

## PREVALENCE OF POTENTIAL ZONOTIC BACTERIAL PATHOGENS ISOLATED FROM HOUSEHOLD PET BIRDS AND THEIR ANTIMICROBIAL PROFILE IN NORTHERN BANGLADESH

Mahmuda Naznin Nupur <sup>1</sup>, Farzana Afroz <sup>1</sup>, Md. Khaled Hossain <sup>1</sup>, S.M. Harun-ur-Rashid <sup>2</sup>, Md. Gausur Rahman <sup>2</sup>, Md Kamruzzaman <sup>3</sup>, Khadija Al Ferdous <sup>4</sup> and Md Atiqul Haque <sup>1,5,\*</sup>

<sup>1</sup>Department of Microbiology, <sup>2</sup>Department of Pathology and Parasitology, <sup>3</sup>Department of Dairy and Poultry Science, <sup>4</sup>Department of Anatomy and Histology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh

<sup>5</sup>Key Lab of Animal Epidemiology and Zoonosis, Ministry of Agriculture and Rural Affairs, College of Veterinary Medicine, China Agricultural University, Beijing, 100193, China

\*Corresponding author: [atique@hstu.ac.bd](mailto:atique@hstu.ac.bd)

### ABSTRACT

Pet bird rearing has become increasingly popular recently, and owners often raise them near to their homes. However, zoonotic infections can spread from pet birds. Thus, this cross-sectional study explored the prevalence of potentially zoonotic bacterial pathogens in household pet birds, risk factors for transmission, and antibiotic susceptibility of isolated bacteria. A total of 240 samples were analyzed, including cloacal swabs (n=80), oral swabs (n=80), and feces (n=80) from pigeons, parrots, budgerigars, and quails. Using cultural and biochemical assays five species of potential zoonotic bacteria namely *E. coli*, *Salmonella* spp., *Shigella* spp., *Klebsiella* spp. and *Staphylococcus* spp. were detected. The prevalence of potential zoonotic bacteria was not statistically significant ( $P>0.05$ ) across age, sex, breed, body weight and diet, while the prevalence was significantly ( $P<0.05$  or  $P<0.01$ ) correlated with hygienic condition and vaccination. The overall prevalence of bacterial isolates from pet birds were *E. coli* (19.6%) *Salmonella* spp. (13.3%), *Shigella* spp. (14.6%), *Klebsiella* spp. (15.4%) and *Staphylococcus* spp. (15%). Quail had the highest prevalence of *E. coli*, *Salmonella* spp., *Shigella* spp., *Klebsiella* spp., and *Staphylococcus* spp., with 33.3%, 25%, 25%, 29.1%, and 29.1%, respectively. Sample-wise prevalence of *Salmonella* spp. and *Staphylococcus* spp. was highly statistically significant ( $P<0.01$ ) in pigeon, while prevalence of *E. coli*, *Shigella* spp., *Klebsiella* spp., and *Staphylococcus* spp. was statistically significant ( $P<0.05$ ) in budgerigar, pigeon, parrot, and quail. The antibiotic sensitivity test with 18 antibiotics demonstrated that bacterial isolates were most sensitive to GEN (83.1%), followed by LEV (81.5%), CIP (80.4%), KAN (77.8%), and CFM (59.3%). Likewise, there was 100% resistance to PG, AMX, BAC followed by CH (98.4%), COX (95.2%), CN (90.5%), TET (77.2%) and VAN (75.7%). The findings of this study suggest that the emergence of multidrug-resistant bacterial isolates into pet birds in Dinajpur, Bangladesh, poses a risk to the wellbeing of both owners and the general populace.

**Keywords:** Pet Bird, Zoonotic Pathogen, Prevalence, Antibiotic, Multi-Drug Resistant

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### 1. INTRODUCTION

The term "Pet bird" refers to wild or exotic birds with good heritability potential that are kept in confinement and raised solely for aesthetic purposes. This includes Passeriformes (such as canaries, finches, and sparrows etc.) and Psittaciformes (parrots, parakeets, budgerigars, love birds etc.) (Zahoor et al. 2018). People, particularly kids, enjoy pet birds, which enhance their life satisfaction. They live in our surroundings and are considered as family members in modern families (Boseret et al. 2013; Akter et al. 2020; Akbari and Asadpour 2022). Across all birds, parrots are favored as pets because of their gregarious and lovable nature, intellect, vivid colors, and capacity to mimic human speech. Parrots have also been tamed since the period of Alexander the Great and the ancient Egyptians, and their market as pets has been financially viable to societies (Akhter et al. 2010; Zahoor et al. 2018). In addition, pigeons were among the earliest tamed birds. They have traditionally been utilized as messenger pigeons, notably during battle (Zahoor et al. 2018). However, this interaction between people and pets may result in the spread of a number of zoonotic infections to the owners, which could have serious consequences for biosafety and human health (Boseret et al. 2013; Akbari and Asadpour 2022). Zoonoses are illnesses and infections that are

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spontaneously spread from vertebrate animals to people. There are over 1415 pathogens that can harm people, and about 61% of these infections are zoonotic (Akhter et al. 2020). Among the zoonotic bacteria associated with pet birds include *Chlamydia psittaci*, *Salmonella* spp., *Mycobacterium* spp., *Campylobacter* spp., *Borrelia burgdorferi*, *Pasteurella* spp., *Klebsiella* spp., *Yersinia* spp., *Pseudomonas* spp., *Aeromonas* spp., *Citrobacter* spp. and *Escherichia coli* (Akhter et al. 2010; Williams et al. 2011; Boseret et al. 2013). Food-borne zoonoses have been linked to highly pathogenic *E. coli* O157:H7 and toxigenic strains of *E. coli* transmitted from wild Passeriformes and pet birds to cattle (Williams et al. 2011; Boseret et al. 2013). In Brazil, enteropathogenic *E. coli* (EPEC) and Shiga toxin-producing *E. coli* (STEC) have been found in the feces of pet birds, posing a zoonotic threat to human health in the home setting (Gioia-Di Chiacchio et al. 2016; Sanches et al. 2017; Gioia-Di Chiacchio et al. 2018). Consequently, zoonotic bacterial pathogens are crucial for pet bird owners and breeders.

In contrast to food-borne zoonoses, pet-associated bacterial zoonoses are a largely unexplored field. Nevertheless, the intimate proximity between household pets and people provides ideal circumstances for bacterial transfer, either by directly (via stroking, kissing, or bodily injury with a diseased or carrier bird) or indirectly (via contamination of food and indoor settings) (Akhter et al. 2020; Damborg et al. 2016). Birds play an important role in the transmission and spread of zoonoses, even over long ranges, because they can travel and serve as natural hosts, reservoirs, amplifying hosts, or liaison hosts for zoonotic agents. Also, in advanced nations, campylobacteriosis and salmonellosis, which are usually spread through food, are most often linked to birds (Contreras et al. 2016). Pet birds, especially member of the psittacine group (e.g. cockatiels, parrots, parakeets and lorries), serve as a large reservoir for *C. psittaci*, the causative agent of psittacosis or 'parrot fever' putting owners, pet store personnel, taxidermists and vets at risk of infection (Damborg et al. 2016). Pigeons are a common type of bird in metropolitan areas and have a significant role in spreading bacteria to free-range poultry. They are also known to contaminate drinking water sources and agricultural harvests with their feces. The potential of zoonotic infections spreading to people through close contact with pigeons at home, in live bird markets, and on farms is high. To date, 110 zoonotic agents have been identified from pigeons (Hosain et al. 2012; Teske et al. 2013; Contreras et al. 2016; Bupasha et al. 2020; Chrobak-Chmiel et al. 2021). The presence of methicillin-resistant staphylococci and other multidrug-resistant (MDR) zoonotic infection in pigeons is worrisome, as these pathogens may disperse across a large geographical region due to extensive distances travelled during racing flights (Chrobak-Chmiel et al. 2021). Importantly, these birds occupy the same habitat as people, domesticated and wild animals, and serve as carriers of a variety of new infections, including toxigenic *E. coli*, *Salmonella* serovars, *Campylobacter* spp., *Cryptococcus* spp., and *C. psittaci* (Teske et al. 2013; Contreras et al. 2016; Bupasha et al. 2020; Chrobak-Chmiel et al. 2021). Also, *C. avium* with zoonotic potential was found in various respiratory disease outbreaks in psittacine birds and pigeons (De Meyst et al. 2022). Migratory quail serve as potential biological and mechanical vectors, contributing to the conservation and spread of several zoonotic pathogens through direct or indirect interaction with poachers or consumers, effective production loss and poor quality of animal-derived foods as well as harming public health and domestic animals (Youssef and Mansour 2014).

Due to the increased contact of pet birds with people and other animals as well as the possibility of zoonotic pathogens carrying by these birds pose a health risk to people particularly young children and immunocompromised individual (Contreras et al. 2016). A key issue influencing the effective treatments of infectious diseases is the emergence of antimicrobial resistance in pathogenic and commensal bacteria, which leads to the production of drug-resistant strains (Dey et al. 2013). To date, very few works have been studied on the prevalence of zoonotic bacterial pathogen from pet birds in Bangladesh. Therefore, the purpose of the current study was to determine the prevalence of potentially zoonotic bacterial pathogens of household pet birds and their associated risk factors for zoonotic transmission and the antibiogram profile pattern of the isolated bacteria.

## 2. MATERIALS AND METHODS

### 2.1 Sampling, Collection and Processing of Samples

The current study was done in Dinajpur district (25.63° N, 88.65° E) of Rangpur division in Bangladesh. A total of 240 samples were collected during July 2016 to June 2017 from various sources, cloacal swab (n=80), oral swab (n=80) and feces (n=80) in different locations across the district. All these samples were collected from pigeon (n=40), 20 parrot (n=20), budgerigar (n=12) and quail (n=8) were maintaining aseptic techniques (Kamal et al. 2018; Haque et al. 2022). Briefly the samples were collected by sterile cotton buds and took into sterile tube containing 1% peptone water. Each sample was marked properly with date, time and sample number. After collection the tube cap was closed and each sample was immediately kept in sterile Ziploc plastic bags, transported in an insulated foam box with cold chain (temperature, 4–6°C) to the Microbiology Laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. Upon arrival, all samples were refrigerated at 4°C until microbiological analysis, which was completed within 24 h after receiving the samples.

## 2.2 Questionnaire Survey

A cross-sectional survey was performed to choose participants for interviews and 9 pet owners were arbitrarily selected (Table S1). Data on the socio-demographic variable including breed, age, sex, body weight, diet, hygienic condition and vaccination history were collected (Supplementary Appendix I, Table S2–S5). The experimental protocols were approved by an Ethical Reviewing Board on Institutional Animal Care and Use Committee at Hajee Mohammad Danesh Science and Technology University (Approved code: HSTU/IRT/3966).

## 2.3 Microbiological Analysis

Microbiological assessment of the samples such as total bacterial count (TBC) was determined using the method described by ISO, 2002. All the glassware used in this study were sterilized by autoclaving at 121°C for 15min and then cooling to 45°C. The system was also used for serial dilution, inoculation and incubation, sub-culture, Gram staining, and identification of isolates. Pure cultures were stored at –80°C in glycerol stocks for further study.

### 2.3.1 Isolation and Identification of Bacterial Isolates

#### 2.3.1.1 Bacteriological Examination

The samples were first cultured into Plate Count Agar (PCA) (HiMedia, India) for TBC. The samples were diluted with distilled water as  $10^{-1}$  to  $10^{-10}$ . Then 50µL of samples were taken and spread in PCA plate following the spread-plate method and incubated at 37°C for 24h. Following incubation, plates with 30–300 colonies were counted, and TBC were expressed as colony-forming units per gram of sample (CFU/g). The number of organisms per ml or per gram of original culture was calculated by multiplying the number of colonies counted by the dilution factor: Number of cells per ml or per gram = number of colonies × Dilution factor/Volume of dilution.

#### 2.3.1.2 Cultural Characterization and Biochemical Test

The processed samples were cultured onto the Nutrient agar (HiMedia, India) to obtain pure colonies. After 24–48 hours of incubation, each culture was subjected to gram stain to differentiate Gram-positive or Gram-negative bacteria. The culture was then grown into selective media such as MacConkey agar (HiMedia, India), Eosin Methylene Blue agar (HiMedia, India), Salmonella Shigella agar (HiMedia India), Staphylococcus Agar No. 110 (HiMedia, India), Blood Agar (HiMedia, India), Mannitol salt agar (HiMedia, India), BGA = Brilliant Green Agar (HiMedia, India) and then following biochemical test were performed : Sugar Fermentation test, Simon Citrate test; Indole test; Triple sugar iron test; Methyl-Red test; Voges-Proskauer test; Catalase test, Oxidase test and Motility Indole Urease test (Table S6 and S7).

## 2.4 Antibiotic Sensitivity Test (AST)

AST profile of all isolates was determined using Kirby-Bauer disk diffusion method following guidelines of the Clinical and Laboratory Standards Institute (CLSI 2018). A panel of 18 commonly used antibiotics were selected for AST consisting of Penicillin G (PG, 10µg), Cloxacillin (COX, 5µg), Amoxicillin (AMX, 30µg), Cefradin (CH, 25µg), Cefalexin (CN, 30µg), Cefixime (CFM, 5 µg), Erythromycin (E, 15 µg), Azithromycin (AZ, 15µg), Ciprofloxacin (CIP, 5µg), Levofloxacin (LEV, 5µg), Colistin (CL, 10µg), Chloramphenicol (C, 30µg), Bacitracin (BAC, 10 µg), Gentamicin (GEN, 10µg), Kanamycin (KAN, 30µg), Neomycin (NEO, 30µg), Tetracycline (TET, 30µg), and Vancomycin (VAN, 30µg). After setting the antimicrobial discs on the freshly inoculated Mueller-Hinton agar, the plates were incubated at 37°C for 24–48h. The positive control was used as *Escherichia coli* ATCC 25922. Isolates were classified as susceptible, intermediate and resistant categories based on the standard according to the Clinical and Laboratory Standards Institution (CLSI 2018).

## 2.5. Statistical Analysis

All data were incorporated into Excel sheets (MS-2016) and analyzed by SPSS software (SPSS-21.0). The prevalence was calculated using descriptive analysis and Chi-square test was done to determine the level of significance. Statistical significance was measured by  $P < 0.05$ .

## 3. RESULTS

### 3.1 Total Bacterial Count (TBC)

The results presented in Table 1 reveal the mean values of the TBC of bird samples in relation to age and sex. In pigeon, the highest TBC was  $10.45 \pm 0.06$  in feces of adult male bird and lowest TBC was  $9.71 \pm 0.41$  in oral swab of female young bird. In parrot the highest TBC was  $10.54 \pm 0.26$  in oral swab of adult male bird and lowest TBC was  $9.10 \pm 0.29$  in oral swab of female adult bird. In budgerigar, the highest TBC was  $10.53 \pm 0.26$  in oral swab of

**Table 1:** Enumeration of TBC in CS, OS and feces sample of pet birds

| Birds                | Parameters       |             | TBC (CFU/g)   |               |              |
|----------------------|------------------|-------------|---------------|---------------|--------------|
|                      | Sex              | Age         | CS (mean±SEM) | OS (mean±SEM) | F (mean±SEM) |
| Pigeon<br>(n=40)     | Male<br>(n=16)   | Young       | 10.34±0.14    | 10.40±0.10    | 10.04±0.49   |
|                      |                  | Adult month | 10.02±0.27    | 9.87±0.38     | 10.45±0.06   |
|                      |                  | P value     | 0.40          | 0.31          | 0.31         |
|                      | Female<br>(n=24) | Young       | 9.71±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         |
| Parrot<br>(n=20)     | Male<br>(n=10)   | Young       | 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<br>(n=10) | Young       | 9.34±0.99     | 10.50±0.07    | 10.29±0.06   |
|                      |                  | Adult       | 10.48±0.16    | 9.10±0.29     | 10.50±0.23   |
|                      |                  | P value     | 0.13          | 0.03*         | 0.57         |
| Budgerigar<br>(n=12) | Male (n=6)       | Young       | 10.47±0.21    | 10.53±0.26    | 10.28±0.22   |
|                      |                  | Adult       | 10.51±0.10    | 10.27±0.22    | 9.82±0.46    |
|                      |                  | P value     | 0.83          | 0.51          | 0.55         |
|                      | Female<br>(n=6)  | Young       | 9.34±0.89     | 10.51±0.07    | 10.28±0.06   |
|                      |                  | Adult       | 10.48±0.16    | 9.09±0.22     | 10.50±0.23   |
|                      |                  | P value     | 0.127         | 0.02*         | 0.57         |
| Quail<br>(n=8)       | Male (n=4)       | Young       | 9.86±0.48     | 10.0±0.09     | 9.64±0.66    |
|                      |                  | Adult       | 10.21±0.32    | 10.51±0.32    | 10.11±0.52   |
|                      |                  | P value     | 0.12          | 0.71          | 0.21         |
|                      | Female<br>(n=4)  | Young       | 9.39±0.57     | 10.35±0.14    | 10.32±0.36   |
|                      |                  | Adult       | 10.52±0.17    | 10.45±0.01    | 9.52±0.14    |
|                      |                  | P value     | 0.19          | 0.66          | 0.19         |

TBC=Total bacterial count, CFU=Colony forming unit, CS=Cloacal swab, OS=Oral swab, F=Feces, SEM=Standard error of mean, \*Significant at P<0.05

young male bird and lowest TBC was 9.09±0.22 in oral swab of female adult bird. In quail, the highest TBC was 10.52±0.17 in cloacal swab of adult female bird and lowest TBC was 9.39±0.57 in oral swab of female young bird. The load of isolated bacteria was not statistically significantly (P>0.05) in all bird, while bacterial load from oral swab was statistically significant (P<0.05) in relation to age of female parrot and budgerigar.

### 3.2 Prevalence of Potential Zoonotic Bacterial Pathogen

Out of 189 isolates, overall, 47 strains of *E. coli* (19.6%), 32 strains of *Salmonella* spp. (13.3%), 35 strains of *Shigella* spp. (14.6%), 39 strains of *Klebsiella* spp. (15.4%) and 36 strains of *Staphylococcus* spp. (15%) were identified by morphological and biochemical tests (Fig. 1 and Table S6). In case of pigeon, the prevalence was *E. coli* (17.5%), *Salmonella* spp. (10.8%), *Shigella* spp. (15.8%), *Klebsiella* spp. (13.3%) and *Staphylococcus* spp. (15%). In case of parrot, the prevalence was *E. coli* (18.3%), *Salmonella* spp. (15%), *Shigella* spp. (11.7%), *Klebsiella* spp. (18.3%) and *Staphylococcus* spp. (13.3%). In case of budgerigar, the prevalence was *E. coli* (19.4%), *Salmonella* spp. (11.1%), *Shigella* spp. (8.3%), *Klebsiella* spp. (13.8%) and *Staphylococcus* spp. (8.3%). In case of quail, the prevalence was *E. coli* (33.3%), *Salmonella* spp. (25%), *Shigella* spp. (25%), *Klebsiella* spp. (29.1%) and *Staphylococcus* spp. (29.1%).

Sample-wise distribution in pigeons showed 23.8%, 28.6% and 47.6% *E. coli*, 23.1%, 7.7% and 69.2% *Salmonella* spp., 26.3%, 15.8% and 57.9% *Shigella* spp., and 11.1%, 66.7% and 22.3% *Staphylococcus* spp. in cloacal swab, oral swab and feces, respectively (Fig. 2A). In parrot, the prevalence was 27.3%, 18.2% and 54.5% of *E. coli*, 33.3%, 11.1% and 55.6% of *Salmonella* spp., 14.3%, 14.3% and 71.4% of *Shigella* spp., 63.6%, 9.1% and 27.3% of *Klebsiella* spp. and 12.5%, 62.5% and 25% of *Staphylococcus* spp. in cloacal swab, oral swab and feces, respectively (Fig. 2B). In budgerigar, the prevalence was 28.6%, and 71.4% of *E. coli*, 25%, and 50% of *Salmonella* spp., and 60%, and 40% of *Klebsiella* spp. in cloacal swab, and feces respectively. Notably, 100% prevalence of *Staphylococcus* spp. and *Shigella* spp. was observed only in oral swab and feces, respectively (Fig. 2C). In quail, the prevalence was 25%, 12.5% and 62.5% of *E. coli*, 33.3%, 16.7% and 50% of *Shigella* spp., 57.1%, 14.3% and 28.6% of *Klebsiella* spp. and 14.3%, 71.4% and 14.3% of *Staphylococcus* spp. in cloacal swab, oral swab and feces, respectively (Fig. 2D). Further, the prevalence of *Salmonella* spp., (P<0.01) *Shigella* spp. (P<0.05) and *Staphylococcus* spp. (P<0.01) were statistically significant while, *E. coli* and *Klebsiella* spp. was not statistically significant (P>0.05) in pigeon. In parrot, prevalence of *Shigella* spp. and *Klebsiella* spp. was statistically significant (P<0.05) while, *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. was not statistically significant (P>0.05). In

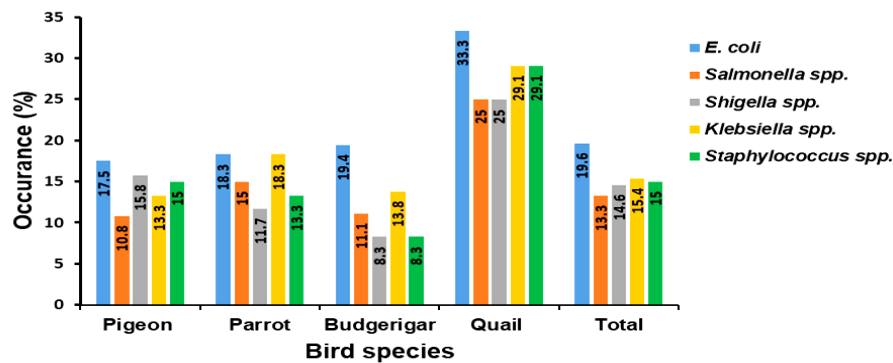


Fig. 1: Overall prevalence of bacterial isolates from pet birds.

budgerigar, prevalence of *E. coli*, *Shigella* spp. and *Staphylococcus* spp. were statistically significant ( $P > 0.05$ ) while, *Salmonella* spp., and *Klebsiella* spp. was not statistically significant ( $P > 0.05$ ). In quail, prevalence of *E. coli*, *Salmonella* spp., *Shigella* spp. and *Klebsiella* spp. was not statistically significant ( $P > 0.05$ ) while, *Staphylococcus* spp. was statistically significant ( $P < 0.05$ ) (Table S8).

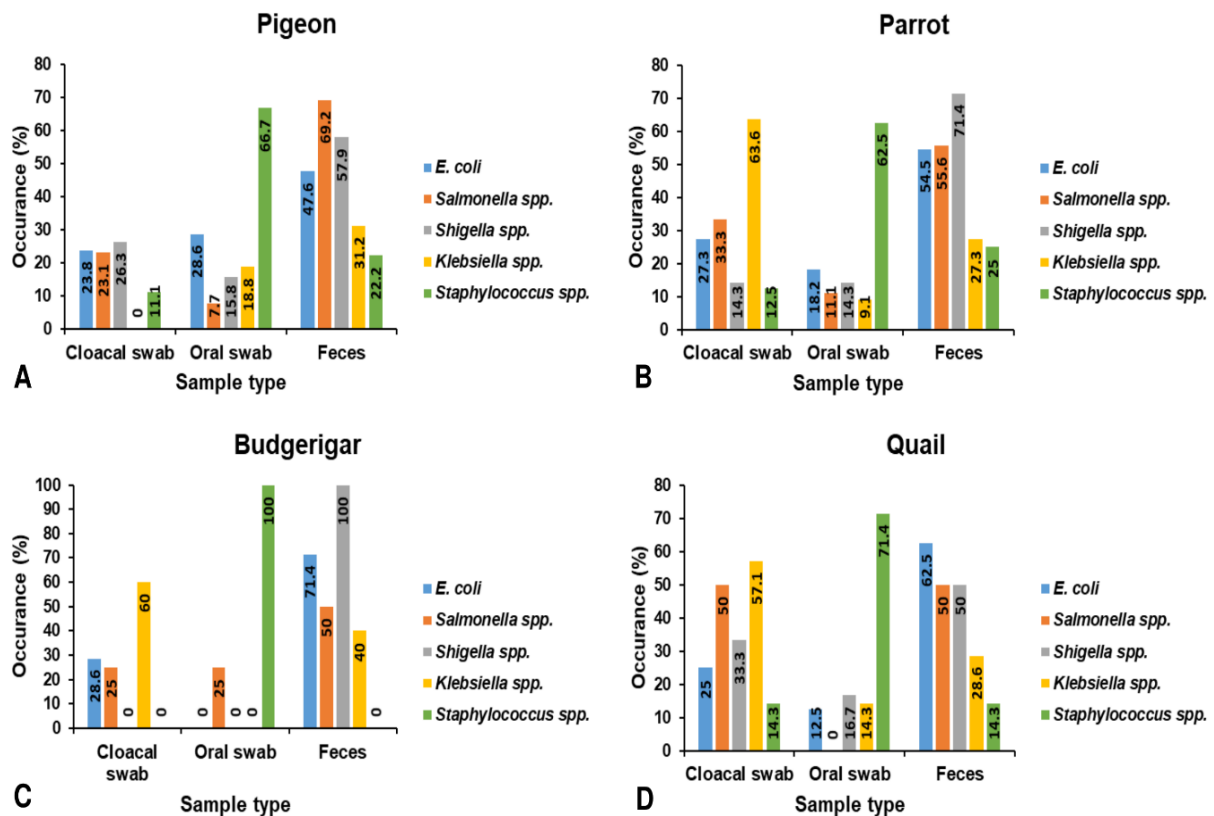


Fig. 2: Sample-wise distribution of bacterial isolate from pet birds.

### 3.3. Risk Factors Associated with Potential Bacterial Zoonotic Pathogen

Tables 2-5 showed the relationship between socio-demographic factors and bacterial prevalence in pigeon, parrot, budgerigar and quail, respectively. This study revealed that the prevalence of bacterial isolates was non-significantly ( $P > 0.05$ ) different across age, sex, breed, body weight and diet. However, among the four breeds of pigeon, the Madina had the highest prevalence rate, with *E. coli* (33.3%), *Salmonella* spp. (38.5%) and *Staphylococcus* spp. (38.9%), while Poter had the lowest prevalence rate with *E. coli* (14.3%), *Salmonella* spp. (7.7%), *Shigella* spp. (21.1%) and *Staphylococcus* spp. (16.7%). The prevalence of bacterial isolates was significantly ( $P < 0.05$  or  $P < 0.01$ ) correlated with hygienic condition and vaccination status in all pet birds.

**Table 2:** Association between the socio-demographic variable and potential bacterial zoonotic pathogen in pigeon

| Parameters         |                    | Prevalence (%)           |                                  |                                |                                  |                                      |
|--------------------|--------------------|--------------------------|----------------------------------|--------------------------------|----------------------------------|--------------------------------------|
|                    |                    | <i>E. coli</i><br>(n=21) | <i>Salmonella</i> spp.<br>(n=13) | <i>Shigella</i> spp.<br>(n=19) | <i>Klebsiella</i> spp.<br>(n=16) | <i>Staphylococcus</i> spp.<br>(n=18) |
| Age                | Young (n=15)       | 9 (42.9)                 | 7 (53.8)                         | 9 (47.4)                       | 6 (37.5)                         | 8 (44.4)                             |
|                    | Adult (n=25)       | 12 (57.1)                | 6 (46.1)                         | 10 (52.6)                      | 10 (62.5)                        | 10 (55.6)                            |
|                    | P-value            | 0.355                    | 0.653                            | 0.746                          | 0.157                            | 0.505                                |
| Sex                | Male (n=16)        | 10 (47.6)                | 7 (53.8)                         | 7 (36.8)                       | 7 (43.8)                         | 8 (44.4)                             |
|                    | Female (n=24)      | 11 (52.4)                | 6 (46.1)                         | 12 (63.2)                      | 9 (56.2)                         | 10 (55.6)                            |
|                    | P-value            | 0.758                    | 0.653                            | 0.105                          | 0.480                            | 0.505                                |
| Breed              | White king (n=10)  | 5 (23.8)                 | 4 (30.8)                         | 4 (21.1)                       | 4 (25.0)                         | 4 (22.2)                             |
|                    | Fantail (n=6)      | 6 (28.6)                 | 3 (23.0)                         | 5 (26.3)                       | 4 (25.0)                         | 4 (22.2)                             |
|                    | Porter (n=12)      | 3 (14.3)                 | 1 (7.7)                          | 4 (21.1)                       | 5 (31.2)                         | 3 (16.7)                             |
|                    | Madina (n=12)      | 7 (33.3)                 | 5 (38.5)                         | 6 (31.5)                       | 3 (18.8)                         | 7 (38.9)                             |
|                    | P-value            | 0.528                    | 0.256                            | 0.856                          | 0.881                            | 0.446                                |
| Body weight        | 250-300 gm (n=11)  | 6 (28.6)                 | 4 (30.8)                         | 6 (31.5)                       | 4 (25.0)                         | 5 (27.8)                             |
|                    | 301-500 gm (n=9)   | 8 (38.1)                 | 3 (23.0)                         | 5 (26.3)                       | 4 (25.0)                         | 6 (33.3)                             |
|                    | 501-1000 gm (n=20) | 7 (33.3)                 | 6 (46.2)                         | 8 (42.1)                       | 8 (50.0)                         | 7 (38.9)                             |
|                    | P-value            | 0.807                    | 0.446                            | 0.575                          | 0.223                            | 0.779                                |
| Diet               | Ready food (n=20)  | 8 (33.1)                 | 6 (46.2)                         | 8 (42.1)                       | 5 (31.2)                         | 5 (27.8)                             |
|                    | Raw food (n=10)    | 6 (28.6)                 | 1 (7.7)                          | 3 (15.8)                       | 3 (18.8)                         | 6 (33.3)                             |
|                    | Both (n=10)        | 7 (33.3)                 | 6 (46.1)                         | 8 (42.1)                       | 8 (50.0)                         | 7 (38.9)                             |
|                    | P-value            | 0.807                    | 0.056                            | 0.139                          | 0.168                            | 0.779                                |
| Hygienic condition | Poor (n=20)        | 14 (66.7)                | 8 (61.5)                         | 11 (57.9)                      | 9 (56.2)                         | 10 (55.6)                            |
|                    | Good (n=12)        | 5 (23.8)                 | 4 (30.8)                         | 5 (26.3)                       | 5 (31.2)                         | 5 (27.8)                             |
|                    | Excellent (n=8)    | 2 (9.5)                  | 1 (7.7)                          | 3 (15.8)                       | 2 (12.5)                         | 3 (16.6)                             |
|                    | P-value            | 0.001**                  | 0.014*                           | 0.016*                         | 0.031*                           | 0.039*                               |
| Vaccination        | Yes (n=30)         | 6 (28.6)                 | 7 (53.8)                         | 5 (26.3)                       | 5 (31.2)                         | 6 (33.3)                             |
|                    | No (n=10)          | 15 (71.4)                | 6 (46.2)                         | 14 (73.7)                      | 11 (68.8)                        | 12 (66.7)                            |
|                    | P-value            | 0.005**                  | 0.695                            | 0.004**                        | 0.034*                           | 0.046*                               |

\* Significant at P<0.05; \*\* Significant at P<0.01

**Table 3:** Association between the socio-demographic variable and potential bacterial zoonotic pathogen in parrot

| Parameters         |                    | Prevalence (%)           |                                 |                               |                                  |                                     |
|--------------------|--------------------|--------------------------|---------------------------------|-------------------------------|----------------------------------|-------------------------------------|
|                    |                    | <i>E. coli</i><br>(n=11) | <i>Salmonella</i> spp.<br>(n=9) | <i>Shigella</i> spp.<br>(n=7) | <i>Klebsiella</i> spp.<br>(n=11) | <i>Staphylococcus</i> spp.<br>(n=8) |
| Age                | Young (n=8)        | 6 (54.5)                 | 4 (44.4)                        | 3 (42.9)                      | 6 (54.5)                         | 3 (37.5)                            |
|                    | Adult (n=12)       | 5 (45.5)                 | 5 (45.6)                        | 4 (57.1)                      | 5 (45.5)                         | 5 (62.5)                            |
|                    | P-value            | 0.670                    | 0.637                           | 0.593                         | 0.670                            | 0.317                               |
| Sex                | Male (n=10)        | 7 (63.6)                 | 5 (45.6)                        | 4 (57.1)                      | 5 (45.5)                         | 4 (50.0)                            |
|                    | Female (n=10)      | 4 (36.4)                 | 4 (44.4)                        | 3 (42.9)                      | 6 (54.5)                         | 4 (50.0)                            |
|                    | P-value            | 0.201                    | 0.637                           | 0.593                         | 0.670                            | 1.00                                |
| Body weight        | 10-500 gm (n=8)    | 6 (54.5)                 | 4 (44.4)                        | 3 (42.9)                      | 6 (54.5)                         | 5 (62.5)                            |
|                    | 501-1000 gm (n=12) | 5 (45.5)                 | 5 (45.6)                        | 4 (57.1)                      | 5 (45.5)                         | 3 (37.5)                            |
|                    | P-value            | 0.670                    | 0.637                           | 0.593                         | 0.670                            | 0.317                               |
| Diet               | Ready food (n=6)   | 4 (36.4)                 | 4 (44.4)                        | 2 (28.6)                      | 4 (36.4)                         | 2 (25.0)                            |
|                    | Raw food (n=10)    | 4 (36.4)                 | 3 (33.3)                        | 4 (57.1)                      | 4 (36.4)                         | 5 (62.5)                            |
|                    | Both (n=4)         | 3 (27.2)                 | 2 (22.2)                        | 1 (14.3)                      | 3 (27.2)                         | 1 (12.5)                            |
|                    | P-value            | 0.873                    | 0.607                           | 0.223                         | 0.873                            | 0.087                               |
| Hygienic condition | Poor (n=10)        | 7 (63.6)                 | 5 (55.6)                        | 5 (71.4)                      | 7 (63.7)                         | 5 (62.5)                            |
|                    | Good (n=7)         | 3 (27.3)                 | 3 (33.3)                        | 2 (28.6)                      | 3 (27.2)                         | 3 (37.5)                            |
|                    | Excellent (n=3)    | 1 (9.1)                  | 1 (11.1)                        | 0 (0.0)                       | 1 (9.1)                          | 0 (0.0)                             |
| P-value            | 0.022*             | 0.135                    | 0.017*                          | 0.022*                        | 0.028*                           |                                     |
| Vaccination        | Yes (n=5)          | 3 (27.3)                 | 3 (33.3)                        | 2 (28.6)                      | 3 (27.3)                         | 2 (25.0)                            |
|                    | No (n=15)          | 8 (72.7)                 | 6 (66.7)                        | 5 (71.4)                      | 8 (72.7)                         | 6 (75.0)                            |
|                    | P-value            | 0.033*                   | 0.157                           | 0.109                         | 0.033*                           | 0.046*                              |

\* Significant at P<0.05; \*\* Significant at P<0.01

In pigeon, prevalence of *E. coli* was highly statistically significant (P<0.01) with hygienic condition and vaccination, while prevalence of other species was statistically significant (P<0.05) with hygienic condition and vaccination. In parrot, the prevalence of *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. was statistically significant

( $P < 0.01$ ) with hygienic condition and vaccination, while *Salmonella* spp. and *Shigella* spp. were not statistically significant ( $P > 0.05$ ) with vaccination. In budgerigar, the prevalence of *E. coli* and *Klebsiella* spp. was statistically significant ( $P < 0.05$ ) with hygienic condition, while prevalence of other species was not statistically significant ( $P > 0.05$ ). Further, the prevalence of *E. coli* was highly statistically significant ( $P < 0.01$ ) with vaccination, while prevalence of other species was not statistically significant ( $P > 0.05$ ). In case of quail, the prevalence of *Shigella* spp. was highly statistically significant ( $P < 0.01$ ) with hygienic condition and the prevalence of *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. was statistically significant ( $P < 0.05$ ), while *Salmonella* spp. was not significant ( $P > 0.05$ ). Further the prevalence of *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. was highly statistically significant ( $P < 0.01$ ) with vaccination and the prevalence of *Salmonella* spp. and *Shigella* spp. was statistically significant ( $P < 0.05$ ).

**Table 4:** Association between the socio-demographic variable and potential bacterial zoonotic pathogen in budgerigar

| Parameters         | Prevalence (%)          |                                 |                               |                                 |                                     |          |
|--------------------|-------------------------|---------------------------------|-------------------------------|---------------------------------|-------------------------------------|----------|
|                    | <i>E. coli</i><br>(n=7) | <i>Salmonella</i> spp.<br>(n=4) | <i>Shigella</i> spp.<br>(n=3) | <i>Klebsiella</i> spp.<br>(n=5) | <i>Staphylococcus</i> spp.<br>(n=3) |          |
| Age                | Young (n=4)             | 4 (57.1)                        | 2 (50.0)                      | 1 (33.3)                        | 3 (60.0)                            | 1 (33.3) |
|                    | Adult (n=8)             | 3 (42.8)                        | 2 (50.0)                      | 2 (66.7)                        | 2 (40.0)                            | 2 (66.7) |
|                    | P-value                 | 0.593                           | 1.00                          | 0.414                           | 0.527                               | 0.414    |
| Sex                | Male (n=6)              | 4 (57.1)                        | 2 (50.0)                      | 2 (66.7)                        | 2 (40.0)                            | 2 (66.7) |
|                    | Female (n=6)            | 3 (42.8)                        | 2 (50.0)                      | 1 (33.3)                        | 3 (60.0)                            | 1 (33.3) |
|                    | P-value                 | 0.593                           | 1.00                          | 0.414                           | 0.527                               | 0.414    |
| Body weight        | 10-25 gm (n=4)          | 3 (42.8)                        | 2 (50.0)                      | 1 (33.3)                        | 2 (40.0)                            | 1 (33.3) |
|                    | 21-50 gm (n=8)          | 4 (57.1)                        | 2 (50.0)                      | 2 (66.7)                        | 3 (60.0)                            | 2 (66.7) |
|                    | P-value                 | 0.593                           | 1.00                          | 0.414                           | 0.527                               | 0.414    |
| Diet               | Ready feed (n=4)        | 4 (57.1)                        | 1 (25.0)                      | 1 (33.3)                        | 1 (20.0)                            | 1 (33.3) |
|                    | Raw feed (n=6)          | 2 (28.6)                        | 2 (50.0)                      | 1 (33.3)                        | 2 (40.0)                            | 1 (33.3) |
|                    | Both (n=2)              | 1 (14.3)                        | 1 (25.0)                      | 1 (33.3)                        | 2 (40.0)                            | 1 (33.3) |
|                    | P-value                 | 0.223                           | 0.687                         | 1.00                            | 0.741                               | 1.00     |
| Hygienic condition | Poor (n=5)              | 5 (71.4)                        | 3 (75.0)                      | 2 (66.7)                        | 4 (80.0)                            | 2 (66.7) |
|                    | Good (n=5)              | 1 (14.3)                        | 1 (25.0)                      | 1 (33.3)                        | 1 (20.0)                            | 1 (33.3) |
|                    | Excellent (n=2)         | 1 (14.3)                        | 0 (0.0)                       | 0 (0.0)                         | 0 (0.0)                             | 0 (0.0)  |
|                    | P-value                 | 0.032*                          | 0.072                         | 0.223                           | 0.020*                              | 0.223    |
| Vaccination        | Yes (n=4)               | 1 (14.3)                        | 1 (25.0)                      | 1 (33.3)                        | 1 (20.0)                            | 1 (33.3) |
|                    | No (n=8)                | 6 (85.7)                        | 3 (75.0)                      | 2 (66.7)                        | 4 (80.0)                            | 2 (66.7) |
|                    | P-value                 | 0.008**                         | 0.157                         | 0.414                           | 0.058                               | 0.414    |

\* Significant at  $P < 0.05$ ; \*\* Significant at  $P < 0.01$

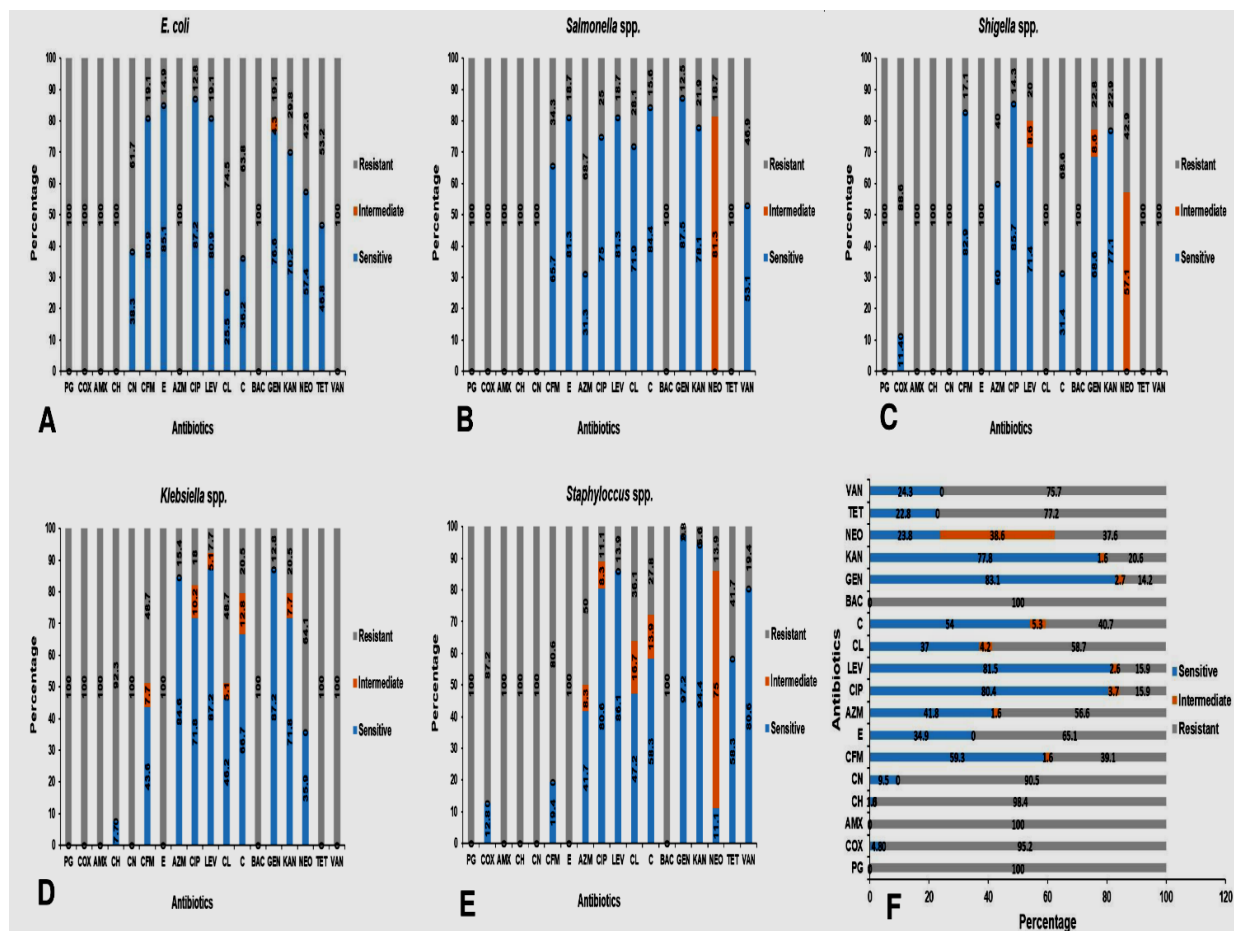
### 3.4 Antibiogram Profile of Isolated Bacteria

Antibiogram profile of 18 antibiotics revealed that all isolates were generally sensitive to GEN (83.1%), LEV (81.5%), CIP (80.4%) and KAN (77.8%) and intermediate sensitive to NEO (11.4%). Further isolated bacteria were generally resistant to PG (100%), AMX (100%), BAC (100%), CH (98.4%), COX (95.2%), CN (90.5%), TET (77.2%) and VAN (75.7%) and isolated resistant bacteria showed 100% multidrug resistance pattern (Fig. 3F, Table S8 and S9). In our study, the isolated *E. coli* was 100% resistance against PG, COX, AMX, CH, BAC and VAN followed by CL (74.5%), C (63.8%), CN (61.7%), TET (53.2%), and sensitive to CIP (87.2%), E (85.1%), LEV & CFM (80.9%), GEN (76.6%), KAN (70.2%), and NEO (57.4%) (Fig. 3A). Again, the isolated *Salmonella* spp. were 100% resistant against PG, COX, AMX, CH, CN, BAC and TET followed by AZM (68.7%), VAN (46.9%), while sensitive to GEN (87.5%), C (84.4%), LEV & E (81.3%), KAN (78.1%), CL (71.9%), CIP (75%), CFM (65.7%), VAN (53.1%) and intermediate sensitive to NEO (81.3%) (Fig. 3B). Further isolated *Shigella* spp. were 100% resistant against PG, AMX, CH, CN, BAC, TET and VAN followed by COX (88.6%), C (68.6%), while sensitive to CIP (85.7%), CFM (82.9%), KAN (77.1%), LEV (71.4%), GEN (68.6%), AZM (60%), and intermediate sensitive to NEO (57.1%) (Fig. 3C). In the present study, isolated *Klebsiella* spp. were 100% resistant against PG, COX, AMX, CN, E, BAC, TET and VAN followed by COX (92.3%), NEO (64.1%), CFM & CL (48.7%) and sensitive to LEV & GEN (87.2%), AZM (84.6%), CIP & KAN (71.8%), (66.7%), CL (46.2%) and CFM (43.6%) (Fig. 3D). Furthermore, isolated *Staphylococcus* spp. showed 100% resistance against PG, AMX, CH, CN, E, and BAC followed by COX (87.2%), CFM (80.6%), AZM (50%), while sensitive to GEN (97.2%), KAN (94.4%), LEV (86.1%), CIP & VAN (80.6%), C & TET (58.3%), and intermediate sensitive to NEO (75%) (Fig. 3E).

**Table 5:** Association between the socio-demographic variable and potential bacterial zoonotic pathogen in quail

| Parameters         |                  | Prevalence (%)       |                              |                            |                              |                                  |
|--------------------|------------------|----------------------|------------------------------|----------------------------|------------------------------|----------------------------------|
|                    |                  | <i>E. coli</i> (n=8) | <i>Salmonella</i> spp. (n=6) | <i>Shigella</i> spp. (n=6) | <i>Klebsiella</i> spp. (n=7) | <i>Staphylococcus</i> spp. (n=7) |
| Age                | Young (n=5)      | 5 (62.5)             | 3 (50.0)                     | 4 (66.7)                   | 4 (57.1)                     | 4(57.1)                          |
|                    | Adult (n=3)      | 3 (37.5)             | 3 (50.0)                     | 2 (33.3)                   | 3 (42.9)                     | 3(42.9)                          |
|                    | P-value          | 0.391                | 0.257                        | 0.257                      | 0.284                        | 0.284                            |
| Sex                | Male (n=4)       | 4 (50.0)             | 4 (66.7)                     | 3 (50.0)                   | 3 (42.9)                     | 3 (75.0)                         |
|                    | Female (n=4)     | 4 (50.0)             | 2 (33.3)                     | 3 (50.0)                   | 4 (57.1)                     | 3 (60.0)                         |
|                    | P-value          | 1.00                 | 0.248                        | 1.00                       | 0.284                        | 0.284                            |
| Body weight        | 70-100 gm (n=5)  | 5 (62.5)             | 3 (50.0)                     | 3 (50.0)                   | 4 (57.1)                     | 5 (62.5)                         |
|                    | 101-300 gm (n=3) | 3 (37.5)             | 3 (50.0)                     | 3 (50.0)                   | 3 (42.9)                     | 1 (50.0)                         |
|                    | P-value          | 0.391                | 1.00                         | 1.00                       | 0.284                        | 0.284                            |
| Diet               | Ready feed (n=2) | 2 (25.0)             | 1 (16.7)                     | 2 (33.3)                   | 2 (28.5)                     | 1 (50.0)                         |
|                    | Raw feed (n=2)   | 2 (25.0)             | 2 (33.3)                     | 2 (33.3)                   | 2 (28.5)                     | 1 (50.0)                         |
|                    | Both (n=4)       | 4 (50.0)             | 3 (50.0)                     | 2 (33.3)                   | 3 (42.9)                     | 3 (75.0)                         |
|                    | P-value          | 0.472                | 0.472                        | 1.00                       | 0.807                        | 0.807                            |
| Hygienic condition | Poor (n=5)       | 5 (62.5)             | 4 (66.7)                     | 5 (83.3)                   | 5 (71.4)                     | 2 (50.0)                         |
|                    | Good (n=3)       | 3 (37.5)             | 2 (33.3)                     | 1 (16.7)                   | 2 (28.5)                     | 2 (100)                          |
|                    | Excellent (n=0)  | 0 (0.0)              | 0 (0.0)                      | 0 (0.0)                    | 0 (0.0)                      | 0 (0.0)                          |
|                    | P-value          | 0.028*               | 0.050                        | 0.005**                    | 0.017*                       | 0.017*                           |
| Vaccination        | Yes (n=1)        | 1 (12.5)             | 1 (16.7)                     | 1 (16.7)                   | 1 (14.3)                     | 1 (14.3)                         |
|                    | No (n=7)         | 7 (87.5)             | 5 (83.3)                     | 5 (83.3)                   | 6 (85.7)                     | 6 (85.7)                         |
|                    | P-value          | 0.033*               | 0.021*                       | 0.021*                     | 0.008**                      | 0.008**                          |

\* Significant at P<0.05; \*\* Significant at P<0.01



**Fig. 3:** Antibigram profiles of bacterial isolates from pet birds.

**Citation:** Nupur MN, Afroz F, Hossain MK, Harun-ur-Rashid SM, Rahman MG, Kamruzzaman M, Ferdous KA and Haque MA, 2023. Prevalence of potential zoonotic bacterial pathogens isolated from household pet birds and their antimicrobial profile in northern Bangladesh. *Agrobiological Records* 11: 28-38. <https://doi.org/10.47278/journal.abr/2023.005>



#### 4. DISCUSSION

The important bacterial pathogens associated with pet birds which are considered as zoonotic include Chlamydia, Salmonella, Mycobacterium, Campylobacter, Pasteurella, Klebsiella, Yersinia, Pseudomonas and *Escherichia coli* (Youssef and Mansour 2014; Zahoor et al. 2018). This study focused on domestic pet birds because they may harbor zoonotic bacteria without symptoms. This study isolated five potential zoonotic bacterial isolates from pigeon, parrots, budgerigar and quail, all of which were resistant to multiple antibiotics. We record overall prevalence of *E. coli*, *Salmonella* spp., *Shigella* spp., *Klebsiella* spp. and *Staphylococcus* spp. was 17.5%, 10.8%, 15.8%, 13.3%, and 15% in pigeon respectively. Further this study revealed the presence of *E. coli* in cloacal swab, oral swab and feces of pigeons ranging from 23.8-47.6%, 18.2-54.5% in parrot, 28.6-71.4% in budgerigar and 12.5-62.5% in quail. Many zoonotic infections are transmitted to humans from caged or pet birds via direct or indirect interaction with the diseased or carrier birds (Akhter et al. 2010). Dey et al. (2013) reported 44.4-86.1% prevalence of *E. coli* in the cloacal swab, foot pads and feces of pigeon. Recent studies reported 50-55% prevalence of *E. coli* in cloacal and oral swab of pigeons, 43.7% prevalence in feces of ornamental bird (Karim et al 2020; Akbari and Asapour 2022). Other studies recorded 60.5% prevalence of *E. coli* in chicken meat, 78.8% prevalence in poultry, 66.6% prevalence in duck, 14.4-24.5% prevalence in psittacine bird, 32.8-64.4% prevalence in parrot, 16.9% prevalence in budgerigar, 37.2-40% prevalence in quail, 10-31.2% prevalence in wild bird, 18.82% prevalence in diarrheic goat and 65% prevalence in animal feed (Hedawy and El-Shorbagy 2007; Akhter et al. 2010; Dey et al. 2013; Ghazi et al. 2014; Youssef and Mansour 2014; Sanches et al. 2017; Sultana et al. 2017; Kamal et al. 2018; Habib et al. 2021). Our study revealed the presence of *Salmonella* spp. in cloacal swab, oral swab and feces of pigeons ranging from 23.1-69.2%, 11.1-55.6% in parrot, 25-50% in budgerigar and 50% in quail. Others studies recorded 22.2-29% prevalence of *Salmonella* spp. in cloacal swab, 30% prevalence in oral swab, 22.8-27.5% prevalence in feces, 33.3% prevalence in loft, 66.6% prevalence in pet store of pigeon, 46.6-50% prevalence in parrot, 21.6-40% prevalence in quail, 5.8-25% prevalence in wild bird and 55% prevalence in animal feed (Pasmans et al. 2004; Hedawy and El-Shorbagy 2007; Kobayashi et al. 2007; Akhter et al. 2010; Hosain et al. 2012; Ghazi et al. 2014; Youssef and Mansour 2014; Sultana et al. 2017; Bupasha et al. 2020; Karim et al 2020; Elgresly et al. 2021). Our study also revealed the presence of *Shigella* spp., *Klebsiella* spp. and *Staphylococcus* spp. in cloacal swab, oral swab and feces of pigeons ranging from 15.8-57.9%, 18.8-31.2% and 11.1-66.7%, 14.3-71.4%, 9.1-63.6%, and 12.5-62.5% in parrot, 100%, 40-60% and 100% in budgerigar and 16.7-50%, 14.3-57.1% and 14.3-71.4% in quail, respectively. Other studies recorded 10% prevalence of *Klebsiella* spp. in wild bird, 3.1-20% prevalence in quail and 46.6% prevalence of *Staphylococcus* spp. in parrot, 19.1-20% prevalence in quail, 5-20% prevalence in wild bird (Akhter et al. 2010; Ghazi et al. 2014; Youssef and Mansour 2014). The variations in the prevalence rate of bacterial pathogens in this study could be attributed to bird health, population density, fecal-oral transmission success, strain differences, flock immune status, cross contamination, climatic conditions, water supply and food variation, geographical location and management strategies like bird rearing, biosecurity and veterinary care (Hosain et al. 2012; Youssef and Mansour 2014; Elgresly et al. 2021)

The antibiotic therapy is one of the most widely used method to treat disease, however, overuse and incorrect administration of antibiotics leads to antimicrobial resistance. Pet birds have the possibility of contributing to the spread and transmission of antibiotic-resistant bacteria to humans. Thus, it is crucial to understand the status of antimicrobial resistance in pet birds and to avoid misuse of antibiotics in the treatment or prevention of disease in these birds (Kaczorek-Lukowska et al. 2021; Akbari and Asadpour 2022). Bacteria can withstand the effect of many antibiotics used in various medications as they generate enzymes and metabolites that either breakdown or help the survival of bacteria through diverse method (Karim et al. 2020). In the present study, 18 different antibiotics were used to perform the antibiogram profiling of *E. coli*, *Salmonella* spp., *Shigella* spp., *Klebsiella* spp. and *Staphylococcus* spp. isolated from household pet birds using the disk diffusion method. Almost all isolates were found to be resistant to at least six antibiotics used in this study along with showing MDR traits. A number of previous studies in Bangladesh reported MDR *E. coli*, *Salmonella* spp. in pigeons (Hosain et al. 2012; Dey et al. 2013; Bupasha et al. 2020). These birds may have acquired MDR strains via eating food and water polluted with human feces and farm waste, misusing antibiotics, and transmitting these bacteria to humans and other animals (Borges et al. 2017; Karim et al. 2020; Chrobak-Chmiel et al. 2021). According to our study, isolated bacteria showed highest sensitivity to GEN (83.1%), followed by LEV (81.5%), CIP (80.4%), KAN (77.8%), and CFM (59.3%). Moreover, 100% resistance to PG, AMX, BAC followed by CH (98.4%), COX (95.2), CN (90.5%), TET (77.2) and VAN (75.7%). Our findings corroborated those of Akhter et al. 2010, Hosain et al. 2012, Dey et al. 2013, Bupasha et al. 2020, Karim et al. 2020, Elgresly et al. 2021 and Akbari and Asadpour 2022, who reported that GEN, KN, CIP and LEV were the most highly sensitive and PG, AMX, TET, E were highly resistant against *E. coli* and *Salmonella* spp. isolated from pigeons, parrots and ornamental birds. This high susceptibility to bacterial isolates in the study area could be attributed to the relatively low use of these drugs in avian disease treatment. However, COX, CH, CN, CL and BAC were resistant to most bacterial pathogens, since all these drugs have been

used in the country for many years in both veterinary and public health. To successfully fight the increasing numbers of drug resistant and MDR bacteria, extensive knowledge of the molecular mechanisms of acquiring antibiotic resistance and updated information regarding current distribution of resistance pattern are required.

**Conclusion:** The close contact between household pet birds and people offers favorable conditions for bacterial transmission and the potential risk for public health posed by drastically increasing MDR of bacteria isolated from these birds must be highlighted. Thus, increased awareness among pet bird owners and the implementation of preventive measures in the pet bird industry is advised to limit the circulation of zoonotic avian bacterial pathogens.

### Supplementary data

Supplementary data/Appendix are available on request from corresponding author.

### Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Author Contribution

Md Atiqul Haque and Md. Khaled Hossain conceived the idea and designed the study. Mahmuda Naznin Nupur conducted the experiments. Mahmuda Naznin Nupur and Farzana Afroz wrote the manuscript, Md Gausur Rahman and Md Kamruzzaman performed statistical analysis. Khadija Al Ferdous, Md Atiqul Haque, Md. Khaled Hossain and S.M. Harun-ur-Rashid were involved in the review, edit and proofreading of the manuscript.

### ORCID

Mahmuda Naznin Nupur <https://orcid.org/0009-0005-2130-1966>  
Farzana Afroz <https://orcid.org/0000-0001-8810-8515>  
Md. Khaled Hossain <https://orcid.org/0000-0002-0138-4523>  
S. M. Harun-Ur Rashid <https://orcid.org/0000-0003-0418-222X>  
Md Gausur Rahman <https://orcid.org/0000-0001-7244-8865>  
Md Kamruzzaman <https://orcid.org/0000-0002-5757-1870>  
Khadija Al Ferdous <https://orcid.org/0000-0002-7622-8824>  
Md Atiqul Haque <https://orcid.org/0000-0002-0978-4484>

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