa Afroz et al., 2013). 86% isolates was resistant to Nalidixic acid in the present study. Some other studies observed 92.5% (Afroj et al., 2011), 72.73-78.3% (Harish and Menezes, 2011; Suman et al., 2017) and 29% (Mannan et al., 2014) resistant isolates in Bangladesh.

Another higher resistant antibiotic for *Salmonella* spp. isolated from bats was Azithromycin (76%). Other studies in Bangladesh observed more or less similar result; 79.49% (Fatema et al., 2016) and 91-95% (Ahsan and Rahman, 2018). Resistance of Chloramphenicol was found in 62% *Salmonella* spp. isolates in bats which is concordant to previous study in Bangladesh; 57.9% (Chiou et al., 2014) but lower resistance was observed in Nepal (27%) and India (28.42%) and higher in China (84%) (Xia et al., 2009). Chloramphenicol is widely used in humans even in common cold in Bangladesh. This practice might have contributed to the high resistance against this drug.

On the other hand, *Salmonella* spp. was found highly sensitive to Gentamicin (72%), Imipenem (69%) followed by Ceftriaxone (66%) and Cefixime (62%). Previous studies also reported high sensitivity to Gentamicin (90-100%) (Hassan et al., 2014; Sigirci et al., 2018), Imipenem (82.1- 100%) (Singh and Cariappa, 2016; Khan et al., 2017), 75.73% in Ceftriaxone and 74.76% in Cefixim (Saleem et al., 2017). In another Bangladeshi study, all the isolates of *Salmonella* spp., in fruit bats showed sensitivity to Tetracycline, Nalidixic acid, Amoxicillin-clavulanic acid, Cefoxitin, Ceftriaxone, Ciprofloxacin, gentamycin, Chloramphenicol and Sulphamethoxazole-trimethoprim (Islam et al., 2013).

5.3 Prevalence of Antimicrobial Resistant *Staphylococcus spp* in Bats

The overall antimicrobial resistance of *Staphylococcus spp*. was found higher than *Salmonella* spp. in the present study (26.29%). In previous studies, 19.11% isolates was found resistant in Nigerian straw colour fruit bats (Akobi et al., 2012) and 20% in Brazil (Moreno et al., 1975). Highest prevalence was found (68%, 63/92) (Vengust et al., 2018)

in Slovenia, 52.8% in *Ile-Ife,* Nigeria (Olatimehin et al., 2018), 39.28% in USA (Heard et al., 1997), 28.6% in Germany (Kristin et al., 2011; Mühldorfer et al., 2011) and 18.45% in Costa Rica (Chaverri, 2006). Lower prevalence of 4-6% were recorded in Gabon (Held et al., 2017) and 9.7% in Poland (Jarzembowski, 2002) in free living bats.

5.3.1 Antimicrobial resistance pattern of *Staphylococcus spp* in Bat

About 56% *Staphylococcus spp*. isolates showed resistance to Ampicillin in the present study. Noticeable amount of resistance was recorded in previous studies; 90% in Slovakia (Vandžurová et al., 2013) and 100% in Bangladesh (Islam et al., 2008; Ahaduzzaman et al., 2014). Other β-lactam groups like penicillin showed 3.7% resistance in Nigeria (Akobi et al., 2012), 67.5-95% in Central & West Africa (Ateba Ngoa et al., 2012; Olalekan et al., 2012; Aiken et al., 2014; Schaumburg et al., 2015) and 68% in Bangladesh (Islam, 2016). Resistance to Clindamycin and Oxacillin among *Staphylococcus spp*. was estimated as 23% and 13% in bats, respectively in the present study; while 31% and 46% resistance was recorded in a previous study of Bangladesh (Islam, 2016). Around 12% Methicillin resistance was observed in *Staphylococcus spp*. isolates in bats in the present study which was supported by a previous study of Bangladesh (12%) (Hossain et al., 2002).

5.4 Prevalence of Antimicrobial Resistant *Escherichia coli* in Bats

Resistant *Escherichia coli* were most frequently (28.18%) isolated from the free ranging bats of Bangladesh among other microorganisms and similar results were observed in USA (Heard et al., 1997), Philippines (Italia et al., 2012), Brazil (Moreno et al., 1975; Pinus and Muller, 1980; Cabal et al., 2015), Indonesia (Graves et al., 1988), Australia (Gordon and Cowling, 2003) and Africa (Adesiyun, 1999; Gopee et al., 2000b; Costa et al., 2008; Nowak et al., 2017).

On the other hand, prevalence of *E.coli* in fruits bats were found as low as 12.6% in India (Banskar et al., 2016), 16.3% in Japan (Obi et al., 2014), 13% in Trinidad and Tobago (Adesiyun et al., 2009), 9.6% in Portugal (Garcês et al., 2017), 17.5% in Costa Rica (Chaverri, 2006), 4.1-5.26% in Philippines (Italia et al., 2012; Hatta et al., 2016), 11-15% in Malaysia (Apun et al., 2011; Bilung et al., 2014).

Where several studies reported prevalence of antimicrobial resistant *E.coli* in the feces of bats (Gordon and FitzGibbon, 1999; Sherley et al., 2000; Prem Anand and Sripathi, 2004), many observed absence of it in bats feces (Jarzembowski, 2002; Daniel et al., 2013; Di Bella et al., 2014; Cláudio et al., 2018).

Migratory habits have been suggested to contribute to the transmission of antibiotic resistant *E. coli* to wildlife in remote areas with other migrating and non-migrating bat species and enables horizontal spread of pathogens within and among bat species (O'shea et al., 2014; Moratelli and Calisher, 2015). The human surroundings and environment in Bangladesh are heavily polluted by resistant bacteria, which might influence the bats fecal flora. Factors that can influence the fecal carriage of bacteria in bats are dependent on the feeding habitats, behaviors and lifestyles of bats.

5.4.1 Antimicrobial resistance pattern of *Escherichia coli* in Bat

Number of isolates of *E. coli* resistant to different antimicrobials was less than 20%; highest resistance observed against Cefepime (16%) and Ampicillin (13%) followed by Cefixime (12%), Ceftriaxone (10%), Ceftriaxone (10%) and Amoxicillin (5%). Different studies also reported a low resistance of E. coli to different antibiotics (around 6%) (Adesiyun et al., 2009; Iovine et al., 2015; Cláudio et al., 2018).

On the other hand, many studies showed noticeable resistance of *E. coli* to several antibiotics (Graves et al., 1988; Gopee et al., 2000b; Mühldorfer, 2013). Similar results have been reported earlier from other species of wild animals (Souza et al., 1999; Sherley et al., 2000; Costa et al., 2008). Higher number of β-lactam antibiotics (Ampicillin, Amoxicillin, Cefixime, Ceftriaxone) was found resistance to *E.coli* in wild birds of Bangladesh (Hasan et al., 2012; Hasan et al., 2014; Rashid et al., 2015).

Bats are common throughout Bangladesh and lives in close proximity to humans (living nearby and under bat roosts) and livestock and share their ecological and dietary traits such as feeding, roosting, and breeding (Epstein et al., 2006; Luby et al., 2009). In overall, Bats may be get microorganisms by their habits of feeding, size of the host, host specificity and geographical location may affect the types and dissemination of bacteria and in its faeces (Gordon and FitzGibbon, 1999; Kingston et al., 2006; Anand et al., 2012). Another reason,

Staphylococcus spp are common on the skin of several mammals (Kloos, 1999), so it is probable that these microorganisms are shared in the digestive tract, primarily due to their high contact/interaction through the skin of their prey. Of the bacteria of the genus *Staphylococcus* such as *S. aureus* have previously been isolated in the Bats (Moreno et al., 1975).

5.5 Prevalence of Antimicrobial Resistant *Salmonella* spp. in water sample

The prevalence of AMR isolates of *Salmonella* spp in water was 75% in Faridpur which was significantly higher (*P*=0.04) than Dhaka (22.2%). The present finding is supported by several previous studies in Bangladesh (Bhatta et al., 2007; Parveen et al., 2008; Acharjee et al., 2011; Kuo et al., 2013). AMR *Salmonella* spp. isolates of water sample was significantly (*P*=0.04) more prevalent in rural area (75%) than that of urban which is concordant with other studies (Seidman et al., 2009; Lévesque et al., 2013). In the rural landscape where wild animals are often exposed to anthropogenic disturbance might show more frequency in pathogen contamination.

5.5.1 Antimicrobial resistance pattern of S*almonella* spp isolates for water samples

Higher resistance to Tetracycline (86%) followed by Amoxicillin-Clavulanic acid (75%), Azithromycin (73%), Ceftriaxone (72%), Ciprofloxacin (71%) were recorded in *Salmonella* spp. isolates recovered from water which is similar to previous report (Acharjee et al., 2011). On the other hand, Cefotaxime & Imipenem (55%) had highest sensitivity followed by Nalidixic Acid & Cefixime (52%) which is more or less comparable to earlier studies (Ahmed et al., 2013; Acharjee et al., 2014).

Rural areas in Bangladesh commonly include stagnant lagoons used for bathing and drinking by people and livestock and these water sources are often contaminated with sewage (Parveen et al., 2008). In addition bats often use this water for drinking purpose; thus, contaminated water could be a possible source of infection (Islam et al., 2013).

5.6. Risk factors associated with the prevalence

The odds of having AMR isolates in bats were found different in different sampling areas with Dhaka and Madaripur having higher risk. Spatial difference also reported by previous

studies (Sherley et al., 2000; Jardine et al., 2012; Talukdar et al., 2013). Differences in risk recognized in the present study might be attributed to the difference in inhabitant's density, improper waste disposal, water supply in the sampled area.

The current study identified season as a risk factor. Prevalence of *Salmonella* spp. and *Staphylococcus spp*. was higher in summer and winter seasons and AMR *E.coli* was higher in summer season (Mahmud et al., 2011). Seasonal variation in risks might be due to changes in feeding habits and geographical location of bats and host affect the types and distribution of bacteria in the gut and so in the faeces (Pinus and Muller, 1980; Gordon and FitzGibbon, 1999; Prem Anand and Sripathi, 2004).

5.7. Limitations of the study

Sample size: Only 369 samples were collected for laboratory examination. Due to limitation of proper time, availability of facilities and limited resources; targeted number of sample collection was not possible.

Sensitivity and specificity: Disk diffusion method is not as sensitive as other detection methods such as minimum inhibitory concentration (MIC). Therefore, some misclassification might have introduced in the results.

Identification of Species: Due to time and budget limitations during the study period; species & serotypes (*Salmonella* spp, *Staphylococcu*s *spp* and *Escherichia coli*) identification of the microorganisms could not be done.

Gene analysis: Virulent gene analysis (VGA) was not done in this study

Chapter-6: Conclusion

Antimicrobial resistance is presently one of the utmost challenges to the global health care because of improper use an antimicrobials substance. Due to shared environment, wild animals are in great danger of having resistant bacteria. In bats species, prevalence of *Salmonella* spp., *Staphylococcus spp* and *Escherichia coli* in *Pteropus medius* & *Rousettus leschenaulti* bats was found 10.04-2.86%, 19.65-40.71% & 31.85-25.71%, respectively. The prevalent of resistance infection of *E. coli* was significantly higher than *Salmonella* spp., & *Staphylococcus spp*. The frequency of fecal samples of bats positive to resistant *Salmonella* spp. was 7.32%, 26.29% samples were positive for resistant *Staphylococcus spp.* and 28.18% for *E. coli* resistant bacteria. Prevalence of AMR isolates of *Salmonella* spp., in water sample was significantly higher in Faridpur than Dhaka. Together *Salmonella* spp., and *E. coli* was higher in urban habitat whereas *Staphylococcus spp*., was higher in rural habitat. This could be owing to sharing their feeding habits, host specificity, forage, geographical location and environment. Multiple antimicrobial resistances were evidenced among *Salmonella* spp., *Staphylococcus spp,* and *E. coli* isolates in small fruits bat faeces & water sample. Resistance against Tetracycline, followed by Nalidixic Acid & Ampicillin was found to a remarkable level in free ranging bats. Although, small mammals like bats do not consume any drug or antimicrobial agents directly but present study found alarming level of multidrug resistant organisms in bats but it is unclear how bats get AMR bacteria, however water contaminated by people and/or livestock may be a source of infection. The antibacterial resistance was observed here in the isolates might be attributed to routine indiscriminate use of those antibacterial agents in field condition in the study areas and/or rapid chromosomal mutations. So, necessary steps should be taken to control or avoid antimicrobial resistance and improve public health threat.

Chapter-7: Recommendations and Future perspective

7.1 Recommendations

Based on the study results, it might be recommended that, to know the exact resistance pattern of antimicrobial agents, advanced and molecular research work should be conducted in a large scale. Contaminated water, feeding habitats, host specificity, geographical location and environment are contaminating with various resistant microorganisms due to improper utilization of antimicrobials. Therefore, awareness programs and training for professionals should be conducted to limit this practice. Antimicrobial resistant drugs should be avoided both in human and animal treatment. To ensure proper disposal of drugs, prescribed withdrawal period of drugs by registered physician and veterinarian should be followed strictly. Antimicrobial susceptibility should be monitored collaborately in order to select appropriate antimicrobials agents for treatment of bacterial infection both in human and animals through one health approach. To develop or increase public awareness and motivation regarding AMR aspects, programs like school kids awareness should be conducted in a regular basis. Associated wild animal species further need to study for antibiogram due to their potentiality of antimicrobial resistance reservoir and a source of transmission.

7.2 Future perspective

This study only covered a limited area of Dhaka, Faridpur & Madaripur districts of Bangladesh. To explore the risk factors infection and transmission dynamics of resistant *Staphylococcus spp*., *E. coli*, and *Salmonella* spp. in small fruit bats, extended study targeting individual animals should be arranged. Moreover, an extended study covering the whole country and wide range of wildlife might add valuable information regarding the transmission dynamics of resistant bacteria.

Beside this, disc diffusion method was applied to assess antimicrobial susceptibility in this study. So, in a future study, we can use other sensitive tests such as double disc synergy test, phenotypic confirmatory disc diffusion test, and E-test to discover the different findings.

A good and effective network system was established between CVASU and Eco- Health Alliance (an organization working at wild animal research) through this study. Besides, present study helped us to develop laboratory skills, field activities, laboratory data management and large-scale data analysis. The extended study might be executed through the developed laboratory facilities and collaboration with different organizations.

Bibliography

Achan, J., Talisuna, A.O., Erhart, A., Yeka, A., Tibenderana, J.K., Baliraine, F.N., Rosenthal, P.J., D'Alessandro, U., 2011. Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. Malaria journal 10 (1), 144.

Acharjee, M., Rahman, F., Beauty, S.A., Feroz, F., Rahman, M.M., Noor, R., 2011. Microbiological Study on Supply Water and Treated Water in Dhaka City. Stamford Journal of Microbiology 1, 4.

Acharjee, M., Rahman, F., Jahan, F., Noor, R., 2014. Bacterial proliferation in municipal water supplied in Mirpur locality of Dhaka city, Bangladesh. Clean–Soil, Air, Water 42, 434-441.

Adeshina, G., Osuagwu, N., Okeke, C., Ehinmidu, J., Bolaji, R., 2009. Prevalence and susceptibility of Salmonella typhi and Salmonella paratyphi in Zaria, Nigeria. International Journal of Health Research 2 (4).

Adesiyun, A., Downes, M., 1999. Prevalence of antimicrobial resistance and enteropathogenic serogroups in Escherichia coli isolates. Veterinarski Arhiv 69 (6), 335-347.

Adesiyun, A.A., 1999. Absence of Escherichia coli O157 in a Survey of Wildlife from Trinidad and Tobago. Journal of Wildlife Diseases 35, 115-120.

Adesiyun, A.A., Stewart-Johnson, A., Thompson, N.N., 2009. Isolation of enteric pathogens from bats in Trinidad. Journal of wildlife diseases 45, 952-961.

Afema, J.A., Sischo, W.M., 2016. Salmonella in Wild Birds Utilizing Protected and Human Impacted Habitats, Uganda. EcoHealth 13, 558-569.

Afroj, S., Ilias, M., Islam, M., Saha, S.K., 2011. Prevalence of ciprofloxacin and nalidixic acid resistant Salmonella enterica serovar Typhi in Bangladesh. Bangladesh Journal of Microbiology 28, 7-11.

Ahaduzzaman, M., Hassan, M.M., Alam, M., Islam, S., Uddin, I., 2014. Antimicrobial resistance pattern against *Staphylococcus aureus* in environmental effluents. Research Journal for Veterinary Practitioners 2 (1), 13-16.

Ahlm, C., Alexeyev, O.A., Elgh, F., Aava, B., Wadell, G., Tarnvik, A., Juto, P., Palo, T., 1997. High prevalence of hantavirus antibodies in bank voles (Clethrionomys glareolus) captured in the vicinity of households afflicted with nephropathia epidemica. The American journal of tropical medicine and hygiene 56, 674-678.

Ahmed, T., Acharjee, M., Rahman, M.S., Meghla, M., Jamal, J., Munshi, S.K., Noor, R., 2013. Microbiological study of drinking water: qualitative and quantitative approach. Asian Journal of Microbiology, Biotechnology & Environmental Sciences 15, 23-458.

Ahsan, S., Rahman, S., 2018. Azithromycin Resistance in Clinical Isolates of *Salmonella* enterica Serovars Typhi and Paratyphi in Bangladesh. Microbial Drug Resistance.

Aiken, A.M., Mutuku, I.M., Sabat, A.J., Akkerboom, V., Mwangi, J., Scott, J.A.G., Morpeth, S.C., Friedrich, A.W., Grundmann, H., 2014. Carriage of *Staphylococcus aureus* in Thika Level 5 Hospital, Kenya: a cross-sectional study. Antimicrobial resistance and infection control 3, 22-22.

Akobi, B., Aboderin, O., Sasaki, T., Shittu, A., 2012. Characterization of Staphylococcus aureus isolates from faecal samples of the Straw-Coloured Fruit Bat (Eidolon

helvum) in Obafemi Awolowo University (OAU), Nigeria. BMC microbiology 12 (1), 279-279.
Al Faruq, A., Hassan, M.M., Uddin, M.M., Rahman, M.L., Rakib, T.M., Alam, M., Islam, A., 2016. Prevalence and multidrug resistance pattern of Salmonella isolated from resident wild birds of Bangladesh. International Journal of One Health, 2, 35-41.
Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K.A., Davies, J., Handelsman, J., 2010. Call of the wild: antibiotic resistance genes in natural environments. Nature Reviews Microbiology 8, 251.
Allocati, N., Petrucci, A., Di Giovanni, P., Masulli, M., Di Ilio, C., De Laurenzi, V., 2016. Bat–man disease transmission: zoonotic pathogens from wildlife reservoirs to human populations. Cell death discovery 2, 16048.
Anand, A.A.P., Chattopadhyay, B., Kandula, S., 2012. Isolation and Characterization of Cellulose-Degrading and Xylanolytic Bacteria from the Short-Nosed Fruit Bat Cynopterus sphinx. Acta Chiropterologica 14, 233-239.
Angulo, F.J., Johnson, K.R., Tauxer, R.V., Cohen, M.L., 2000. Origins and consequences of antimicrobial-resistant nontyphoidal Salmonella: implications for the use of fluoroquinolones in food animals. Microbial Drug Resistance 6, 77-83.
Anon, 2016. Bangladesh Meteorological Department. December.
Antunes, P.c., Réu, C., Sousa, J.C., Peixe, L.s., Pestana, N., 2003. Incidence of Salmonella from poultry products and their susceptibility to antimicrobial agents. International Journal of Food Microbiology 82, 97-103.
Apun, K., Kho, K., Chong, Y., Hashimatul, F., Abdullah, M., Rahman, M., Samuel, L., 2011. Detection of *Escherichia coli* O157: H7 in wildlife from disturbed habitats in Sarawak, Malaysia. Research Journal of Microbiology 6, 132.
Arata, A.A., Vaughn, J.B., Newell, K.W., Barth, R.A., Gracian, M., 1968. *Salmonella* and *Shigella* infections in bats in selected areas of Colombia. The American Journal of Tropical Medicine and Hygiene 17, 92-95.
Ateba Ngoa, U., Schaumburg, F., Adegnika, A.A., Kösters, K., Möller, T., Fernandes, J.F., Alabi, A., Issifou, S., Becker, K., Grobusch, M.P., Kremsner, P.G., Lell, B., 2012. Epidemiology and population structure of *Staphylococcus aureus* in various population groups from a rural and semi urban area in Gabon, Central Africa. Acta Tropica 124, 42-47.
Azad, M.A.K., Rahman, Z., Amin, M.N., 2013. A study on Antimicrobial Resistance: recent trend in Armed Forces of Bangladesh. Journal of Armed Forces Medical College, Bangladesh 9, 03-09.
Bai, Y., Urushadze, L., Osikowicz, L., McKee, C., Kuzmin, I., Kandaurov, A., Babuadze, G., Natradze, I., Imnadze, P., Kosoy, M., 2017. Molecular survey of bacterial zoonotic agents in bats from the country of Georgia (Caucasus). PloS one 12, e0171175.
Baldy-Chudzik, K., Mackiewicz, P., Stosik, M., 2008. Phylogenetic background, virulence gene profiles, and genomic diversity in commensal *Escherichia coli* isolated from ten mammal species living in one zoo. Veterinary microbiology 131, 173-184.
Bannerman, T.L., 2007. Staphylococcus, Micrococcus and other catalase-positive cocci. Manual of clinical microbiology, 390-411.

Banskar, S., Bhute, S.S., Suryavanshi, M.V., Punekar, S., Shouche, Y.S., 2016. Microbiome analysis reveals the abundance of bacterial pathogens in *Rousettus leschenaultii* guano. Scientific reports 6, 36948.

Baquero, F., 2001. Low-level antibacterial resistance: a gateway to clinical resistance. Drug Resistance Updates 4, 93-105.

Baquero, F., Martínez, J.-L., Cantón, R., 2008. Antibiotics and antibiotic resistance in water environments. Current opinion in biotechnology 19, 260-265.

Bassett, E.J., Keith, M.S., Armelagos, G.J., Martin, D.L., Villanueva, A.R., 1980. Tetracycline-labeled human bone from ancient Sudanese Nubia (AD 350). Science 209, 1532-1534.

Bates, P.J.J., Harrison, D.L., 2000. Bats of the Indian subcontinent. ETI, University of Amsterdam.

Bauer, A., Kirby, W., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology 45, 493-496.

Bengtsson-Palme, J., 2017. Antibiotic resistance in the food supply chain: where can sequencing and metagenomics aid risk assessment? Current Opinion in Food Science 14, 66-71.

Bennett, P., 2008. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. British journal of pharmacology 153, S347-S357.

Berg, G., Eberl, L., Hartmann, A., 2005. The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. Environmental Microbiology 7, 1673-1685.

Bhatta, D.R., Bangtrakulnonth, A., Tishyadhigama, P., Saroj, S.D., Bandekar, J.R., Hendriksen, R.S., Kapadnis, B.P., 2007. Serotyping, PCR, phage-typing and antibiotic sensitivity testing of Salmonella serovars isolated from urban drinking water supply systems of Nepal. Letters in Applied Microbiology 44 (6), 588-594.

Bilung, L.M., Ling, K.K., Apun, K., Abdullah, M.T., Rahman, M.A., Ming, C.Y., Benjamen, A., Chong, Y.L., Hashim, H.F., 2014. Occurrence of *Escherichia coli* in Wildlife from Different Habitats of Sarawak, Malaysia. Borneo Journal of Resource Science and Technology 4, 19-27.

Boerlin, P., Kuhnert, P., Hüssy, D., Schaellibaum, M., 2003. Methods for identification of Staphylococcus aureus isolates in cases of bovine mastitis. Journal of clinical microbiology 41, 767-771.

Bondarczuk, K., Markowicz, A., Piotrowska-Seget, Z., 2016. The urgent need for risk assessment on the antibiotic resistance spread via sewage sludge land application. Environment international 87, 49-55.

Bonnedahl, J., Drobni, M., Gauthier-Clerc, M., Hernandez, J., Granholm, S., Kayser, Y., Melhus, Å., Kahlmeter, G., Waldenström, J., Johansson, A., 2009. Dissemination of *Escherichia coli* with CTX-M type ESBL between humans and yellow-legged gulls in the south of France. PloS one 4, e5958.

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

Brown, A.E., 2009. Benson´ s. Microbiological applications: laboratory manual in general microbiology, New York, NY: McGraw-Hill higher educational. xiv, 413 p. / 27 cm .. Edition 9a. edition (No. 579 E76).

Brygoo, E., Simond, J., Mayoux, A., 1971. The pathogenic enterobacteria of Pteropus rufus (Megachiroptera) in Madagascar. Comptes rendus des seances de la Societe de biologie et de ses filiales 165, 1793-1795.

Buchholz, U., Bernard, H., Werber, D., Böhmer, M.M., Remschmidt, C., Wilking, H., Deleré, Y., an der Heiden, M., Adlhoch, C., Dreesman, J., 2011. German outbreak of Escherichia coli O104: H4 associated with sprouts. New England Journal of Medicine 365, 1763-1770.

Buxton, A., Fraser, G., 1977. Animal microbiology. Volume 1: immunology, bacteriology, mycology, diseases of fish and laboratory methods. Blackwell Scientific Publications.

Cabal, A., Pereira, M.J., Aguiar, L.M.S., Domínguez, L., Fonseca, C., Álvarez, J., Drexler, J.F., Gortázar, C., 2015. Direct Detection of Escherichia coli Virulence Genes by Real-Time PCR in Fecal Samples from Bats in Brazil. Journal of Wildlife Diseases 51, 942-945.

Cabrita, J., Rodrigues, J., Braganca, F., Morgado, C., Pires, I., Gonçalves, A.P., 1992. Prevalence, biotypes, plasmid profile and antimicrobial resistance of Campylobacter isolated from wild and domestic animals from northeast Portugal. Journal of Applied Bacteriology 73, 279-285.

Calisher, C.H., Childs, J.E., Field, H.E., Holmes, K.V., Schountz, T., 2006. Bats: important reservoir hosts of emerging viruses. Clinical microbiology reviews 19, 531-545.

Caprioli, A., Morabito, S., Brugère, H., Oswald, E., 2005. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. Veterinary research 36, 289-311.

Carrique-Mas, J., Davies, R., 2008. Sampling and bacteriological detection of Salmonella in poultry and poultry premises: a review. Revue scientifique et technique 27, 665.

Carter, G.R., Chengappa, M., Roberts, A.W., Claus, G.W., Rikihisa, Y., 1995. Essentials of veterinary microbiology.

Carter, G.R., Cole Jr, J.R., 2012. Diagnostic procedure in veterinary bacteriology and mycology. Academic Press.

Cassel-Beraud, A., Richard, C., 1988. The aerobic intestinal flora of the microchiropteran bat Chaerephon pumila in Madagascar. Bulletin de la Societe de pathologie exotique et de ses filiales 81, 806-810.

CDC, 2013. National *Salmonella* surveillance annual summary 2009. Centers for Disease Control and Prevention, Atlanta, GA.

Cernicchiaro, N., Pearl, D., McEwen, S., Harpster, L., Homan, H., Linz, G., LeJeune, J., 2012. Association of wild bird density and farm management factors with the prevalence of E. coli O157 in dairy herds in Ohio (2007–2009). Zoonoses and public health 59, 320-329.

Chain, E., Florey, H.W., Gardner, A.D., Heatley, N.G., Jennings, M.A., Orr-Ewing, J., Sanders, A.G., 1940. Penicillin as a chemotherapeutic agent. The lancet 236, 226-228.

Chakravarthy, A., Yeshwanth, H., Kumar, L.V., Kumar, N.P., 2009. Giant Indian fruit bat Pteropus giganteus Brunnich Roost in Karnataka, South India: A case for preservation as a heritage site . Tiger paper (FAO) 36.

Chang, Q., Wang, W., Regev-Yochay, G., Lipsitch, M., Hanage, W.P., 2015. Antibiotics in agriculture and the risk to human health: how worried should we be? Evolutionary applications 8, 240-247.

Chaverri, G., 2006. Aerobic bacterial flora from the digestive tract of the common vampire bat, Desmodus rotundus (Chiroptera: Phyllostomidae). Revista de biologia tropical 54, 717-724.

Chayani, N., Tiwari, S., Sarangi, G., Mallick, B., Mohapatra, A., Paty, B., Das, P., 2009. Role of azithromycin against clinical isolates of family enterobacteriaceae: a comparison of its minimum inhibitory concentration by three different methods. Indian journal of medical microbiology 27, 107.

Cheesbrough, M., 2006. District laboratory practice in tropical countries. Cambridge university press.

Chiou, C.-S., Lauderdale, T.-L., Phung, D.C., Watanabe, H., Kuo, J.-C., Wang, P.-J., Liu, Y.-Y., Liang, S.-Y., Chen, P.-C., 2014. Antimicrobial Resistance in Salmonella enterica Serovar Typhi Isolates from Bangladesh, Indonesia, Taiwan, and Vietnam. Antimicrobial Agents and Chemotherapy 58, 6501-6507.

Cláudio, V.C., Gonzalez, I., Barbosa, G., Rocha, V., Moratelli, R., Rassy, F., 2018. Bacteria richness and antibiotic-resistance in bats from a protected area in the Atlantic Forest of Southeastern Brazil. PloS one 13, e0203411.

Cleveland, A.G., 2009. A Guide to the Mammals of South-east Asia. Journal of Mammalogy 90, 779.

Clewell, D.B., 2014. Antibiotic resistance plasmids in bacteria. eLS, 17 February. John Wiley & Sons, Ltd (Ed.). doi:10.1002/9780470015902.a0001491.pub3.

CLSI, 2007. Performance standards for antimicrobial susceptibility testing, 17th informational supplement. M100–S17. Wayne, PA; Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, .

CLSI, 2008. Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement M100-S18. Wayne, PA: Clinical and Laboratory Standards Institute.

CLSI., 2011. CLSI Performance standards for antimicrobial susceptibility testing. Nineteenth informational supplement CLSI document M100-S21 Wayne, PA: Clinical and Laboratory Standards Institute.

Cockerill, F.R., 2010. Performance standards for antimicrobial susceptibility testing. Approved Standard M100-S20.

Cole, D., Drum, D., Stalknecht, D., White, D.G., Lee, M.D., Ayers, S., Sobsey, M., Maurer, J.J., 2005. Free-living Canada geese and antimicrobial resistance. Emerging infectious diseases 11, 935-938.

Collignon, P., 2015. Antibiotic resistance: are we all doomed? Internal medicine journal 45, 1109-1115.

Corn, J.L., Manning, E.J., Sreevatsan, S., Fischer, J.R., 2005. Isolation of Mycobacterium avium subsp. paratuberculosis from free-ranging birds and mammals on livestock premises. Applied and environmental microbiology 71, 6963-6967.

Costa, D., Poeta, P., Sáenz, Y., Vinué, L., Coelho, A.C., Matos, M., Rojo-Bezares, B., Rodrigues, J., Torres, C., 2008. Mechanisms of Antibiotic Resistance in

Escherichia coli Isolates Recovered from Wild Animals. Microbial Drug Resistance 14, 71-77.
Cowan, S.T., 2003. Cowan and Steel's manual for the identification of medical bacteria. Cambridge university press.
Cox, G., Wright, G.D., 2013. Intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. International Journal of Medical Microbiology 303, 287-292.
Cox, T., Smythe, L., Leung, L.-P., 2005. Flying foxes as carriers of pathogenic Leptospira species. Journal of wildlife diseases 41, 753-757.
Cucarella, C., Tormo, M.Á., Úbeda, C., Trotonda, M.P., Monzón, M., Peris, C., Amorena, B., Lasa, Í., Penadés, J.R., 2004. Role of biofilm-associated protein bap in the pathogenesis of bovine Staphylococcus aureus. Infection and immunity 72, 2177-2185.
D'Costa, V.M., King, C.E., Kalan, L., Morar, M., Sung, W.W., Schwarz, C., Froese, D., Zazula, G., Calmels, F., Debruyne, R., 2011. Antibiotic resistance is ancient. Nature 477, 457.
Daffner, B., 2001. Causes of morbidity and mortality in British bat species and prevalence of selected zoonotic pathogens. Thesis for MSc in Wild Animal Health, University of London.
Daniel, D.S., Ng, Y.K., Chua, E.L., Arumugam, Y., Wong, W.L., Kumaran, J.V., 2013. Isolation and identification of gastrointestinal microbiota from the short-nosed fruit bat Cynopterus brachyotis brachyotis. Microbiological Research 168, 485-496.
Dargatz, D.A., Kopral, C.A., Erdman, M.M., Fedorka-Cray, P.J., 2016. Prevalence and antimicrobial resistance of Salmonella isolated from cattle feces in United States feedlots in 2011. Foodborne pathogens and disease 13, 483-489.
David, M.Z., Daum, R.S., 2010. Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. Clinical microbiology reviews 23, 616-687.
Davies, J., Davies, D., 2010. Origins and evolution of antibiotic resistance. Microbiology and molecular biology reviews 74, 417-433.
Davison, J., 1999. Genetic exchange between bacteria in the environment. Plasmid 42, 73-91.
De Boeck, H., Miwanda, B., Lunguya-Metila, O., Muyembe-Tamfum, J.-J., Stobberingh, E., Glupczynski, Y., Jacobs, J., 2012. ESBL-positive Enterobacteria isolates in drinking water. Emerging infectious diseases 18, 1019.
De Medici, D., Croci, L., Delibato, E., Di Pasquale, S., Filetici, E., Toti, L., 2003. Evaluation of DNA extraction methods for use in combination with SYBR green I real-time PCR to detect Salmonella enterica serotype enteritidis in poultry. Applied and environmental microbiology 69, 3456-3461.
De Oliveira, L.P., Silva, V., Cirqueira, M., 2011. Study of *Staphylococcus aureus* in raw and pasteurized milk consumed in the Reconcavo area of the State of Bahia, Brazil. Journal of Food Processing and Technology 2.
De Sousa, C.P., 2006. *Escherichia coli* as a specialized bacterial pathogen. Rev biol cienc Terra 2, 341-352.
Di Bella, C., Piraino, C., Caracappa, S., Fornasari, L., Violani, C., Zava, B., 2014. Enteric microflora in Italian chiroptera. Journal of Mountain Ecology 7.

Dohoo, I.R., Martin, W., Stryhn, H., 2003. Veterinary epidemiologic research. AVC Incorporated Charlottetown, Canada.

Douglas, W., Waltman, R., Gast, K., Mallinson, E., 1998. Salmonellosis. A laboratory manual for the isolation and identification of avian pathogens. American Association of Avian Pathologists, Kenett Square, 4-14.

Eggert, M., Stüber, E., Heurich, M., Fredriksson-Ahomaa, M., Burgos, Y., Beutin, L., Märtlbauer, E., 2013. Detection and characterization of Shiga toxin-producing Escherichia coli in faeces and lymphatic tissue of free-ranging deer. Epidemiology & Infection 141, 251-259.

Eick, G.N., Jacobs, D.S., Matthee, C.A., 2005. A nuclear DNA phylogenetic perspective on the evolution of echolocation and historical biogeography of extant bats (Chiroptera). Molecular Biology and Evolution 22, 1869-1886.

Epstein, J.H., Field, H.E., Luby, S., Pulliam, J.R., Daszak, P., 2006. Nipah virus: impact, origins, and causes of emergence. Current Infectious Disease Reports 8, 59-65.

Epstein, J.H., Quan, P.-L., Briese, T., Street, C., Jabado, O., Conlan, S., Khan, S.A., Verdugo, D., Hossain, M.J., Hutchison, S.K., 2010. Identification of GBV-D, a novel GB-like flavivirus from old world frugivorous bats (*Pteropus giganteus*) in Bangladesh. PLoS pathogens 6, e1000972.

Everard, C.O., Tota, B., Bassett, D., Ali, C., 1979. Salmonella in wildlife from Trinidad and Grenada, WI. Journal of Wildlife Diseases 15, 213-219.

Ewers, C., Guenther, S., Wieler, L.H., Schierack, P., 2009. Mallard ducks–a waterfowl species with high risk of distributing *Escherichia coli* pathogenic for humans. Environmental Microbiology Reports 1, 510-517.

Ewing, W.H., 1986. Edwards and Ewing's identification of Enterobacteriaceae. Elsevier Science Publishing Co. Inc.

Fair, R.J., Tor, Y., 2014. Antibiotics and bacterial resistance in the 21st century. Perspectives in medicinal chemistry 6, PMC. S14459.

Falkow, S., 1975. Infectious multiple drug resistance. Infectious multiple drug resistance.

Fatema, K., Trisha, T.A., Morshed, A.M.A., Pk, M.M.U., Akter, T., Jannat, B., Datta, S., 2016. An Epidemiological Study of Antibiotic Resistance of Salmonella typhi and Salmonella paratyphi-A from Clinical Samples in Dhaka City. Journal of Applied Life Sciences International, 8(4): 1-5; Article no.JALSI.27965 ISSN: 2394-1103.

Feder, I., Wallace, F.M., Gray, J.T., Fratamico, P., Fedorka-Cray, P.J., Pearce, R.A., Call, J.E., Perrine, R., Luchansky, J.B., 2003. Isolation of Escherichia coli O157: H7 from intact colon fecal samples of swine. Emerging infectious diseases 9, 380.

Finch, R.G., Greenwood, D., Whitley, R.J., Norrby, S.R., 2010. Antibiotic and chemotherapy e-book. Elsevier Health Sciences.

Foley, S., Lynne, A., 2008. Food animal-associated Salmonella challenges: pathogenicity and antimicrobial resistance. Journal of animal science 86, E173-E187.

Forbes, B.A., Sahm, D.F., Weissfeld, A.S., 2002. Diagnostic microbiology. Bailey & Scott1s Diagnostic Microbiology 11, 11-14.

Francois, P., Huyghe, A., Charbonnier, Y., Bento, M., Herzig, S., Topolski, I., Fleury, B., Lew, D., Vaudaux, P., Harbarth, S., 2005. Use of an automated multiple-locus, variable-number tandem repeat-based method for rapid and high-throughput genotyping of Staphylococcus aureus isolates. Journal of clinical microbiology 43, 3346-3355.

Frost, L.S., Leplae, R., Summers, A.O., Toussaint, A., 2005. Mobile genetic elements: the agents of open source evolution. Nature Reviews Microbiology 3, 722.

Gao, J., Stewart, G.C., 2004. Regulatory elements of the *Staphylococcus aureus* protein A (Spa) promoter. Journal of bacteriology 186, 3738-3748.

Garcês, A., Correia, S., Amorim, F., Pereira, J.E., Igrejas, G., Poeta, P., 2017. First report on extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* from European free-tailed bats (*Tadarida teniotis*) in Portugal: A one-health approach of a hidden contamination problem. Journal of Hazardous Materials, Elsevier, pp: 6; Dec 23.

Garrod, L.P., O'Grady, F., 1971. Antibiotic and chemotherapy. Antibiotic and chemotherapy.

Ghaly, T.M., Chow, L., Asher, A.J., Waldron, L.S., Gillings, M.R., 2017. Evolution of class 1 integrons: Mobilization and dispersal via food-borne bacteria. PloS one 12, e0179169.

Ghasemian, A., Peerayeh, S.N., Bakhshi, B., Mirzaee, M., 2014. Biofilm formation in multi-drug resistant Staphylococcus aureus isolates from hospitalized patients. Bangladesh Journal of Infectious Diseases 1, 27-31.

Gherna, R.L., Reddy, C., 2007. Culture preservation. Methods for General and Molecular Microbiology, Third Edition. American Society of Microbiology, 1019-1034.

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

Gopee, N.V., Adesiyun, A.A., Caesar, K., 2000a. A longitudinal study of *Escherichia coli* strains isolated from captive mammals, birds, and reptiles in Trinidad. Journal of Zoo and Wildlife Medicine, 353-360.

Gopee, N.V., Adesiyun, A.A., Caesar, K., 2000b. Retrospective and longitudinal study of Salmonellosis in captive wildlife in Trinidad. Journal of Wildlife diseases 36, 284-293.

Gordon, D.M., Cowling, A., 2003. The distribution and genetic structure of Escherichia coli in Australian vertebrates: host and geographic effects. Microbiology 149, 3575-3586.

Gordon, D.M., FitzGibbon, F., 1999. The distribution of enteric bacteria from Australian mammals: host and geographical effects. Microbiology 145 (Pt 10), 2663-2671.

Goveas, S.W., Miranda, E.C., Seena, S., Sridhar, K.R., 2006. Observations on guano and bolus of Indian flying fox, *Pteropus giganteus*. Current Science 90, 160-162.

Graham, P.L., Lin, S.X., Larson, E.L., 2006. A US population-based survey of Staphylococcus aureus colonization. Annals of internal medicine 144, 318-325.

Graveland, H., Wagenaar, J.A., Bergs, K., Heesterbeek, H., Heederik, D., 2011. Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. PloS one 6, e16830.

Graves, S.R., Kennelly-Merrit, S.A., Tidemann, C.R., Rawlinson, P.A., Harvey, K.J., Thornton, I.W., 1988. Antibiotic-resistance patterns of enteric bacteria of wild mammals on the Krakatau islands and west Java, Indonesia. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 322, 339-353.

Grema, H.A., Geidam, Y.A., Suleiman, A., Gulani, I.A., Birma, R.B., 2015. Multi-Drug Resistant Bacteria Isolated from Fish and Fish Handlers in Maiduguri, Nigeria. International Journal of Animal and Veterinary Advances 7, 49-54.

Guardabassi, L., Dalsgaard, A., 2002. Occurrence and fate of antibiotic resistant bacteria in sewage. Environmental project, (722).

Guenther, S., Bethe, A., Fruth, A., Semmler, T., Ulrich, R.G., Wieler, L.H., Ewers, C., 2012. Frequent combination of antimicrobial multiresistance and extraintestinal pathogenicity in Escherichia coli isolates from urban rats (*Rattus norvegicus*) in Berlin, Germany. PLoS One 7, e50331.

Guenther, S., Ewers, C., Wieler, L.H., 2011. Extended-spectrum beta-lactamases producing E. coli in wildlife, yet another form of environmental pollution? Frontiers in microbiology 2, 246.

Gulraiz, T.L., Javid, A., Hussain, S.M., Shahbaz, M., Daud, S., 2017. Microbial Analysis of Indian Flying Fox (*Pteropus giganteus*) Ejecta Collected from Two Public Parks in Lahore, Pakistan. Pakistan Journal of Zoology 49.

Hafsa Afroz, M., Manjur Hossain, M., Fakruddin, M., Afzal Hossain, Z.U.M.K., 2013. Prevalence and antibiotic susceptibility patterns of bloodstream *Salmonella* infections in a tertiary care hospital, Dhaka. Journal of Medical Sciences 13, 360-366.

Hahn, M.B., Epstein, J.H., Gurley, E.S., Islam, M.S., Luby, S.P., Daszak, P., Patz, J.A., 2014. Roosting behaviour and habitat selection of *Pteropus giganteus* reveal potential links to Nipah virus epidemiology. Journal of Applied Ecology 51, 376-387.

Harish, B., Menezes, G., 2011. Preserving efficacy of chloramphenicol against typhoid fever in a tertiary care hospital, India. In, Regional Health Forum, 93.

Hasan, B., Islam, K., Ahsan, M., Hossain, Z., Rashid, M., Talukder, B., Ahmed, K.U., Olsen, B., Kashem, M.A., 2014. Fecal carriage of multi-drug resistant and extended spectrum β-lactamases producing *E. coli* in household pigeons, Bangladesh. Veterinary microbiology 168, 221-224.

Hasan, B., Sandegren, L., Melhus, Å., Drobni, M., Hernandez, J., Waldenström, J., Alam, M., Olsen, B., 2012. Antimicrobial drug–resistant Escherichia coli in wild birds and free-range poultry, Bangladesh. Emerging infectious diseases 18, 2055.

Hasan, R., Acharjee, M., Noor, R., 2016. Prevalence of vancomycin resistant *Staphylococcus aureus* (VRSA) in methicillin resistant *S. aureus* (MRSA) strains isolated from burn wound infections. Tzu Chi Medical Journal 28, 49-53.

Hassan, M., Ahaduzzaman, M., Alam, M., Bari, M., Amin, K., Faruq, A., 2015. Antimicrobial resistance pattern against E. coli and Salmonella spp. in environmental effluents. International Journal of Natural Sciences 5, 52-58.

Hassan, M.M., Amin, K.B., Ahaduzzaman, M., Alam, M., Al Faruk, M.S., Uddin, I., 2014. Antimicrobial resistance pattern against E. coli and Salmonella in layer poultry. Research Journal for Veterinary Practitioners, 2(2), 30-35.

Hatta, Y., Omatsu, T., Tsuchiaka, S., Katayama, Y., Taniguchi, S., Masangkay, J.S., Puentespina Jr, R., Eres, E., Cosico, E., Une, Y., 2016. Detection of Campylobacter jejuni in rectal swab samples from Rousettus amplexicaudatus in the Philippines. Journal of Veterinary Medical Science 78, 1347-1350.

Hayman, D., Bowen, R., Cryan, P., McCracken, G., O'shea, T., Peel, A., Gilbert, A., Webb, C., Wood, J., 2013. Ecology of zoonotic infectious diseases in bats: current knowledge and future directions. Zoonoses and Public Health 60, 2-21.

Hazeleger, W.C., Jacobs-Reitsma, W.F., Lina, P.H., De Boer, A.G., Bosch, T., Van Hoek, A.H., Beumer, R.R., 2018. Wild, insectivorous bats might be carriers of *Campylobacter spp*. PloS one 13, e0190647.

Heard, D.J., De Young, J.L., Goodyear, B., Ellis, G.A., 1997. Comparative rectal bacterial flora of four species of flying fox (*Pteropus sp*.). Journal of Zoo and Wildlife Medicine, 471-475.

Held, J., Gmeiner, M., Mordmüller, B., Matsiégui, P.-B., Schaer, J., Eckerle, I., Weber, N., Matuschewski, K., Bletz, S., Schaumburg, F., 2017. Bats are rare reservoirs of Staphylococcus aureus complex in Gabon. Infection, Genetics and Evolution 47, 118-120.

Hendriksen, R., 2003. A global Salmonella surveillance and laboratory support project of the World Health Organization: Laboratory Protocols Level 1 Training Course Identification of Salmonella 4th Ed. April 2003.

Henry, R., Galbraith, P., Coutts, S., Prosser, T., Boyce, J., McCarthy, D.T., 2018. What's the risk? Identifying potential human pathogens within grey-headed flying foxes faeces. PloS one 13, e0191301.

Henzler, D., Opitz, H., 1992. The role of mice in the epizootiology of *Salmonella* enteritidis infection on chicken layer farms. Avian diseases, 625-631.

Hilton, A.C., Willis, R.J., Hickie, S.J., 2002. Isolation of *Salmonella* from urban wild brown rats (*Rattus norvegicus*) in the West Midlands, UK. International Journal of Environmental Health Research 12, 163-168.

Hoelzer, K., Switt, A.I.M., Wiedmann, M., 2011. Animal contact as a source of human non-typhoidal salmonellosis. Veterinary research 42, 34.

Holt, J.G., Krieg, N.R., Sneath, P.H., Staley, J.T., Williams, S.T., 1994. Group 19. Regular, nonsporing Gram-positive rods. Bergey's manual of determinative bacteriology, 9th ed. Williams & Wilkins Co., Baltimore, Md, 566.

Hossain, M., Chowdhury, E., Islam, M., Haider, M., Hossain, M., 2006. Avian salmonella infection: isolation and identification of organisms and histopathological study. Bangladesh Journal of Veterinary Medicine 4, 7-12.

Hossain, M., Shamsuzzaman, A., Musa, A., Ahmed, R., Tariquzzaman, M., 2002. Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolated at Mymensingh Medical College Hospital. Bangladesh Medical Review 28, 9-12.

Hughes, V.M., Datta, N., 1983. Conjugative plasmids in bacteria of the 'pre-antibiotic'era. Nature 302, 725.

Hunter, A., 1998. OIE Manual of Standards for Diagnostic Tests and Vaccines, List A and B diseases of mammals, birds and bees, 3rd edition. Tropical Animal Health and Production 30, 158-158.

Hutson, A.M., Mickleburgh, S.P., 2001. Microchiropteran bats: global status survey and conservation action plan. IUCN.

Iovine, R.d.O., Dejuste, C., Miranda, F., Filoni, C., Bueno, M.G., Carvalho, V.M.d., 2015. Isolation of *Escherichia coli* and *Salmonella* spp. from free-ranging wild animals. Brazilian Journal of Microbiology 46, 1257-1263.

Islam, A., Mikolon, A., Mikoleit, M., Ahmed, D., Khan, S.U., Sharker, M.Y., Hossain, M.J., Islam, A., Epstein, J.H., Zeidner, N., 2013. Isolation of *Salmonella* virchow from a fruit bat (*Pteropus giganteus*). EcoHealth 10, 348-351.

Islam, A., Nath, A. D., Islam, K., Islam, S., Chakma, S., Hossain, M. B., & Hassan, M. M.,, 2016. Isolation, identification and antimicrobial resistance profile of *Staphylococcus aureus* in Cockroaches (*Periplaneta americana*). Journal of Advanced Veterinary and Animal Research 3, 221-228.

Islam, M., Alam, M., Choudhury, M., Kobayashi, N., Ahmed, M., 2008. Determination of minimum inhibitory concentration (MIC) of cloxacillin for selected isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) with their antibiogram. Bangladesh Journal of Veterinary Medicine 6, 121-126.

Italia, J.T., Rovira, H.G., Masangkay, J.S., Yoshikawa, Y., Perez, M.T.M., Reyes, A.W.B., Baticados, W.N., 2012. Conventional isolation and polymerase chain reaction assay for detection of Escherichia coli O157: H7 from intestines of philippine bats. Veterinarski arhiv 82, 283-294.

IUCN, B., 2000. Red Book of threatened mammals of Bangladesh. IUCN-The World Conservation Union 294.

Jardine, C.M., Janecko, N., Allan, M., Boerlin, P., Chalmers, G., Kozak, G., McEwen, S.A., Reid-Smith, R.J., 2012. Antimicrobial resistance in *Escherichia coli* isolated from raccoons (Procyon lotor) in southern Ontario, Canada. Applied and environmental microbiology, (AEM), 78, 3873-3879.00705-00712.

Jarzembowski, T., 2002. Commensal aerobic bacterial flora of the gastrointestinal tract of Pipistrellus nathusii (Chiroptera: Vespertilionidae): lack of Escherichia coli in fecal samples. Acta chiropterologica 4, 99-106.

Jay, J.M., Loessner, M.J., Golden, D.A., 2005. *Staphylococcal* gastroenteritis. Modern food microbiology, 545-566.

Jevons, M.P., 1961. "Celbenin"-resistant staphylococci. British medical journal 1, 124.

Johnning, A., Moore, E.R., Svensson-Stadler, L., Shouche, Y.S., Larsson, D.J., Kristiansson, E., 2013. The acquired genetic mechanisms of a multi-resistant bacterium isolated from a treatment plant receiving wastewater from antibiotic production. Applied and environmental microbiology, AEM. 02141-02113.

Jones, Y.E., McLaren, I.M., Wray, C., 2000. Laboratory aspects of *Salmonella*. Salmonella in domestic animals, 393.

Kamins, A.O., Restif, O., Ntiamoa-Baidu, Y., Suu-Ire, R., Hayman, D.T., Cunningham, A.A., Wood, J.L., Rowcliffe, J.M., 2011. Uncovering the fruit bat bushmeat commodity chain and the true extent of fruit bat hunting in Ghana, West Africa. Biological conservation 144, 3000-3008.

Kang, C.-I., Song, J.-H., 2013. Antimicrobial resistance in Asia: current epidemiology and clinical implications. Infection & chemotherapy 45, 22-31.

Kaper, J.B., Nataro, J.P., Mobley, H.L., 2004. Pathogenic escherichia coli. Nature reviews microbiology 2, 123.

Karmi, M., 2013. Prevalence of methicillin-resistant *Staphylococcus aureus* in poultry meat in Qena, Egypt. Veterinary World 6.

Kateete, D.P., Kimani, C.N., Katabazi, F.A., Okeng, A., Okee, M.S., Nanteza, A., Joloba, M.L., Najjuka, F.C., 2010. Identification of *Staphylococcus aureus*: DNase and

Mannitol salt agar improve the efficiency of the tube coagulase test. Annals of clinical microbiology and antimicrobials 9, 23.

Khan, A.M., Jannat, F.T., Ahasan, M.F., 2017. Antimicrobial sensitivity patterns among sepsis patients in intensive care unit of Dhaka Medical College Hospital, Bangladesh. Journal of Molecular Studies and Medicine Research 2, 80-87.

Khan, M.A.R., 1982. Wildlife of Bangladesh: a checklist. University of Dhaka.

Khan, M.A.R., 2001. Status and distribution of bats in Bangladesh with notes on their ecology. Zoos' Print Journal 16, 479-483.

KHMNH, N., Rahman, M., Nasiruddin, K., Akhtar, F., Khan, M., Islam, M., 2005. Antibiotic sensitivity of Escherichia coli isolated from water and its relation with plasmid profile analysis. Pakistan Journal of Biological Sciences 8, 1610-1613.

Kingston, T., Lim, B., Zubaid, A., 2006. Bats of Krau Wildlife Reserve. University Kebangsaan Malaysia. Penerbit (UKM), Bangi 1.

Klite, P., 1965. Intestinal bacterial flora and transit time of three neotropical bat species. Journal of Bacteriology 90, 375-379.

Kloos, W.E., 1999. *Staphylococcus* and *Micrococcus*. Manual of clinical microbiology, 264-282.

Ko, K.S., Lee, J.-Y., Suh, J.Y., Oh, W.S., Peck, K.R., Lee, N.Y., Song, J.-H., 2005. Distribution of major genotypes among methicillin-resistant Staphylococcus aureus clones in Asian countries. Journal of Clinical Microbiology 43, 421-426.

Krawiec, M., Kuczkowski, M., Kruszewicz, A.G., Wieliczko, A., 2015. Prevalence and genetic characteristics of *Salmonella* in free-living birds in Poland. BMC veterinary research 11 (1), 15.

Krieg, N.R., Padgett, P.J., 2011. Phenotypic and physiological characterization methods. Methods in microbiology. Elsevier, 15-60.

Kristin, M., Stephanie, S., Gudrun, W., 2011. Diseases in free-ranging bats from Germany. BMC Veterinary Research.

Krystufek, B., 2009. Indian flying fox Pteropus giganteus colony in Peradeniya Botanical Gardens, Sri Lanka. Hystrix, the Italian Journal of Mammalogy 20.

Kumar, R., Prasad, A., 2010. Detection of E. coli and Staphylococcus in milk and milk products in and around Pantnagar. Veterinary World 3, 495.

Kuo, C.W., Hao Huang, K., Hsu, B.M., Tsai, H.L., Tseng, S.F., Shen, T.Y., Kao, P.M., Shen, S.M., Chen, J.S., 2013. Isolation and identification of Salmonella spp. in environmental water by molecular technology in Taiwan. In, EGU General Assembly Conference Abstracts.

Langton, K.P., Henderson, P.J., Herbert, R.B., 2005. Antibiotic resistance: multidrug efflux proteins, a common transport mechanism? Natural product reports 22, 439-451.

Lee, M., Lee, H., Ryu, P., 2001. Public health risks: Chemical and antibiotic residues-review. Asian-Australasian Journal of Animal Sciences 14, 402-413.

Leon-Velarde, C.G., Cai, H.Y., Larkin, C., Bell-Rogers, P., Stevens, R.W.C., Odumeru, J.A., 2004. Evaluation of methods for the identification of Salmonella enterica serotype Typhimurium DT104 from poultry environmental samples. Journal of microbiological methods 58, 79-86.

Leung, D.T., Das, S.K., Malek, M., Ahmed, D., Khanam, F., Qadri, F., Faruque, A., Ryan, E.T., 2013. Non-typhoidal Salmonella gastroenteritis at a diarrheal hospital in

Dhaka, Bangladesh, 1996–2011. The American journal of tropical medicine and hygiene 88, 661-669.

Lévesque, S., Fournier, E., Carrier, N., Frost, E., Arbeit, R.D., Michaud, S., 2013. Campylobacteriosis in Urban versus Rural Areas: A Case-Case Study Integrated with Molecular Typing to Validate Risk Factors and to Attribute Sources of Infection. PLOS ONE 8, e83731.

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

Lilly, A.A., Mehlman, P.T., Doran, D., 2002. Intestinal parasites in gorillas, chimpanzees, and humans at Mondika research site, Dzanga-Ndoki National Park, Central African Republic. International Journal of Primatology 23, 555-573.

Lin, J., Biyela, P., 2005. Convergent acquisition of antibiotic resistance determinants amongst the Enterobacteriaceae isolates of the Mhlathuze River, KwaZulu-Natal (RSA). Water Sa 31, 257-260.

Lipsitch, M., Singer, R.S., Levin, B.R., 2002. Antibiotics in agriculture: When is it time to close the barn door? Proceedings of the National Academy of Sciences 99, 5752-5754.

Literak, I., Dolejska, M., Radimersky, T., Klimes, J., Friedman, M., Aarestrup, F.M., Hasman, H., Cizek, A., 2010. Antimicrobial-resistant faecal *Escherichia coli* in wild mammals in central Europe: multiresistant *Escherichia coli* producing extended-spectrum beta-lactamases in wild boars. Journal of applied microbiology 108, 1702-1711.

Livermore, D.M., 2009. Has the era of untreatable infections arrived? Journal of Antimicrobial Chemotherapy 64, i29-i36.

Loncarevic, S., Jørgensen, H., Løvseth, A., Mathisen, T., Rørvik, L., 2005. Diversity of Staphylococcus aureus enterotoxin types within single samples of raw milk and raw milk products. Journal of applied microbiology 98, 344-350.

Luby, S.P., Hossain, M.J., Gurley, E.S., Ahmed, B.-N., Banu, S., Khan, S.U., Homaira, N., Rota, P.A., Rollin, P.E., Comer, J.A., 2009. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001–2007. Emerging infectious diseases 15, 1229.

Luis, A.D., Hayman, D.T., O'Shea, T.J., Cryan, P.M., Gilbert, A.T., Pulliam, J.R., Mills, J.N., Timonin, M.E., Willis, C.K., Cunningham, A.A., 2013. A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? Proceedings of the Royal Society B: Biological Sciences 280, 20122753.

Lwanga, S.K., Lemeshow, S., Organization, W.H., 1991. Sample size determination in health studies: a practical manual / S. K. Lwanga and S. Lemeshow. Geneva : World Health Organization, p: 80

Mac Faddin, J.F., 1976. Biochemical tests for identification of medical bacteria. Williams & Wilkins Co.

Mahmood-ul-Hassan, M., Nameer, P., 2006. Diversity, role and threats to the survival of bats in Pakistan. Journal of Animal and Plant Sciences 16, 38-42.

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

Mahmud, T., Hassan, M.M., Alam, M., Khan, M.M., Bari, M.S., Islam, A., 2013. Prevalence and multidrug-resistant pattern of Salmonella from the eggs and egg-storing trays of retail markets of Bangladesh. population. International Journal of One Health, 2, 7-12.

Makovec, J.A., Ruegg, D.P.L., 2003. Antimicrobial resistance of bacteria isolated from dairy cow milk samples submitted for bacterial culture: 8,905 samples (1994–2001). Journal of the American Veterinary Medical Association 222, 1582-1589.

Mannan, A., Shohel, M., Rajia, S., Mahmud, N.U., Kabir, S., Hasan, I., 2014. A cross sectional study on antibiotic resistance pattern of Salmonella typhi clinical isolates from Bangladesh. Asian Pacific journal of tropical biomedicine 4, 306-311.

March, s.B., Ratnam, s., 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157: H7 associated with hemorrhagic colitis. Journal of clinical microbiology 23, 869-872.

Marshall, B.M., Levy, S.B., 2011. Food animals and antimicrobials: impacts on human health. Clinical microbiology reviews 24, 718-733.

Martínez, J.L., 2008. Antibiotics and antibiotic resistance genes in natural environments. Science 321, 365-367.

Martínez, J.L., 2012. Natural antibiotic resistance and contamination by antibiotic resistance determinants: the two ages in the evolution of resistance to antimicrobials. Frontiers in microbiology 3, 1.

Matuschek, E., Brown, D.F., Kahlmeter, G., 2014. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clinical Microbiology and Infection 20, O255-O266.

McCormick, J.B., 1998. Epidemiology of emerging/re-emerging antimicrobial-resistant bacterial pathogens. Current opinion in microbiology 1, 125-129.

Mead, P.S., Griffin, P.M., 1998. *Escherichia coli* O157: H7. The Lancet 352, 1207-1212.

Mediavilla, J.R., Chen, L., Uhlemann, A.-C., Hanson, B.M., Rosenthal, M., Stanak, K., Koll, B., Fries, B.C., Armellino, D., Schilling, M.E., 2012. Methicillin-susceptible *Staphylococcus* aureus ST398, New York and New Jersey, USA. Emerging infectious diseases 18, 700.

Mellon, M., Benbrook, C., Benbrook, K.L., 2001. Hogging it. Estimates of antimicrobial abuse in livestock, 7-9.

Merchant, I., Packer, R., 1967. The genus corynebacterium. Veterinary Bacteriology and Virology. The Iowa State University Press, IA, USA, 425-440.

Michael, G.B., Simoneti, R., Costa, M.d., Cardoso, M., 2003. Comparison of different selective enrichment steps to isolate *Salmonella* sp. from feces of finishing swine. Brazilian Journal of Microbiology 34, 138-142.

Mickleburgh, S., Waylen, K., Racey, P., 2009. Bats as bushmeat: a global review. Oryx 43, 217-234.

Mickleburgh, S.P., Hutson, A.M., Racey, P.A., 2002. A review of the global conservation status of bats. Oryx 36, 18-34.

Millán, J., Aduriz, G., Moreno, B., Juste, R., Barral, M., 2004. *Salmonella* isolates from wild birds and mammals in the Basque Country (Spain). Revue Scientifique et Technique-Office International des Epizooties 23, 905-912.

Mitsuhashi, S., 1993. Drug resistance in bacteria: history, genetics and biochemistry. Journal of international medical research 21, 1-14.
Mølbak, K., 2004. Spread of resistant bacteria and resistance genes from animals to humans–the public health consequences. Journal of Veterinary Medicine, Series B 51, 364-369.
Molur, S., Srinivasulu, C., Bates, P., Francis, C., 2012. *Pteropus giganteus*. IUCN.
Moratelli, R., Calisher, C.H., 2015. Bats and zoonotic viruses: can we confidently link bats with emerging deadly viruses? Memórias do Instituto Oswaldo Cruz 110, 1-22.
Moreno, G., Lopes, C., Seabra, E., Pavan, C., Correa, A., 1975. Bacteriological study of the intestinal flora of bats (Desmodus rotundus)(author's transl). Arquivos do Instituto Biologico 42, 229-232.
Morrison, M.L., Block, W.M., Strickland, M.D., Collier, B.A., Peterson, M.J., 2008. Wildlife study design. Springer Science & Business Media, Springer, May 25
Mühldorfer, K., 2013. Bats and bacterial pathogens: a review. Zoonoses and Public Health 60, 93-103.
Mühldorfer, K., Speck, S., Kurth, A., Lesnik, R., Freuling, C., Müller, T., Kramer-Schadt, S., Wibbelt, G., 2011. Diseases and causes of death in European bats: dynamics in disease susceptibility and infection rates. PLoS One 6, e29773.
Muscarella, R., Fleming, T.H., 2007. The role of frugivorous bats in tropical forest succession. Biological reviews 82, 573-590.
Musgrove, M., Jones, D., Northcutt, J., Cox, N., Harrison, M., Fedorka-Cray, P., Ladely, S., 2006. Antimicrobial resistance in *Salmonella* and *Escherichia coli* isolated from commercial shell eggs. Poultry science 85, 1665-1669.
Muthaiyan, A., Limayem, A., Ricke, S.C., 2011. Antimicrobial strategies for limiting bacterial contaminants in fuel bioethanol fermentations. Progress in Energy and Combustion Science 37, 351-370.
Nataro, J.P., Kaper, J.B., 1998. Diarrheagenic escherichia coli. Clinical microbiology reviews 11, 142-201.
Nelson, M.L., Dinardo, A., Hochberg, J., Armelagos, G.J., 2010. Brief communication: mass spectroscopic characterization of tetracycline in the skeletal remains of an ancient population from Sudanese Nubia 350–550 CE. American journal of physical anthropology 143, 151-154.
Nesa, M., Khan, M., Alam, M., 2011. Isolation, identification and characterization of *Salmonella* serovars from diarrhoeic stool samples of human. Bangl J Vet Med 9, 85-93.
Ngoa, U.A., Schaumburg, F., Adegnika, A.A., Kösters, K., Möller, T., Fernandes, J.F., Alabi, A., Issifou, S., Becker, K., Grobusch, M.P., 2012. Epidemiology and population structure of *Staphylococcus aureus* in various population groups from a rural and semi urban area in Gabon, Central Africa. Acta tropica 124, 42-47.
Nizeyi, J.B., Mwebe, R., Nanteza, A., Cranfield, M.R., Kalema, G.R., Graczyk, T.K., 1999. Cryptosporidium sp. and Giardia sp. infections in mountain gorillas (Gorilla gorilla beringei) of the Bwindi Impenetrable National Park, Uganda. The Journal of parasitology, 1084-1088.
Nkogwe, C., Raletobana, J., Stewart-Johnson, A., Suepaul, S., Adesiyun, A., 2011. Frequency of detection of *Escherichia coli*, *Salmonella* spp., and *Campylobacter*

spp. in the faeces of wild rats (*Rattus* spp.) in Trinidad and Tobago. Veterinary medicine international 2011.

Nowak, K., Fahr, J., Weber, N., Lübke-Becker, A., Semmler, T., Weiss, S., Mombouli, J.-V., Wieler, L.H., Guenther, S., Leendertz, F.H., 2017. Highly diverse and antimicrobial susceptible Escherichia coli display a naïve bacterial population in fruit bats from the Republic of Congo. PloS one 12, e0178146.

O'Brien, T.F., 1987. Resistance of bacteria to antibacterial agents: report of Task Force 2. Reviews of infectious diseases 9, S244-S260.

O'shea, T.J., Cryan, P.M., Cunningham, A.A., Fooks, A.R., Hayman, D.T., Luis, A.D., Peel, A.J., Plowright, R.K., Wood, J.L., 2014. Bat flight and zoonotic viruses. Emerging infectious diseases 20, 741.

O'Shea, T.J., Neubaum, D.J., Neubaum, M.A., Cryan, P.M., Ellison, L.E., Stanley, T.R., Rupprecht, C.E., Pape, W.J., Bowen, R.A., 2011. Bat ecology and public health surveillance for rabies in an urbanizing region of Colorado. Urban Ecosystems 14, 665-697.

Obi, T., Chibana, M., Taira, C., Nakayama, A., Miyazaki, K., Takase, K., Nakamura, I., Miyamoto, A., Kawamoto, Y., 2014. Antimicrobial Susceptibility in Enterobacteriaceae Recovered from Okinawa Least Horseshoe Bat Rhinolophus pumilus. Wildlife Biology 20, 64-66.

Okeke, I.N., Klugman, K.P., Bhutta, Z.A., Duse, A.G., Jenkins, P., O'Brien, T.F., Pablos-Mendez, A., Laxminarayan, R., 2005. Antimicrobial resistance in developing countries. Part II: strategies for containment. The Lancet infectious diseases 5, 568-580.

Olalekan, A. O., Schaumburg, F., Nurjadi, D., Dike, A. E., Ojurongbe, O., Kolawole, D. O., & Zanger, P. (2012). Clonal expansion accounts for an excess of antimicrobial resistance in Staphylococcus aureus colonising HIV-positive individuals in Lagos, Nigeria. International journal of antimicrobial agents, 40(3), 268-272.

Olatimehin, A., Shittu, A.O., Onwugamba, F.C., Mellmann, A., Becker, K., Schaumburg, F., 2018. Staphylococcus aureus Complex in the Straw-Colored Fruit Bat (*Eidolon helvum*) in Nigeria. Frontiers in microbiology 9, 162-162.

Oluduro, A.O., 2012. Antibiotic-resistant commensal Escherichia coli in faecal droplets from bats and poultry in Nigeria. Veterinaria Italiana, 48 (3), 297-308.

Openshaw, J.J., Hegde, S., Sazzad, H.M., Khan, S.U., Hossain, M.J., Epstein, J.H., Daszak, P., Gurley, E.S., Luby, S.P., 2017. Bat hunting and bat–human interactions in Bangladeshi villages: Implications for zoonotic disease transmission and bat conservation. Transboundary and emerging diseases 64, 1287-1293.

Osterblad, M., Norrdahl, K., Korpimaki, E., Huovinen, P., 2001. Antibiotic resistance. How wild are wild mammals? Nature 409, 37-38.

Pacha, R., Clark, G., Williams, E., Carter, A., Scheffelmaier, J., Debusschere, P., 1987. Small rodents and other mammals associated with mountain meadows as reservoirs of *Giardia spp*. and *Campylobacter spp*. Applied and environmental microbiology 53, 1574-1579.

Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J., Griffin, P.M., 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerging infectious diseases 19, 407.

Pal, C., Bengtsson-Palme, J., Kristiansson, E., Larsson, D.J., 2016. The structure and diversity of human, animal and environmental resistomes. Microbiome 4, 54.
Pao, S., Patel, D., Kalantari, A., Tritschler, J.P., Wildeus, S., Sayre, B.L., 2005. Detection of Salmonella strains and Escherichia coli O157: H7 in feces of small ruminants and their isolation with various media. Applied and environmental microbiology 71, 2158-2161.
Park, S.-H., Ryu, S., Kang, D.-H., 2012. Development of an improved selective and differential medium for the isolation of *Salmonella* spp. Journal of clinical microbiology, Journal of clinical microbiology (JCM)-01228-01212.
Parveen, S., Ahmed, M.S.U., Nasreen, T., 2008. Microbial Contamination of Water in Around Dhaka City. Bangladesh Journal of Scientific and Industrial Research 43, 4.
Pathak, S., Gaur, A., Bhattacherjee, J., 1993. Distribution and antibiotic resistance among aerobic heterotrophic bacteria from rivers in relation to pollution. Journal of Environmental Science & Health Part A 28, 73-87.
Pedersen, K., Clark, L., Andelt, W.F., Salman, M., 2006. Prevalence of Shiga toxin–producing Escherichia coli and Salmonella enterica in rock pigeons captured in Fort collins, Colorado. Journal of wildlife diseases 42, 46-55.
Pelisser, M.R., Klein, C.S., Ascoli, K.R., Zotti, T.R., Arisi, A.C.M., 2009. Ocurrence of Staphylococcus aureus and multiplex pcr detection of classic enterotoxin genes in cheese and meat products. Brazilian Journal of Microbiology 40, 145-148.
Piddock, L.J., 1996. Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic-resistant bacteria that infect man and compromise antimicrobial chemotherapy? Journal of Antimicrobial Chemotherapy 38, 1-3.
Pinus, M., Muller, H.E., 1980. [Enterobacteria of bats (Chiroptera) (author's transl)]. Zentralblatt fur Bakteriologie. 1. Abt. Originale. A: Medizinische Mikrobiologie, Infektionskrankheiten und Parasitologie 247, 315-322.
Plowright, R.K., Eby, P., Hudson, P.J., Smith, I.L., Westcott, D., Bryden, W.L., Middleton, D., Reid, P.A., McFarlane, R.A., Martin, G., 2015. Ecological dynamics of emerging bat virus spillover. Proceedings of the Royal Society B: Biological Sciences, Jan 7; 282 (1798), 20142124.
Plowright, R.K., Foley, P., Field, H.E., Dobson, A.P., Foley, J.E., Eby, P., Daszak, P., 2011. Urban habituation, ecological connectivity and epidemic dampening: the emergence of Hendra virus from flying foxes (Pteropus spp.). Proceedings of the Royal Society of London B: Biological Sciences 278, 3703-3712.
Pongdee, T., Li, J.T., 2018. evaluation and Management of Penicillin Allergy. In, Mayo Clinic Proceedings, 101-107.
Popoff, M., Le Minor, L., 2001. Antigenic formulas of the Salmonella serovars, WHO Collaborating Centre for Reference and Research on Salmonella. World Health Organ.
Prakash, P., Gupta, N., 2005. Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. Indian journal of physiology and pharmacology 49, 125.
Prem Anand, A.A., Sripathi, K., 2004. Digestion of cellulose and xylan by symbiotic bacteria in the intestine of the Indian flying fox (*Pteropus giganteus*). Comparative

biochemistry and physiology. Part A, Molecular & integrative physiology 139 (1), 65-69.
Putturu, R., Thirtham, M., Eevuri, T.R., 2013. Antimicrobial sensitivity and resistance of Salmonella Enteritidis isolated from natural samples. Veterinary world 6, 185-188.
Radhouani, H., Poeta, P., Goncalves, A., Pacheco, R., Sargo, R., Igrejas, G., 2012. Wild birds as biological indicators of environmental pollution: antimicrobial resistance patterns of Escherichia coli and enterococci isolated from common buzzards (Buteo buteo). Journal of medical microbiology 61, 837-843.
Radhouani, H., Silva, N., Poeta, P., Torres, C., Correia, S., Igrejas, G., 2014. Potential impact of antimicrobial resistance in wildlife, environment and human health. Frontiers in microbiology 5, 23.
Rashid, M., Rakib, M.M., Hasan, B., 2015. Antimicrobial-resistant and ESBL-producing Escherichia coli in different ecological niches in Bangladesh. Infection ecology & epidemiology 5, 26712-26712.
Reyes, Guerrero Rovira, H., Saavedra Masangkay, J., Juan Ramirez, T., Yoshikawa, Y., Navarra Baticados, W., 2011. Polymerase chain reaction assay and conventional isolation of Salmonella spp. from Philippine bats. Acta Scientiae Veterinariae 39.
Riley, L.W., Remis, R.S., Helgerson, S.D., McGee, H.B., Wells, J.G., Davis, B.R., Hebert, R.J., Olcott, E.S., Johnson, L.M., Hargrett, N.T., 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. New England Journal of Medicine 308, 681-685.
Roberson, J., Fox, L., Hancock, D., Besser, T., 1992. Evaluation of methods for differentiation of coagulase-positive staphylococci. Journal of Clinical Microbiology 30, 3217-3219.
Roess, A.A., Winch, P.J., Ali, N.A., Akhter, A., Afroz, D., El Arifeen, S., Darmstadt, G.L., Baqui, A.H., Group, B.P.S., 2013. Animal husbandry practices in rural Bangladesh: potential risk factors for antimicrobial drug resistance and emerging diseases. The American journal of tropical medicine and hygiene 89, 965-970.
Rohinishree, Y.S., Negi, P.S., 2011. Detection, identification and characterization of staphylococci in street vend foods. In, Food Nutrition Science, Vol. 2, pp. 304-313.
Rowe, B., Ward, L.R., Threlfall, E.J., 1997. Multidrug-resistant *Salmonella* typhi: a worldwide epidemic. Clinical Infectious Diseases 24, S106-S109.
Rózalska, B., Radzicki, G., Sadowska, B., Markowski, J., Rudnicka, W., 1998. Aerobic microflora of Myotis myotis [Borkhausen, 1797] and Barbastella barbastellus [Schreber, 1774]. Bulletin of the Polish Academy of Sciences. Biological Sciences 46.
Rushdy, A.A., Salama, M.S., Othman, A.S., 2007. Detection of methicillin/oxacillin resistant *Staphylococcus aureus* isolated from some clinical hospitals in Cairo using Meca/Nuc genes and antibiotic susceptibility profile. Int Journal of Agriculture Biology 9, 800.
Saga, T., Yamaguchi, K., 2009. History of antimicrobial agents and resistant bacteria. Japan Medical Association — Journal, 52 (2), pp:103-108.
Saleem, M., Arshad, A., Qayyum, M., 2017. Changing Trends in Antibiogram and Molecular Analysis of Quinolone Resistant Salmonella typhi Isolates in Pakistan. journal of infectious diseases and treatment, 3, 1.

Saleha, A., Zunita, Z., 2010. Methicillin resistant Staphylococcus aureus (MRSA): An emerging veterinary and zoonotic pathogen of public health concern and some studies in Malaysia. Journal of Animal and Veterinary Advances 9, 1094-1098.
Sanchez, S., Hofacre, C.L., Lee, M.D., Maurer, J.J., Doyle, M.P., 2002. Animal sources of salmonellosis in humans. Journal of the American Veterinary Medical Association 221, 492-497.
Santiviago, C.A., Toro, C.S., Bucarey, S.A., Mora, G.C., 2001. A chromosomal region surrounding the ompD porin gene marks a genetic difference between Salmonella typhi and the majority of *Salmonella* serovars. Microbiology 147, 1897-1907.
Sarker, S.U., Sarker, N.J., 1988. Wildlife of Bangladesh: A Systematic List with Status, Distribution, and Habitat. SU Sarker.
Sarmah, A.K., Meyer, M.T., Boxall, A.B., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65, 725-759.
Sasaki, Y., Goshima, T., Mori, T., Murakami, M., Haruna, M., Ito, K., Yamada, Y., 2013. Prevalence and antimicrobial susceptibility of foodborne bacteria in wild boars (Sus scrofa) and wild deer (*Cervus nippon*) in Japan. Foodborne pathogens and disease 10, 985-991.
Sayah, R.S., Kaneene, J.B., Johnson, Y., Miller, R., 2005. Patterns of antimicrobial resistance observed in Escherichia coli isolates obtained from domestic-and wild-animal fecal samples, human septage, and surface water. Applied and environmental microbiology 71, 1394-1404.
Schaumburg, F., Alabi, A., Peters, G., Becker, K., 2014. New epidemiology of S taphylococcus aureus infection in A frica. Clinical Microbiology and Infection 20, 589-596.
Schaumburg, F., Pauly, M., Anoh, E., Mossoun, A., Wiersma, L., Schubert, G., Flammen, A., Alabi, A.S., Muyembe-Tamfum, J.-J., Grobusch, M.P., 2015. Staphylococcus aureus complex from animals and humans in three remote African regions. Clinical Microbiology and Infection 21, 345. e341-345. e348.
Schipper, J., Chanson, J.S., Chiozza, F., Cox, N.A., Hoffmann, M., Katariya, V., Lamoreux, J., Rodrigues, A.S., Stuart, S.N., Temple, H.J., 2008. The status of the world's land and marine mammals: diversity, threat, and knowledge. Science 322, 225-230.
Schönenbrücher, V., Mallinson, E.T., Bülte, M., 2008. A comparison of standard cultural methods for the detection of foodborne Salmonella species including three new chromogenic plating media. International journal of food microbiology 123, 61-66.
Seguin, B., Boucaud-Maitre, Y., Quenin, P., Lorgue, G., 1986. Epidemiologic evaluation of a sample of 91 rats (*Rattus norvegicus*) captured in the sewers of Lyon. Zentralblatt fur Bakteriologie, Mikrobiologie, und Hygiene. Series A, Medical microbiology, infectious diseases, virology, parasitology 261, 539-546.
Seidman, J.C., Anitha K, P., Kanungo, R., Bourgeois, A.L., Coles, C.L., 2009. Risk factors for antibiotic-resistant E. coli in children in a rural area. Epidemiology and infection 137, 879-888.
Seltzer, C.E., Ndangalasi, H.J., Cordeiro, N.J., 2013. Seed dispersal in the dark: shedding light on the role of fruit bats in Africa. Biotropica 45, 450-456.

Shanahan, P., Karamat, K., Thomson, C., Amyes, S., 2000. Characterization of multi-drug resistant Salmonella typhi isolated from Pakistan. Epidemiology & Infection 124, 9-16.

Sherley, M., Gordon, D.M., Collignon, P.J., 2000. Variations in antibiotic resistance profile in Enterobacteriaceae isolated from wild Australian mammals. Environmental Microbiology 2, 620-631.

Shoemaker, N., Vlamakis, H., Hayes, K., Salyers, A., 2001. Evidence for Extensive Resistance Gene Transfer amongBacteroides spp. and among Bacteroides and Other Genera in the Human Colon. Applied and environmental microbiology 67, 561-568.

Sigirci, B.D., Celik, B., Kahraman, B., Bagcigil, A.F., Ak, S., 2018. Tetracycline Resistance of Enterobacteriaceae Isolated From Feces of Synanthropic Birds. Journal of Exotic Pet Medicine.

Silley, P., 2012. Susceptibility testing methods, resistance and breakpoints: what do these terms really mean? Revue Scientifique et Technique-OIE 31, 33.

Simjee, S., Silley, P., Werling, H., Bywater, R., 2007. Potential confusion regarding the term 'resistance'in epidemiological surveys. Journal of antimicrobial chemotherapy 61, 228-229.

Simmons, N.B., 2005. Order chiroptera. Mammal species of the world: a taxonomic and geographic reference, 312-529.

Singh, L., Cariappa, M.P., 2016. Blood culture isolates and antibiogram of Salmonella: Experience of a tertiary care hospital. Medical Journal, Armed Forces India 72, 281-284.

Singh, P., Prakash, A., 2008. Isolation of Escherichia coli, Staphylococcus aureus and Listeria monocytogenes from milk products sold under market conditions at Agra region. Acta Agriculturae Slovenica 92, 83-88.

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

Smythe, L.D., Field, H.E., Barnett, L.J., Smith, C., Dohnt, M.F., Symonds, M.L., Moore, M.R., Rolfe, P., 2002. Leptospiral antibodies in flying foxes in Australia. Journal of wildlife diseases 38, 182-186.

Snyder, L., Champness, W., Champness, W., 2013. Molecular genetics of bacteria. American Society of Microbiology, Washington DC. .

So, A.D., Shah, T.A., Roach, S., Ling Chee, Y., Nachman, K.E., 2015. An integrated systems approach is needed to ensure the sustainability of antibiotic effectiveness for both humans and animals. The Journal of Law, Medicine & Ethics 43, 38-45.

Soria, M.A., Bueno, D.J., 2016. Culture based methods to detect Salmonella from different poultry products. Food microbiology: Fundamentals, challenges and health implications, 57-86.

Sørum, H., Sunde, M., 2001. Resistance to antibiotics in the normal flora of animals. Veterinary research 32, 227-241.

Souza, V., Rocha, M., Valera, A., Eguiarte, L.E., 1999. Genetic structure of natural populations of Escherichia coli in wild hosts on different continents. Applied and environmental microbiology 65, 3373-3385.

Spellberg, B., Hansen, G.R., Kar, A., Cordova, C.D., Price, L.B., Johnson, J.R., 2016. Antibiotic resistance in humans and animals. National Academy of Medicine.

Srinivasulu, C., Racey, P.A., Mistry, S., 2010. A key to the bats (Mammalia: Chiroptera) of South Asia. Journal of Threatened Taxa 2, 1001-1076.

Stanton, T.B., 2013. A call for antibiotic alternatives research. Trends in Microbiology 21, 111-113.

Stedt, J., Bonnedahl, J., Hernandez, J., Waldenström, J., McMahon, B.J., Tolf, C., Olsen, B., Drobni, M., 2015. Carriage of CTX-M type extended spectrum β-lactamases (ESBLs) in gulls across Europe. Acta Veterinaria Scandinavica 57, 74.

Streicker, D.G., Allgeier, J.E., 2016. Foraging choices of vampire bats in diverse landscapes: potential implications for land-use change and disease transmission. Journal of applied ecology 53, 1280-1288.

Sudhakaran, M., Doss, P.S., 2012. Food and foraging preferences of three pteropodid bats in southern India. Journal of Threatened Taxa 4, pp: 2295-2303.

Suman, M.-u.A., Siddique, M.A., Shamsuzzaman, S.M., Khandakar, A.R., Khondaker, F.A., Sumi, S.A., Jahan, R., 2017. Detection Of Mutated gyrA Gene From Nalidixic Acid Resistant *Salmonella Typhi* And Paratyphi A Isolated From Enteric Fever Patients In A Tertiary Care Hospital Of Bangladesh. 2017 10, 5.

Talukdar, P.K., Rahman, M., Rahman, M., Nabi, A., Islam, Z., Hoque, M.M., Endtz, H.P., Islam, M.A., 2013. Antimicrobial resistance, virulence factors and genetic diversity of *Escherichia coli* isolates from household water supply in Dhaka, Bangladesh. PloS one 8, e61090-e61090.

Tang, Z.H., Cao, M., Sheng, L.X., Ma, X.F., Walsh, A., Zhang, S.Y., 2008. Seed dispersal of Morus macroura (Moraceae) by two frugivorous bats in Xishuangbanna, SW China. Biotropica 40, 127-131.

Tanwar, J., Das, S., Fatima, Z., Hameed, S., 2014. Multidrug resistance: an emerging crisis. Interdisciplinary perspectives on infectious diseases 2014.

Tanzin, T., KHMNH, N., Zahan, M.N., Parvej, M.S., Zesmin, K., Rahman, M.T., 2016. Antibiotic resistance profile of bacteria isolated from raw milk samples of cattle and buffaloes. Journal of Advanced Veterinary and Animal Research 3, 62-67.

Tenaillon, O., Skurnik, D., Picard, B., Denamur, E., 2010. The population genetics of commensal *Escherichia coli*. Nature Reviews Microbiology 8, 207.

Thaker, H., Brahmbhatt, M., Nayak, J., Thaker, H.C., 2013. Isolation and identification of Staphylococcus aureus from milk and milk products and their drug resistance patterns in Anand, Gujarat. Veterinary World 6, 10-13.

Thaller, M.C., Migliore, L., Marquez, C., Tapia, W., Cedeño, V., Rossolini, G.M., Gentile, G., 2010. Tracking acquired antibiotic resistance in commensal bacteria of Galapagos land iguanas: no man, no resistance. PloS one 5, e8989.

Thapa, S., 2014. A checklist of mammals of Nepal. Journal of Threatened Taxa 6, 6061-6072.

Thrusfield, M., Christley, R., 2005. Veterinary epidemiology. Wiley Online Library, Oxford: Blackwell science.

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

Toma, A., Deyno, S., 2015. Overview on Mechanisms of Antibacterial Resistance. Indian Journal of Research in Pharmacy and Biotechnology, 2 (1), 27.

Tuohy, M.J., Procop, G.W., Washington, J.A., 2000. Antimicrobial susceptibility of Abiotrophia adiacens and Abiotrophia defectiva. Diagnostic microbiology and infectious disease 38, 189-191.

Uchil, R.R., Kohli, G.S., Katekhaye, V.M., Swami, O.C., 2014. Strategies to combat antimicrobial resistance. Journal of clinical and diagnostic research : Journal of Clinical and Diagnostic Research for doctors, 8 (7), ME01-ME04.

Udikovic-Kolic, N., Wichmann, F., Broderick, N.A., Handelsman, J., 2014. Bloom of resident antibiotic-resistant bacteria in soil following manure fertilization. Proceedings of the National Academy of Sciences 111, 15202-15207.

Union, I., 2014. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. Brussels.

Uzzau, S., Brown, D.J., Wallis, T., Rubino, S., Leori, G., Bernard, S., Casadesús, J., Platt, D.J., Olsen, J.E., 2000. Host adapted serotypes of *Salmonella* enterica. Epidemiology & Infection 125, 229-255.

Van Cleef, B.A., Monnet, D.L., Voss, A., Krziwanek, K., Allerberger, F., Struelens, M., Zemlickova, H., Skov, R.L., Vuopio-Varkila, J., Cuny, C., 2011. Livestock-associated methicillin-resistant *Staphylococcus aureus* in humans, Europe. Emerging infectious diseases 17, 502.

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

Van Dongen, W.F., White, J., Brandl, H.B., Moodley, Y., Merkling, T., Leclaire, S., Blanchard, P., Danchin, É., Hatch, S.A., Wagner, R.H., 2013. Age-related differences in the cloacal microbiota of a wild bird species. Bmc Ecology 13, 11.

Vandžurová, A., Bačkor, P., Javorský, P., Pristaš, P., 2013. *Staphylococcus nepalensis* in the guano of bats (Mammalia). Veterinary Microbiology 164, 116-121.

Veldman, K., van Tulden, P., Kant, A., Testerink, J., Mevius, D., 2013. Characteristics of cefotaxime resistant E. coli from wild birds in The Netherlands. Applied and environmental microbiology, Applied and Environmental Microbiology, Dec, 79 (24): 7556–7561.

Vengust, M., Knapic, T., Weese, J.S., 2018. The fecal bacterial microbiota of bats; Slovenia. PloS one 13, e0196728.

Virpari, P., Nayak, J., Brahmbhatt, M., Thaker, H., 2013. Study on isolation, molecular detection of virulence gene and antibiotic sensitivity pattern of Escherichia coli isolated from milk and milk products. Veterinary World 6(8).

Vlahović, K., Matica, B., Bata, I., Pavlak, M., Pavičić, Ž., Popović, M., Nejedli, S., Dovč, A., 2004. *Campylobacter*, *salmonella* and *chlamydia* in free-living birds of Croatia. European Journal of Wildlife Research 50, 127-132.

Von Eiff, C., Becker, K., Machka, K., Stammer, H., Peters, G., 2001. Nasal carriage as a source of Staphylococcus aureus bacteremia. New England Journal of Medicine 344, 11-16.

Vora, N.M., Osinubi, M., Wallace, R.M., Aman-Oloniyo, A., Gbadegesin, Y.H., Sebastian, Y.K., Saliman, O.A., Niezgoda, M., Davis, L., Recuenco, S., 2014. Assessment of potential zoonotic disease exposure and illness related to an annual bat festival--Idanre, Nigeria. MMWR. Morbidity and mortality weekly report 63, 334-334.

Walker, S., Molur, S., 2003. Summary of the Status of South Asian Chiroptera, Extracted from the CAMP 2002 Report, Zoo Outreach Organization, CBSG. South Asia and WILD, Coimbatore, India.

Wallace, J., Cheasty, T., Jones, K., 1997. Isolation of Vero cytotoxin-producing Escherichia coli O157 from wild birds. Journal of Applied Microbiology 82, 399-404.

Walsh, C., 2003. Antibiotics: actions, origins, resistance. American Society for Microbiology (ASM), Nov; 13(11): 3059–3060.

Walther, B., Friedrich, A., Brunnberg, L., Wieler, L., Lübke-Becker, A., 2006. Methicillin-resistant Staphylococcus aureus (MRSA) in veterinary medicine: a" new emerging pathogen"? Berliner und Munchener Tierarztliche Wochenschrift 119, 222-232.

Wayne, P., 2011. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing.

White, D.G., Ayers, S., Maurer, J.J., Thayer, S.G., Hofacre, C., 2003. Antimicrobial susceptibilities of Staphylococcus aureus isolated from commercial broilers in northeastern Georgia. Avian diseases 47, 203-210.

White, P.L., Schlosser, W., Benson, C.E., Maddox, C., Hogue, A., 1997. Environmental survey by manure drag sampling for Salmonella Enteritidis in chicken layer houses. Journal of Food Protection 60, 1189-1193.

WHO, 2014. Antimicrobial resistance: global report on surveillance. World Health Organization.

WHO, 2017. Global action plan on antimicrobial resistance. 2015.

Wichelhaus, T., Schulze, J., Hunfeld, K., Schäfer, V., Brade, V., 1997. Clonal heterogeneity, distribution, and pathogenicity of methicillin-resistant *Staphylococcus aureus*. European Journal of Clinical Microbiology and Infectious Diseases 16, 893-897.

Wiegand, I., Hilpert, K., Hancock, R.E., 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols 3, 163.

Wikler, M.A., 2007. Performance standards for antimicrobial susceptibility testing: Seventeenth informational supplement. Clinical and Laboratory Standards Institute.

Wilson, D.E., Reeder, D.M., 2005. Mammal species of the world: a taxonomic and geographic reference. Johns Hopkins University Press.

Winn, W.C., 2006. Koneman's color atlas and textbook of diagnostic microbiology. Lippincott williams & wilkins Pheladelphia.

Wong, R., Hägg, U., Samaranayake, L., Yuen, M., Seneviratne, C., Kao, R., 2010. Antimicrobial activity of Chinese medicine herbs against common bacteria in oral biofilm. A pilot study. International journal of oral and maxillofacial surgery 39, 599-605.

Wong, S., Lau, S., Woo, P., Yuen, K.Y., 2007. Bats as a continuing source of emerging infections in humans. Reviews in medical virology 17, 67-91.

Wood, J.L., Leach, M., Waldman, L., MacGregor, H., Fooks, A.R., Jones, K.E., Restif, O., Dechmann, D., Hayman, D.T., Baker, K.S., 2012. A framework for the study of zoonotic disease emergence and its drivers: spillover of bat pathogens as a case

study. philosophical transactions of the royal society B: Biological sciences 367, 2881-2892.
Wright, G.D., 2007. The antibiotic resistome: the nexus of chemical and genetic diversity. Nature Reviews Microbiology 5, 175.
Xia, S., Hendriksen, R.S., Xie, Z., Huang, L., Zhang, J., Guo, W., Xu, B., Ran, L., Aarestrup, F.M., 2009. Molecular Characterization and Antimicrobial Susceptibility of Salmonella Isolates from Infections in Humans in Henan Province, China. Journal of Clinical Microbiology 47, 401-409.
Zhang, Y., 2007. Mechanisms of antibiotic resistance in the microbial world. Baltimore, USA.
Zhao, S., White, D., Friedman, S., Glenn, A., Blickenstaff, K., Ayers, S., Abbott, J., Hall-Robinson, E., McDermott, P., 2008. Antimicrobial resistance in Salmonella enterica serovar Heidelberg isolates from retail meats, including poultry, from 2002 to 2006. Applied and environmental microbiology 74 (21), 6656-6662.

Appendix-I

A Questionnaire to study the epidemiology of antimicrobial resistance pattern of *Salmonella* spp., *Staphylococcus* spp., and *E. coli* isolates in free ranging Bats

Roost Id No.: Date: / /2017

Location/Districts: Location types:

GPS Coordinate

Latitude: Longitude:

Bats Species: Scientific name:

Fecal consistency: Roosting pattern: clumped/with satellites

Roost types:

Elevation of the roost:

Odour: yes/No

Drinking water source: Lagoon/Lake/Pond Proximity to river:

Tree: Human settlements:

Topography: Ridge, upper slope, valley bottom

Sign of human access: Garbage, hunting information

Any others findings:

Signature of the sample collector

Appendix-II

Protocol of Media Preparation for Bacteriological Culture and Biochemical Confirmation

1. Buffer Peptone Water (BPW) Media (Oxoid, UK)

It is a pre-enrichment medium to be used prior to selective enrichment for the isolation of Bacterial. An amount of 20 grams of powder was added to 1 liter of distilled water which has a low mineral content/conductivity. Then it was mixed well, heated and boiled for 1 minutes and distributed into final containers as 5ml in per falcon tube. Sterilization was done by autoclaving at 121°C for 15 minutes. Our final product was Light straw colored liquid solution. After cooling to normal temperature it was then stored in a refrigerator for the final use.

2. Xylose Lysine Dextrose (XLD) Media (Oxoid, UK)

It is a selective medium for the isolation of Salmonellae and Shigella from clinical specimens. An amount of 53g powder was suspended into 1 liter of distilled water. It was then heated with frequent agitation until the medium boils but it was not overheating. After that, it was transferred immediately to a water bath at 50°C. Then, it was poured into sterile Petri dishes 4mm thickness as soon as the medium has cooled. The prepared medium was a red colored gel. Each of the Petri dishes was then stored in the refrigerator with an aluminum foil wrapping for further use.

3. Mannitol Salt Agar (MSA) Media (Oxoid, UK)

It is a selective medium for the isolation of presumptive pathogenic *Staphylococci*. Most of the other bacteria are inhibited, with the exception of a few halophilic species. An amount of 111 grams of powder was added to 1L of distilled water, which has a low mineral content/conductivity. Then it was mixed well, heated and boiled for 1 minutes and distributed into Petridis with a 4mm thickness. Sterilization was done by autoclaving at 121°C for 15 minutes. Our final product was red colored gel solution. After cooling to

normal temperature it was then stored in a refrigerator with an aluminum foil wrapping for the final use.

4. Baird Parker Agar (BPA) broth (HiMedia Laboratories):

Baird Parker Agar Base is recommended for the isolation and enumeration of coagulase positive staphylococci from food and clinical samples. Suspend 63.0 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 50 ml concentrated Egg Yolk Emulsion (FD045) and 3 ml sterile 3.5% Potassium Tellurite solution (FD047) or 50 ml Egg Yolk Tellurite Emulsion (FD046). For additional selectivity, if desired add rehydrated contents of 1 vial of BP Sulpha Supplement (FD069). Alternatively, 1 vial of Fibrinogen Plasma Trypsin Inhibitor Supplement (FD195) may be used per 90 ml medium in place of Egg yolk Tellurite Emulsion (FD046) for identification of coagulase, positive *Staphylococci*. Mix well and pour into sterile Petri plates.

5. MacConkey Agar (MA) Media (Oxoid, UK)

It is a differential medium for the isolation of Coliforms and intestinal pathogens in biological specimens. An amount of 52 grams of powder was added to 1 liter of distilled water, which has a low mineral content/conductivity. Then it was mixed well, heated and boiled for 1 minutes and distributed into Petridis with a 4mm thickness. Sterilization was done by autoclaving at 121°C for 15 minutes. Our ending product was dark red colored gel solution. After cooling to normal temperature it was then stored in a refrigerator with an aluminum foil wrapping for the final use.

6. Eosin Methylene Blue (EMB) Agar Media (Oxoid, UK)

It is the isolation medium for the differentiation of the Enterobacteriaceae. For its preparation, an amount of 37.5 grams powder was added to 1 liter of distilled water, which has a low mineral content/conductivity. Then it was mixed well, heated and boiled for 1 minutes and distributed into Petridis with a 4mm thickness. Sterilization was done by autoclaving at 121°C for 15 minutes. Our final product was dark purple colored gel

solution. After cooling to normal temperature it was then stored in a refrigerator with an aluminum foil wrapping for the final use.

7. Blood Agar (BA) Media (Oxoid, UK)

It is a non-selective and general-purpose medium which may be enriched with blood or serum which is suitable for the cultivation of fastidious pathogens and other microorganisms. An amount of 40 grams blood agar powder was suspended in 1 liter distilled water and heated to bring to the boil to dissolve the medium completely. The suspending medium was then sterilized by autoclaving at 121°c for 15 minutes and then cooled at 45-50°c and kept it at the room temperature. For 5% sheep blood agar preparation, an amount of 5ml defibrinated sheep blood was added to the prepared 100 ml of blood agar base and poured into Petridis aseptically. The Petri dishes containing medium were allowed to dry before storing in a refrigerator for the future use.

8. Brain Heart Infusion Broth (BHIB) Media (Oxoid, UK)

It is a highly nutritious fermentation medium recommended for the cultivation of Streptococci, Neisseria, and other fastidious organisms. An amount of 37 grams of powder was added to 1liter of distilled water, which has a low mineral content and/or conductivity. Then it was mixed well, heated and boiled for 1 minutes and distributed into falcon tubes. Sterilization was done by autoclaving at 121°C for 15 minutes. Our final product was the straw-colored solution. After cooling to normal temperature it was then stored in a refrigerator for the final use.

9. Muller-Hinton Agar (MHA) Media (Oxoid, UK)

It is an antimicrobial susceptibility testing medium which may be used in internationally recognized standard procedures. An amount of 38 grams powder was added to 1 liter of distilled water, which has a low mineral content/conductivity. Then it was mixed well, heated and boiled for 1 minutes and distributed into Petridis with a 4mm thickness. Sterilization was done by autoclaving at 121°C for 15 minutes. Our final product was straw colored gel solution. After cooling to normal temperature it was then stored in a refrigerator with an aluminum foil wrapping for the final use.

10. Triple Sugar Iron broth (Oxoid, UK):

It is a composite medium for the differentiation of Enterobacteriaceae by three sugar fermentations and hydrogen sulfide production. An amount of 65 grams of powder was added to 1 liter of distilled water, which has a low mineral content/conductivity. Then it was mixed well, heated and boiled for 1 minutes and distributed into falcon tube. Sterilization was done by autoclaving at 121°C for 15 minutes. Our final product was straw colored gel solution. Then we had allowed the medium to set in sloped form with a butt about 1 inch deep. After complete formation of butt, it was then stored in a refrigerator with an aluminum foil wrapping for the final use.

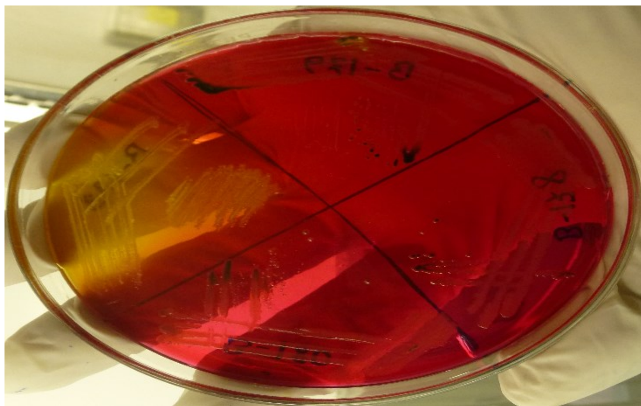
Appendix-III

Pictorial Presentation

Figure 7: Roost of Bats in different habitats

Figure 8: Fecal sample collection from different roosting site

Figure 9: Inoculation sample in different media



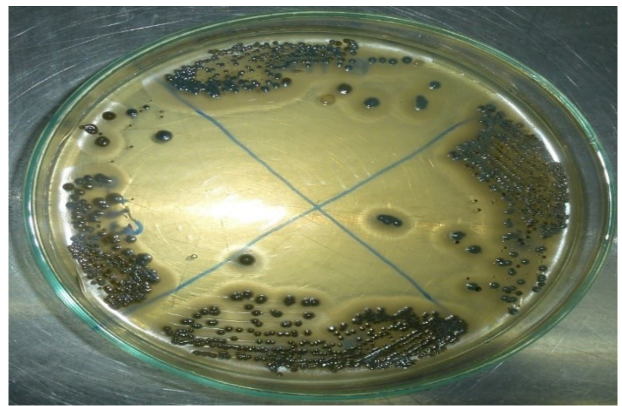
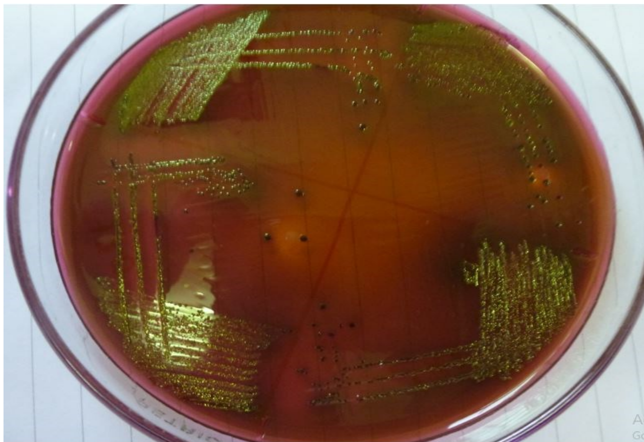


Figure 10: *Salmonella* spp.,

Figure 11: *Staphylococcus spp*



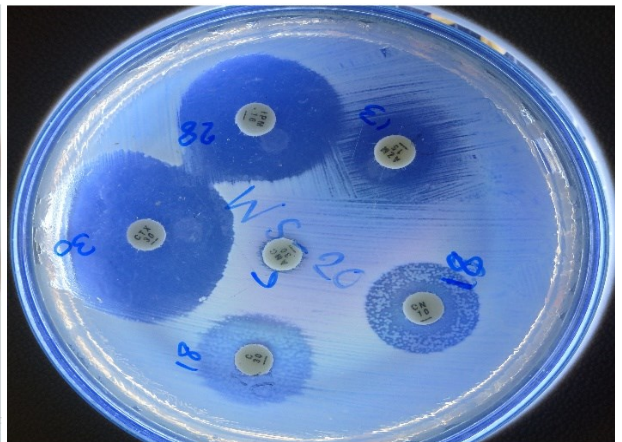
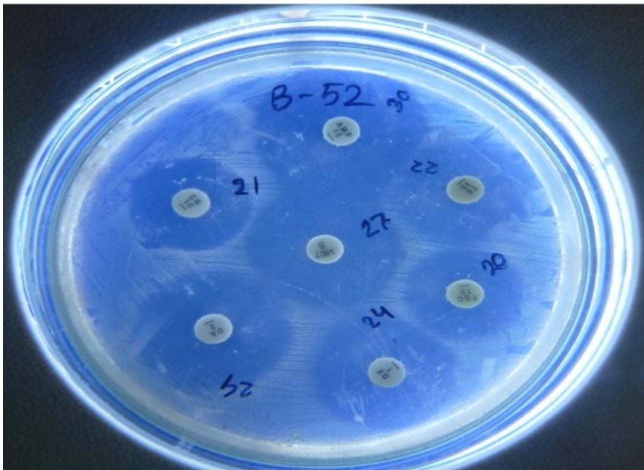


Figure 12: *Escherichia coli*

Figure 13: Antibiotics resistance test



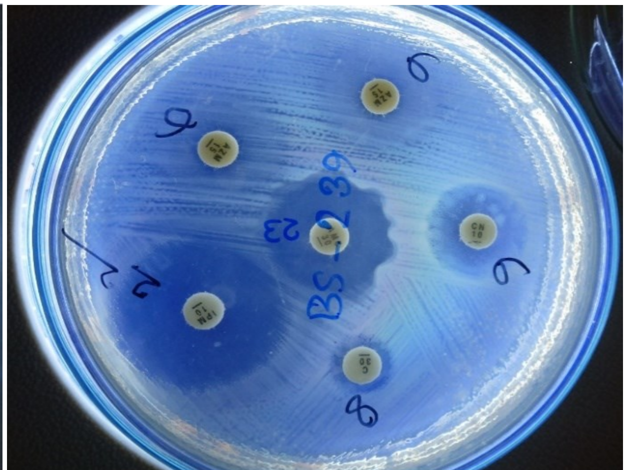


Figure 14: Antibiogram test by disc diffusion method

Brief Biography

Md. Helal Uddin was born in Chauddagram, Cumilla village of Cumilla district, to Md. Kalu Miah, a Retired Govt. service holder, and Joshnara Begum. He is the youngest of six children. He passed the Secondary School Certificate (SSC) examination as a student of Debidwar Reaz Uddin pilot High School, Cumilla in 2007. He completed Higher Secondary Certificate (HSC) from Feni Govt. College, Feni in 2009. Then he signed up at Chittagong Veterinary and Animal Sciences University (CVASU) in Doctors of Veterinary Medicine (DVM) and received DVM degree with CGPA 3.56 (Scale 4.0). Now, he is a candidate for the degree of MS in Epidemiology under the Department of Medicine and Surgery, Faculty of Veterinary Medicine (FVM), CVASU. He has published research articles & conference papers in national & international peer-reviewed journals. His favorite hobby is reading Al-Quran, books and research articles. He has immense interest to work in epidemiology of bacteria, virus and fungal microorganisms of free-living and captive wildlife species from disease ecology, evolution, and conservation points of view.