PREVALENCE OF POTENTIALL ZOONOTIC BACTERIA FROM HOUSEHOLD PET BIRDS AND THEIR ANTIMICROBIAL RESISTANCE AT DINAJPUR DISTRICT OF BANGLADESH

A THESIS

BY

MAHMUDA NAZNIN NUPUR

REGISTRATION NO. 1605136

SEMESTER: JANUARY–JUNE, 2017

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MASTER OF SCIENCE (M.S.)
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HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
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JUNE, 2017

Dedicated To My Beloved Parents

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The author
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ABSTRACT

A cross sectional study was conducted to ascertain the prevalence of zoonotic bacterial pathogen in pet birds (pigeon, parrot, budgerigar or love birds & quail). The study was done in selected areas of sadar upazilla at Dinajpur district during the period of July 2016 to June 2017). A total of 81 pet birds including 40 pigeons, 20 parrots, 12 budgerigar or love birds and 9 quails were selected and samples were collected in relation to different socio demographic variables (age, sex, breed, body weight, diet history, hygienic condition & vaccination). A total of 243 samples consist of Cloacal swab (81), Oral swab (81) and Feces (81) were examined and 5 types of potential zoonotic bacteria were isolated from pet birds. The organisms were isolated by using standard microbiological method. The results were determined by the average microbial load in plate count agar. In case of pigeon, highest total viable count (TVC) was found in male (young) 10.40±0.10 cfu/g and the lowest TVC was found in female (young) 9.71 ±0.41 cfu/g. In case of parrot, the highest TVC was found in male (young) 10.54±0.26 cfu/g and the lowest TVC was found in female (adult) 9.09±0.29 cfu/g. In case of budgerigar or love birds the highest TVC was found in male (young) 10.53±0.26 cfu/g and the lowest TVC was found in female (adult) 9.09±0.29 cfu/g. In case of quail, highest TVC was found in female (adult) 10.63±0.17 cfu/g and the lowest TVC was found in female (young) 9.37±0.57 cfu/g. The overall prevalence of potential zoonotic pathogens in pigeon out of

120 samples, *E. coli* (17.5%), *Salmonella* spp (10.83%), *Shigella* spp (15.83%), *Klebsiella* spp *(*13.13%) and *Staphylococcus* spp (15%) were found respectively. Out of 60 samples (Cloacal swab=20, Oral swab=20 and Feces=20) of parrot, the prevalence was *E. coli* (18.33%), *Salmonella* spp (15%), *Shigella* spp (11.67%), *Klebsiella* (18.33%) spp and *Staphylococcus* spp (11.67%) respectively. In case of budgerigar out of 36 samples (12 Cloacal swab, 12 Oral swab and 12 Feces) the prevalence were found *E. coli* (19.44%), *Salmonella* spp (19.44%), *Shigella* spp (8.33%), *Klebsiella* spp (13.88%) and *Staphylococcus* spp (8.33%) respectively. In case of quail Out of 27 samples (9 Cloacal swab, 9 Oral swab and 9 Feces) the prevalence were found *E. coli* (77.78%), *Salmonella* spp (66.67%), *Shigella* spp (66.67%), *Klebsiella* spp (77.78%) and *Staphylococcus* spp (66.67%) respectively. The prevalecne of isolated bacteria was statistically significant ($P<0.05$) in relation to diet and hygienic condtion in pigeon. Again the prevalence of isolated bacteria was statistically significant ($P<0.05$) in relation to age, bodyweight, hygienic condition and vaccination in budgerigar and in relation to hygienic condition in quail respectively. On the other hand the prevalence of isolated bacteria in pigeon was not statistically significant ($P>0.05$) in relation to age, sex, bodyweight, breed and vaccination. In case of Parrot the prevalence of isolated bactria was not statistically significant ($P>0.05$) in relation to all socio-demographic variables studied. In case of love bird the prevalence of isolated bacteria was statistically significant ($P<0.05$) in relation to age, sex, breed and body weight. On antibiotic sensitivity test 18 antibiotics were used against five isolated bacteria. Gram negative bacteria i.e *E. coli*, *Salmonella* spp, *Shigella* spp, *Klebsiella* spp were sensitive to Erythromycin, Cephalexin, Gentamycin, Chloramphenicol, Kanamycin, Cefixime, Cefradine, Ciprofloxacine and Levofloxacine whereas gram positive bacteria i.e *Staphylococcus* spp was sensitive to Ciprofloxacin, Gentamycin, Kanamycin, Vancomycin and chloramphenicol. Isolated bacteria were resistant to Penicillin G,

Azithromycin, Amoxycillin, Cloxacillin, Bacitracin, Tetracycline, Collistin and Neomycin. Since, pet birds play a vital role in the transmission and maintenance of zoonotic pathogens leads to interactions of public health concern. Pet birds infections with zoonotic potential should not be overlooked, considering the major health impact on the population including children.
Key words: Potentially zoonotic pathogen, pet bird, prevalence, antimicrobial resistance, colony forming unit

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LIST OF ABBREVIATIONS AND SYMBOLS

-	:	Negative
%	:	Percentage
/	:	Per
<	:	Less than
>	:	Greater than
+	:	Positive
µg	:	Microgram

µl	:	Micro liter
^0C	:	Degree of Celsius
CFU	:	Colony forming units
E. coli	:	*Escherichia coli*
e.g	:	Example
EMB	:	Eosin Methylene Blue
et al.	:	Associated
Etc	:	Etcetera
Fig.	:	Figure
Gm	:	Grams
H_2S	:	Hydrogen sulfide
Hrs	:	Hours
HSTU	:	Hajee Mohammad Danesh Science and Technology University
KOH	:	Potassium hydroxide
L	:	Lactose
MC	:	MacConkey Agar
Min	:	Minutes
MI	:	Milliliter
MIU	:	Motility Indole Urease
Ml	:	Milliliter
ML	:	Maltose
Mm	:	Millimeter
MN	:	Mannitol
MR	:	Methyl Red
MSA	:	Mannitol Salt Agar

LIST OF ABBREVIATIONS AND SYMBOLS (Contd.)

N	:	Number
NA	:	Nutrient agar
NB	:	Nutrient broth
-	:	Negative
No.	:	Number

NS	:	Non-significance
PBS	:	Phosphate Buffer Saline
R	:	Resistant
S	:	Sucrose
S	:	Sensitive
Sec	:	Second
SL.	:	Serial
spp.	:	Species
Sq	:	Square
SSA	:	Salmonella Shigella Agar
TCC	:	Total coliform count
TSS	:	Total Salmonella-Shigella count
TSI	:	Triple sugar iron
TVC	:	Total viable count
VP	:	Voges Proskaur

CHAPTER 1

INTRODUCTION

The term “Pet bird” designates birds housed and bred for an exclusively ornamental use. Pet birds are the source of recreation for human especially children. According to encyclopedia of flora and fauna of Bangladesh, birds, (Volume 26), 650 species of birds belong to 295 genera and 64 families have been recorded in Bangladesh. Birds are very important wild creatures, as they help in pest control, pollination, cleaning the environment as scavenger as well as an important ecological indicator (Ali and Ripley, 1983). Unlike cats and dogs, birds are not typically considered domesticated animals even when bred in captivity. This is due in part to the fact that many bird species produced for the pet trade are only one or two generations removed from the wild and, as such, retain most if not all of their wild instincts and behaviours (Davis 1998; Graham 1998). In addition, many bird species that are bred and traded as companion animals also remain physically indistinguishable from their wild counterparts, with the few exceptions of those birds which have been hybridised or selectively bred to express colour mutations (Engebretson, 2006). Birds were first caged for their beauty more than 4,000 years ago. Before that, birds had been associated with human settlements, but as dinner, not pets. Egyptian hieroglyphics depict what appear to be first pet birds, including doves and parrots. It is unclear when they were first domesticated, if at all. Whether birds are domesticated or not depends on the definition of the word domestic and the kind of bird to choose as a companion (Animal Hospitals USA, 2017).

The current study was focused on birds mostly kept as pets (pigeon, parrots, budgerigar or love birds & quails) and pigeons held and bred as pets or for sport and reared for food. Parrots are a well-defined group of birds that are so distinctive (small to medium sized with stout, hooked bills and a moveable upper mandible) range in size from the relatively small budgerigars (*Melopsittacus undulatus*), cockatiels (*Nymphicus hollandicus*) and lovebirds (*Agapornis* spp), and medium-sized conures

(*Aratinga* spp), amazons (*Amazona* spp) and African greys (*Psittacus erithacus*) to large-sized cockatoos (*Cacatua* spp) and macaws (*Ara* spp) (Gill 1990). “Parrot” is a term commonly used to refer to any of the more than 350 species belonging to the Psittaciformes order comprising three families: Psittacoidea (‘true” parrots), Cacatuoidea (cockatoos), and Strigopoidea (New Zealand parrots). These species are found worldwide in subtropical and tropical climates, but there are species living in temperate latitudes, high altitudes (e.g., Patagonia, Tierra del Fuego, New Zealand) and even sub-Antarctic islands (Bradshaw and Engebretson, 2013). Parrots are excellent companion animals, and can form close, affectionate bonds with their owners. They are popular as pets due to their sociable and affectionate nature, intelligence, bright colors, and ability to imitate with human voices (Akhter *et al,* 2010). Parrots are well known for their perceptiveness, brilliant and spectacular plumage, strong tongues, curved beaks, and zygodactyl feet (two digits facing forward and two facing backward) that allow these birds to be formidable climbers. Some species are very long lived; there are recorded ages beyond 100 years such as, Amazon yellow-naped (*Amazona auropalliata*) (Montgomery, 2011).

The budgerigar (*Melopsittacus undulatus*) or “budgie” is a small bird of the psittacine group (parakeets) that enjoys immense popularity as a cage bird pet. It was originally introduced into North America and Europe over 100 years ago from the arid regions of Australia. During the past 50 years it has been bred extensively in captivity and has become thoroughly adapted to domestication (Steiner and Davis, 1981). A lovebird is one of nine species of the genus *Agapornis* (Greek: *agape* 'love'; *ornis* 'bird'). They are a social and affectionate small parrot. Eight species are native to the African continent, and the grey-headed lovebird is native to Madagascar. Their name comes from the parrots' strong, monogamous pair bonding and the long periods which paired birds spend sitting together. Some species are kept as pets, and several color mutations were selectively bred in aviculture. Lovebirds are 13 to 17 cm

(5 to 7 in) in length and 40 to 60 g (1 ½ to 2 oz) in weight. Their average lifespan is 10 to 20 years (Alderton, 2003).

Pigeons had been associated with human society both as a source of food and as cage birds from a longtime. The pigeon played a range of important roles in ancient cultures, including messenger, food, pet, religious icon, medicine, and navigation aid (Thomas *et al,* 2013). Pigeons are first mentioned in Mesopotamian records over 5,000 years ago, and are documented in most subsequent developed cultures of the region (Johnson & Janiga 1995) and they are the earliest domestic birds and one of the earliest domestic animals (Hansell 1998). Pigeon (*Columba livia*) is a plump and rounded-bodied bird of the family *Columbidae,* order Columbiformes. Out of 8600 nos. of known species of birds, 289 species are of pigeon worldwide, among them 30 species were found in Indian sub-continent. They are reared scientifically in Belgium and England, as the people used to exhibit the flock in racing competition (Dutta, 2013). Pigeons are domesticated birds which widely distributed the entire world. They have adapted to life in urban, suburban and rural environment and have close communication with humans. In Bangladesh, there are 17 species of pigeons, of which 2 are migratory (Dey *et al,* 2013). The weather and vast areas of crop field along with housing premises of Bangladesh are suitable for pigeon farming (Asaduzzaman *et al.,* 2009). According to Agricultural Sample Survey in 2013, Bangladesh had a population of 10.8 million pigeons of which 11% were kept on what is termed commercial farms that were however not identified. Farmers in rural areas rear pigeons for family nutrition and to sell in the markets for money. Some people rear pigeons in cages at their houses for recreation. Now a day's most people are engaged with pigeon rearing as second occupation even student. Pigeon are rather prolific and there is a lot of demand of squab meat in the market due to its delicacy and taste (Paul *et al.*, 2015).

Quails are small game birds that are now used for commercial production of eggs and meat. They attain rapid sexual maturity have shorter incubation period and can produce up to four generations per annum,

therefore making them the most suitable and effective poultry (Rahman *et al.,* 2016). There are two species of quails suitable for breeding, *viz*., the Japanese quail (*Coturnix coturnix japonica*) and the American or common quail (*Coturnix coturnix*). Japanese quails belong to Phasianidae family and are migratory birds which migrate between Asia and Europe (Onyewuchi et al., 2013, Saidu *et al.,* 2014). Quail meat in some countries considered as a good food for all ages due to its high meat yield, little shrinkage during cooking, fast cooking and serving and also due to their delicacy and low level of cholesterol. In addition, quail meat is tender and fortified with nutrients (Hamad *et al.,* 2012). In our country many quail farms has been established in different districts and today a considerable numbers of live quail birds are sell in local birds markets for human consumption.

Zoonotic diseases are those infections that can be transmitted between animals and humans with or without vectors. There are approximately 1500 pathogens, which are known to infect humans and 61% of these cause zoonotic diseases (Cantas and Suer, 2014). Birds are susceptible to many bacterial diseases common to humans and domestic animals (Broman, et al., 2002) and also to other potentially infectious microorganisms, including protozoa and viruses (Benskin, *et al.,* 2009). Many zoonotic diseases are transferred from cage or pet birds to human through direct or indirect contact of the diseased or carrier birds. Birds participate effectively in the transmission and spread of zoonoses, even over great distances, by acting as natural hosts, reservoirs and amplifying or liaison hosts for zoonotic agents because of their ability to fly. Furthermore, birds are the reservoir of the most widely reported classical foodborne zoonotic agents in developed countries (campylobacteriosis and salmonellosis) (Contreras *et al,* 2016). Parrots are often suffered from many bacterial diseases with often involvement of normal flora or environmental pathogens mainlyby *E. coli, Klebsiella, Salmonella, Pasteurella, Pseudomonas, Aeromonas* and *Citrobacter* (Altman and Robert, 1997). Close contact of humans with pigeons at home, live bird markets and farms bear the risk of transmission of

zoonotic infections (Hosain *et al,* 2012). Many potential infections of humans silently exist in pigeons which are not apparent. They have the potential for transmission of over 30 diseases to humans plus another ten to domestic animals (Weber, 1979). Transmission of infectious agents from diseased pigeons to poultry has been described (Alexander et al., 1985) and approximately 110 zoonotic agents have so far been isolated from pigeons (Haag-Wackernagel, 2011). Bacterial pathogens including *Salmonella* serovars, *Campylobacter* spp. and *Chlamydophila* (*Chlamydia*) *psittaci* have in common that they show a wide host range including humans and pigeons (Teske *et al,* 2013). Migratory quail act as possible (biological and/or mechanical) vectors playing role in the ecology and circulation of some zoonotic pathogen threatening human health and domestic animals. These zoonotic pathogens cause losses of efficient production and quality of food of animal origin (Ahmed and Mansour, 2014). Migratory quails pose a risk of transmission of many zoonotic diseases to hunters or consumers who handle or eat these birds by either direct or indirect contact (Smith, 1999).

Very few works have been studied on the prevalence of zoonotic bacterial pathogen from pet birds in Bangladesh and the present study, therefore, was undertaken with the following objectives-

i. To determine the prevalence of potentially zoonotic bacterial pathogens of pet birds (Pigeon, Parrot, Budgerigar or Love birds and Quail).
ii. To assess the risk factors associated with zoonotic transmission in relation to birds age, sex, breed, diet, hygienic condition and vaccination.
iii. To determine the antimicrobial susceptibility of isolated pathogens to 18 antibiotics of human and veterinary importance.

CHAPTER: 2

REVIEW OF LITERATURE

The review of literatures related to the present study is pinpointed briefly presented under the following headings

2.1 Bacterial zoonotic diseases of pet birds

Dipineto *et al*. (2017) reported that avian species are considered as the main reservoir of *Campylobacter* spp. However, few data are available on the presence of this microorganism in pet birds. This study was therefore performed to determine the prevalence of *Campylobacter* spp. in pet birds bred in southern Italy. Faecal samples were collected from 88 cages housing different species of pet birds and examined by bacteriological culture and polymerase chain reaction. A total of 13.6% of the cage samples were positive for *Campylobacter coli*. Other *Campylobacter* spp. was not found. The study shows that *E. coli* can be isolated from the cages of apparently healthy pet birds, which should therefore be considered as potential carriers of *E. coli* and a possible source of infection for humans and companion animals.

Saifullah *et al.* (2016) determined that the prevalence of *Salmonella* in cloacal swabs and pharyngeal swabs of apparently healthy pigeons sold in the live bird markets and villages in and around Bangladesh Agricultural University Campus, Mymensingh, Bangladesh. They examined about 50 samples, comprised of cloacal swabs (n=24) and pharyngeal swabs (n=26) were collected. They were processed the samples and *Salmonella* was isolated through a series of conventional bacteriological techniques and biochemical tests followed by polymerase chain reaction (PCR). The prevalence rate of *Salmonella* was found to be 37.5% (n=9/24) in cloacal swabs and 30.77% (n=8/26) in pharyngeal swabs with an overall prevalence rate of 34% (n=17/50). The prevalence rate of *Salmonella* pigeon varied slightly among locations; 34.62% (n=9/26) in live bird markets, and 33.33% (n=8/24) in villages. Molecular detection of 17 *Salmonella* isolates obtained from biochemical test was

performed by genus specific PCR, where all of them amplified a region of 496-bp segment of the *histidine transport operon* gene. Antibiogram study revealed multi-drug resistant traits in most of the isolates tested. They were found resistant against Ampicillin (88.23%) followed by Cephalexin (82.35%). The rate of sensitivity of the isolates to Ciprofloxacin was 100% followed by Azithromycin (82.35%), Gentamicin (76.47%) and Nalidixic acid (76.47%).

Elisângela *et al.* (2016) They were examined total of 167 individual cloacal swabs were collected from apparently healthy psittacines, who were housed in the local Wildlife Rehabilitation Center (Centro de Triagem de Animais Selvagens - CETAS) in Fortaleza, CE, Brazil. Initially, samples were submitted to the microbiological procedure, with the following steps pre-enrichment, selective enrichment and plating. They were performed biochemical tests used to the identify the species of enterobacteria. The samples with biochemical profile of *Salmonella* spp. were submitted to slide agglutination test using polyvalent "O" serum anti-*Salmonella*. To perform the antibiotic susceptibility testing, all the strains isolated were cultured in BHI broth, and then streaked in MacConkey agar.

Abbas (2016) investigated that isolation of bacteria from birds he ovsered that, the result revealed that isolation rate was (63%) for *Staphylococcus aureus*, *(*66%) for *Streptococcus* group D, (49%) for *Escherichia coli,* (6%) for *E coliO157*, (11%) for *Salmonella* sp, (18%) for *Shigella*, (14%) for *Vibrio* sp., (10%) for *Aeromonas* sp., (8%) for *Plesimonas shigelloides*, (30%) for *Klebsiella* sp., (2%) for *Nocardia* sp.

Ashraf *et al.* (2015) determined to isolate Salmonellae from 579 birds (348 chickens, 104 ducks, 30 turkeys, 50 quail, 30 pigeons and 17 geese) from 4 Egyptian Governorates. The Samples collected from internal organs (liver, cecum, spleen and heart) were examined bacteriologically and serologically. Sixty-three (10.9%) out of 579 birds were found

positive while 516 (89.1%) birds were negative for Salmonella isolation. The number and percentage of positive chickens, ducks, turkeys, quails, pigeons and geese were 43 (12.4%), 10 (9.6%), 3 (10%), 5 (10%), 2 (6.7%) and 0 (0%) respectively. In this study, were also isolated *S. typhimurium, S. apeyeme, S. kentucky, S. daula*, *S. newport, S. Tamale, S. molade, S. colindale, S. lexington, S. bargny, S. enteritidis, S. papuana, S. labadi, S. santiago*, *S. magherafelt, S. rechovot, S. takoradi, S. angers* and *S. shubra* from chickens.

Abo-Amer and Shobrak (2015) observed that the isolation and molecular characterization of multidrug-resistant *Salmonella, Shigella* and *Proteus* from domestic Birds. *Salmonella*, *Shigella* and *Proteus* were isolated from different types of healthy domestic birds (n=42) collected from different places. Bacterial isolates were recovered from cloacal swabs of birds by non-selective and selective pre-enrichment technique. One hundred and sixty six bacterial isolates were screened for antibiotic susceptibility such as Cefaclor, Oxacillin, Ampicillin, Chloramphenicol, Cephalexin, Neomycin, Colistin, Ciprofloxacin, Oxytetracycline, Norfloxacin, Lincomycin, Gentamycin, Amoxicillin, Enrofloxacin and Piperacillin.

Caballero *et al.* (2015) reported that the isolation and molecular identification of potentially pathogenic *Escherichia coli* and *Campylobacter jejuni* in feral pigeons. They were determined isolate and detect strains of diarrheagenic *Escherichia coli* and *Campylobacter jejuni* of urban feral pigeons. They examined that microbiological isolation of *E. coli* strains in selective agar, and *Campylobacter* by filtration method. They were also mentioned molecular identification of diarrheagenic pathotypes of *E.coli* and *Campylobacter jejuni* was performed by PCR. Twenty-two parks were sampled and 16 colonies of *Campylobacter* spp. were isolated and 102 colonies of *E. coli* were isolated and the 5.88% resulted as Enteropathogenic (EPEC) type and 0.98% as Shiga toxin-producing *E. coli* (STEC).

Suelen *et al.* (2015) demonstrated that the occurrence of *Salmonella* sp. and *Escherichia coli* in free-living and captive wild birds. The objective of this study was to assess the occurrence of *Salmonella* sp. and *Escherichia coli* in free-living and captive wild birds in the city of Guarapuava, PR, Brazil. Their study showed that bacterial isolation was carried out by means of cloacal swabs, with 69.38% birds positive for *E. coli* and 22.32% for *Salmonella* sp. From the total of birds, 143 showed co-infection with *Salmonella* sp. and *E. coli.* Coliforms showed the greatest occurrence of *E. coli* (82.33%). Falconiformes showed the greatest number of negative birds (57.9%). These results demonstrate that birds that were analyzed may carry and spread these enterobacteria, and preventive measures for human exposure should be determined, as these microorganisms are public health concerns.

Hassan and Bakeet (2014) conducted that *E. coli* is a major pathogen of commercially produced poultry all over the world, causing colibacillosis and contributing high significantly to economic losses. They were performed isolation, serotyping, virulence factors and antimicrobial susceptibility test were characterized for avian pathogenic *E. coli* strains that isolated from tissues of the infected pigeons and from non-hatched pigeon eggs in Assiut Province. They were examined about 124 samples (87 pigeons and 37 non-hatched pigeon eggs) for *E. coli* infection. The samples include liver and kidney tissues of diseased and freshly dead pigeons plus yolk of 20 infertile eggs and yolk sac of dead-in-shell embryos of the non-hatched pigeon eggs. Twenty three bacterial isolates were identified, from which 19 isolates for *E. coli* and one isolate for each *Enterobacter agglomeranns, Enterobacter cloacae, Hafnia alvei and Serratia marcescens* from tissues of infected pigeons while samples of pigeon eggs were found negative for *E. coli* isolation. Eight serogroups were identified among sixteen of pigeon *E. coli* isolates, however, 3 isolates were nontyped.

Maysa *et al.* (2013) isolated *E. coli* from wild birds and human. Six *Salmonella* isolates from wild birds were serotyped into *Salmonella enteritidis (S. enteritidis),* S. *typhimurium*, *S. haifa*, *S. chester* and *S. muenster*, while those two isolates of human were identified into *S. typhimurium* and *S. entertidis*. Eight *E. coli* serotypes; belonged to O127: K63, O128:K67and O26:K60 strains from wild birds and human; were subjected to RAPD-PCR.

Magda *et al.* (2013) studied that the prevalence of *Enterobacteriacea* in wild Birds and Humans at Sharkia Province, with Special reference to the Genetic relationship between *E. coli* and *Salmonella* isolates determined by Protein Profile Analysis they were isolated microorganisms revealed the recovery of *Escherichia coli*, *Salmonella spp, Enterobacter cloacae*, *Enterobacter agglomerans*, *Enterobacter aerogens*, *Enterobacter hafnia, Citrobacter freundii*, *Citrobacter diversus*, *Klebsiella ozaenae*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Proteus mirabilis*, and *Proteus rettegri* at different percentages from the examined wild bird.

Lydia Teske *et al.* (2013) examined epidemiological investigations on the possible risk of distribution of zoonotic bacteria through apparently healthy homing pigeons. They determined two samplings were performed during the racing season in summer (1242 adult and 1164 juvenile pigeons) and two during winter (1074 adult pigeons). Each sampling was accompanied by a questionnaire to identify risk factors for positive lofts. Between 0.9 and 3.7%, 13.1 and 23.7%, and 12.8 and 42.6% of lofts were tested positive by cultural methods or polymerase chain reaction for Salmonella Typhimurium var. Copenhagen, Campylobacter jejuni and C. psittaci, respectively. The detection rate of C. psittaci was twice as high in samples from juvenile pigeons (29.1%) compared with samples from adult pigeons (15.0%, PB0.001). No other influence of age or season was detected.

Md. Sahadat *et al.* (2012) determined that the prevalence of *Salmonella* spp. in apparently healthy pigeons at the live bird markets, farms and villages in the Mymensingh district of Bangladesh. They were collocted cloacal swabs (n = 36), foot pads (n = 36) and feces (n = 40) of pigeons and inoculated onto various culture media for isolation of *Salmonella*. They were identified *Salmonella* from culture positive samples was performed by cultural characteristics, Gram's staining and biochemical tests. The prevalence of *Salmonella* in cloacal swabs, foot pads and feces was 22.22%, 58.33% and 27.50%, respectively. The overall prevalence of *Salmonella* in pigeons was 35.71%. Pigeons at live bird markets, farms and villages showed a 40.48%, 20% and 30% prevalence of *Salmonella*, respectively. Antibiotic sensitivity tests of the *Salmonella* was performed by the disc diffusion method against 10 randomly used antibiotics. The highest rate of resistance was found with amoxicillin (90%) followed by ampicillin (80%), erythromycin (80%) and tetracycline (60%). The highest rate of sensitivity was recorded to ciprofloxacin (80%) followed by sulphamethoxazole (70%), chloramphenicol (60%), kanamycin (60%), gentamicin (60%) and nalidixic acid (60%).

Rahmani *et al.* (2011) investigated *Salmonella* infection in birds kept in parks and pet shops. They obsereved samples contained cloacal swabs from large birds, freshly-dropped feces from small birds and, infrequently, carcasses. All samples were cultured for the isolation and identification of *Salmonella* serovars according to standard procedures. They were found that the antimicrobial susceptibility of the isolates was determined to a panel of 30 antimicrobial agents using the agar disc diffusion method. In total, 19 Salmonella isolates (2.8%) were identified. Samples that were positive for Salmonella originated from canaries (10 out of 62, 16.1%), pigeons (5 out of 139, 3.6%), psittacines (3out of 130, 2.3%), and eagles (1 out of 2, 50%). All Salmonella isolates were susceptible to danofloxacin, norfloxacin, levofloxacin, amikacin,

gentamicin, and tobramycin. Resistance to other antibacterial agents was variable and ranged from 0-57.9%.

Aleya Begum and S. Sehrin (2011) examined total of 60 pigeons, *Columba livia* (25 males and 35 females) for ectoparasites. All the birds were infected (100%) by 10 species of
ectoparasites. The ectoparasite comprised lice: 60 (100%) *Menopon gallinae*, 28 (46.66%) *Menacanthus stramineus*, 43 (71.66%) *Colpocephalum turbinatum*, 60 (100%) *Columbicola columbae*, 31 (51.66%) *Lipeurus caponis*, 19 (31.66%) *Goniocotes gallinae*, 28 (46.66%) *Chelopistes meleagridis*; fleas: five (8.33%) *Echidnophaga gallinacean*; flies: 38 (63.33%) *Pseudolynchia canariensis* and eight (13.33%) of mites *Dermanyssus gallinae*. Serious damage was observed in wing feathers (31.74%). The females had a higher intensity (30.11) of infestation than the males (29.04). The ectoparasites were removed from the pigeons throughout the year. The overall intensity of infestation was highest during summer (40.69) and lowest during winter (21.94).

J. Akhter *et al.* (2010) conducted to isolate and identify the micro-flora from apparently healthy caged parrots. A total of 45 samples (oral swabs, cloacal swabs and feces) were collected from five types of caged parrots. The bacteria isolated in this study from different types of caged parrots were *E. coli* (64.44%), *Salmonella* spp. (46.67%), *Staphylococcus* spp. (46.67%), *Pasteurella* spp. (33.33%), *Proteus* spp. (6.67%) and some unidentified Gram-positive and Gram-negative bacteria. However, the antibiotics of fluoroquinolone group such as ciprofloxacin, norfloxacin and enrofloxacin showed moderate to high sensitivity against almost all the bacterial isolates. Of these, ciprofloxacin was found to be consistently highly sensitive to all the bacterial isolates.

Belén Vázquez *et al.* (2010) reported that the screening for several potential pathogens in feral Pigeons. They were determined the pathogens with the zoonotic potential to infect humans, such as

Campylobacter jejuni, Campylobacter coli and *Chlamydophila psittaci*, can be found in feral pigeons. They were demonstrated a high prevalence of *Chlamydophila psittaci* (52.6%) and *Campylobacter jejuni* (69.1%) among the birds captured. In contrast, *Campylobacter coli* was rarely detected (1.1%).

Kerri Pedersen *et al.* (2006) studied that the prevalence of shiga toxin–producing escherichia coli and salmonella enterica in rock pigeons. Their findings of this study was suggested that Prevalence of STEC and S. enterica was estimated by bacteriologic culture of cloacal swabs collected from pigeons trapped at urban and dairy locations in and around fort Collins, Colorado from January to November 2003. They found that the Presumptive *E. coli* isolates were tested for the presence of virulence genes SLT-1, SLT-2, eae, hlyA, K1, CNF-1, CNF-2, and LT using polymerase chain reaction. Shiga toxins were not isolated from any of 406 samples from pigeons, but virulence genes typically associated with disease in humans were identified in isolates from 7.9% (95% CI: 5.5% to 10.9%) of captured pigeons.

Thomas R. Raffel *et al.* (2002) analyzed that the prevalence of *Bordetella avium* infection in selected wild and domesticated birds. A survey of the prevalence of *B. Avium* in wild and domesticated birds was conducted from June 1998 to January 2000, using tracheal cultures and serology of 237 blood samples from 61 species, 100 individuals From 41 species had antibodies against *B. avium* as determined with a microtiter agglutination Test. Nine isolates of *B. avium* were cultured from 128 tracheal samples.

2.2 Public health significance of zoonotic diseases in pet birds

Ludovico Dipineto *et al*. (2017) reported that avian species are considered as the main reservoir of *Campylobacter* spp. However, few data are available on the presence of this microorganism in pet birds.

This study was therefore performed to determine the prevalence of *Campylobacter* spp. in pet birds bred in southern Italy. Faecal samples were collected from 88 cages housing different species of pet birds and examined by bacteriological culture and polymerase chain reaction. A total of 13.6% of the cage samples were positive for *Campylobacter coli*. Other *Campylobacter* spp. was not found. The study shows that *C. coli* can be isolated from the cages of apparently healthy pet birds, which should therefore be considered as potential carriers of *C. coli* and a possible source of infection for humans and companion animals.

Ghazi *et al.* (2017) examined cloacal swabs and drooping samples of 48 wild birds, including 16sparrows, 6 Brewer's blackbirds (Songbirds /starling),7 black crows (raven) ,10 Doves , 5 quail . 2 Parrots (parakeets), 1 Eagles and 1 hawk were collected and subjected for isolation of potentially pathogenic bacteria between Marchand June 2013 from different sites at 3 districts located in Amran governorate (Yemen). Eleven bacterial species in 14 genera were isolated from wild birds. They were isolated *E.coli* (15/48; 31.25%), *Salmonella Typhimurium*. (6/48; 12 %), *Proteus mirabilis* (6/48; 12 %), *Proteus vulgaris* (4/48; 8 %), *Citrobacter frundii* (6/48; 12 %), *Klebsiella pneumonia* (5/48; 10%) , *Pseudomonas aeruginosa* (5/48; 8%) , *Campylobacter jejuni* (4/48; 8%) , *Staphylococcus aureus* (4/48; 8%), *Enterococci* (7/48;14%) and *Clostridium perfringens* (6/48;12%) from examined wild birds.

T. K. Paul *et al.* (2015) studied on pigeon diseases at Khulna sadar and surrounding private farms was done to determine the occurrence of the common pigeon diseases. They were examined 502 diseased of pigeon. According to age, they were classified into three categories squab (1-2 weeks), young (30-90 days) and adult (>90days). Those diseases were identified clinically by postmortem examination and laboratory testing, were carried out in veterinary hospital at Khulna from March 2013 to February 2014. Out of 502, 20.32% were salmonellosis,

18. 92% were pigeon pox, 11.95% were canker. Pigeon pox found high level in June-July. Among other diseases parasitic infestation (31.67%) was more prevalent. Disease varies significantly (P<0.01) with season, where summer (57.37%) is more prevalent. Rate of diseases (salmonellosis and pigeon pox) affection significantly (P<0.01) varies with age. Young are more susceptible with salmonellosis and pigeon pox. This study was done first time at Khulna and right time to take necessary steps saving pigeon farming.

Ludovico Dipineto *et al.* (2014) analyzed Prevalence of enteropathogenic bacteria in common quail (Coturnix coturnix). They determined that the prevalence of enteropathogenic bacteria (i.e. Campylobacter spp., shigatoxin-producing Escherichia coli, Salmonella spp.) in common quail (Coturnix coturnix). They were obtained 70 common quails were collected during the hunting season in the Campania region (southern Italy). They were found that the present study showed a prevalence of 21.4% and 5.7% for Campylobacter spp. and shigatoxin-producing E. coli, respectively. No Salmonella spp. was isolated.

Rubel K. Dey *et al.* (2013) determined that the prevalence of antimicrobial resistant *Escherichia coli* in pigeon a total of 112 samples such as cloacal swabs (n=36), foot pads (n=36) and feces (n=40) were collected from pigeon aseptically. Samples were enriched in nutrient broth and then streaked onto Eosine Methylene Blue agar, Salmonella-Shigella agar, MacConkey agar and blood agar. They were performed cultural and biochemical characteristics of bacterial isolates of pigeon were indicative of *E. coli*. The prevalence of *E. coli* in cloacal swabs, foot pads and feces samples were 86.11%, 44.44% and 77.50%, respectively. The overall prevalence of *E. coli* in pigeon was 69.64% (78 of 122 samples were found positive for *E. coli*). The antibiotic sensitivity pattern showed that *E. coli* isolates were sensitive to erythromycin, ciprofloxacin, kanamycin, nalidixic acid and resistant to amoxicillin, tetracycline and

sulphamethaxazole. It may be concluded that pigeons from Mymensingh locality in Bangladesh carry multidrug resistant *E. coli*.

Pwaveno *et al.* (2013) conducted to elucidate the prevalence of *Cryptosporidium* oocysts in birds in Zaria, Nigeria. A total of 890 faecal samples comprising 132, 305 and 453 from wild, local and exotic birds respectively from different parts of Zaria were examined using the formol-ether concentration technique with safranin–methylene blue stain and auramine phenol stain using light microscopy and fluorescent microscopy respectively. The total prevalence rate was 7.4%. However, Samaru had the highest prevalence rate of 20.6% and Tudun Wada the lowest rate of 2.8%. They were differentiate the prevalence rates between the different localities of Zaria was found to be statistically significant ($P<0.001$). Among the different birds sampled, local birds had the highest prevalence rate of 9.5% followed by exotic birds 6.6% and the wild ones with 5.3%. The difference was not statistically significant ($P>0.05$). In Tudun Wada, where the different sexes were noted, there was no significant statistical difference ($P>0.05$) in the prevalence rate between male and female birds and none between the different species of wild birds sampled ($P>0.05$)

Kriaek *et al.* (2012) examined total of 411 samples from birds of different species originating from all counties of the Republic of Croatia have been tested for the presence of Chlamydophila psittaci. They were conducted in pet stores, breeders' aviaries, in a specialized bird clinic and in zoos. The testing included 177 parrots, 169 pigeons, 58 canaries and 7 finches. For the detection of specific C. psittaci antigen a commercial ELISA kit was used- IDEIATM PCE Chlamydia (DAKO Cytomation Ltd., United Kingdom). The samples that were non-specifically positive or doubtful in the ELISA test (a total of 26 samples) were analyzed also by means of polymerase chain reaction (PCR). Diagnostic ELISA method found a total of 17.03% birds positive for chlamydiosis, and after additional testing by PCR a total of 12.65%

positive ones were found. According to bird species, the most frequently positive ones were canaries and pigeons (15.52% and 13.02%), and according to the sampling location most of the positive birds were found in pet stores (16.52%), but a high percentage of positive samples were also found in breeders' aviaries (11.76%).

Madani *et al*. (2011) investigated that Avian chlamydiosis is one of the most important infectious diseases of birds. Despite the rapid growth of exotic bird populations in Iran, there is little or no information on the specific infections that these types of birds carry. In this study, they were isolated methods used in cell culture to study occurrence of infection in pet birds. Samples from the conjunctiva, choana, and cloaca and/or droppings were provided from 17 birds of different species.

Ahmed *et al.* (2011) investigated Prevalence of some zoonotic bacteria in wild birds in Kirkuk city. They were examined to isolated some of zoonotic bacteria from different organs of native wild birds included 21 individuals of House Sparrow, 15 individuals of White- Cheeked Bulbul, 20 individuals of Collared Dove and 20 individuals of Rock Dove. Samples of liver, kidney, blood and content of middle intestine of individual birds in Kirkuk city. They showed that the results of many zoonotic bacteria included *Listeria monocytogenes, Salmonella* spp., *Shigella* Spp., *Brucella abortus* and *Campylobacter* Spp.

Ajayi and Egbebi. (2011) reported that the antibiotic sucseptibility of *Salmonella Typhi* and *Klebsiella Pneumoniae* from poultry and local birds in Ado-Ekiti, Ekiti-State, Nigeria. They were recovered of sixty-four strains of *S. typhi and* 77 strain of *K. pneumoniae* from 120 poultry birds while 100 strains of *S. typhi* and 90 strains of *K. pneumoniae* were isolated 150 local birds. All the isolates were screened for their antibiotic susceptibility to the following antibiotics using the agar disk diffusion technique: augmentin (25µg), cotrimoxazole (25µg), ofloxacin ((25µg), gentamicin (10µg), nitrofurantoin (200µg), nalidixic-acid (30µg),

amoxicillin (25µg) and tetracycline (25µg). The frequency of antibiotic-resistance from poultry birds ranged between 87.5% and 98.4% for *S. typhi and* 53.2% to 100% for *K. pneumoniae.* In addition, the frequency of antibiotic resistance among the isolates from local birds ranged between 39% and 100% for *S. typhi* and 28% to 88% among *K. pneumoniae.*

Clare McW. H. Benskin *et al.* (2009) examined on wild birds as potential vectors of disease has received recent renewed empirical interest, especially regarding human health. Understanding the spread of bacterial pathogens in wild birds may serve as a useful model for examining the spread of other disease organisms, both amongst birds, and from birds to other taxa. Information regarding the normal gastrointestinal bacterial flora is limited for the majority of wild bird species, with the few well-studied examples concentrating on bacteria that are zoonotic and/or relate to avian species of commercial interest. However, most studies are limited by small sample sizes, the frequent absence of longitudinal data, and the constraints of using selective techniques to isolate specific pathogens. The pathogenic genera found in the gut are often those suspected to exist in the birds' habitat, and although correlations are made between bacterial pathogens in the avian gut and those found in their foraging grounds, little is known about the effect of the pathogen on the host, unless the causative organism is lethal. In this review, we provide an overview of the main bacterial pathogens isolated from birds.

Sara et al. 2008 determined the prevalence of *Salmonella* in a population of house sparrows, which are commonly found around poultry houses, and to characterize the obtained *Salmonella* isolates via serotyping, multiplex polymerase chain reaction (PCR), and antibiotic resistance analysis. Samples of visceral organs (gastrointestinal tract, liver, and heart) from 470 house sparrows were subjected to culture and the results show that 18 (3.8%) were positive for *Salmonella* of the 18 *Salmonella* isolates characterized, the most predominant serovars were

Salmonella typhimurium and *S*. Enteritidis (9 and 8 cases each, respectively), whereas only 1 serovar belonged to *S*. Montevideo. All 9 *S*. *Typhimurium* serovars were positive for *rfb*J, *flj*B, *inv*A, and *fli*C genes based on multiplex PCR assay. In the case of *S*. enteritidis serovars, PCR generated amplification products for *spv* and *sef*A genes, and a random sequence (specific for the genus *Salmonella*) in all 8 samples. All the *Salmonella* isolates were sensitive to norfloxacin, flumequine, ampicillin, and sultrim, and 35% were resistant to lincospectin (the most prevalent resistance).

Dzoma and Mulenga (2007) examined that 880 aviary bird case files, including pathology, biopsy, and clinical findings was conducted at the University of Zimbabwe Veterinary Pathology Laboratory and Hospital for the period 1986- 2004. The ages of the birds ranged from hatchling to 40 years old. Ninety-two percent of these were psittacines while the remainder were passerines. Among the psittacines, the Lovebird and the African Grey parrot had the greatest prevalence rates of 46% and 17% respectively. The passerines included the threatened Lady Gouldian finch, the canary and the mynah. Infections accounted for the greatest cause of mortality at 64%. Some causes of mortality had a species biased distribution, with the lovebird succumbing mostly to the psittacine beak and feather disease (PBFD) virus and aspergillosis.

Alenka D. *et al* (2007) reported on pathological findings and interpretation of the results of diagnostic tests, obtained at chlamydial infection in a flock of parrots. In a two week period, a high mortality in one flock of budgerigars (*Melopsittacus undulatus*) was reported. They were examined adults as well young older than 14 d died. The laboratory investigation confirmed the infection with *Chlamydophila psittaci.* In the same period two members of the owner's family showed signs of atypical pneumonia. The owner decided to eliminate the whole flock. Samples of blood and swabs from cloacal were taken before the birds were euthanized.

Heddema *et al.* (2006) investigated that the feral rock dove is an abundant bird species that can harbor *Chlamydophila psittaci*. They were determined the prevalence and genotype of *C. psittaci* in fresh fecal samples from feral pigeons in Amsterdam, The Netherlands. The prevalence was 7.9% overall (26/331; 95% confidence interval, 5 to 11). Ten genotyped PCR-positive samples were all genotype B.

Zdenek Hubalek (2004) observed that pathogenic microorganisms by migratory birds is of concern. They were isolated many species of microorganisms pathogenic to homeothermic vertebrates including humans have been associated with free-living migratory birds. They were identified *Campylobacter jejuni, Salmonella enterica, Pasteurella multocida, Mycobacterium avium, Candida* spp.,from the samples. The efficiency of dispersal of pathogenic microorganisms depends on a wide variety of biotic and abiotic factors affecting the survival of the agent in, or disappearance from, a habitat or ecosystem in a new geographic area.

Kapperud and Olav Rosef (1983) examined that Cloacal swabs were collected from 540 wild-living birds and cultured for *Campylobacterfetus s*ubsp. *jejuni, Yersinia* spp., and *Salmonella* spp. They were detected carrier rates as follows: *C. fetus* subsp. *jejuni*, 28.4%; *Yersinia* spp., 1.2%; and *Salmonella* spp., 0.8%. All birds were apparently healthy when captured. *C. fetus* subsp. *jejuni* was isolated from 11 of the 40 bird species examined. These isolates were identified as *S. typhimurium*, *S. indiana*, and *S. djugu.* Their results indicate that *campylobacters* are a normal component of the intestinal flora in several bird species, whereas *Salmonella* and *Yersinia* carriers are more sporadic.

CHAPTER 3
MATERIALS AND METHOD

The present study was carried out of pet birds (Pigeon, Parrot, Budgerigar or Love birds & Quail) under the sadar of Dinajpur district and samples were taken in the bacteriology laboratory of the department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh for the identification of bacteria by different microbiological methods.

3.1 Materials

3.1.1 Study area

The research work was undertaken in different areas of sadar upazilla at the district of Dinajpur. sadar at the district of Dinajpur. Samples (Cloacal swab, Oral swab, & Feces) were collected from those selected area of Dinajpur district.

The study was directed during the period from July 2016 to June 2017.

3.1.2 Specimen Collection and Transplant

A total of 243 samples (cloacal swab, oral swab & feces) were collected from pet birds (pigeon, parrot, budgerigar or love birds & quail) by sterile cotton buds and took into sterile tube containing with 1% peptone water. Each sample was marked properly with date, time and sample number. After collection of those samples in a tube closed the cap and taken to the laboratory for microbiological investigation.

3.1.3 Instrument and apparatus

At research laboratory, Department of microbiology, Hajee Mohammad Danesh Science and Technology University. Used various kinds of apparatus, which are given below:-

1) Distilled water
2) Sterile bent glass or plastic spreader rods.
3) Micropipette
4) Spirit lamp

5) Labeling tape
6) Experimental test tube
7) Stopper of test tube
8) Petri dish
9) Conical flask.
10) Slide
11) Microscope
12) Cotton, Immersion Oil, Toothpick
13) Autoclave
14) Incubator
15) Jar ,Beaker, Cylinder
16) Electric Balance
17) Filter paper
18) Spirit lamp
19) Refrigerator
20) Marking pen
21) Bacteriological loop etc

3.1.4 Media

3.1.4.1 Bacteriological media

1. Nutrient agar
2. Mac Conkey agar
3. Salmonella-Shigela Agar
4. Mannitol Salt Agar
5. Mueller Hinton agar

3.1.4.2 Media for Biochemical test

1) Indole Broth
2) Methyl Red Broth
3) Voges-proskauer Broth
4) Simmon's citrate Agar

5) Triple sugar iron agar
6) Motility Indole Ureas (MIU)

3.1.5 Reagent

1. Crystal violet dye
2. Grams iodine
3. Alcohol
4. Safranin
5. Saline
6. Iodine solution
7. Phosphate Buffer Solution (PBS)
8. Kovac's reagent
9. Methyl- red solution
10. 3% H_2O_2
11. P – Amino dimethylanilin oxalate
12. Phenol red

3.1.6 Media used for culture

For the isolation of bacteria the enrichment, streak and spread plate methods were followed by alkaline peptone water, Nutrient agar, MacConkey agar, EMB agar, DCA agar, SS agar and MSA medium. All the samples were diluted with distilled water as 10^{-1}, 10^{-2},$10^{3,}10^{4}$,$10^{5}$and 10^{-6}. The all dilution cultured primarily spread in nutrient Agar at 37ºC for 18-24 h, then sub-cultured onto the MacConkey, SS agar, EMB agar, DCA agar & MSA agar by streak plate method (Cheesbrough, 1985) to observe the colony characteristic and colony morphology of *E. coli, Salmonella spp, Staphylococcus spp* and *Shigella spp* was repeatedly sub-cultured onto MacConkey agar, SS agar, EMB agar, DCA agar and MSA agar until the pure culture morphology (shape, size, surface texture, edge and elevation, color, opacity etc). The organisms showing with homogenous colonies were obtained.

3.1.6.1 Plate Count Agar:

Plate Count Agar (PCA), also called Standard Methods Agar (SMA), is a microbiological growth medium commonly used to assess or to monitor "total" or viable bacterial growth of a sample. PCA is not a selective medium (Cheesbrough, 1985).

3.1.6.2 Nutrient Agar:

Nutrient agar is used for cultivating of non-fastidious microorganisms (Cheesbrough, 1985).

3.1.6.3 MacConkeys Agar:

A differential medium for the isolation of coliforms and intestinal pathogens in water and biological specimens (Cheesbrough, 1985).

3.1.6.4 Salmonella Shigella Agar:

Salmonella Shigella Agar is used as a selectivemedium for *Salmonella spp* which causes enhancement of the growth of *Salmonella spp* (Cheesbrough, 1985).

3.1.6.5 Mannitol Salt Agar (MSA):

Each isolate was streaked on MSA and incubated at 37^0C for overnight. Next day demonstrated morphological characteristics of the bacterial colonies .When the *Staphylococcus spp* is present then the plate was yellow color (Cheesbrough, 1985).

3.2 Methods

3.2.1 Questionaire

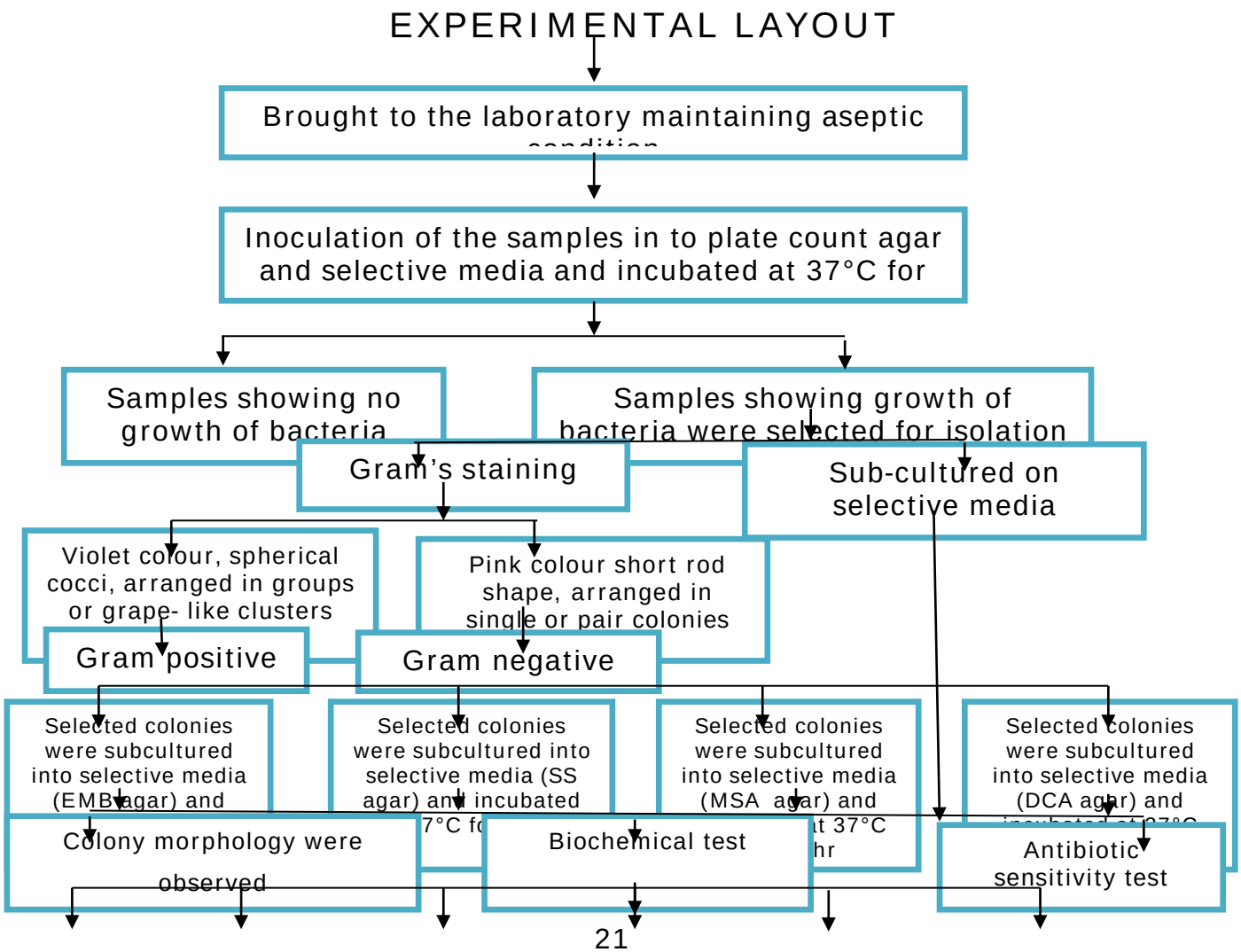
A structured, pre-coded questionnaire focusing on the information of pet birds and pet owners related to the transmission of zoonotic infection was written in English and converted to Bengali whenever needed during data collection. Socio demographic variables of the study population such as age, sex, breed, body weight, diet and hygienic condition were included in this questionnaire.

3.2.2 Plan of the experiment work at a glance:

All of those samples were collected from different areas of Dinajpur sadar with a thermoflask containing ice in sterile diluent with peptone water. Then all of the samples were transferred to the microbiological laboratory of dependent of Microbiology, HSTU, Dinajpur, Bangladesh. Appropriate amount of samples were primarily inoculated into Nutrient agar, Plate count agar for determining the density of bacterial and fungal profiles in pet birds that they carry and obtained their total viable count (TVC). Subsequently Nutrient agar, Blood agar, SS agar, MacConkey agar were employed and specific biochemical tests were done for the isolation and identification of bacteria. At last performed antibiotic sensitivity test with the pure isolated organisms.

3.2.3 Experimental layout

The process of isolation and identification is presented on



TSI slant	MIU test	Indole test	MR test	VP test	Citrate Utilization test

Fig 1: The schematically illustration of layout of

3.2

Proper care was taken during the sampling procedure to prevent contamination of sample. The samples tube were completely filled at the time of sampling. After brought to the laboratory the samples tubes were shaken to mix with peptone water. After that 1ml sample was taken from each sample for ten-fold serial dilution (10^{-1} to 10^{-10}) and 50 µg samples were placed on nutrient agar, plate count agar using spread plate method. The plating was done in the laminar air flow to maintain aseptic conditions and the medium were then incubated at 37° C for 24 h.

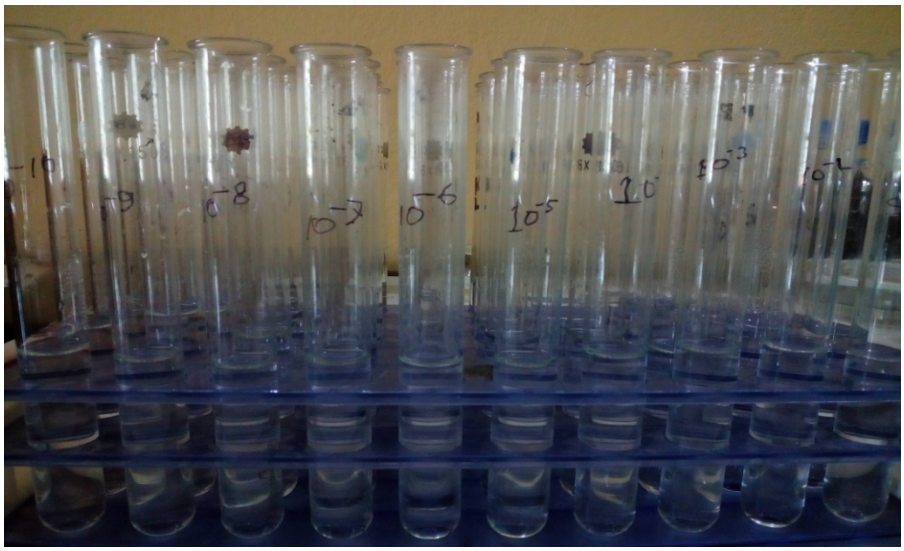


Fig: 2 Ten-fold serial dilution (10^{-1} to 10^{-10})

Fig: 3 Collection of oral swab from Parrot

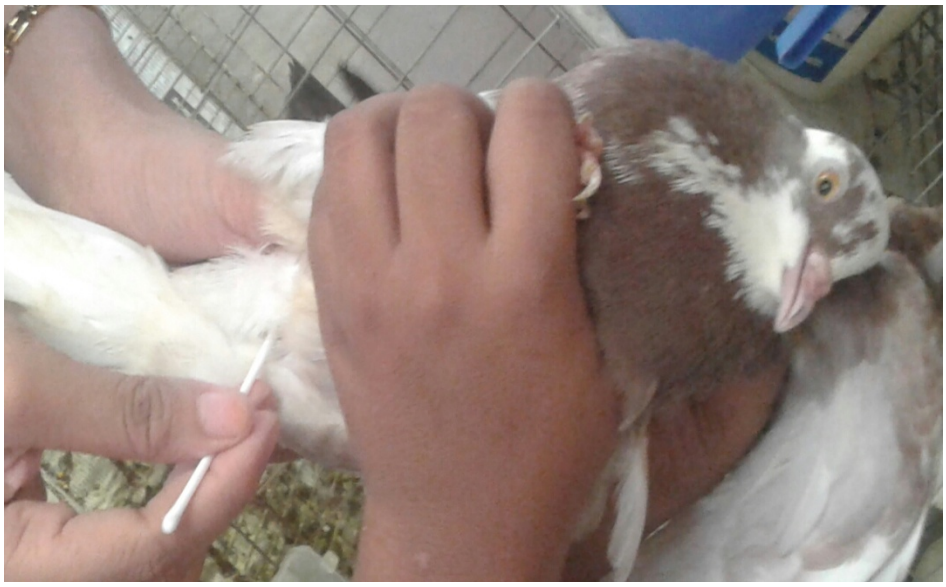


Fig: 4 Collection of cloacal swab from Pigeon

Fig: 5 Collection of oral swab from Quail

3.2.5 Preparation of culture media

3.2.5.1.1 Nutrient Broth

Thirteen grams of dehydrated nutrient broth was suspended into 1000 ml of distilled water and boil to dissolve it completely. The solution was then distributed in tubes, stopper with cotton plugs and sterilized in autoclaving at 121° C and 1.2 kg/cm2 pressure for 15 minutes. The sterility of the medium was checked by incubating at 37° C for overnight and stored at 4° C in aerator for further use (Cater 1979).

3.2.5.1.2 Peptone broth

Peptone water is a microbial growth medium composed of peptic digest of animal tissue and sodium chloride. The pH of the media is 7.2±0.2 at 25° and is rich in tryptophan. Peptone water is also a non-selective broth media which can be used as a primary enrichment media for the growth of bacteria. (HIMEDIA and Baird et al)

3.2.6 Solid media

3.2.6.1 Plate count agar

Seventeen grams of plate count agar powder was suspended in 1000 ml of cold distilled water in a flask and heated to boiling for dissolving the medium completely. The medium was then sterilized by autoclaving. After autoclaving, the medium was poured into each sterile petri dish and allowed to solidify. After solidification of the medium in the petri dishes, these were incubated at 37^0C for overnight to check their sterility and used for culture characterization (Carter, 1979).

3.2.6.2 Nutrient agar

Twenty eight grams of nutrient agar powder (Hi-media, India) was suspended in 1000 ml of cold distilled water in a flask and heated to boiling for dissolving the medium completely. The medium was then sterilized by autoclaving. After autoclaving, the medium was poured into each sterile petridish and allowed to solidify. After solidification of the medium in the petridishes, these were incubated at 37^0C for overnight to check their sterility and used for culture characterization (Carter, 1979).

3.2.6.3 Salmonella Shigella Agar

Salmonella Shigella (SS) agar is the selective medium for the isolation of *Salmonella* and *Shigella*. 63.0 grams SS agar powder was dissolved in 1000 ml of distilled water. It was mixed well until a homogeneous suspension is obtained. It was heated with frequent agitation and boiled for one minute. It did not sterilized by autoclaved. It was cooled to 45ºC

and 50° C and distributed in Petri plates and allow the medium to solidify partially uncovered. (HIMEDIA and Leifson et al, 1935)

3.2.6.4 Eosin methylene blue agar

Thirty six grams of EMB agar base (Hi-media, India) was added to 1000 ml of distilled water in a conical flask and heated until boiling to dissolve the medium completely. After sterilization by autoclaving, the medium was poured in 10 ml quantities in sterile glass petridishes (medium sized) and in 15 ml quantities in sterile glass petridishes (large sized) to form a thick layer therein. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petridishes partially removed. The sterility of the medium was judged and used or stored at 4°C in refrigerator for future use (Carter, 1979).

3.2.6.5 MacConkey agar

51.50 grams of dehydrated Bacto-MacConkey agar (Difco) was suspended in 1000 ml of cold distilled water taken in a conical flask and was heated up to boiling to dissolve the medium completely. After sterilization by autoclaving, the medium was poured in 10 ml quantities in sterile glass petridishes (medium sized) and in 15 ml quantities in sterile glass petridishes (large sized) to form a thick layer therein. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petridishes partially removed. The sterility of the medium was judged and used for cultural characterization or stored at 4°C in refrigerator for future use (Carter, 1979).

3.2.6.6 Mannitol Salt Agar:

111 grams Mannitol Salt Agar base powder was added to 1000 ml of distilled water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at 1.2 kg/cm^2 pressure and 121° C for 15 minutes. After autoclaving the medium was put into water bath at 45^0- 50^{0}C to decrease the temperature. Then medium was poured in 10 ml quantities in sterile glass petridishes (medium sized) and in 15 ml quantities in sterile glass Petridishes (large sized) to form thick layer there in. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the Petridishes partially removed. The sterility of the medium was checked by incubating at 37°C for overnight. The sterile medium was used for cultural characterization or stored at 4°C in refrigerator for future use. Petridishes, these were incubated at 37° C for overnight to check their sterility and used for cultural characterization or stored at 4°C in refrigerator for future use (Cater 1979).

3.2.6.7 Deoxycholate Citrate Agar (DCA)

Deoxycholate Citrate Agar is a selective medium recommended for the isolation of enteric pathogens particularly *Salmonella* and *Shigella* species in accordance with European Pharmacopoeia. 69.02 grams of DCA powder was suspended in 1000 ml of purified/distilled water. It was heated to boiling to dissolve the medium completely. Avoided autoclaved and it was cooled to 50°C and poured into sterile Petri plates (HIMEDIA and Leifson et al, 1935)

3.2.6.8 Mueller Hinton Agar

Mueller Hinton Agar is used in antimicrobial susceptibility testing by the disk diffusion method. The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species. Other media were subsequently developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria* species, but it became widely used in the

determination of sulphonamide resistance of gonococci and other organisms. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing. Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel. Suspend 38 grams of medium in one liter of distilled water. Mix well. Heat agitating frequently and boil for about one minute. Dispense and sterilize in autoclave at 116 - 121°C (15 lbs.sp) for 15 minutes. Cool to 45° or 50° C and add defibrinated blood if desired. The blood mixture should be chocolated by heating to 80° C for 10 minutes if Neisseria development is desired. DO NOT OVER HEAT. To re-melt the cold medium, heat as briefly as possible. (Carter, 1979).

3.2.7 Reagents preparation

3.2.7.1 Methyl Red-Voges Proskauer broth

A quantity of 3.4 grams of Bacto MR-VP medium was dissolved in 250 ml of distilled water dispensed in 2 ml amount in each test tube and then the test tubes were autoclaved. After autoclaving, the tubes containing medium were incubated at 37°C for overnight to check their sterility and used for biochemical characterization or stored at 4°C in refrigerator for future use (Cheesbrough, 1984).

3.2.7.2 Methyl red solution

The indicator methyl red (MR) solution was prepared by dissolving 0.1 gm of Bacto methyl red (Difco) in 300 ml of 95% alcohol and diluting this to 500 ml with the addition of 200 ml of distilled water.

3.2.7.3 Alpha-naphthol solution

Alpha-naphthol solution was prepared by dissolving 5 grams of 1-naphthol in 100 ml of 95% ethyl alcohol.

3.2.7.4 Potassium hydroxide solution

Potassium hydroxide (KOH) solution was prepared by adding 40 grams of potassium hydroxide crystals in 100 ml of cold distilled water.

3.2.7.5 Kovac's reagent

This solution was prepared by mixing 25 ml of concentrated Hydrochloric acid in 75 ml of amyl alcohol and to this mixture 5 grams of paradimethyl-aminohenzyldehide crystals were added. This was then kept in a flask equipped with rubber cork for future use (Merchant and Packer, 1967).

3.2.7.6 Phosphate buffered saline solution

For preparation of Phosphate buffered saline (PBS) solution, 8 gram of sodium chloride (NaCl), 2.89 gram of disodium hydrogen phosphate (Na_2HPO_4, $12H_2O$), 0.2 gram of potassium chloride (KCl) and 0.2 gram of potassium hydrogen phosphate were suspended in 1000 ml of distilled water. The solution was heated to dissolve completely. The solution was then sterilized by autoclave at 121 °C maintaining a pressure of 15 pounds per square inch for 15 minutes and stored at refrigerator until use. The pH of the solution was measured by a pH meter and maintained at 7.0-7.2 (Cheesbrough, 1984).

3.2.8 Morphological characterization of organisms by Gram's staining method

The most widely used staining procedure in microbiology is the gram stain ,discovered by the Danish scientist and physician Hans Christian

Joachim Gram in 1884,Gram staining is a differential staining technique that differentiates bacteria into two groups :gram- positives and gram-negatives. The procedure is based on the ability of microorganisms to retain color of the stains used during the gram stain reaction. Gram-negative bacteria are decolorized by the alcohol, losing the color of the primary stain, purple .Gram-positive bacteria are not decolorized by alcohol and will remain as purple .After decolorization step, and a counterstain is used to impart a pink color to the decolorized gram-negative organisms.

3.2.8.1 Preparation of Gram's staining solution:

The four solutions needed for the Gram staining procedure.

- Crystal violet
- Gram's iodine
- 95% alcohol
- Safranin

3.2.8.2 Gram staining procedure:

1. Obtain clean glass slides.
2. Using sterile technique, prepared a smears of each of the organisms. Did this by placing a drop of water on the slide, and then transferring each organisms separately to the drop water with a sterile, cooled loop .Mixed and speeded organism by means of a circular motion of the inoculating loop.
3. Allowed smears to air –dry and then heat fixed in the usual manner.
4. Gently flooded smears with crystal violet and let stood for 1 min gently washed with tap water8

5. Gently flooded smears with Grams iodine mordant and let stood for 1 min .Gently washed with tap water.
6. Decolorized with 95% ethyl alcohol .Gently washed with tap water.
7. Counter stain with safranin for 30 sec.
8. Gently washed with tap water.
9. Examined under oil immersion. (James G. Cuppuccion , Natalie Sherman ,1996*)*

3.2.8.3 Biochemical test:

Several types of biochemical tests were performed in this study.

1) Oxidase test
2) Catalase test
3) Indole test
4) MR Test
5) Voges-proskauer test
6) Simmon's citrate
7) Triple sugar iron agar
8) MIU test

3.2.8.4 Oxidase test

The oxidase test uses Kovac's reagent (a 1% [wt/vol] solution of N, N, N', N' –tetramethyl-ρ-phenylenediamine dihydrochloride) to detect the presence of cytochrome c in a bacterial organism's respiratory chain; if the oxidase reagent is catalyzed, it turns purple. The oxidase test can be performed on filter paper or on a swab (Cheesbrough, 1985).

3.2.8.5 Catalase test

This test was used to differentiate bacteria which produce the enzyme catalase .To perform this test, a small colony of good growth pure culture of test organism was smeared on a slide .Then one drop of catalase reagent (3%H_2O_2) was added on the smear. The slide was observed for bubbles formation. Formation of bubble within few seconds was the

indication of positive test while the absence of bubble formation indicated negative result (Cheesbrough, 1985)

3.2.8.6 Indole test

Two milliliter of peptone water was inoculated with the 5 ml of bacterial culture and incubated at 37°c for 48 hours. Kovac's reagents (0.5ml) were added, shake well and examined after 1 minute. A red color in the reagent layer indicated Indole test positive. In negative case there is no development of red color (Cheesbrough, 1985).

3.2.8.7 Methyl Red test (MR)

Sterile MR-VP broth was inoculated with the test organism and following incubation at 37°c for 24 hours. if the organism would ferment glucose via the mixed acid fermentation pathway like lactic, acetic, which decreases the PH ,hence upon the addition of the indicator methyl red the broth becomes red in color and yellow color indicated a negative result (Cheesbrough, 1985).

3.2.8.8 Voges-Proskauer test (VP)

Voges Proskauer Test If the organism would ferment glucose via the butylenes glycol pathway, an intermediate product, acetyl methyl carbinol or acetone which is neutral is converted to diacetyl upon the addition of the VP Reagent –B (40% KOH with 0.3% creatine) in the presence of VP Reagent A (5% alpha- naphthol in abs. methyl alcohol). Diacetyl is red in color. Negative is yellow color (Cheesbrough, 1985).

3.2.8.9 Simmon's Citrate Agar (SCA)

This tube medium is used to identify Gram negative enteric bacilli based on the ability of the organisms to utilize citrate s the sole source of carbon. (Citrate utilization test).The organism which utilizes citrate as its source of carbon degrades it to ammonia and subsequently converts it to ammonium hydroxide. The pH of the medium is then increased and this is indicated by a change in color from green to blue (Cheesbrough, 1985).

3.2.8.10 Triple Sugar Iron Agar (TSI)

This tube medium is used to identify Gram negative enteric bacilli based on the following biochemical characteristics (Cheesbrough, 1985):

- Glucose fermentation – indicated by yellow butt
- Lactose fermentation – indicated by yellow slant
- Hydrogen sulfide production – indicated by blackening of the medium
- Gas production – indicated by presence of a crack, bubble or gas space
- pH indicator – phenol red
- Hydrogen sulfide indicator – ferric ammonium citrate with sodium thiosulfate.

3.2.8.11 Mortility Indole Urease (MIU) test:

MIU medium is a semisolid medium used in the qualitative determination of motility, production of indole and ornithine decarboxylase. MIU medium is used for the differentiation of the Family Enterobacteriaceae. The organisms tested must ferment glucose for proper performance of the medium. MIU medium contains dextrose as fermentable sugar, ornithine as an amino acid, bromcresol purple as pH indicator, casein peptone as a source of tryptophan, and other essential nutrients for growth. The medium contains a small amount of agar allowing for detection of motility (Cheesbrough, 1985).

3.2.9 Antibiotic sensitivity test:

The antibiotic resistance was determined by Kirby-Bauer disc diffusion technique using Mueller-Hinton agar (Difco), according to the recommendations of National Committee for Clinical Laboratory Standards (CLSI 2011). After overnight incubation at 37 °C, the diameter in millimeters of the zones of inhibition around each of the antimicrobial discs was recorded and categorized as resistant or sensitive in accordance with company recommendations. (Cappuccino 2005).

Table: 01. Antimicrobial agents with their disc concentration:

Antimicrobial agents	Symbol	Disc concentration (µg/disc)
Cefixime	CFM	5 (µg/disc)
Tetracycline	TE	30 (µg/disc)
Chloramphenicol	C	30 (µg/disc)
Ciprofloxacin	CIP	5 (µg/disc)
Levofloxacin	LE	5 (µg/disc)
Azithromycin	AZM	15 (µg/disc)
Erythromycin	E	15 (µg/disc)
Penicillin G	P	10 (µg/disc)
Neomycin	N	30 (µg/disc)
Vancomycin	VA	30 (µg/disc)
Cephalexin	CN	30 (µg/disc)
Kanamycin	K	30 (µg/disc)
Amoxycillin	AMX	30 (µg/disc)
Cloxacillin	COX	1 (µg/disc)
Bacitracin	B	10 (µg/disc)
Gentamicin	GEN	10 (µg/disc)
Cefradine	CH	25 (µg/disc)
Collistin	CL	10 (µg/disc)

3.2.10 Statistical analysis:

Data were analyzed using SPSS for Windows (version 21.0). Prevalence of bacterial isolates was expressed in simple descriptive statistics such as means and standard deviation. For cfu/gm values, one-sample test was

used to test if there is any statistical association between different bird samples and isolated pathogens.

CHAPTER: 04
RESULTS

The present study was designed to microbial assessment and detection of micro-organism from Cloacal swab, Oral swab, and Feces in Sadar at Dinajpur district from household pet birds (pigeon, parrot, budgerigar or lovebirds and quail). The samples were collected from different place and transferred to the bacteriological laboratory of the department of Microbiology, HSTU, Dinajpur. For this a total 243 (Cloacal swab, Oral swab and Feces) samples were collected from different ages of birds tested for the microbiological examination. Socio demographic variable of study population in case of (pigeon, parrot, budgerigar or love birds & quail) shows under the following table.

Table-02 (a): Socio demographic variable of study population (in case of pigeon)

Socio demographic profile		Frequency	Percentage (%)
1.Age	Young(0-4 months)	15	37.5 %
	Adult (>4 months)	25	62.5%
2.Sex	Male	16	40%
	Female	24	60%
3. Body weight	250-300 gm	11	27.5%
	450-500 gm	9	22.5%
	500-1000 gm	20	50%
4.Breed	White king	10	25%
	Fantail	6	15%
	Poter	12	30%
	Madina	12	30%
5.Diet	Ready food	20	50 %
	Raw food	10	25%
	Both	10	25%
6. Hygienic condition	Good	20	50%
	Poor	12	30%
	Excellent	8	20%
7. Vaccination	Yes	30	75%
	No	10	25%

8. Total	40	100%

Table-02 (b): Socio demographic variable of study population (in case of parrot)

Socio demographic profile		Frequency	Percentage (%)
1.Age	Young(0-3 months)	8	40%
	Adult (>3months)	12	60%
2.Sex	Male	10	50%
	Female	10	50%
3. Body weight	(10 gm-500 gm)	8	40%
	(550-1000 gm)	12	60%
4.Diet	Ready food	6	30%
	Raw food	10	50%
	Both	4	20%
4. Hygienic condition	Good	7	35%
	Poor	10	50%
	Excellent	3	15%
6. Vaccination	Yes	5	25%
	No	15	75%

Table-02 (c): Socio demographic variable of study population (in case of budgerigar or love birds)

Socio demographic profile		Frequency	Percentage (%)
1.Age	Young (1-4 months)	4	33.33%
	Adult ((>4 months)	8	66.67 %
2.Sex	Male	6	50%
	Female	6	50%
3. Body weight	(10-20 gm)	4	33.33%
	(30-40gm)	8	66.67%
4.Diet	Ready food	4	33.33%
	Raw food	6	50%
	Both	2	16.66%
6. Hygienic	Good	5	41.66%
	Poor	5	41.66%

condition	Excellent	2	16.66%
7. Vaccination	Yes	4	33.33%
	No	8	66.67%

Table-02 (d): Socio demographic variable of study population (in case of quail)

Socio demographic profile		Frequency	Percentage (%)
1.Age	Young(<3 months)	5	62.5%
	Adult (3-6 months)	3	38.5%
2.Sex	Male	4	50%
	Female	4	50%
3. Body weight	(70-100 gm)	6	75%
	(100-140gm)	2	25%
3.Diet	Ready food	2	25%
	Raw food	2	25%
	Both	4	50%
4. Hygienic condition	Good	4	50%
	Poor	2	25%
	Excellent	2	25%
5. Vaccination	Yes	2	25%
	No	6	75%

4.1 Sex and age wise distribution of Total Viable Count of zoonotic bacterial pathogen

4.1.1 Result of Total Viable Count (TVC)

The result presented in table 03 (a, b, c, & d) reveals that the mean values of the total viable count (TVC) of bird samples.

Table 03 (a): Sex and age wise distribution of Total Viable Count of zoonotic bacterial pathogen from Pigeon:

Pigeon	Total viable count			
Sex	Age	Cloacal swab(Mean±SEM)	Oral swab(Mean±SEM)	Feces(Mean±SEM)
Male	Young (<4 month)	10.34±0.14	10.40±0.10	10.04±0.49
	Adult	10.02±0.27	9.87±0.38	10.45±0.06
	P	0.40	0.31	0.31
Female	Young (<4 month)	9.7164±0.41	10.02±0.32	9.92±0.20
	Adult	10.38±0.13	10.26±0.16	10.20±0.20
	P value	0.75	0.47	0.36

Legends:
SEM= Standerd Error Mean
cfu= colony forming unit

Table 03 (a) shown that the total viable count of pigeon in case of cloacal swab (male) 10.34±0.14 cfu/g (young), 10.02±0.27 cfu/g (adult), (female) 9.7164±0.41 cfu/g (young), 10.38±0.13 cfu/g (adult), oral swab (male) 10.40±0.10 cfu/g (young), 9.87±0.38 (adult), female 10.02±0.32 cfu/g (young), 10.38±0.13 (adult) & feces (male) 10.45±0.09 cfu/g (young), 10.45±0.06 cfu/g (adult), female 9.92±0.20 (young), 10.20±0.20 (adult).

Table 03 (b): Sex and age wise distribution of Total Viable Count of zoonotic bacterial pathogen from Parrot.

Parrot	Total viable count			
Sex	Age	Cloacal swab(Mean±SEM)	Oral swab(Mean±SEM)	Feces (Mean±SEM)
Male	Young (<3 month)	10.47±0.21	10.54±0.26	10.28±0.22
	Adult	10.51±0.09	10.26±0.22	9.81±0.46
	P value	0.83	0.51	0.55
Female	Young (<3 month)	9.34±0.99	10.50±0.07	10.29±0.06
	Adult	10.48±0.16	9.09±0.29	10.50±0.23
	P	0.13	0.03	0.57

	value			

Legends:
SEM= Standerd Error Mean
cfu= colony forming unit

Table 03 (b) shown that the total viable count of parrot in case of cloacal swab (male) 10.47±0.21cfu/g (young), 10.51±0.09 cfu/g (adult), (female) 9.34±0.99 cfu/g (young), 10.48±0.16 cfu/g (adult), oral swab (male) 10.54±0.26 cfu/g (young), 10.26±0.22 (adult), female 10.50±0.07 cfu/g (young), 9.09±0.29 (adult) and feces (male) 10.28±0.22 cfu/g (young), 9.81±0.46 cfu/g (adult), female 10.29±0.06 (young), 10.50±0.23 (adult).

Table 03 (c): Sex and age wise distribution of Total Viable Count of zoonotic bacterial pathogen from budgerigar or love birds

Budgerigar or love birds	Total viable count			
Sex	Age	Cloacal swab(Mean±SEM)	Oral swab(Mean±SEM)	Feces (Mean±SEM)
Male	Young (<4 month)	10.47±0.21	10.53±0.26	10.28±0.22
	Adult (>4 month)	10.51±0.10	10.27±0.22	9.82±0.46
	P value	0.83	0.51	0.55
Female	Young (<3 month)	9.34±0.89	10.50±0.07	10.28±0.06
	Adult (>3 month)	10.48±0.16	9.09±0.29	10.50±0.23
	P value	0.127	0.03	0.57

Legends:

SEM= Standerd Error Mean
cfu= colony forming unit

Table 03 (c) shown that the total viable count of budgerigar & love birds in case of cloacal swab (male) 10.47±0.21cfu/g (young), 10.51±0.10 cfu/g (adult), (female) 9.34±0.89 cfu/g (young), 10.48±0.16 cfu/g (adult), oral swab (male) 10.53±0.26 cfu/g (young), 10.27±0.22 (adult), (female) 10.50±0.07 cfu/g (young), 9.09±0.29 (adult) and feces (male) 10.28±0.22 cfu/g (young), 9.82±0.46 (adult), female 10.28±0.06 (young), 10.50±0.23 (adult).

Table 03 (d): Sex and age wise distribution of Total Viable Count of zoonotic bacterial pathogen from quail

Quail	Total viable count			
Sex	Age	Cloacal swab(Mean±SEM)	Oral swab(Mean±SEM)	Feces (Mean±SEM)
Male	Adult	9.86±0.48	10±0.09	9.64±0.66
	P value	0.00	0.00	0.001
Female	Young (<3 month)	9.37±0.57	10.35±0.14	10.32±0.36
	Adult (>3	10.63±0.17	10.45±0.01	9.52±0.14
	P value	0.19	0.66	0.19

Legends:
SEM= Standerd Error Mean
cfu= colony forming unit

Table 03 (d) shown that the total viable count of quail in case of cloacal swab (male) 9.86±0.48 cfu/g (adult), (female) 9.37±0.57 cfu/g (young), 10.63±0.17 cfu/g (adult), oral swab (male) 10±0.09 cfu/g (adult), female 10.35±0.14 cfu/g (young), 10.45±0.01 (adult) & feces (male) 9.64±0.66cfu/g (adult), female 10.32±0.36 (young), 9.52±0.14 (adult).

4.2 Identification of organism by different bacteriological methods:

4.2.1 Results of Cultural Examination:

The cultural characteristics of *E. coli, Staphylococcus, Salmonella* spp., *Shigella* spp., and *Klebsiella* on various selective media are presented in table 4.

Table 4: The result of cultural characteristics of the organisms which are isolated from cloacal swab, oral swab, & feces of household pet birds.

Serial No	Name of bacteria	Name of media	Colony Characteristics
01	*E. coli*	Nutrient Agar	Produce large, mucoid, white colony
		Mac-Conkey's Agar	Produce large mucoid rose pink colony
		EMB agar	Produce metallic sheen (greenish black) colony
02	*Staphylococcus* spp.	Mannitol salt agar	Produce yellow colony, may have yellow halo around colonies
		Blood Agar	Produce β-hemolytic colony
03	*Salmonella* spp.	S.S agar	Produce opaque, smooth, round with black centered colony

04	*Klebsiella* spp.	EMB Agar	Produce smooth, mucoid lactose fermenting pink colour colony
05	*Shigella* spp.	DCA	Produce colourless (lactose negative) colonies

4.2.1.1 Plate count agar:

Plate count agar plates spread with the samples revealed the growth of bacteria after 24 hours of incubation at 370C aerobically and were indicated by the growth of circular, small smooth, convex, greenies and gray white or yellowish colonies.

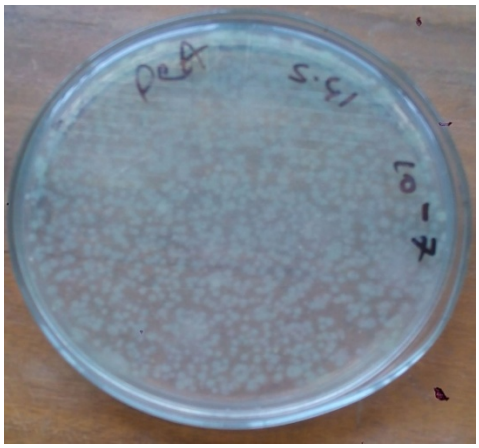


Plate 4.1: Culture of organism on Plate Count Agar

4.2.1.2 Nutrient Agar:

Nutrient agar plates streaked separately with the organisms revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically

and were indicated by the growth of circular, small smooth, convex and gray white or yellowish colonies.

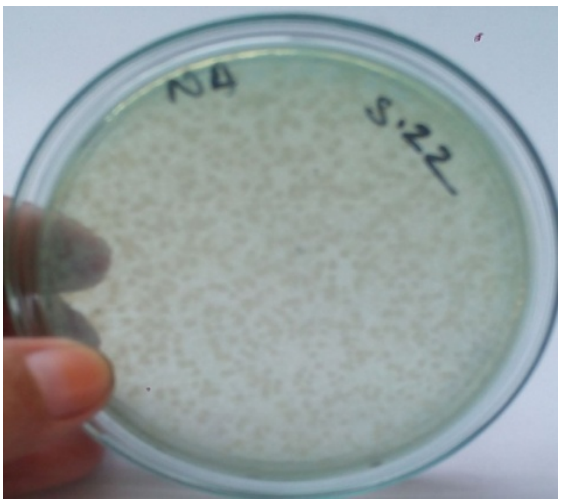


Plate 4.2: Culture of organism on Nutrient's Agar

4.2.1.3 MacConkey Agar:

Mac Conkey Agar plates streaked separately with the organisms revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically and were indicated the colorless colonies after prolonged incubation pink color colonies.

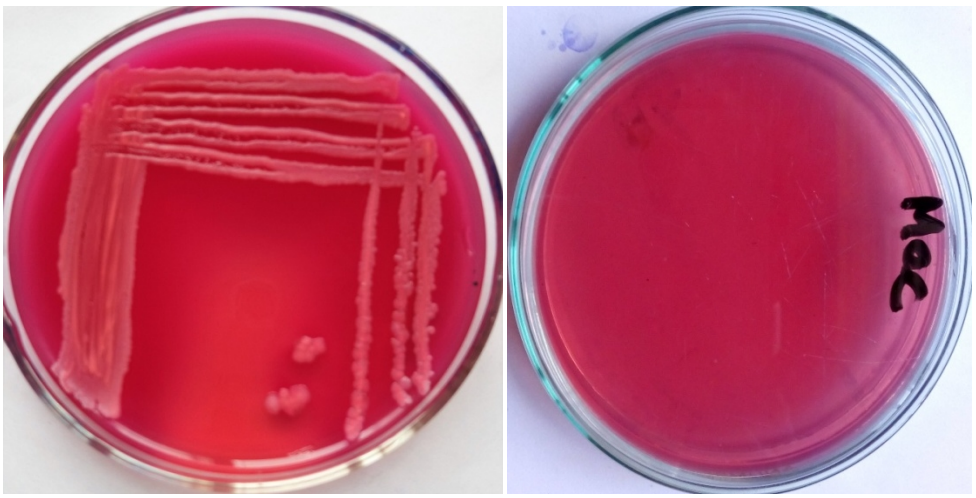


Plate 4.3 : Culture of *E. coli* organism on Mac Conkey Agar

4.2.1.4 Eosin methylene blue (EMB) agar:

EMB agar plates streaked separately with the organism revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically. The growth was indicated by smooth, circular, black color colonies with metallic sheen on the agar plate.

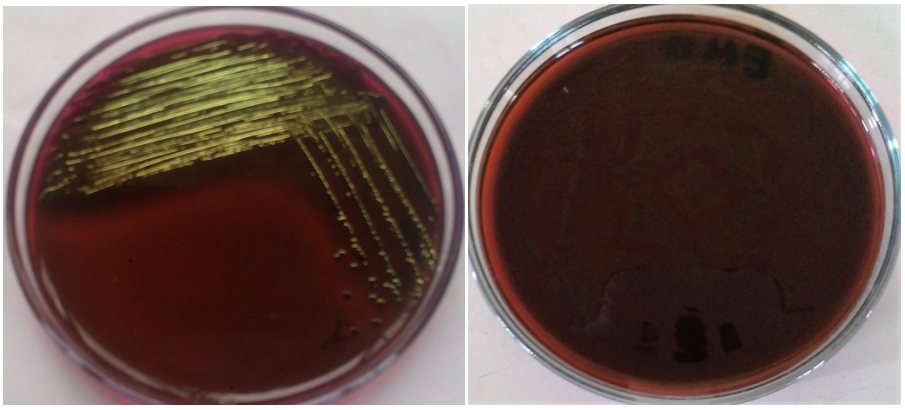


Plate 4.4 : Culture of *E. coli* on EMB Agar plates

4.2.1.5 Eosin methylene blue (EMB) agar

EMB agar plates streaked separately with the organism revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically. The growth was indicated by smooth, Characteristic mucoid lactose-fermenting and pink colored colonies.

Plate 4.5 : Culture of *Klebsiella* spp on EMB Agar plates

4.2.1.6 Salmonella-Shigella Agar:

Salmonella-Shigella Agarplates streaked separately with the organisms revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically and were indicated by the clear by the clear, black center colony, transparent.

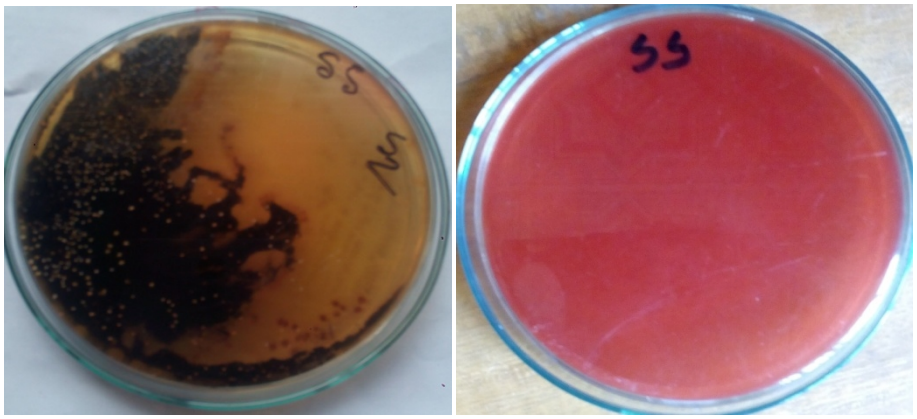


Plate 4.6: Culture of *Salmonella* spp. on Salmonella-Shigella Agar

4.2.1.7 Mannitol Salt Agar:

Mannitol salt agar plates streaked separately with the organisms revealed the growth of bacteria after 24 hours of incubation at 37^0C

aerobically and were indicated by the yellow colony, may have yellow halo around colony.

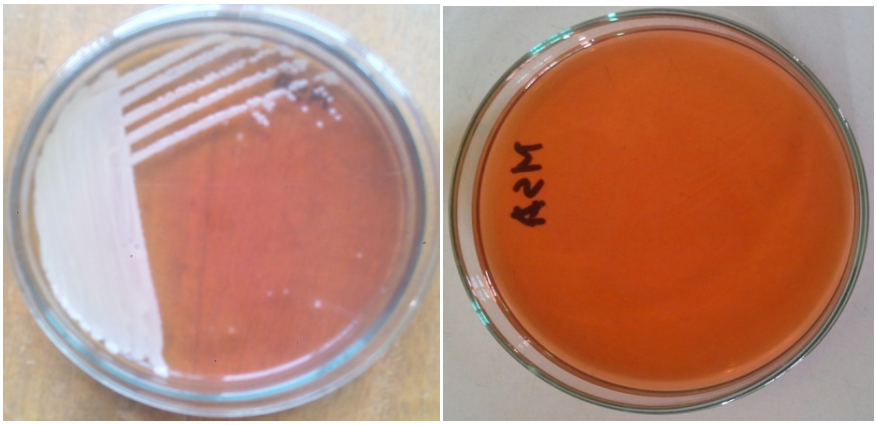


Plate 4.7: Culture of Staphyloccus spp. on Manitol Salt Agar

4.2.1.8 Blood Agar (BA):

Blood agar plates streaked separately with the organisms revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically and were indicated by the β-hemolytic colony.

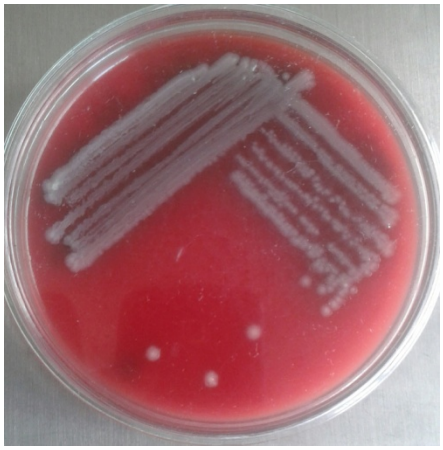


Plate 4.8: Culture of *Staphyloccus* spp. on Blood Agar (BA)

4.2.1.9 Desoxycholate Citrate Agar (DCA)

DCA agar plate streaked separately with the organism revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically. The growth was indicated by colourless (lactose-negative) colonies.

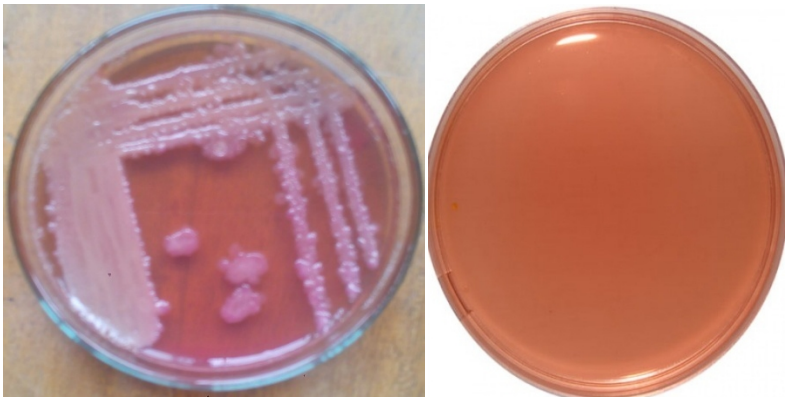


Plate 4.9: Culture of *Shigella* spp. on Desoxycholate Citrate Agar (DCA)

4.2.2 Microscopic examination:

4.2.1.2 Gram staining results of *E. coli*

The microscopic examination of Gram's stained smears from MacConkey and EMB agar revealed Gram-negative, pink colored, small rodshaped organisms arranged in single, pairs or short chain

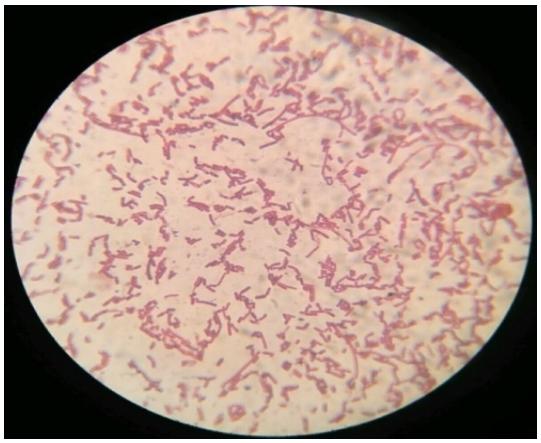


Plate 4.10: Gram negative *E. coli*

4.2.1.3 Gram staining results of *Klebsiella* spp.

The microscopic examination of Gram's stained smears from EMB agar revealed Gram-negative, pink colored, small rod shaped organisms arranged in single, pairs or short chain.

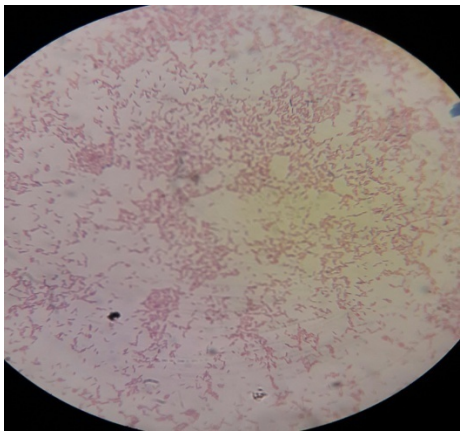


Plate 4.11: Gram negative *Klebsiella* spp.

4.2.1.4 Gram staining results of *Salmonella* spp.

The microscopic examination of Gram's stained smears from SS agar revealed Gram-negative, pink colored, small rod shaped organisms arranged in single, pairs or short chain.

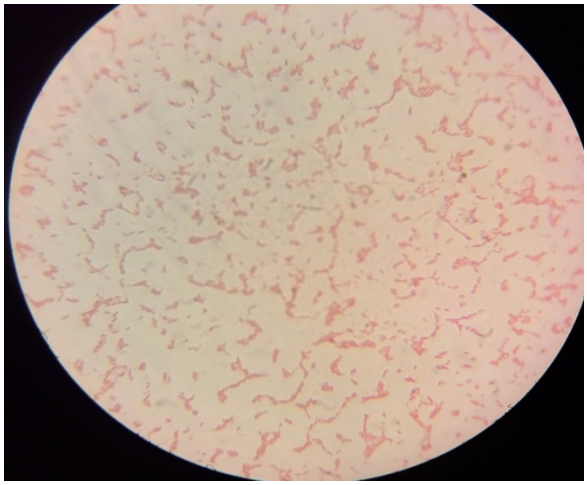


Plate 4.12: Gram negative *Salmonella* spp.

4.2.1.5 Gram staining results of *Shigella* spp.

The microscopic examination of Gram's stained smears from DCA agar revealed Gram-negative, pink colored, small rod shaped organisms arranged in single, pairs or short chain.

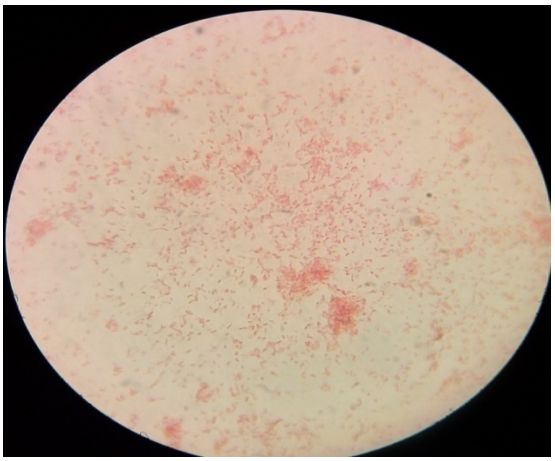


Plate 4.13: Gram negative *Shigella* spp.

4.2.1.6 Gram staining results of *Staphylococcus* spp.

The microscopic examination of Gram's stained smears from MSA agar revealed Gram-positive, cocci shaped organisms arranged in single, pairs or short chain.

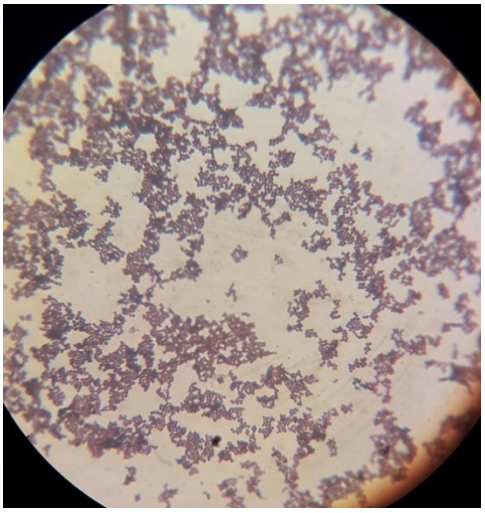


Plate 4.14: Gram positive *Staphylococcus* spp.

4.2.3 Biochemical Results:

Table 5 :Result of biochemical test Enrichment results of the representative test isolates

Serial No	Ind	MR	VP	SC	TSI	MIU	Result
1	+	+	-	-	YY	+	*E.coli*
2	-	+	+	+	YR	+	*Salmonella* spp
3	-	+	-	-	YR	+	*Staphylococcus* spp
4	-	-	-	-	YY	+	*Shigella* spp
5	-	+	-	+	YY	-	*Klebsiella* spp

[A= Acid, + = positive, - = negative, YY= Yellow Yellow, YR= Yellow Red, Ind= Indole, MR= Methyl Red, VP= Voges-Proskaur, SC= Simmons Citrate, TSI= Triple Sugar Iron, MIU= Motility Indole Urease]

4.2.3.1 Methyl Red

The *E. coli, Salmonella* spp*, Shigella* spp, *Staphylococcus* were positive and *Klebsiella* spp was negative for methyl red test.

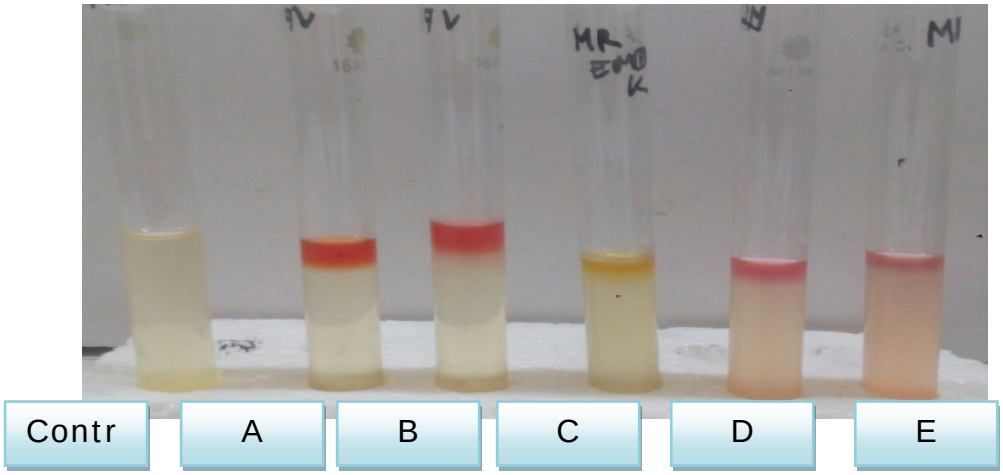


Plate 4.15: MR test results (right) A= *E.* coli (positive), B= *Shigella* spp (positive), C= *Klebsiella* spp *(*negative*),* D=*Salmonella* spp *(*positive), E*= Staphylococcus* spp (positive) and uninoculated control (left).

4.2.3.2 Voges-Proskauer Test

The *E.coli, Salmonella* spp, *Shigella* spp, *Staphylococcus spp, Bacillus* spp were negative and *Klebsiella* spp was positive for voges-proskauer test.

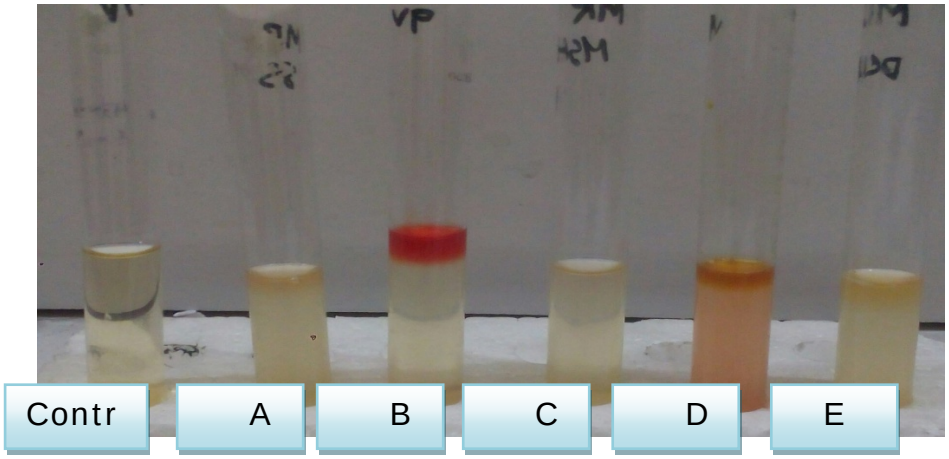


Plate 4.16: VP test results (right) A= *E. coli* (negative), B= *Klebsiella* spp (positive), C= *Shigella* spp (negative*)*, D= *Staphylococcus* spp (positive), E*= Salmonella* spp (negative) and uninoculated control (left).

4.2.3.3 Indole Test

The *E.coli was* positive and *Salmonella* spp, *Shigella* spp *Staphylococcus, Klebsiella* spp were negative for indole test.

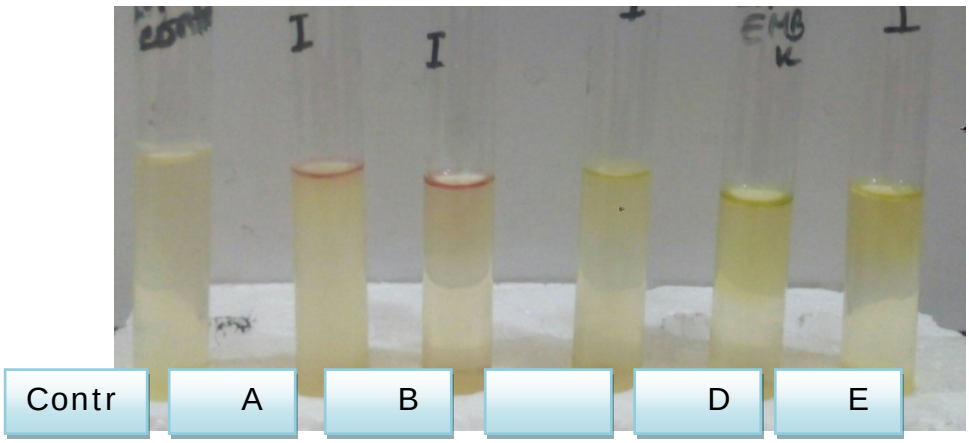


Plate 4.17: Indole test results (right) A= *E.* coli (positive), B= *Shigella* spp (positive), C= *Salmonella* spp (negative*)*, D= *Klebsiella* spp (negative), E *= Staphylococcus* spp (negative) and uninoculated control (left).

4.2.3.4 Simmons Citrate

The *E.coli; Shigella* spp *Staphylococcus* spp were negative and *Salmonella* spp and *Klebsiella* spp were positive for simmons citrate test.

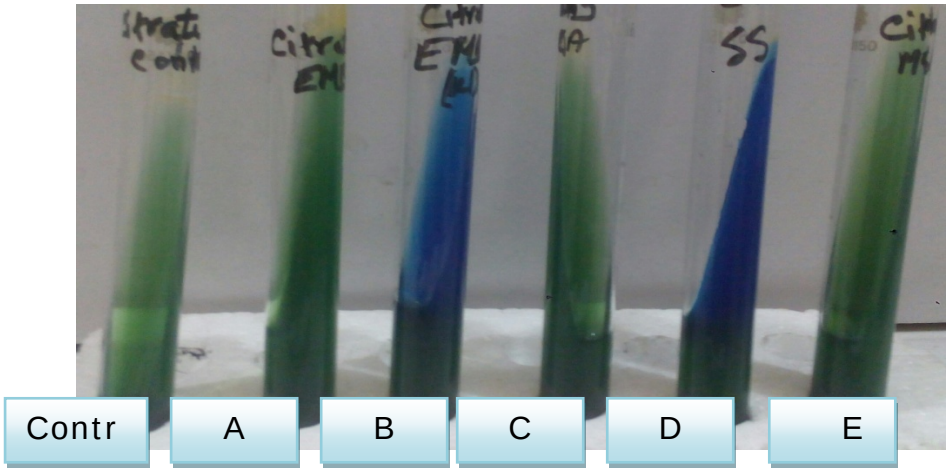


Plate 4.18: Citrate utilization test results (right) A= *E. coli* (negative), B= *Klebsiella* spp (positive), C= *Shigella* spp (negative*)*, D= *Salmonella* spp (positive), E *= Staphylococcus* spp (negative) and uninoculated control (left).

4.2.3.5 Triple Sugar Iron (TSI) Test for *E. coli*

On TSI agar slant, *E. coli* isolates produced acid (yellow) and gas in the butt, hydrogen sulfide gas was absent in both butt and slant and the acidic reaction in the slant (Plate- 4.19).

4.2.3.6 Triple Sugar Iron (TSI) Test for *Salmonella* spp

On TSI agar slant, *Salmonella* spp. isolates produced acid (yellow) in the butt, gas and hydrogen sulfide gas was absent in both butt and slant and produce alkaline reaction in the slant (Plate-4.19).

4.2.3.7 Triple Sugar Iron (TSI) Test for *Shigella* spp

On TSI agar slant, *Shigella* spp. isolates produced acidic (yellow) and gas in the butt, hydrogen sulfide gas was present in both butt and slant and the alkaline reaction in the slant (Plate-4.19).

4.2.3.8 Triple Sugar Iron (TSI) Test for *Staphylococcus spp*

On TSI agar slant, *Staphylococcus* spp. isolates produced acidic (yellow) colour butt, and red colour slant (plate- 4.19).

4.2.3.9 Triple Sugar Iron (TSI) Test for *Klebsiella spp*

On TSI agar slant, *Klebsiella* spp. isolates produced acid (yellow) and gas in the butt, hydrogen sulfide gas was absent in both butt and slant and the acidic reaction in the slant. (Plate-4.19).

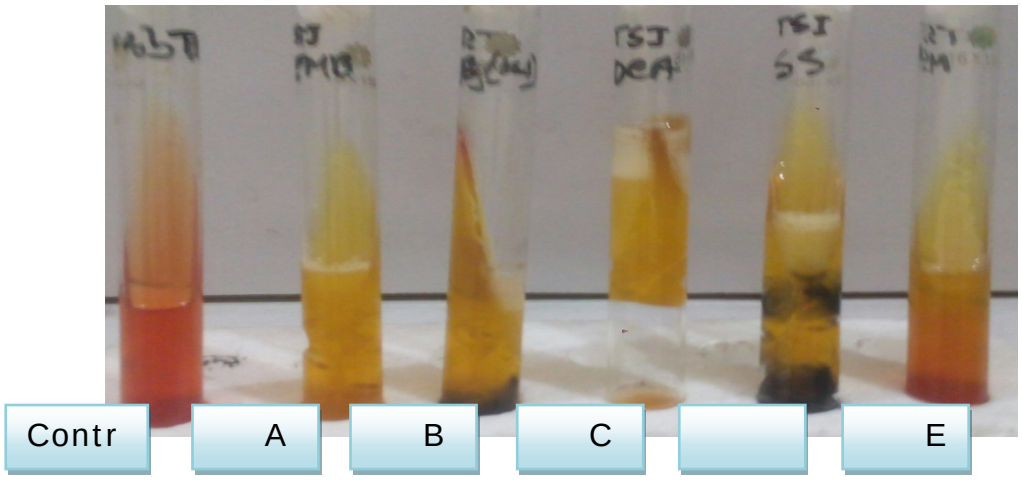


Plate 4.19: TSI test results (right) A= *E.* coli B= *Klebsiella* spp, C= *Shigella* spp, D= *Salmonlla* spp, E = *Staphylococcus* spp and uninoculated control (left).

4.2.3.10 Motility indole urease Test:

The *E.coli,* was positive and *Salmonella* spp, *Shigella* spp, *Staphylococcus* and *Klebsiella* spp were negative for motility indole urease test.

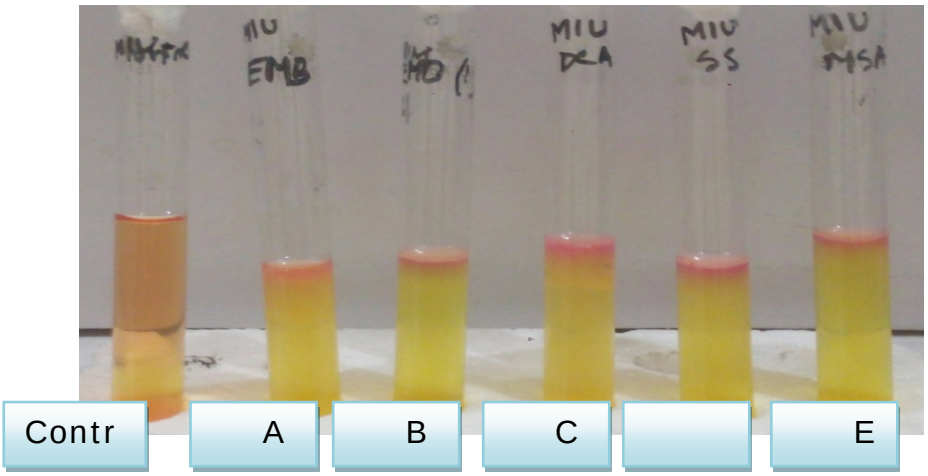


Plate 4.20: MIU test results (right) A= *E. coli* (negative), B= *Klebsiella* spp (Negative), C= *Shigella* spp (negative*)*, D= *Salmonella* spp (negative), E *= Staphylococcus* spp (positive) and uninoculated control (left).

4.3 Percentage distribution of socio demographic variable in bacterial pathogens

Table-06 (a): Association between the socio-demographic variable and bacterial zoonotic pathogen in case of pigeon

Species	Parameters		Bacterial zoonotic pathogen				
			E. coli	*Salmonella* spp	*Shigella* spp	*Klebsiella* spp	*Staphylococcus* spp
	Age	Young (<3 month)	9 (56.25 %)	7(43.75 %)	9 (56.25%)	6(37.5%)	8(50%)
		Adult (>3 month)	12(50%)	6(25%)	10 (41.67%)	10 (41.67%)	10(41.67 %)
		$\chi 2$	0.150	1.538	0.819	0.69	0.269
		P-value	0.70	0.21	0.37	0.79	0.60
	Sex	Male	10 (62.5%)	6 (37.5%)	7 (43.75%)	7 (43.75%)	8(50%)
		Female	11 (45.83 %)	7 (29.16 %)	12(50%)	9(37.5%)	10(41.66 %)
		$\chi 2$	1.07	0.30	0.15	0.156	0.269

Pigeon		P-value	0.30	0.58	0.70	0.69	0.60
	Breed	White king	5(50%)	4 (40%)	4(40%)	4(40%)	4(40%)
		Fantail	6(100%)	3 (50%)	5(83.33%)	4(66.67%)	4(66.67%)
		Porter	3(25%)	1 (8.33%)	4(33.33%)	5(41.66%)	3(25%)
		Madina	7(58.33%)	5 (42.66%)	6(50%)	3(25%)	7(58.33%)
		$\chi 2$	9.26	4.75	4.31	2.91	4.04
		P-value	0.026	0.19	0.23	0.40	0.26
	Body weight	(250-300 gm)	6(54.54%)	4 (36.36%)	6(54.54%)	3(27.27%)	5(45.45%)
		(450-500 gm)	8(66.67%)	3 (25%)	5(41.67%)	4(33.33%)	6(50%)
		(500-1000 gm)	7(41.17%)	6 (35.29%)	8(47.05%)	9(52.94%)	7(41.17%)
		$\chi 2$	1.86	0.443	0.384	2.15	0.223
		P-value	0.39	0.80	0.83	0.34	0.90
	Diet	Ready food	8 (80%)	6 (60%)	8(80%)	5(50%)	5(50%)
		Raw food	6 (40%)	1(6.67%)	3(20%)	3(20%)	6(40%)
		Both	7 (46.67%)	6 (40%)	8(53.33%)	8(53.33%)	7(46.67%)
		$\chi 2$	4.177	8.395	8.989	4.028	0.269
		P-value	0.124	0.015	0.011	0.133	0.874
	Hygien-ic condition	Good	12(80%)	6(40%)	8(53.33%)	6(40%)	8(53.33%)
		Poor	4(23.52%)	4(23.52%)	7(41.17%)	6(35.29%)	6(35.29%)
		Excellent	5(62.5%)	3(37.5%)	4(50%)	4(50%)	4(50%)
		$\chi 2$	10.591	1.099	0.497	0.490	1.149
		P-value	0.005	0.577	0.780	0.783	0.563
	Vacc in-ation	Yes	13(65%)	7(35%)	10(50%)	7(35%)	10(50%)
		No	8(40%)	6(30%)	9(45%)	9(45%)	8(40%)
		$\chi 2$	2.506	0.114	0.100	0.417	0.404
		P-value	0.113	0.736	0.752	0.519	0.525

Legends:
$\chi 2$=Chi square
%=Percentage

Table-06 (b): Association between the socio-demographic variable and bacterial zoonotic pathogen in case of parrot

Species	Parameters		Bacterial zoonotic pathogen				
			E. coli	*Salmonella* spp	*Shigella* spp	*Klebsiella* spp	*Staphylococcus* spp
Parrot	Age	Young (<3 month)	6(75%)	4(50%)	2(25%)	6(75%)	2(25%)
		Adult (>3 month)	5(41.67%)	5(41.67%)	5(41.67%)	5(41.67%)	5(41.67%)
		$\chi 2$	2.155	0.135	0.586	2.155	0.586
		P-value	0.142	0.713	0.444	0.142	0.444
	Sex	Male	7(70%)	5(50%)	4(40%)	4(40%)	3(30%)
		Female	4(40%)	4(40%)	3(30%)	7(70%)	4(40%)
		$\chi 2$	1.818	0.202	0.220	1.818	0.220
		P-value	0.178	0.653	0.639	0.178	0.639
	Body weight	(10-500 gm)	6(75%)	4(50%)	2(25%)	6(75%)	2(25%)
		(550-1000 gm)	5(41.67%)	5(41.67%)	5(41.67%)	5(41.67%)	5(41.67%)
		$\chi 2$	2.155	0.135	0.586	2.155	0.586
		P-value	0.142	0.715	0.444	0.142	0.444
	Diet	Ready food	4(66.67%)	4(66.67%)	2(33.33%)	4(66.67%)	2(33.33%)
		Raw food	4(40%)	3(30%)	4(40%)	4(40%)	5(50%)
		Both	3(75%)	2(50%)	1(25%)	3(75%)	0(0%)
		$\chi 2$	1.886	2.088	0.293	1.886	3.150
		P-value	0.390	0.352	0.864	0.390	0.207
	Hygien-ic condition	Good	4(57.14%)	5(71.42%)	3(42.85%)	4(57.14%)	2(28.57%)
		Poor	5(50%)	3(30%)	3(30%)	5(50%)	5(50%)
		Excellent	2(66.67%)	1(33.33%)	1(33.33%)	2(66.67%)	0(0%)
		$\chi 2$	0.279	3.050	0.304	0.279	2.732
		P-	0.870	0.218	0.859	0.870	0.255

		value					
	Vaccin-ation	Yes	4(80%)	3(60%)	2(40%)	3(60%)	1(20%)
		No	7(46.67%)	6(40%)	5(33.33%)	8(53.33%)	6(40%)
		$\chi 2$	1.684	0.606	0.073	0.067	0.659
		P-value	0.194	0.436	0.787	0.795	0.417

Legends:
$\chi 2$=Chi square
%=Percentage

Table-06 (c): Association between the socio-demographic variable and bacterial zoonotic infection in case of budgerigar or love birds

Species	Parameters		Bacterial zoonotic infection				
			E. coli	*Salmonella* spp	*Shigella* spp	*Klebsiella* spp	*Staphylococcus* spp
Budgeri-gar or Love birds	Age	Young (<4 month)	4(100%)	2(50%)	0(0%)	1(25%)	1(25%)
		Adult (>4 month)	3(37.5%)	5(62.5%)	3(37.5%)	4(50%)	2(25%)
		$\chi 2$	4.286	0.171	2.000	0.686	0.000
		P-value	0.038	0.679	0.157	0.408	1.000
	Sex	Male	4(66.67%)	3(50%)	1 (16.67%)	2 (33.33%)	2(33.33%)
		Female	3(50%)	4(66.67%)	2 (33.33%)	3(50%)	1(16.67%)
		$\chi 2$	0.343	0.343	0.444	0.343	0.444
		P-value	0.558	0.558	0.505	0.558	0.505
	Body weight	(10-20 gm)	4(100%)	2(50%)	0(0%)	1(25%)	1(25%)
		(30-40 gm)	3(37.5%)	5(62.5%)	3(37.5%)	4(50%)	2(25%)
		$\chi 2$	4.286	0.171	2.000	0.686	0.000
		P-	0.038	0.679	0.157	0.408	1.000

		value					
	Diet	Ready food	4(100%)	2(50%)	0(0%)	1(25%)	1(25%)
		Raw food	2(33.33%)	3(50%)	3(50%)	2(33.33%)	1(16.67%)
		Both	1(50%)	2(100%)	0(0%)	2(100%)	1(50%)
		$\chi 2$	4.457	1.714	4.000	3.429	0.889
		P-value	0.108	0.424	0.135	0.180	0.641
	Hygien-ic condition	Good	5(100%)	2(40%)	0(0%)	2(40%)	1(20%)
		Poor	1(20%)	3(60%)	3(60%)	1(20%)	1(20%)
		Excellent	1(50%)	2(100%)	0(0%)	2(100%)	1(50%)
		$\chi 2$	6.651	2.126	5.600	3.771	0.800
		P-value	0.036	0.345	0.061	0.152	0.670
	Vaccin-ation	Yes	4(100%)	2(50%)	0(0%)	1(25%)	1(25%)
		No	3(37.5%)	5(62.5%)	3(37.5%)	4(50%)	2(25%)
		$\chi 2$	4.286	0.171	2.000	0.686	0.000
		P-value	0.038	0.679	0.157	0.408	1.000

Legends:
$\chi 2$=Chi square
%=Percentage

Table-06 (d): Association between the socio-demographic variable and bacterial zoonotic infection in case of quail

Species	Parameters		Bacterial zoonotic pathogen				
			E. coli	*Salmonella* spp	*Shigella* spp	*Klebsiella* spp	*Staphylococcus* spp
	Age	Young (<3 month)	5(71.42%)	4(57.14%)	4(57.14%)	6(85.71%)	4(57.14%)
		Adult	2(100	2(100%	2(100	1(50%)	2(100%)

Quail		(>3 month)	%)	)	%)		
		$\chi 2$	0.735	1.286	1.286	1.148	1.286
		P-value	0.391	0.257	0.257	0.284	0.257
	Sex	Male	3(75%)	3(75%)	2(50%)	3(75%)	3(75%)
		Female	4(80%)	3(60%)	4(80%)	4(80%)	3(60%)
		$\chi 2$	0.032	0.225	0.900	0.032	0.225
		P-value	0.858	0.635	0.343	0.858	0.635
	Body weight	(70-100 gm)	6(75%)	5(62.5 %)	5(62.5 %)	6(75%)	5(62.5%)
		(100-140 gm)	1(100 %)	1(100%)	1(100 %)	1(100 %)	1(100%)
		$\chi 2$	0.321	0.562	0.562	0.321	0.562
		P-value	0.571	0.453	0.453	0.571	0.453
	Diet	Ready food	3(100 %)	3(100%)	2(66.67%)	3(100 %)	2(66.67%)
		Raw food	1(50%)	1(50%)	1(50%)	1(50%)	1(50%)
		Both	3(75%)	2(50%)	3(75%)	3(75%)	3(75%)
		$\chi 2$	1.768	2.250	0.375	1.768	0.375
		P-value	0.413	0.325	0.829	0.413	0.829
	Hygie-nic condition	Good	4(100 %)	3(75%)	3(75%)	4(100 %)	2(50%)
		Poor	1(50%)	2(100%)	1(50%)	0(0%)	2(100%)
		Excellent	2(66.67%)	1(33.33 %)	2(66.67%)	3(33.33%)	1(66.67%)
		$\chi 2$	2.250	2.625	0.375	9.000	1.500
		P-value	0.325	0.269	0.829	0.011	0.472
	Vacci-nation	Yes	3(100 %)	3(100%)	2(66.67%)	3(100 %)	2(66.67%)
		No	4(66.67%)	3(50%)	4(66.67%)	4(66.67%)	4(66.67%)
		$\chi 2$	1.286	2.250	0.000	1.286	0.000
		P-value	0.257	0.134	1.000	0.257	1.000

Legends:
$\chi 2$=Chi square
%=Percentage

Table 06. (a, b, c, & d) were summarized that the socio demographic variable and bacterial pathogen for (pigeon, parrot, budgerigar or love birds & quail). In case of pigeon hygienic condition and breed were statistically significant value (<0.05) for *E. coli*. The other kinds of values were not statistically significant. In case of parrot, all variables were not significant for isolated pathogens. In case of budgerigar or love birds for *E. coli*. these variables such as age, body weight, hygienic condition and vaccination were also significant value is (<0.05). In case of quail all variables were not significant.

4.4 Prevalance of zoonotic bacterial pathogens:

Different samples of (cloacal swab. Oral swab & feces) were collected from pigeon, parrot, budgerigar or love birds & quail. Bacteria were isolated from (cloacal swab. Oral swab & feces) of pet birds. Out of the isolated organisms the ranking percentage is as follows Table no:7 (a, b, c & d).

Table 07 (a): Prevalence (Disease & percentage) of bacterial zoonotic pathogens in household in case of pigeon

Samples of pigeon	*E. coli*	*Salmonella* spp	*Shigella* spp	*Klebsiella* spp	*Staphylococcus* spp
Cloacal swab (40)	5 (12.5%)	3(7.5%)	5(12.5%)	8(20%)	2(5%)
Oral swab (40)	6(15%)	1(2.5%)	3(7.5%)	3(7.5%)	12(30%)
Feces (40)	10(25%)	9(22.5%)	11(27.5%)	5(12.5%)	4(10%)
Total=120	21 (17.5%)	13(10.83%)	19(15.83%)	16(13.33%)	18(15%)

Table 07 (a). Table 07 (a) Showed that the prevalence of bacterial pathogens in case of pigeon, *E. coli* (17.5%), *Salmonella* spp (10.83%), *Shigella* spp (15.83%), *Klebsiella* spp (16.33%), and *Staphylococcus* spp ((15%) were found respectively.

Table 07 (b): Prevalence (Disease & percentage) of bacterial zoonotic pathogens in household in case of parrot

Samples of parrot	*E. coli*	*Salmonella* spp	*Shigella* spp	*Klebsiella* spp	*Staphylococcus* spp
Cloacal	3 (15%)	3(15%)	1(5%)	7(35%)	1(5%)

swab (20)					
Oral swab (20)	2(10%)	1(5%)	1(5%)	1(5%)	4(20%)
Feces (20)	6(30%)	5(25%)	5(25%)	3(15%)	2(10%)
Total=60	11(18.33%)	9(15%)	7(11.67%)	11(18.33%)	7(11.67%)

Table 07 (b). Table 07 (b) Showed that the prevalence of bacterial pathogens in case of parrot, were found *E. coli* (18.33), *Salmonella* spp (15%), *Shigella* spp (11.67%), *Klebsiella* spp (18.3%) and *Staphylococcus* spp (11.67%) respectively.

Table 07 (c): Prevalence (Disease & percentage) of bacterial zoonotic pathogens in case of budgerigar or love birds

Samples of (budgerigar or love birds)	*E. coli*	*Salmonella* spp	*Shigella* spp	*Klebsiella* spp	*Staphylococcus* spp
Cloacal swab (12)	2(16.67%)	2(16.67%)	0(0%)	3(25%)	0(0%)
Oral swab (12)	0(0%)	1(8.33%)	0(0%)	0(0%)	3(25%)
Feces (12)	5(41.67%)	4(33.33%)	3(25%)	2(16.67%)	0(0%)
Total=36	7(19.44%)	7(19.44%)	3(8.33%)	5(13.88%)	3(8.33%)

Table 07 (c). Table 07 (c) presented that the prevalence of bacterial pathogens found in case of budgerigar or love birds were *E. coli* (19.44%), *Salmonella* spp (19,44%), *Shigella* spp (8.33%), *Klebsiella* spp (13.88%) and Staphylococcus spp (8.33%) respectively.

Table 07 (d): Prevalence (Disease & percentage) of bacterial zoonotic pathogens in household pet birds (in case of quail)

Samples of quail	*E. coli*	*Salmonella* spp	*Shigella* spp	*Klebsiella* spp	*Staphylococcus* spp
Cloacal swab (9)	2(22.22%)	3(33.33%)	2(22.22%)	4(44.44%)	1(11.11%)
Oral swab (9)	1(11.11%)	0(0%)	1(11.11%)	1(11.11%)	3(33.33%)
Feces (9)	4(44.44%)	3(33.33%)	3(33.33%)	2(22.22%)	2(22.22%)

Total=27	7(77.78%)	6(66.67%)	6(66.67%)	7(77.78%)	6(66.67%)

Table 07 (d). Table 07 (d) Showed that the prevalence of bacterial pathogens found in case of budgerigar or love birds were *E. coli* (77.78%), *Salmonella* spp (66.67%), *Shigella* spp (66.67%), *Klebsiella* spp (77.78%) and *Staphylococcus* spp (66.67%) respectively.

4.5 Antibiotic Sensitivity Test

Antimicrobial susceptibility testing was performed using Muller-Hinton agar (Mumbai, India) plates as recommended by the Clinical and Laboratory Standards Institute. A total five isolated organisms (*E.coli*, *Salmonella* spp, *Shigell* spp*, Klebsiella* spp *& Staphjylococcus spp)* are obtained from defferent samples of pet birds (pigeon, parrot, budgerigar or love birds & quail) were subjected to Antibiotic Sensitivity patern showed in below.

Table no.8 Antibiotic sensitivity patterrn of the isolated bacteria.

Antibacterial agents	*E. coli*		*Salmonella* spp		*Shigella* spp		*Klebsiella* spp		*Staphylococcus*	
	Zone of inhibiti-on	Outcome	Zone of inhibition	Outcome	Zone of inhibition	Outcome	Zone of inhibition	Outcome	Zone of inhibition	Out Come
Cefixime (5µg)	21	S	23	S	15	R	0	R	0	R
Tetracycline (5µg)	10	R	0	R	0	R	0	R	10	R
Cloramphenicol (30µg)	25	S	22	S	21	S	19	S	20	S
Ciprofloxacin (5µg)	0	R	26	S	20	S	34	S	17	R
Levofloxacin (5µg)	10	R	26	S	16	I	20	S	18	I
Azithromycin (15µg)	0	R	13	R	11	R	17	R	14	R
Erythromycin (15µg)	12	R	8	R	0	R	0	R	0	R
Penicillin G (10µg)	0	R	0	R	0	R	0	R	0	R

Neomycin (30µg)	17	S	16	I	14	I	10	R	15	I
Vancomycin (30µg)	0	R	13	R	0	R	0	R	12	R
Cephalexin (30µg)	10	R	0	R	0	R	0	R	17	S
Kanamycin (30 µg)	15	S	17	S	14	S	14	S	16	S
Amoxycillin (30µg)	0	R	0	R	0	R	0	R	0	R
Cloxacillin (1µg)	0	R	0	R	0	R	0	R	11	R
Bacitracin (10µg)	0	R	0	R	0	R	0	R	O	R
Gentamycin (10µg)	16	S	15	S	14	I	17	S	18	S
Cefradine (25µg)	0	R	0	R	0	R	0	R	7	R
Collistin (10µg)	11	S	13	S	9	R	11	S	9	R

Source: CLSIFDA- 2013 table –update pdf [Note: S=Sensitive, R=Resistant, I=I , n termediate].

Table 8 (a). Results of antibiotic sensitivity test of *E. coli* (n=46)

Antibacterial agents	No. and persentages of isolates		
	Sensitive	Intermediate	Resistance
1. Cefixime (5µg)	39.13%	0%	60.87%
2. Tetracycline (5µg)	47.82%	0%	60.87%
3. Cloramphenicol (30µg)	39.13%	0%	60.87%
4. Ciprofloxacin (5µg)	0%	0%	100%
5. Levofloxacin (5µg)	26.08%	0%	73.98%
6. Azithromycin (15µg)	0%	0%	100%
7. Erythromycin (15µg)	86.95%	0%	13.05%
8. Penicillin G (10µg)	0%	0%	100%
9. Neomycin (30µg)	56.52%	0%	43.48%
10. Vancomycin (30µg)	0%	0%	100%

11.	Cephalexin (30µg)	89.14%	0%	10.86%
12.	Kanamycin (30 µg)	28.26%	0%	71.74%
13.	Amoxycillin (30µg)	0%	0%	100%
14.	Cloxacillin (1µg)	0%	0%	100%
15.	Bacitracin (10µg)	0%	0%	100%
16.	Gentamycin (10µg)	26.08%	0%	73.92%
17.	Cefradine (25µg)	0%	0%	100%
18.	Collistin (10µg)	23.91%	0%	76.09%

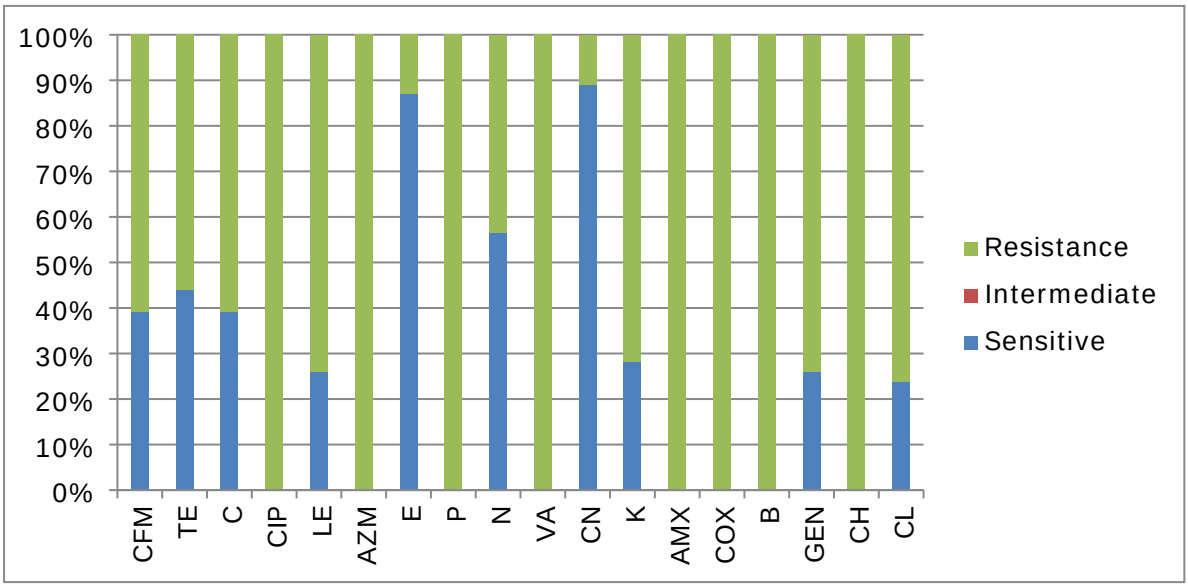


Fig 6(a): Column diagram presenting antibiotic sensitivity test of isolated *E. coli.*

Table 8 (b). Results of antibiotic sensitivity test of *Salmonella* spp (n=35)

Antibacterial agents	No. and persentages of isolates		
	Sensitive	Intermediate	Resistance
1. Cefixime (5µg)	65.71%	0%	34.29%
2. Tetracycline (5µg)	0%	0%	100%
3. Cloramphenicol (30µg)	85.71%	0%	14.29%
4. Ciprofloxacin (5µg)	74.28%	0%	25.72%
5. Levofloxacin (5µg)	57.14%	0%	42.86%

6. Azithromycin (15µg)	31.42%	0%	68.58%
7. Erythromycin (15µg)	80%	0%	20%
8. Penicillin G (10µg)	0%	0%	100%
9. Neomycin (30µg)	0%	82.85%	17.15%
10. Vancomycin (30µg)	51.42%	0%	48.58%
11. Cephalexin (30µg)	0%	0%	100%
12. Kanamycin (30 µg)	77.14%	0%	22.86%
13. Amoxycillin (30µg)	0%	0%	100%
14. Cloxacillin (1µg)	0%	0%	100%
15. Bacitracin (10µg)	0%	0%	100%
16. Gentamycin (10µg)	85.71%	0%	14.29%
17. Cefradine (25µg)	0%	0%	100%
18. Collistin (10µg)	74.28%	0%	25.72%

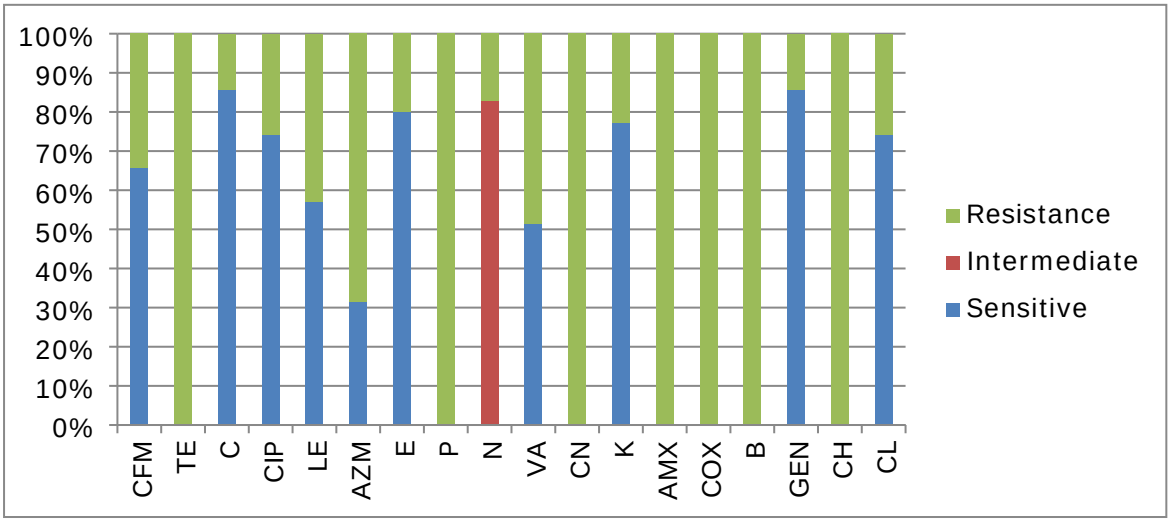


Fig 6 (b): Column diagram presenting antibiotic sensitivity test of isolated *Salmonella* spp.

Table 8 (c). Results of antibiotic sensitivity test of *Shigella* (n=36)

Antibacterial agents	No. and persentages of isolates		
	Sensitive	Intermediate	Resistance
1. Cefixime (5µg)	83.33%	0%	16.67%

2. Tetracycline (5µg)	0%	0%	100%
3. Cloramphenicol (30µg)	30.55%	0%	69.45%
4. Ciprofloxacin (5µg)	83.33%	0%	16.67%
5. Levofloxacin (5µg)	0%	44.44%	55.56%
6. Azithromycin (15µg)	61.1%	0%	39.9%
7. Erythromycin (15µg)	0%	0%	100%
8. Penicillin G (10µg)	0%	0%	100%
9. Neomycin (30µg)	0%	58.33%	41.67%
10. Vancomycin (30µg)	0%	0%	100%
11. Cephalexin (30µg)	0%	0%	100%
12. Kanamycin (30 µg)	38.88%	0%	61.12%
13. Amoxycillin (30µg)	0%	0%	100%
14. Cloxacillin (1µg)	0%	0%	100%
15. Bacitracin (10µg)	0%	0%	100%
16. Gentamycin (10µg)	0%	33.33%	66.67%
17. Cefradine (25µg)	0%	0%	100%
18. Collistin (10µg)	0%	0%	100%

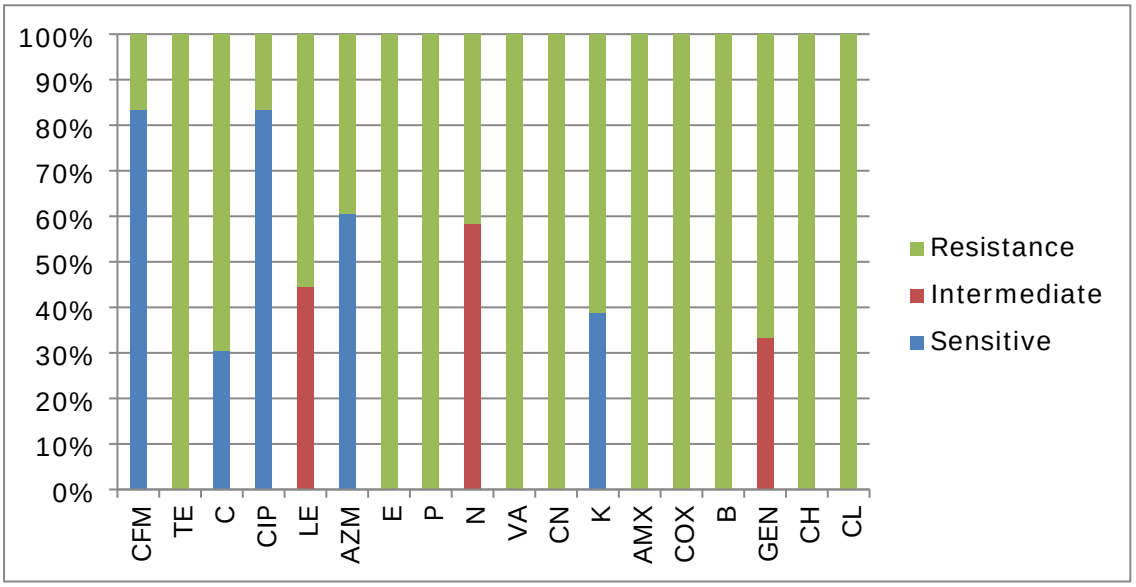


Fig 6(c): Column diagram presenting antibiotic sensitivity test of isolated *Shigella* spp.

Table 8 (d). Results of antibiotic sensitivity test of *Klebsiella* (n=39)

Antibacterial agents	No. and persentages of isolates		
	Sensitive	Intermediate	Resistance
1. Cefixime (5µg)	0%	0%	100%
2. Tetracycline (5µg)	0%	0%	100%
3. Cloramphenicol (30µg)	94.43%	0%	5.57%
4. Ciprofloxacin (5µg)	71.79%	0%	28.21%
5. Levofloxacin (5µg)	92.30%	0%	7.70%
6. Azithromycin (15µg)	86.1%	0%	13.9%
7. Erythromycin (15µg)	0%	0%	100%
8. Penicillin G (10µg)	0%	0%	100%
9. Neomycin (30µg)	35.64%	0%	74.36%
10. Vancomycin (30µg)	0%	0%	100%
11. Cephalexin (30µg)	0%	0%	100%
12. Kanamycin (30 µg)	53.84%	0%	46.16%
13. Amoxycillin (30µg)	0%	0%	100%
14. Cloxacillin (1µg)	0%	0%	100%
15. Bacitracin (10µg)	0%	0%	100%
16. Gentamycin (10µg)	87.17%	0%	12.83%
17. Cefradine (25µg)	97.43%	0%	2.77%
18. Collistin (10µg)	46.15%	0%	53.85%

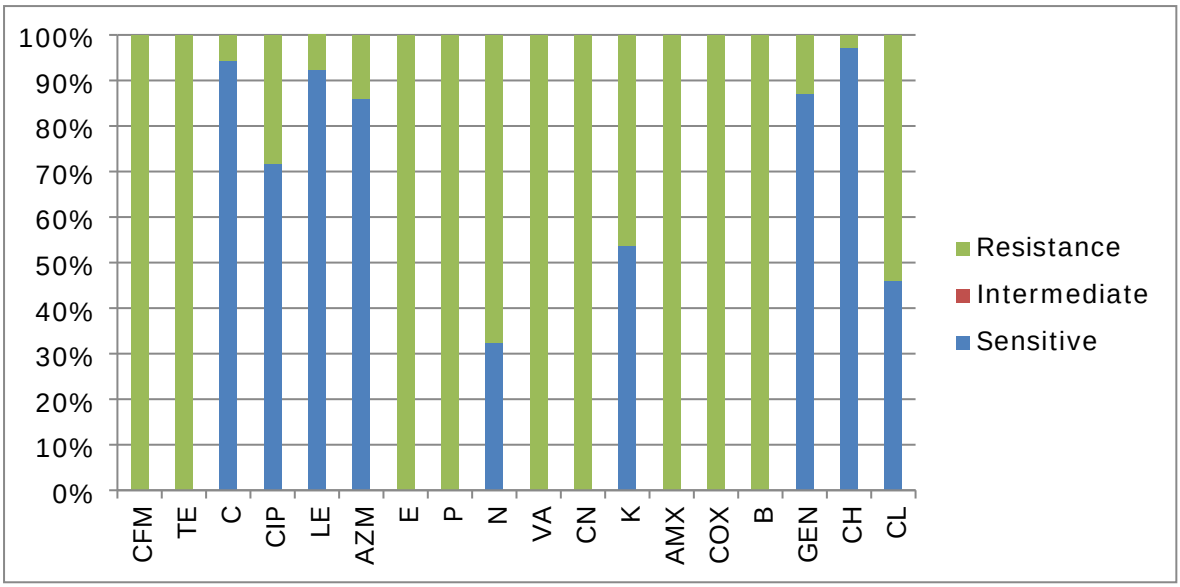


Fig 6(d): Column diagram presenting antibiotic sensitivity test of isolated *Klebsiella* spp.

Table 8 (e). Results of antibiotic sensitivity test of *Staphylococcus* spp (n=34)

Antibacterial agents	No. and persentages of isolates		
	Sensitive	Intermediate	Resistance
1. Cefixime (5µg)	0%	0%	100%
2. Tetracycline (5µg)	58.82%	0%	41.18%
3. Cloramphenicol (30µg)	70%	0%	29.42%
4. Ciprofloxacin (5µg)	100%	0%	0%
5. Levofloxacin (5µg)	0%	52.9%	47.06%
6. Azithromycin (15µg)	41.17%	0%	58.53%
7. Erythromycin (15µg)	0%	0%	100%
8. Penicillin G (10µg)	0%	0%	100%
9. Neomycin (30µg)	0%	88.23%	11.77%
10. Vancomycin (30µg)	82.35%	0%	17.65%
11. Cephalexin (30µg)	70%	0%	29.42%
12. Kanamycin (30 µg)	94.11%	0%	5.89%
13. Amoxycillin (30µg)	0%	0%	100%

14.	Cloxacillin (1µg)	64.70%	0%	35.3%
15.	Bacitracin (10µg)	0%	0%	100%
16.	Gentamycin (10µg)	97.05%	0%	2.95%
17.	Cefradine (25µg)	14.70%	0%	85.30%
18.	Collistin (10µg)	52.94%	0%	47.06%

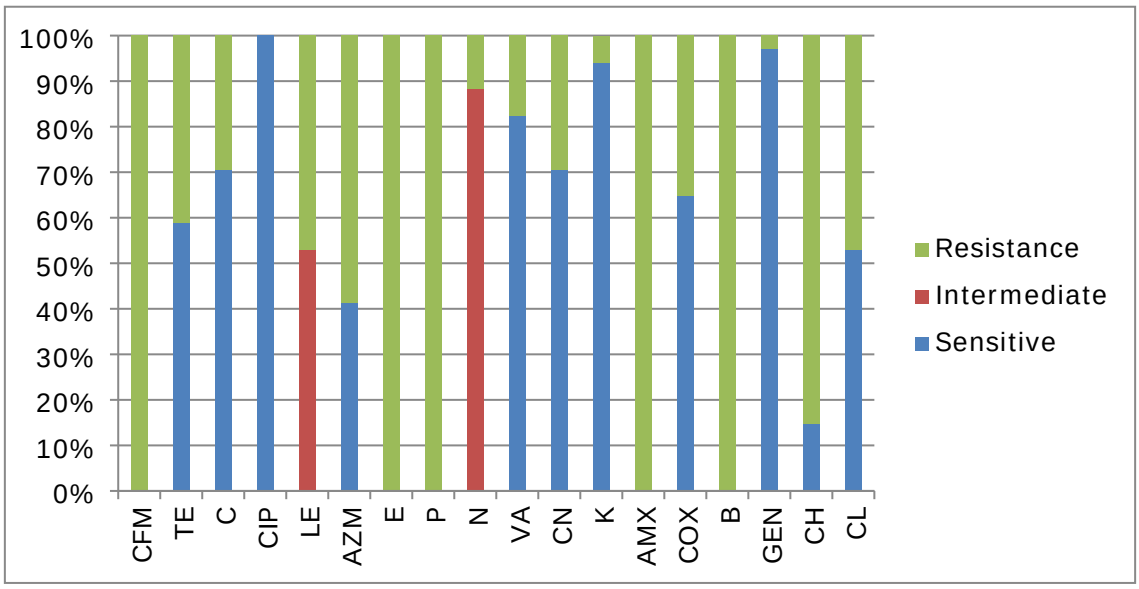


Fig 6(e): Column diagram presenting antibiotic sensitivity test of isolated *Staphylococcus* spp.

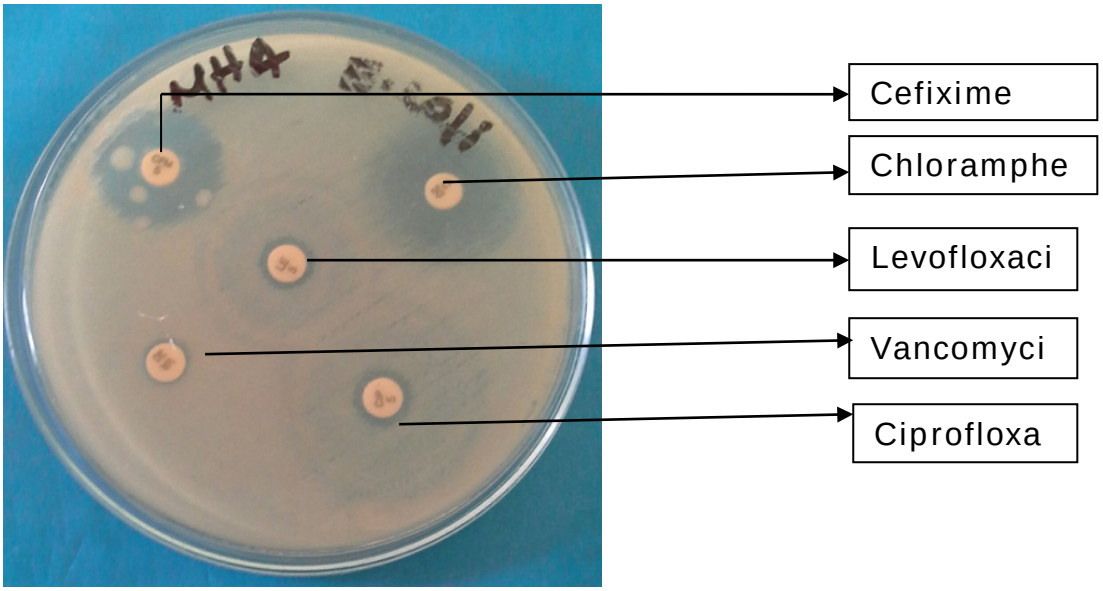


Plate 4.21: Antibiotic sensitivity test for *E.coli* on Mueller-Hinton agar.

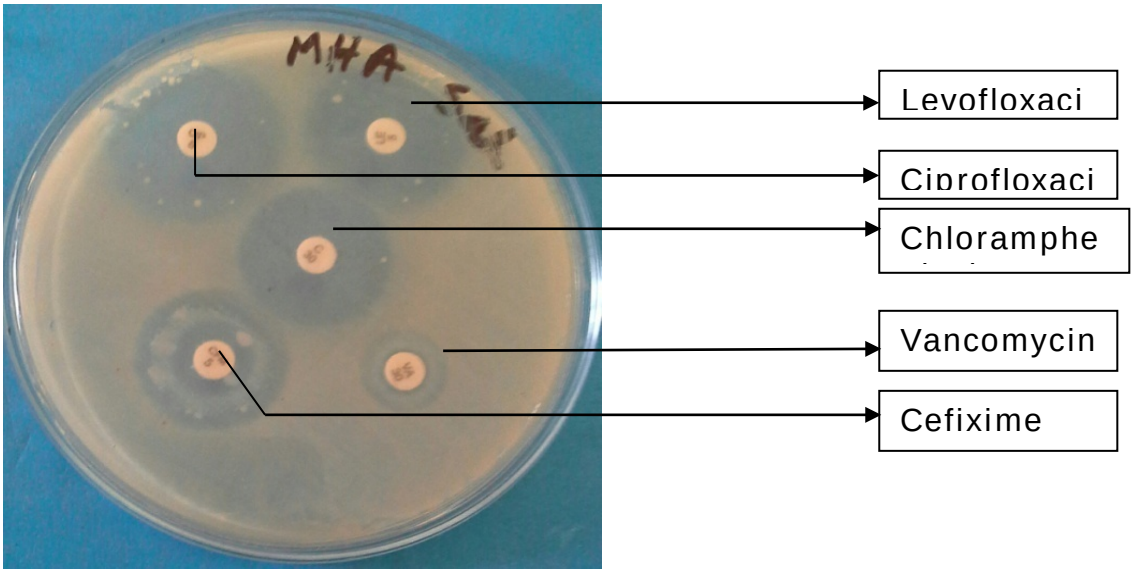


Plate 4.22: Antibiotic sensitivity test for *Salmonella* spp on Mueller-Hinton agar.

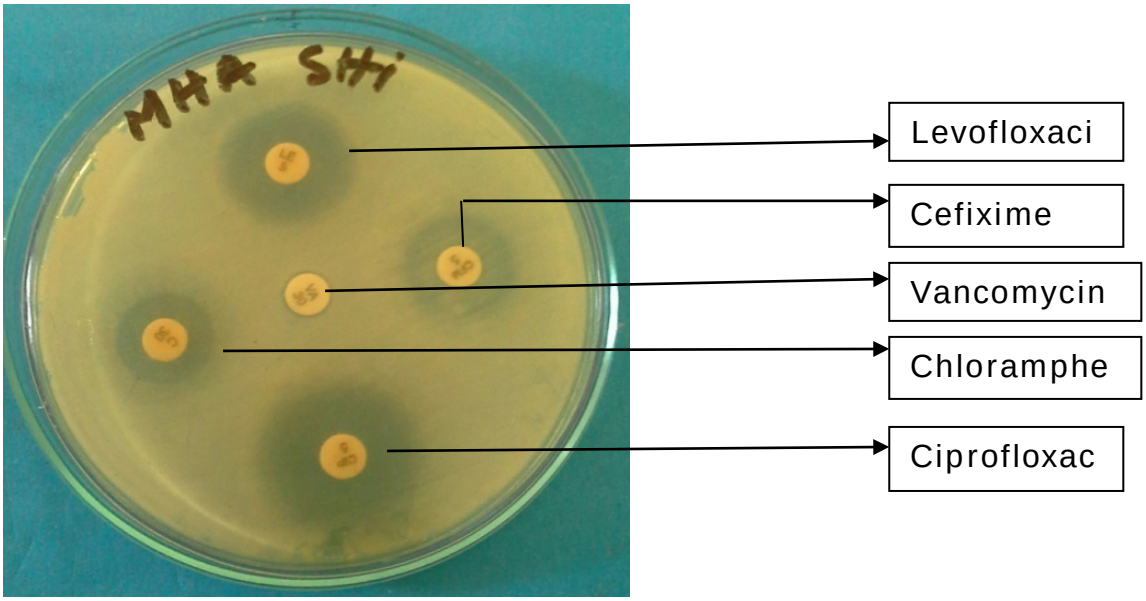


Plate 4.23: Antibiotic sensitivity test for *Sigella* spp on Mueller-Hinton agar.

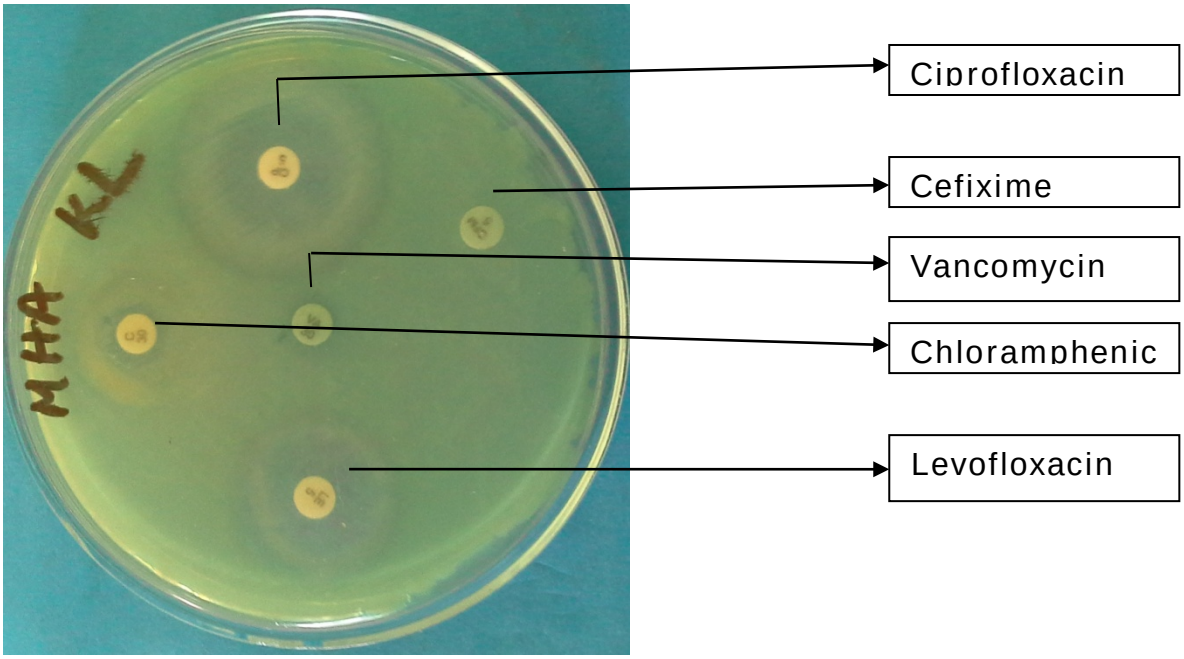


Plate 4.24: Antibiotic sensitivity test for *Klebsiella* spp on Mueller-Hinton agar.

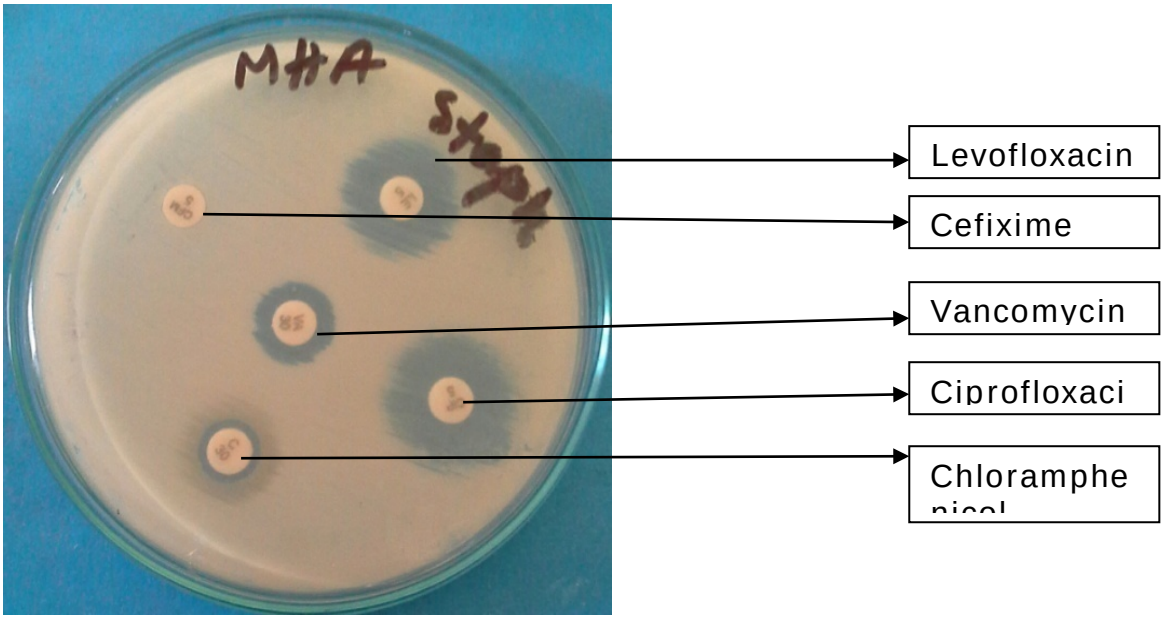


Plate 4.25: Antibiotic sensitivity test for *Staphylococcus* spp on Mueller-Hinton agar.

CHAPTER: 5
DISCUSSION

Birds are found all over the world. They are popular as pets due to their sociable and affectionate nature, intelligence, bright colors, and ability to imitate with human voices. Economically birds can be beneficial to communities as sources of income from the pet trade. Parrot, Pigeon, Budgerigar and quail are the most popular of all pet bird species. Pet birds are the source of recreation for human especially children.

The present study was conducted to bacterial analysis of domestic pet birds (pigeon, parrot, budgerigar or love birds and quail). This study determined the total viable counts, isolation and identification of bacteria and the antibiotic sensitivity test. In this study, a total of 243 samples of four bird orders were analyzed.

The total viable counts of pigeon in case of cloacal swab (male) 10.34±0.14 cfu/g (young), 10.02±0.27 cfu/g (adult), (female) 9.7164±0.41 cfu/g (young), 10.38±0.13 cfu/g (adult), oral swab (male) 10.40±0.10 cfu/g (young), 9.87±0.38 (adult), female 10.02±0.32 cfu/g (young), 10.38±0.13 (adult) & feces (male) 10.45±0.09 cfu/g (young), 10.45±0.06 cfu/g (adult), female 9.92±0.20 (young), 10.20±0.20 (adult).

The total viable counts of budgerigar or love birds in case of cloacal swab (male) 10.47±0.21cfu/g (young), 10.51±0.10 cfu/g (adult), (female) 9.34±0.89 cfu/g (young), 10.48±0.16 cfu/g (adult), oral swab (male) 10.53±0.26 cfu/g (young), 10.27±0.22 (adult), (female) 10.50±0.07 cfu/g (young), 9.09±0.29 (adult) & feces (male) 10.28±0.22 cfu/g (young), 9.82±0.46 (adult), female 10.28±0.06 (young), 10.50±0.23 (adult).

The total viable counts of parrot in case of cloacal swab (male) 10.47±0.21cfu/g (young), 10.51±0.09 cfu/g (adult), (female) 9.34±0.99 cfu/g (young), 10.48±0.16 cfu/g (adult), oral swab (male) 10.54±0.26 cfu/g (young), 10.26±0.22 (adult), female 10.50±0.07 cfu/g (young), 9.09±0.29 (adult) & feces (male) 10.28±0.22 cfu/g (young), 9.81±0.46 cfu/g (adult), female 10.29±0.06 (young), 10.50±0.23 (adult).

The total viable counts of quail in case of cloacal swab (male) 9.86±0.48 cfu/g (adult), (female) 9.37±0.57 cfu/g (young), 10.63±0.17 cfu/g (adult),

oral swab (male) 10±0.09 cfu/g (adult), female 10.35±0.14 cfu/g (young), 10.45±0.01 (adult) & feces (male) 9.64±0.66cfu/g (adult), female 10.32±0.36 (young), 9.52±0.14 (adult).

The present study showed that, the overall prevalence of isolated organism from pigeon were *E. coli* (17.5%), *Salmonella* spp (10.83%), *Shigella* spp (15.83%), *Klebsiella* spp (13.33%) and *Staphylococcus* spp (15%) respectively. This findings are somewhat differ from the previous results of Dey *et al*. (2013) and Hosain *et al*. (2012) who reported higher prevalence of *E. coli* (69.64%) and *Salmonella* spp (35.71%) in pigeon wheras the prevalence of *Salmonella* spp is (22.22%) in cloacal swab (Hosain et al. 2012) supports the current finding. Again this finding are agreed with Akbarmehr (2010) who reported (15.55%) *Salmonella* spp in pigeon and Pasmans *et al.* (2004) and Kobayashi *et al.* (2007) who reported (22.8%) and (5.8%) *Salmonella* in fecal sample and cloacal swab respectively.

Pravalence rate of *Salmonella* spp influenced by the effects of bird density fecal oral transmission, strain variation immunity of the bird and geographical distribution. (Carraminana *et al.*) 2006. Eggs and poultry are most common routes of *Salmonellla* spp transmission and pigeon meat may be contaminated with *Salmonella* spp pathogen if unhygienic condition during handling and processing of meat (Hossain *et al.*2012).

The present study showed that, the overall prevalence of isolated organism from parrot were *E. coli* (18.33%), *Salmonella* spp (15%), *Shigella* spp (11.67%), *Klebsiella* spp (18.33%) and *Staphylococcus* spp (11.67%) respectively. The present findings are agreed with Akhter *et al.* (2010) who reported that *E. coli* (64.44%), *Salmonella* spp. (46.67%), *Staphylococcus* spp. (46.67%), *Proteus* spp. (6.67%) and *Pasteurella* spp. (33.33%) from parrot samples respectively.

The present study showed that, the overall prevalence of isolated organism from budgerigar were, *E. coli* (19.44%), *Salmonella* spp

(19.44%), *Shigella* spp (8.33%), *Klebsiella* spp (13.88%) and *Staphylococcus* spp (8.33%) respectively.

In this study examined that the overall prevalence of isolated organism from quail were, *E. coli* (77.78%), *Salmonella* spp (66.67%), *Shigella* spp (66.67%), *Klebsiella* spp (77.78%) and *Staphylococcus* spp (66.67%) respectively whereas Ghazi *et al.* (2014) reported lower prevalence of *E.coli* (31.25%), *Staphylococcus aureus* (8%), *Klebsiella pneumonia* (10%) and *Salmonella Typhimurium* (12%) respectively in wild quails. On the other hand Ahmed and Monsour (2014) reported that the prevalence of *E. coli* and *Salmonella* spp was (37.2%) and (24.4%) in migratory quail. This variation due to the density of birds in flock, lack of biosecurity measures, limited access of veterinary care, lack of proper knowledge on quail rearing and shedding of pathogesn in the domestic quail farming in the research area. Domestic quail plays an important role in the transmission of pathogens which may cause clinical disease in animal and human population. *E. coli* and *Salmonella* spp. are the most potential pathogens causing food poisoning and posing a zoonotic hazard Ahmed and Mansour (2014).

Prevalence of isolated bacteria in pigeon was not statistically significantly in relation to breed and hygienic condition ($p>0.05$). In case of Parrot the prevalence of isolated bacteria was not statistically significant in relation to age, sex, breed, body weight ($p>0.05$). In case of love bird the prevalence of isolated pathogens was not statistically significant in relation to age, sex, breed and body weight ($p>0.05$). On the other hand the prevalence of isolated bacteria was statistically signification in relation to diet and hygienic condition in pigeon ($P<0.05$). Again the prevalence of isolated bacteria was statistically significant in relation to age, bodyweight, hygienic condition and vaccination in budgerigar ($P<0.05$) whereas the prevalence was statistically significant in relation to hygienic condition in quail ($P<0.05$).

The practice of antimicrobials in veterinary medication as food animal growth promoting agent and in humans through the earlier decade has

resulted in massive stress for supporting antimicrobial resistance amongst bacterial pathogens worldwide (Hakanen *et al*., 2001).

In our study, all of the isolated *E. coli* was resistance against Ciprofloxacin (100%), Azithromycin (100%), Penicillin G (100%), Vancomycin (100%), Amoxycillin (100%), Cloxacillin (100%), Cefradine (100%), Bacitracin(100%), Cefixime (60.87%), Tetracycline (60.87%), Levofloxacin (73.98%), Kanamycin (71.74%), Gentamycin (73.92%) and Collistin (76.09%) and sensitive to Erythromycin (86.95%), Cephalexin (99.14%). This finding are agreed with Dey *et al*. (2013) who reported that isolated *E. coli* were found sensitive to erythromycin (100%), ciprofloxacin (80%), nalidixic acid (80%), chloramphenicol (70%) and kanamycin (70%) and resistance against sulphamethoxazole (90%), tetracycline (90%), amoxicillin (70%).

In our study, isolated *Salmonlla* spp. was showed resistance against Tetracycline (100%), Cefradine (100%), Bacitracin (100%), Cloxacillin (100%), Amoxycillin (100%), Cephalexin(100%), Penicillin G (100%), Azithromycin (68.58%), Vancomycin (48.58%), Levofloxacin (25.72%) and sensitive to Gentamycin (85.71%), Cloramphenicol (85.71%), Erythromycin (80%), Kanamycin (77.14%), Ciprofloxacin (74.28%). This result was supported with Md. Sahadat Hosain *et al*. (2012) who found that isolated *Salmonella* were sensitive to ciprofloxacin followed by sulphamethoxazole (70%), chloramphenicol (60%), kanamycin (60%), gentamicin (60%) and nalidixic acid (60%) and resistant to amoxicillin (90%), followed by ampicillin (80%), erythromycin (80%) and tetracycline (60%).

Isolated *Shigella* spp. were resistance against Tetracycline(100%), Erythromycin(100%), Amoxycillin (100%), Cephalexin(100%), Cloxacillin (100%), Vancomycin(100%), Penicillin G(100%), Bacitracin (100%), Cefradine(100%), Collistin(100%), Chloramphenicol (69.45%), Gentamycin (66.67%), Kanamycin (61.12%), Levofloxacin (55.56%) and sensitive to Cefixime (83.33%), Ciprofloxacin (83.33%) while Neomycin

(58.33%), Levofloxacin (44.44%), Gentamycin (33.33%) were found intermediate.

In the present study, isolated *Klebsiella* spp. were resistance against Cefixime (100%), Tetracycline (100%), Penicillin G (100%),Vancomycin (100%), Cephalexin (100%), Amoxycillin (100%), Cloxacillin (100%), Bacitracin (100%),Neomycin (74.36%), Collistin (53.85%), Kanamycin (46.16%) and sensitive to Cefradine (97.43%), Cloramphenicol (94.43%), Levofloxacin (92.30%), Gentamycin (87.17%), Ciprofloxacin (71.79%).

Isolated *Staphylococcus* spp were resistance against Cefixime (100%),Erythromycin(100%), Penicillin G (100%), Amoxycillin (100%), Bacitracin (100%), Cefradine (85.30%), Azithromycin (58.53%), Collistin (47.06%), Levofloxacin (47.06%), Tetracycline (41.18%) and sensitive to Ciprofloxacin (100%), Gentamycin (97.05%), Kanamycin (94.11%), Vancomycin (82.35%), Cloramphenicol (70%) while Neomycin (88.23%), Levofloxacin (52.9%) were found intermediate.

Pet birds play an important role in the dissemination of pathogenic organisms such as Salmonellosis causes gastroenteritis in humans and animal, being the most important reported zoonotic disease bacterial food-borne disease. *Staphylococcus aureus* is the bacteria involved in food poisoning causing gastroenteritis from ingestion of enterrotoxins in contaminated food. *Escherichia coli* is the most common food borne zoonotic pathogen causing various disease in both animals and humans.

Thus, the results of this study may help pet clinicians to interpret microbiological culture and sensitivity results in pigeon, parrot, budgerigar or love birds, quails and other pet birds as well.

CHAPTER: 6
CONCLUSION

Overall, the present study investigated the cloacal swab, oral swab & feces of household pet birds which had been found of potentially pathogenic bacteial isolates. *Escherichia coli, Salmonella* spp.*, Staphylococcus* spp.*, Klebsiella* spp*. and Shigella* spp. were isolated from the cloacal swab, oral swab and feces samples of birds. The present study showed that, the prevalence of both potentially pathogenic Gram negative bacteria (*Escherichia coli, Salmonella* spp, *Klebsiella* spp, and *Shigella* spp.) and Gram positive bacteria *(Staphylococcus aureus* spp) were relatively high in healthy pet birds. These birds are able to spread and transmit bacterial pathogens and zoonotic disease to human and animals. Therefore; control measures should be considered to prevent transmission of such bacteria and zoonotic disease.

In the context of this study, it may be concluded that,

i. The presence of *E.coli*, *Salmonella* spp*, Staphylococcus* spp., *Klebsiella* spp and *Shigella* spp in most of the samples are public health concern.
ii. Total viable count was successfully performed from different samples of pet birds.
iii. High counts of bacteria in most of the samples indicate that many bacterial zoonotic disease were transmitted to the humans through pet birds and it also accelerated the public health threat.
iv. The antibiotic resistance properties of isolated bacteria can cause serious health hazards because of ineffective treatment of the sufferers by the commonly prescribed antibiotics.

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APPENDICES

APPENDIX 1

Composition of Different Media

1. Nutrient agar (Hi Media)

Ingredients:

g/L
Peptic digest of animal tissue 5.0
Sodium chloride 5.0
Beef extract 1.5
Yeast extract 1.5
Final pH (at 25^{0}C) 7.4 ± 0.2

2. Eosine methylene blue Agar (Hi Media)

Ingredients:

g/L
Peptic digest of animal tissue 10
Lactose 5.0
Sucrose 5.0
Dipotassium phosphate 2.0
Eosin - Y 0.40
Methylene blue 0.065
Agar 20.0
Final pH (at 25^{0}C) 7.2 ± 0.2

3. MacConkey agar (Hi-media)

Ingredients:
g/L
Peptic digest of animal tissue
17.0
Protease peptone
3.0
Lactose monohydrate
10
Bile salt
1.5
Sodium chloride
5.0
Agar-agar
15.0
Neutral red
0.03
Final pH (at 25^{0}C)
7.1 ± 0.2

4. Deoxycholate Citrate Agar
Component
Amount (g/L)
Meat peptone
10.000
Beef extract
10.000
Lactose monohydrate
10.000
Sodium citrate
20.000
Neutral red
0.020
Sodium deoxycholate
5.000
Ferric citrate
1.000
Agar
13.500

5. Thiosulfate-citrate-bile salts-sucrose agar
g/L

Yeast extract
Proteose Peptone
10.0
Sodium thiosulfate
10.0
Sodium citrate
10.0
Ox gall
5.0
Sodium cholate
3.0
Saccharose
20.0
Sodium chloride
10.0
Ferric citrate
1.0
Bromothymol blue
0.04
Thymol blue
0.04
Agar
15.0

6. Simmon's Citrate Agar

Component
Amount (g/L)
Magnesium sulphate
0.2
Ammoniun dihydrogen phosphate
1.0
Dipotassium phosphate
1.0
Sodium citrate
2.0
Sodium chloride
5.0
Bacto agar
15.0
Bacto bromo thymol blue
0.08

7. Mueller Hinton Agar

Component	Amount (g/L)
Beef infusion	300.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000
Final pH(at 25°C)	7.3±0.1

8. TSI agar (Hi Media)

Ingredients:

g/L
Peptic digest of animal tissue 10.00
Casein enzymic hydrolysate 10.00
Yeast extract 3.00
Beef extract 3.00
Lactose 10.00
Sucrose 10.00
Dextrose 1.00
Sodium chloride 5.00
Ferrous sulphate 0.20
Sodium thiosulphate 0.30
Phenol red 0.024
Agar 12.00
Final pH(at 25°C) 7.4 ± 0.2

9. MIU medium base (Hi Media)

Ingredients:

g/L	
Casein enzymic hydrolysate	10.00
Dextrose	1.00
Sodium chloride	5.00
Phenol Red	0.01
Agar	2.00
Final pH(at 25°C)	6.8 ± 0.2

10. MR-VP medium (Hi Media)

Ingredients:

g/L	
Buffered peptone	7.00
Dextrose	5.00
Dipotassium phosphate	5.00
Final pH (at 25°C)	6.9 ± 0.2

11. Sugar media

Ingredients:

g/L	
a. Peptone water	
Bacto-peptone	10.0 gm
Sodium chloride	5.00 gm
0.5% phenol red	0. 10 ml
Distilled water	1000 ml

b. Sugar solutions

Individul sugar
5.00 gm
Distilled water
100 ml

c. Sugar media preparation

Pepton water
4.50 ml
Sugar solution
0.50 ml

12. Peptone water

Ingredients:
g/L
Peptone
1.00 gm
Distilled water
1000 ml

APPENDIX 2

Preparation of reagents

1. Kovacs reagent

P-dimethyl aminobenzal dehyde 5 gm
Amylalcoho l75 gm
Conc.HCL 25 ml

2. V-P reagent 1

5% alpha –naptholin absolute ethyl alcohol

3. V-P reagent 2

40%potassium hydroxide containing 0.3 creatine. The ingredients were dissolved by heating gently over steam bath. When in solution add 0.05gm of cotton blue dye.

4. Phosphate buffered solution

Sodium chloride 8 gm
Disodium hydrogen phosphate 2.8 gm
Potassium chloride 0.2 gm
Potassium hydrogen phosphate 0.2 gm
Distilled water to make 1000 ml

5. Methyl red solution

Methyl red
0.05 gm
Ethanol (absolute)
28 ml
Distilled water
22 ml

6. Phenol red solution

0.2% aqueous solution of phenol red

7. Potassium hydroxide solution

40% aqueous solution of KOH

8. Gram stain solution

- Stock crystal violet

Crystal violet
10 gm
Ethyl alcohol (95%)
1000 ml

- Stock oxalate solution

Ammonium oxalate
1 gm
Distilled water
1000 ml

- Lugols iodine solution

Iodine crystal
1 gm
potassium iodide
2 gm

 Ethyl alcohol
250 ml

 Acetone
250 ml

 Counterstain

Safranine
2.5 ml
Ethyl alcohol (95%)
100 ml

Safranine working solution
The stock safranine is diluted 1:4 with distilled water.

"Questionnaire Sheet"

Date:.....

Serial No:

1. Particulars of the Owner:

i. Name:.....ii.

Village:.....

iii. Upazila:.....iv.

District:.....v. Mobile:.....

2. Economic status:

i. Poo Mid ss High class

3. Particulars of the Bird:

i. Number of Birds.....

ii. Name of the species.....

iii. Sex: M ale

iii. Age:- .....

iv. Color:-.....

v. Breed:-.....

4. Season:

i. Summer sea P eason Winter season

5. Management System:

i. Housing:-

a) Cage sys eral c) Other

ii. Diet History:

a)Ready food:-..... Loose feed:-

..... b) How many times you feed your pet Birds?..... times/per day

iii. Cleaning system:-

a) Do you clean your pet birds house r ? Ye No

b) Do you wash your your pet birds cage e ay? No

c) Do clean your Birds feces everyday or r ly? No

iv. Biosecurity and Sanitary Condition:-

a) Do you wash any disinfectant for cle ? Y No

6. Medication history:-

i. Vaccination:- No

If yes which vaccine:-

.....

.....

Vaccination interval:-

.....

.....

ii. Do you wash any antihelmenthic for de ng? No

7. Diseases history:

i. Type of disease occurred teria V P al

Parasitic other

ii. Name of the disease:.....
.....
iii. Treatment given☐s ☐ No

8. Sample collection:

i. Type of sample:-

a) Fece☐ Clo☐wab ☐Nasal sw☐
Oral swab
Esophageal s☐ Occu☐scharge ☐edding material

Signature of investigator