CHITTAGONG VETERINARY AND ANIMAL SCIENCES UNIVERSITY

Prevalence and antibiogram of *E. coli* and *Salmonella* spp. isolates in small fruits bat (*Rousettus leschenaulti*) and associated public health risk in Bangladesh

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ABSTRACT

Bats are playing significant role to global ecosystem through seed dispersal, pollination and insect control. Bats also act as a career of wide range pathogens including *Salmonella* spp. and *Escherichia coli*. The current changing trends in natural habitats and deforestation, bats often come in close contact with human, posing threats to public health. Detection of zoonotic pathogens carried by bats is utmost important for understanding disease ecology and for developing preventive measures. Antimicrobial resistance in pathogenic bacteria in free ranging wildlife and environment is a major concern in current decades. *Salmonella* spp. and *E coli* are commensal entero-bacteriaceae in a broad range of hosts. They can cause many diseases. A cross-sectional study was conducted to determine the prevalence of *Salmonella* spp. and *E coli* in *Rousettus leschenaulti* fruit bats in Bangladesh during July and December, 2013. Fresh environmental fecal sample were collected from two roosting sites of Rajbari district in Bangladesh. Samples were collected by using sterile polyvinyl sheet beneath the roosting site with sterile swabs stick and putted in transport media. Samples were transferred in ice eskey and stored 4^0C. *Salmonella* spp. (8.17%; N=49) and *E coli* (34.7%; N=49) were isolated from samples obtained and. These pathogens were significantly more prevalent in the roost close to human vicinity (16% & 48% versus 0% & 20.82 %,). Disk diffusion methods were used to assess antibiogram of isolated pathogens. Both *Salmonella* spp. and *E coli* isolates had attained 100% resistance to amoxicillin and erythromycin, however remained sensitive 100% to ciprofloxacin and 70% to Enrofloxacin. The close interface between bat, human and production animal may responsible to have higher prevalence of *Sallmonella spp* and *E coli.* The anthropological investigation about the common practices of adjacent community people has revealed a potential public health risk.

Keywords:

Salmonella, E coli, Fruits bat, Antimicrobial resistance, Human vicinity, Public health

CHAPTER I

INTRODUCTION

Bats (Order: Chiroptera) are the only mammals capable of true sustainable flight and one of the most diverse and species rich mammals on the earth (Kingston *et al.,* 2010). They assist in the regulation of insect populations in their habitats, pollination of flowers and dispersal of seeds of economically important tress, and these ecological roles support forest regeneration and maintenance (Kunz *et al.,* 2011). Due to rapid fragmentation of habitat and decline of natural forest, bats are often roosting near the human settlements (Hann *et al.,* 2013). Moreover, it makes them more deepened to forage on commercially planted economic crops for their food (Boyles *et al.,* 2013).

Bats are proved natural reservoir of broad ranges pathogens including virus and bacteria and others (Calisher *et al.,* 2006; Wood *et al.,* 2012; Muhldorfer *et al.,* 2012). However, their close with human habitation has increased the chance of emerging and reemerging infections (Wong *et al.,* 2007).The association of bats and human is closer in densely populated country like Bangladesh (Epstein *et al.,* 2010). In recent decades special attention has taken on these flying mammals as vectors of zoonotic pathogens (Luis *et al.,* 2013).

The bat species *Rousettus leschenaulti* is grouped under the suborder Megachiroptera, and it is the most widely distributed fulvous fruits in Bangladesh. The bat is found partial in the forest of Sundarban as well as near the human habitation having green space (Khan, 2001, Srinivasulu *et al.,* 2010). The prime habitat in of Rousettus bat in urban area is formation of colony in old temple, remote house, thatched building or in banana of *Fiscus* tree (Khan, 2001). The bat often eat fresh fruits from banana, manago, guyava, papaya, Monkey Jack (*Artocarpus lacucha*), Cotton tree (*Bombax ceiba*) , Indian rose chestnut (*Mesua ferrea*) Indian Fig (*Ficus racemosa*) which also recognized as human food. Therefore, there is chance of bat-human interaction in Bangladesh. Although E. Coli and *Salmonella* spp are normal flora of a wide range of mammals and bird there are some recent evidence of transmission in human (Elangovan *et al.,* 2002; Stefanraj *et al.,* 2010). *Salmonella* and E coli in different bat species is reported ranges 4-13% and 27-74% respectively in earlier study (Adesiyun *et al.,* (2010).

Antibiotic resistance is a global challenge that impacts all pharmaceutically used antibiotics (Bhullar *et al.,* 2012). Antibiotic use for clinical, veterinary and agricultural practices provides the major selective pressure for emergence and persistence of acquired resistance

determinants (Thaller *et al.,* 2010). The resistant bacteria of production animal and human often transmitted to the environmental organism though there is a little chance of exposure in nature. A growing body of evidence implicates environmental organisms as reservoirs of these resistance genes; Antibiotic resistance, evolving and spreading among bacterial pathogens, poses a serious threat to human health. However, resistance has also been found in the absence of antibiotic exposure, such as in bacteria from wildlife (Wellington *et al.,* 2013; Tacao *et al.,* 2012).

Besides, due to large range of migration and flying close to the human settlement, the public health implications of bat activity are important (Rabinotiz *et al.,* 2013). Though there are some study has been conducted on antibiotic resistance in production animal, no study yet has been conducted in any free ranging wildlife in Bangladesh except Hasan *et al.,* (2012). So, this study investigated the prevalence of *Salmonella* and *E coli* in bat with antibiogram. The risk of public health and influence of human vicinity near the roosting site were the cross products objectives of the study.

CHAPTER II
REVIEW OF LITERATURE

2.1. *Salmonella* and *Escherichia coli*

Salmonella have been known and responsible for causing diseases in human and animal since it was discovered by Dr Daniel Salmon. *Salmonella* Like other Enterobacteriaceae, are motile, non spore forming and facilitative anaerobes. *Salmonella* reduce nitrates to nitrites, ferment glucose and negative in oxidase (Yan *et al.,* 2008).

Salmonella consists of two species – *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* consists of six subspecies (ssp.) under which there are 2500 serovars [11] The subspecies of *S. enterica* being divided as *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp.*houtenae* (IV), *and S. enterica* subsp. *indica* (VI) (Popoff and Gheesling, 2003 ; Popoff *et al.,* 2001 ; Tindall *et al.,* 2005).

All *Salmonella* strains are serologically classified using Kauffmann-White scheme **(**Popoff and Gheesling, 2003 ; Popoff *et al.,* 2001 ; Tindall *et al.,* 2005). The majority of the *Salmonella* serotypes belong to *S. enterica* subsp. *enterica* (about 60%), followed by subspecies *salamae* (20%), *diarizonae* (13 %), *arizonae* (3.8 %), *houtenae* (2.8%) and *indica* (0.45%).Only (0.8%) belong to the second species *Salmonella bongori* . Strains that belong to *S. enterica* subsp. (*S. enterica* subsp. *entericae*), are frequently pathogenic to humans and mammals while those belonging to subspecies II*,* IIIa, IIIb, IV, VI and *Salmonella bongori* are usually isolated from reptiles and other cold- blooded animals (Brenner *et al.,* 2000).

2.2. *Salmonella* and *E coli in* production animal

Salmonella are widely distributed in the animal kingdom, including a wide range of wild and domestic animals and can be excreted in their feces. The degree of host adaptation varies between *Salmonella* serotypes and affects the pathogenicity for man and animals (Tsolis *et al.,* 2011). For epidemiological reasons, it is common to place the *Salmonella* into three groups depending on their pathogenic reactions. The first group of serotypes is infectious and host adapted to only humans. These include serotypes such as *S. typhi*, *S. paratyphi* A and *S. paratyphi* C. This group includes the organisms associated with typhoid and the paratyphoid fevers, which are the most serious of all the diseases caused by *Salmonella*. The second group

is host adapted serotypes to animals, although some of these may also be human pathogens. Included are *S. gallinarum* (poultry), *S. dublin* (cattle), *S. abortus-ovis* (sheep), and *S. choleraesuis* (swine). The third group is unadapted serotypes with no host preference. All these serotypes are potentially pathogenic for humans and animals and they include most food borne serotypes. However, foods of animal origin, especially poultry and poultry products, including eggs, have been consistently implicated in sporadic cases and outbreaks of human salmonellosis, and chicken products are widely acknowledged to be a significant reservoir for *Salmonella.* They have frequently been incriminated as a source of *Salmonella* contamination and consequently thought to be major sources of the pathogen in humans Furthermore, one of the commonest causes of *Salmonella* infection reported in humans has been through the handling of raw poultry carcasses and products, together with the consumption of undercooked poultry meat (Panisello *et al* ., 2000)

The incidence of *Salmonella* in poultry has been well determined in many countries such as (United States, Belgium, UK, Malaysia, Spain and Japan), and the level of contamination by *Salmonella* ranged from 20% to 89% from total poultry population (Capita *et al.,* 2000).

Motile zoonotic *Salmonella* serovar from poultry farm was isolated in Bangladesh. But there is no specific data of outbreak related to food born Salmonellosis (Barua *et al.,* 2012).

2.3. *Salmonella* and *E coli* in wild animal

Wide range of wild animal both from captivity and free range are evidence by different authors to antibiotic resistance bacterial infection. Antibiotic resistant enterobactericace 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; Graczyk *et al.,* 2002; Lilly *et al.,* 2002) . There is also evidence of antibiotic resistenat E. coli in Gorilla in Gabon. Antibiotic resistance to E. coli has been detected in wild and captive Iberian lynx Lynx pardinus and Atlantic bottlenose dolphins Tursiops truncatus [Ref], as well as a wide range of wild fish, bird's mammals. Antibiotic resistant *Salmonella* spp, E. coli and Clostridium perfingens also reporded at overall 8% in captive zoo of South Africa (Ref) Antimicrobial Resistance in Escherichia coli isolates from Swine and other Wild Small Mammals also reported from Canada. Multidrug resistance *E coli* isolates also reported in small mammals in central Europe (Dolejska *et al.,* 2012). High frequency of antibiotic resistance E. Coli, *Salmonella* Spp and Campylobacter in wild rodent of Trinidad Tobago also reported at (Comfort Nkogwe,2011) *Salmonella* spp in with antibiotic resistant properties also reported in wild bird, reptiles and pet animal from Trinidad

(Seepersadsingh & Adesiyun, 2003). Thirty two *Salmonella enterica* isolates were collected from wild birds in northern England between February 2005 and October 2006, of which 29 were *S. enterica* serovar Typhimurium (*S*. Typhimurium);

The antibiotic resistant bacterial infection causes in captive and free ranging wild animal due to exposure of human generated waste water and Environmental contamination with fecal material from domesticated animals and pets Additionally use of antibiotics in broad ranges in veterinary, medical and agricultural purposes also considered as a risk factor in wild animal.

2.4. *Salmonella* and *E coli* in Bats:

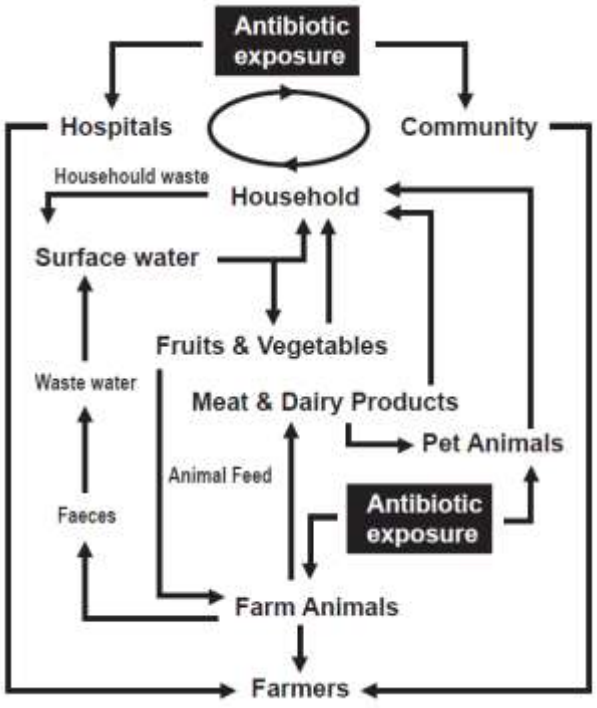
Bats are one of the most widely distributed mammals in the world, and they are reservoirs or carriers of several zoonoses. A study was conducted to detect *Salmonella* spp., Escherichia coli, and Campylobacter spp in both fruits and insectivores bat. The study found 13% positive to *Salmonella* spp and 13% to E. coli. Among the isolated 82% shows antibiotic resistance with one or two antibiotics. And the prevalence of resistant strains was comparatively high to Erythromycin (61%) and streptomycin (27%) but lower to gentamycin (0%) and 2% in sulphamethozaxole/trimethoprim (Adesiyun *et al.,* 201)

Antibiotic resistance Staphylococcus aureus at low frequency also reported from Straw color fruits bat from Nigeria (Akobi *et al.,* 2012). *Yarsinia pestis* in bat also reported from Germany but there is no evidence of antibiotic resistance on Y. pestis.

A study in Germany reported 17% investigated bat dies from bacterial diseases but most of them are opportunistic type bacteria. Pasteurella spp., here identified in 7% Primary bacterial pathogens like *Salmonella* enterica serovar Typhimurium, S. Enteritidis and Yersinia pseudo tuberculosis [22] were identified in almost 12% of affected bats. Some of the bacterial species (e.g. Burkholderia sp., Cedecea davisae and Clostridium sordellii) are newly described in bats.

2. 5. Antibiotic resistance:

Antibiotic resistance is a global problem in public health and is growing around the world (WHO, 2010). Antibiotics have been used for 70 years but during the last decade some treatments have become ineffective and this may lead to spread of some infections in the future. Antimicrobial resistance (AMR) is created by use of antibiotics in a wrong way and develops when a microorganism have mutated or acquired inappropriate use of antibiotics in human and veterinary medicine leads to higher frequencies of AMR (Rosen *et al.,* 2011).



Antibiotics are often used in animals. Transfer to human's food of these antibiotics can affect the safety of the meat, milk, and eggs produced and can be the source of superbugs. The resistant bacteria in animals can transfer to humans by three pathways, consumption of meat or other food, direct contact with animals or through the environment. The figure shows the transfer ways of antibiotic resistance between human, animals and environment (Marshal *et al.,* 2011)

2.6. The spread of resistance among environmental bacteria

Antibiotic resistance is not only found in pathogenic bacteria but also in environmental organisms inhabiting terrestrial and aquatic habitats. The occurrence of resistant bacteria in nature may have originated from antibiotic producing organisms, as suggested by the evidence that in 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). However, higher numbers of resistant bacteria occur in polluted habitats compared with unpolluted habitats (Muneisa *et al.,* 2013), indicating that humans have contributed substantially to the increased proportion of resistant bacteria occurring in the environment. 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 resistance genes are transferred from environmental bacteria to human pathogens. The ability of resistant bacteria and resistance 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 resistance genes in the commensal microflora of animals and their transmission to humans *via* the food chain (Marshal *et al.,* 2011). Similarly, drinking and bathing water could represent a source for the acquisition of resistant bacteria in humans. However, further studies are necessary to validate this hypothesis. 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 resistance 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 (balance) of natural microbial communities (Muthiyan *et al.,* 2011).

Table: 1 Number of *Salmonella* isolates from bat and their frequency in different country in the world

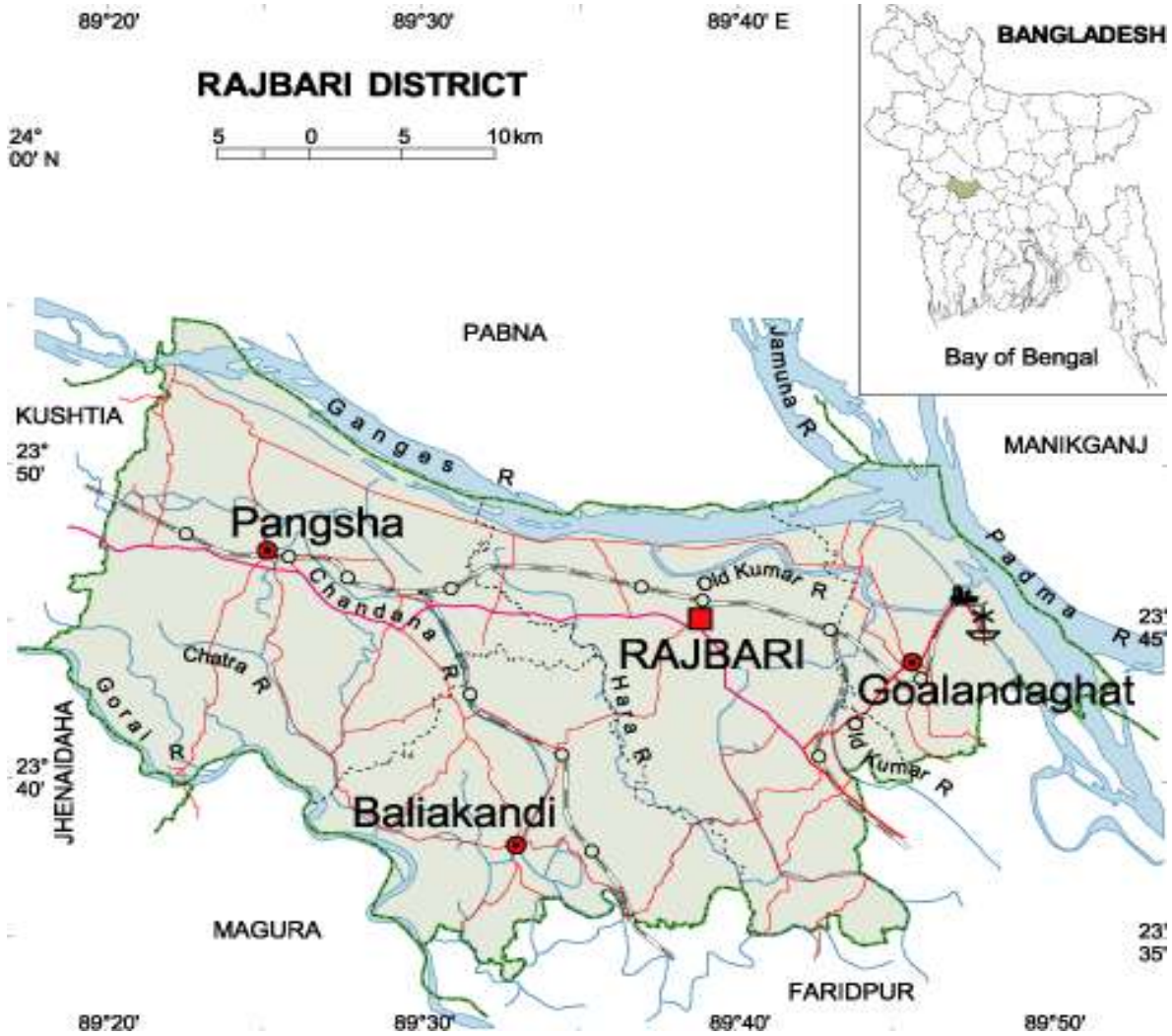
Bat family	Isolated bacteria	Prevalence %	Source	Authour	Author
Vespertilionidae	*Salmonella* spp.	2/46	Intestine	Philippines	Reyes et al. (2011
	Salmonella spp.	1/73	Heart blood	UK	Daffner (2001)
	Salmonella Enteritidis, *Salmonella* Typhimuriumb	3/486	Organ sample	Germany	Mu¨ hldorfer et al. (2011)
Molossidae	*Salmonella* Spp	2/37	Fecal	Trinidad	Adesiyun et al. (2009)
Nectarivorous	*Salmonella* sp.	1/47	Faces	Brazil	de Souza et al. (2002
Noctilionidae	*Salmonella* Moladeb, *Salmonella* Rubislawb	1/11	Gastrointestinal tract	Trinidad	Adesiyun et al. (2009)
Pteropodidae	*Salmonella* Virchow	3/302	Fecal	Bangladesh	Islam *et al.,* 2013

CHAPTER III

MATERIALS AND METHODS

1.1 Study area and bat roost:

Bat roosting sites of Goalanda and Pangsha upazilla under the Rajbari districts were selected purposively for the study. The first roosting site is located about 10 km away from human establishments and the second one located very close to human establishments. 700-1000 bats were observed in the roost. Rajbari district is recognized for previous Nipah encephalitis outbreaks and falls under the Nipah belt in Bangladesh. The study sites are consist of mostly low land and are surrounded by the Padma River. The area have full of green vegetation of different tall and short tree species.



1.2 Description of *Rousettus leschenaulti*

Rousettus leschenaulti, a small fruits bat under the genus of Pteropodidae was taken as reference population for the study. This species is very widely distributed in South Asia, southern China and Southeast Asia. It is presently known from Chittagong, Dhaka, Khulna and Sylhet divisions in Bangladesh. This species is found in a variety of habitats ranging from tropical moist forest to urban environments. Roosts in colonies ranging from a few to several thousands of individuals in caves, old and ruined buildings, forts and disused tunnels (Khan, 2002; Bates and Helgen, 2013).

1.3 Study design:

A cross sectional study was conducted to estimate the prevalence of *Salmonella* spp and *Escherichia coli* in small *Rousettus leschenaulti* with associated public health risk and asses antibiogram of isolated pathogen.

1.4 Study period:

The study was conducted between July and December 2013 during internship period.

1.5 Sample Collection and shipment:

A total of 49 environmental fecal samples were collected from ground of roosting sites (r1= 25 and r2=24). A record keeping sheet was used to note the information related to roost size, habitat characteristics and other ecological factors.

A sterilized polyvinyl sheet was used beneath the roosting site for fresh fecal sample collection. A sterile swabs stick (BD falcon scwab stick, Thomas scientific, USA) was used to collect the f samples in a screw caped vial containing 10 ml of Aimes transport media (Oxoid ^{TM}) .Three individual swabs each contained 1-2 gm of fecal sample pooled together and vial were given with unique identity number. Samples obtained then were transferred to the laboratory of PRTC, Chittagong Veterinary and Animal Sciences University through ice eskies as quickly as possible and stored in 4^0 C. 1.7 Recording of roosting character:

1.6 Data collection:

Data were noted in a structured record keeping sheet. Information included location, character of roosting site, presence of water body, estimated population, vegetation type, sign of human disturbances, hunting information etc. (also see in Appendix 1). The common

practices of the community people were also recorded. Information included basic information, common practice of raw vegetable consumption, consumption of green fruits, water use for domestic animal and other purposes. Face to face interview to the adult person from each family of 20 was included. The purpose of study was briefly informed for ethical reason.

1.7 Laboratory evaluation:

1.7.1 Media used

Nutrient agar (Oxoid Ltd) was used for enriching bacterial growth of the samples. We have used five selective media for isolation of bacteria after enrichment. The MacConkey and EMB agars were used for isolating *E. coli.* On the other hand, XLD agar (Oxoid Ltd), SS agar (Mareck) and TSI agar (Oxoid Ltd.) were used for *Salmonella* isolating. Muller Hinton agar (Biotech) was used for antibiogram of isolates.

1.7.2 Culture and biochemical test procedures

I. *E. coli*

One ml of fecal suspension was inoculated in a screw cap test tube containing 10 ml of nutrient broth and incubated at 37^0 C for 24 hours. Then samples were streaked on Mc Conkey agar and incubated overnight. After the incubation, the colony was observed. The pink colony indicated E coli positive. Sub culture was then performed on EMB agar at 37^0 C for 24 hrs. The characteristics metallic sheen colony was suggestive E. coli positive. Positive samples were further confirmed by gram staining and microscopic examination.

II. *Salmonella*

One ml of fecal suspension was inoculated in a screw cap test tube containing 10 ml of nutrient broth and incubated at 37^0 C for 24 hours. Then samples were streaked on XLD and SS agar and incubated overnight. After the incubation, the colony was observed. The colony with black center in XLD and blackish growth in SS agar were considered as presumptive *Salmonella* positive. Sub culture was then performed on TSI agar at 37^0 C for 24 hrs. The blackish growths on TSI slant were suggestive *Salmonella spp.*

1.7.3 Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing for *Salmonella* and E coli isolates was performed by using antimicrobial disk (Oxoid, Thomas Scientific, USA) through micro disk diffusion technique according to Clinical and Laboratory Standards Institute (CLSI, 2010). Mueller Hinton agar was prepared in petri dishes as per manufacturer instruction.

Antibiotics were selected for susceptibility testing included
GEN: Gentamicin (10mcg), AML: Amoxicillin (30mcg), CIP: Ciprofloxacin (5mcg), OTC: Oxytetracycline (30mcg), ENR: Enrofloxacin (5mcg), CLS: Colistin (10mcg), NEO: Neomycin (30mcg), ERT: Erythromycin (15mcg), SXT: Sulphamethaxole and PFN: Pefloxacin (5mcg). Growth inhibition zone were measured and interpreted as per instruction given by CLSI, (2007).

1.8 Data Analysis:

Field and Laboratory data were entered, stored and cleaned in the MS excel 2007 programme before exporting to STATA-11 for analysis. Descriptive data analysis will be performed to the frequency and distribution of *Salmonella* infection and antibiogram against Sal isolates. Significance test (χ^2 tests) was applied and p value of ≤ 0.05 was used.

CHAPTER IV

RESULTS

3.1 Prevalence of *Salmonella* and *E coli* in fruits bat:

Table 2: Prevalence of *Salmonella* & Escherichia coli in samples of Rousettus bat

Bat roost	*Salmonella* spp			*E coli*			
	No of sample tested	Positive (%)	95% CI	Sample tested	Positive (%)[2]	95% CI	*p*-value(2 tailed)
Roost 1	25	4 (16%)	4-36.1	25	12 (48.0)	27.8-68.7	0.015
Roost 2	24	0 (0.0)	0-14	24	5(20.82)	7.1-42.2	
overall	49	4 (8.17%)	2.2-19.6	49	15(34.7%)	18.3-45.4	0.018
p-value (2 tailed χ^2 test)	0.04			0.05			

Overall prevalence of *Salmonella* and Escherichia coli was 8.17% and 34.7% respectively and the prevalence of E coli was significantly higher than *Salmonella* (p=<0.001). Four samples were turned out as *Salmonella* positive in roost 1 but none was in samples obtain from roost-2. The prevalence of Escherichia coli was 48% in roost 1 and 20.8% in roost 2 and the result was significantly differed from each other (p= 0.05).

3.2: Antibiogram of *Salmonella* and E coli isolated from samples of fruits bat

Table: 3 Antibiogram of *Salmonella* isolated from fecal sample of *Rousettus* bat.

Sample No.	CIP 5µg	ENR 5µg	AML 10µg	CLS 10µg	OTC 30µg	GEN	SXT 25µg	ERT 15µg	PFN	NEO
Sal 13	S	S	R	R	R	R	I	R	S	R
Sal 16	S	S	R	I	I	S	S	R	S	S
Sal 17	S	S	R	R	S	I	S	R	S	S
Sal 19	S	S	R	R	S	S	S	R	I	I

R=Resistance I=Intermediate S=Sensitive. CIP=Ciprofloxacin, ENR=Enrofloxacin, AML=Amoxicillin, CLS = Colistin, OTC= Oxytetracycline, GEN=Gentamycin, SXT= Cotrimoxazole, ERT = Erythromycin, NEO= Neomycin, PFN=Pefloxacin,

Table: 4 Antibiogram of *E. coli* isolated from fecal sample of Rousettus bat.

Sample No.	CIP 5µg	ENR 5µg	AML 10µg	CLS 10µg	OTC 30µg	GEN	SXT 25µg	ERT 15µg	PFN	NEO
EC	S	I	R	I	R	R	R	R	S	R
EC	R	R	R	R	R	S	R	R	R	S
EC	S	S	R	R	R	S	R	R	S	I
EC	I	R	R	I	R	R	R	R	R	S
EC	R	R	R	I	R	I	R	R	R	S
EC	S	S	R	I	R	S	R	R	R	S
EC	R	R	R	I	R	I	R	R	R	I
EC	S	R	R	I	I	S	R	R	I	S
EC	S	S	R	I	R	S	R	R	I	S
EC	S	S	I	R	S	S	S	R	S	S
EC	S	S	R	R	S	S	S	R	S	S
EC	S	S	R	I	R	S	R	R	S	S
EC	S	I	R	R	S	S	I	R	S	S
EC	S	S	R	S	S	S	S	R	S	I
EC	S	S	R	S	R	I	S	R	S	S
EC	S	S	R	R	R	S	R	R	S	S
EC	R	S	R	S	R	S	R	I	R	S

R=Resistance I=Intermediate S=Sensitive. CIP=Ciprofloxacin, ENR=Enrofloxacin, AML=Amoxicillin, CLS = Colistin, OTC= Oxytetracycline, GEN=Gentamycin, SXT= Cotrimoxazole , ERT = Erythromycin, NEO= Neomycin, PFN=Pefloxacin,

All most all *Salmonella* had resistance to Amoxicillin (100%) and Erythromycin (94%) of *Salmonella spp* but contrarily most of the isolates had sensitive to Pefloxacin (53%) and SXT (72%) see Table 2. Most of the E coli isolates had sensitive to Ciprofloxacin (70%), Gentamycin (70%) and Neofloxacin (70%). However, most of the isolates got resistance to Amoxicillin(100%), Cotrimoxazole(70%) and Erythromycin (94%) (table 4 & 5)

Table 5: Prevalence percentage of antibiogram on *Salmonella* and *E coli* isolated to different antibiotic within positive samples.

Name of the antibiotic	Pattern	*Salmonella* spp	Positive %	*E coli*	Positive %
Ciprofloxacin	Resistance	0	0	4	23.53
	Intermediate	0	0	1	5.88
	Sensitive	4	100	12	70.59
Enrofloxacin	Resistance	0	0	5	29.41
	Intermediate	0	0	2	11.76
	Sensitive	4	100	10	58.82
Amoxicillin	Resistance	4	100	16	94.12
	Intermediate	0	0	1	5.88
	Sensitive	0	0	0	0.00
Colistine	Resistance	3	75	6	35.29
	Intermediate	1	25	8	47.06
	Sensitive	0	0	3	17.65
Oxytetracycline	Resistance	1	25	12	70.59
	Intermediate	1	25	1	5.88
	Sensitive	2	50	4	23.53
Gentamycin	Resistance	1	25	2	11.76
	Intermediate	1	25	3	17.65
	Sensitive	2	50	12	70.59
Cotrimoxazole	Resistance	0	0	12	70.59
	Intermediate	1	25	1	5.88
	Sensitive	3	75	4	23.53
Neomycin	Resistance	1	25	1	5.88
	Intermediate	2	50	3	17.65
	Sensitive	1	25	13	76.47
Pefloxacin	Resistance	0	0	6	35.29
	Intermediate	1	25	2	11.76
	Sensitive	3	75	9	52.94
Erythromycin	Resistance	4	100	16	94.12
	Intermediate	0	0	0	0.00
	Sensitive	0	0	1	5.88

CHAPTER V

DISCUSSIONS

The present study was the first epidemiology and antibiogram investigation in south Asian countries with an aim of estimate the prevalence of *Salmonella* and *E coli* in small fruits bat (*Rousettus leschenaulti*) and antibiogram of pathogen. The investigation of associated public health risks and influence of human settlement was the cross products objectives of the study.

Prevalence of *Salmonella spp* Rousettus bat was 8.17% which more or less correspondence to studies (Adeseuyan *et al.,* 2010; Reyes *et al.,* 2011; Muhldorfer *et al.,* 2011; Souza *et al.,* 2002). A study in Trinidad was conducted by Adesiyun *et al.,* (2010) to investigate the prevalence of Salmonellosis i in different species of bats from gastrointestinal tract. The author recorded overall 6.67% prevalence of *Salmonella* in small fruits bats and lower (3.1%) in insectivore's bats. Our current study finding is consistent with this study. 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 by bats Muhldorfer et al. (2011); Souza et al. (2002); were recorded 17% and 34% *Salmonella* infection respectively. Jardine *et al., (*2012) conducted study on rodents near the pig farm in found higher prevalence of *Salmonella* (7.3%) and E coli (24.95%) in Canada. Daffner (2001) also conducted a study in UK to determine the prevalence of *Salmonella* in Vespertilonidae bat and reported 1.36% from blood samples of heart. We were unable to serotype the *Salmonella* spp. However, *Salmonella* Virchow has been reported from Indian flying fox (*Pteropus giganteus*) in Bangladesh (Islam *et al.,* 2013) and wild aquatic bird in Australia (Hoque *et al.,* 2012).

Average of 34.7% of E coli was estimated in Bats in our study. Our results are in the line with other study (Adeseuyan *et al.,* 2010). However the lower and higher prevalence compared with the prevalence recorded earlier. Literak *et al.,* (2011) were conducted a study in Small terrestrial mammals (Rodentia, Insectivora) in a suburban and forest environment and recorded an average of 57.4% E coli in free ranging wild animal. Hasan *et al.,* (2012) reported a higher prevalence of 73.3% of *E coli* in wild birds from Bangladesh. Donegen *et*

al., (20133) 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% (Kozak et al, 2012; Audsiyan et al, 2011), wild birds ranges from 13%-47% (Cernicchiaro *et al.,* 2009; Veldman *et al.,* 2012).

The average prevalence of E coli in roost 1 (48%) was significantly higher compared to other (20.8%). The roosting site 1 of our study was very close to the human settlement and sign of human disturbances also observed. The close vicinity to human settlement affects the emergence of pathogen in free ranging wild life (Skurnik *et al.,* 2006, Alexzander *et al.,* 2010). It is also reported that the human behavior adjacent to the wildlife habitat have an important role in pathogen transmission between environment and wildlife (Brearly *et al.,* 2013). In the urban landscape where wild animal are often exposed to anthropogenic disturbance show more frequency in pathogen contamination. 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. So the influence of human disturbances and behavior is the possible causes of high prevalence in studied roosting site(Skurnik *et al.,* 2006).

A total of 4 *Salmonella* spp and 17 *E. coli* isolates (from 49 fecal samples showed reduced susceptibility to one or multiples antimicrobials. 4 isolates of *Salmonella* spp and 12 isolates of *E coli* were obtained from roost one adjacent to the human settlement, whereas only 5 isolates of *E coli* and none of *Salmonella* from roost 2 which is far away from human habitation. . The prevalence of resistance to antimicrobial agents among E. coli and *Salmonella* was reported as 57.1% found in fruit-eating bats at the Emperor Valley Zoo in Trinidad (Gopee *et al.,* 2000) which is more or less consistent to our study findings. The high prevalence of resistance is comparable to results from different studies on bat in other countries (Sherley *et al.,* 2000; Costa *et al.,* 2008). It has also been reported that resistance to antimicrobial agents among wildlife species may vary locally and may be linked to the use of antibiotics in veterinary, clinical and agricultural field (Rolland *et al.,* 1985; Sherley *et al.,* 2000)

The isolates of *Salmonella* and *E coli* are most commonly resistance to Amoxicillin and Erythromycin at almost 100% (Figure1-2) and Tetracycline at 60-80%. The high prevalence

of resistance to antimicrobial agents among bat isolates of E. coli and *Salmonella* spp detected in the current study is comparable to the frequency of resistance reported poultry, wild bird, cattle, pet animal and captive and free-ranging wildlife, 95.6– 99.6% (Adesiyun and Downes, 1999; Gopee *et al.,* 2000) but considerably lower than resistant rates reported from Dairy cattle in Bangladesh (Islam *et al.,* 2008). The transmission of *Salmonella* and *E coli* in between wild animal, farm animal and human is reported by (Zaho *et al.,* 2011). So the transmission of resistance bacteria may also play crucial role in high prevalence. However, the *E coli* isolate shows more resistance nature to Colistin sulphate and cotrimoxazole than *Salmonella* spp. Although the resistance pattern of *Salmonella and E coli* against this two antibiotic is not reported in previous studies of AMR(anti-microbial research) in wild small mammals (Travis et al.,2006) the trends is more close to wild boar study in Poland (Literak *et al.,* 2010).

Amoxicillin and Tetracycline resistance was by far the most common type of resistance observed in the wild small-mammal isolates and was significantly associated with farm origin. This is not surprising since tetracycline is often used as a first-line antimicrobial in disease prevention and growth promotion in food animals and its widespread use has likely contributed to high rates of resistance (Okekey *et al.,* 2005). The frequency of tetracycline resistance in the poultry farm in Bangladesh reported as 70-100% and amoxicillin near about 77-100%. A British study which found that 97% of *E. coli* isolates from similar animal species (bank voles and wood mice) were resistant to amoxicillin-clavulanate (Jardine *et al.,* 2011) but our study strongly contrast with an study of Kozak *et al.,* (2012) in case of amoxicillin resistance. Since tetracycline resistance genes are located on mobile genetic elements, they are transmissible between bacteria and it is likely that either the wild small mammals exposed to bacteria from farm animal were colonized by these bacteria or their resident flora acquired tetracycline resistance determinants from these bacteria through horizontal gene transfer. Since AMR can be selected by antimicrobials in feed (Ref), it is also possible that *the isolates of our study* from bats were directly or indirectly exposed to selection pressure through animal feed containing antimicrobials, such as tetracycline. However, The *E coli* isolates showing enhance resistant pattern in case of OTC, SXT and PFN than *Salmonella* in our study. The causes may be due to frequent use of this antibiotics and wide distribution of *E coli* in natural habitat (Jardine *et al.,* 2012)

Surprisingly our study found the Ciprofloxacin and Enrofloxacin as completely sensitive to *Salmonella* isolates and partial (80-90%) sensitive to *E coli* isolates.

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 NTS and 2.46% patient from TS (Leung et al. 2013). Villages in rural Bangladesh commonly include stagnant lagoons used for bathing and drinking by people and associated livestock and these water sources are often contaminated with sewage (Parveen et al. 2008). In addition bats are often using the common source of this water for drinking purpose; thus, contaminated water is a possible source of infection (Islam *et al.,* 2012). Through our structured questionnaire survey among 20 community people representing from each of 20 families near the study area, we revealed that 32.4% people use to take bath in ponds and 73% use the pond water for their domestic use. Most of them (88.4%) people provide the pond water to their livestock while only 7.21% people boiled before serving to their animal. 56.67% people are involve in growing backyard vegetable and fruits while 11% of them often take fresh fruits and vegetables without washing. The Rousettus bat often forages in commercial fruits like papaya and Guava and Human infection with *Salmonella Typhi* in Bangladesh has been associated with eating papaya (Ram et al. 2007). So, it could be potential risk to eating the contaminated with bat excreta during feeding activity.

CHAPTER VI
CONCLUSION

In conclusion the prevalence of *Salmonella* and E coli was small fruits bat is 8.17 % and 34.7% respectively. The E coli infection was significantly higher than *Salmonella* spp. The roost near to the human vicinity has significant high prevalence of bacterial infection in the studied roost. Multiple antimicrobial resistances were evidenced among *Salmonella* and E coli isolates in small fruits bat. Amoxicillin and erythromycin become resistance at significant level in free ranging bat while Ciprofloxacin and Enrofloxacin appeared too sensitive. The practices of adjacent community people linked to infection of antimicrobial resistance bacteria. The high prevalence of *Salmonella* and E coli in bats could be threat to public health.

CHAPTER VII

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APPENDIX 1

ROOST CHARACTERISTICS DATA SHEET

Date: June 30, 2013

Roost location (general area): Rural area	Species in roost Rousettus spp.	Lat/Long of roost: Elevation of roost:
How close can the roost be approached: Very close (beneath the roost)	Estimate percentage of roost perimeter which can be accessed: 100%	Describe bats' reaction to approach of researchers: Flying and screaming
Describe roosting pattern (clumped/with satellites?): Clumped	Describe roost trees/vegetation (type, condition, species roosting differences): Mango, domur and banana	Describe signs of human access (e.g. trails, garbage, hunting information): Hunting present, disturbance by kids

# trees within roosting area above 5 meters tall: 8	# dead trees above 5 m: 0	# trees with roosting bats: none
Estimate area containing roosting bats: Two room of old buildings	Topography (e.g. ridge, upper slope, orientation of slope, valley bottom):	Nearness to river and description (e.g. width, gradient): Padma river very close (10 m)
Human settlements (how close, how many): 20 miter a village having more than 10 house near the roost		Roost permanent or changing? Temporal pattern of change? Location of alternative roost sites? Permanent

APPENDIX 2

Questionnaire to the residence near bat roost

Questionnaire type: close ended

Selection of respondent: random

Question	Response			
Does these bat have access in your house	Often	Sometime	Never	Don't know
What types of food they take? What do you think?	Insect	Fruits	Don't know	Both
What type of fruits they taken?	Open ended			
Do you take those rest fruits from the orchard	Always	Sometime	Never	sporadic
Do you wash the green vegetables harvest near the roost before raw eating	Always	Never	Sometimes	Sporadic
Is there anyone who hunt the bat and eat	No	Yes	If yes	
What is the purpose of hunting	Medicinal	Food	Selling	other

APPENDIX 3

Picture


Figure: bat roosting site and Rousettus bat in the roost in Rajbari Bangladesh.


Figure: Fruits eating by bats and Sample collection from ground of the roosting site.

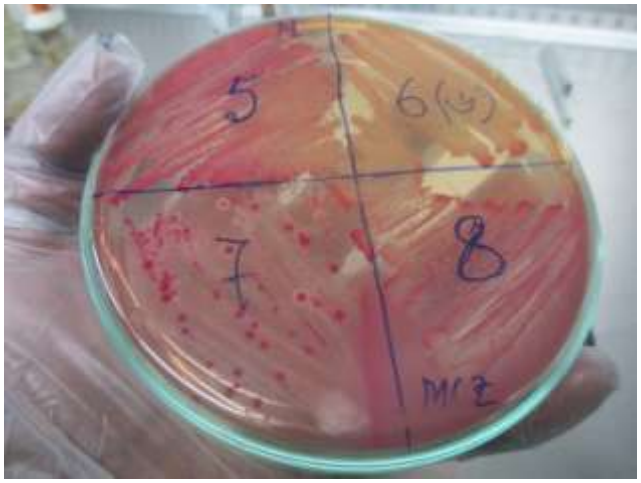

Figure: Growth of *Salmonella* in XLD and antibiotic resistance test of E coli