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IMPACT OF MICROBIAL COMMUNITIES OF PUBLIC HEALTH IMPORTANCE IN WILD BIRD DROPPINGS – A COMPLICATION IN URBAN LANDSCAPES

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ABSTRACT : Identification of potentially pathogenic bacteria to human health in the environment is essential to manage ecosystem health. The study was conducted surrounding a Lake, in the city of Kandy, a highly urbanized tourist destination in Central Sri Lanka. Fifty samples of fresh bird droppings from House Crow (15), Little Cormorant (20), Rock Pigeon (05), Little Egret (05) and Black – crowned Night Heron (05) were collected at five different points using sterile swabs. After pretreatment with saline, 50 µL from each was spread on LB and M17 agar media. Plates were incubated at 37 °C for 24 hours. Colony counts were taken. Different morphotypes were subjected to gram staining. Isolated organisms were identified through PCR based 16S rDNA sequencing. Highest count (CFU/mL) was obtained from House Crow (5.5×10^3) while the lowest obtained from Rock Pigeon (3.6×10^3). Twenty different bacteria were isolated with 88% being Gram-negative. Shannon and Margalef's indices were 2.357 and 2.934, respectively. *Enterobacter soli, Enterobacter cloacae, Escherichia coli, Staphylococcus saprophyticus, Sporosarcina koreensis, Staphylococcus sciuri, Sporosarcina aquimarina* were observed in all bird species. *Enterobacter hormaechei* and *Klebsiella quasipneumoniae* were observed in House Crow. Accordingly, birds can act as reservoirs for pathogenic bacteria and could transmit them to human through bird droppings.

KEY WORDS: wild birds, bird droppings, 16S rDNA sequencing, pathogenic bacteria

INTRODUCTION

With the growing human populations, cities are expanding rapidly, and urbanization represents one of the most intense anthropogenic activities of natural systems, strongly affecting ecosystems and species including birds (Rouffaer *et al.*, 2017). The urbanization, induces a number of substantial environmental modifications such as loss and fragmentation of natural habitats (Er *et al*, 2005), increase of the environmental pollution including soil, water, air and changes in the food resources in the environment (Gottdenker *et al.*,2014; Teyssier *et al.*, 2018). This can create diverse interfaces between wildlife and human and these interfaces are critical points of cross – species transmission and emergence of pathogenic agents (Hassel *et al.*, 2017). This led to emergence of zoonotic pathogens in human as the increased contact between wildlife and human. However, it depends on the interactions between humans and infected animal reservoir, vector hosts or their environment. The land use change has a potential impact on disease dynamics by changing the abundance, demography, behavior, immune response, movement, and contact between host species and vectors (Gottdenker *et al.*, 2014).

Wildlife has existed in urban areas for as long as humans have lived in settlements (Soulsbury and White, 2015). As a result of loss of the natural habitats, wild birds frequently used the urban and periurban areas as their habitats (Ebani, 2016). However, this led to homogenization of urban communities which resulted a limited number of bird species in a high density, such as House Crow, Rock Pigeon, and House Sparrow (Ebani, 2016; Rouffaer, 2017). Wild birds have been ranked as vectors for zoonotic pathogens for number of human and animal diseases through direct or indirect transmission.

The gastrointestinal tract of birds as well as other animals facilitates a habitat to large number of microbes. This community of microbes harboured within the gastrointestinal tract of animals – 'the gut microbiome' – is an important determinant of host health, immunity, and many other physiological functions (Perry *et al.* 2017). In this regard, different pathogenic bacteria and some viruses can be inhabited within the wild bird gut and can enter to the environment through bird fecal matter (Tsiodras, 2008; Keesing, 2010). Therefore, fecal samples are the best approach for sampling the microbial gut communities of wild birds (Grond, 2017; Videvall, 2017).

Mostly members of the family Enterobacteriaceae, including, *Salmonella* sp., *Escherichia coli, Enterobacter aerogenes* and *Enterobacter cloacae,* and *Klebsiella pneumoniae* in wild bird droppings can act as direct or opportunistic pathogens to human. They possess a potential health hazard to the people who use these contaminated areas such as school children, tourists, and municipal workers (Vlahovi,2010).

Many studies have focused on the link between the human activities and the species interactions worldwide (Bradley, 2008). However, there are no published reports on the composition of the fecal microbial communities in wild bird species of public health importance in urban landscape in South Asian tropical region. The city of Kandy is the cultural capital in Sri Lanka and is a world heritage site which consider as a major tourist and religious destination among locals and foreigners. The city lies at an elevation of 465 meters (1,526 ft.) above sea level and in between several mountain regions. About 0.12 million populations reside and about 0.1 million are move daily within the Kandy Municipal Council area and more than 100,000 of vehicles move daily within the area (Premasiri, 2010).

The selected study site, Kandy Lake is an artificial lake in the center of the Kandy city and has the highest attraction of people including tourists. The trees around the Kandy Lake is occupied by thousands of birds ranging in to different species and the surrounding area including the walkways are fully covered with droppings of these birds which has the potential to cause severe health issues in human. The air quality in this area is poor due to the fecal dust. These particles are easily inhalable to people who use this area every day. These have the potential to cause respiratory illnesses such as Asthma, lung infections and skin allergies etc. These droppings persist in the environment especially on trees and adjacent ground areas for a long period of time there by increasing the exposure time of the fecal contaminants to human. When considering about the air quality in Kandy city including the study site, a previous study confirmed that there were number of opportunistic pathogenic bacteria (Weerasundara *et al.*, 2017) used the habitat for their survival. The airborne transmission of this bacteria can cause significant health impact on people. According to Madamarandawala *et al.*, 2019, there is a possible effect on the air quality specially focusing on microbial air quality to the respiratory health of preschool children in Kandy city and surrounding area.

Therefore, the aim of this study was to identify the microbial composition in bird droppings collected from most common wild bird species around the Kandy Lake. The identification and the characterization of the bacteria were based on the culture and by DNA sequencing. We mainly focused on the identification of the potentially pathogenic bacteria in bird droppings which can cause health hazards to human and to provide evidence that bird droppings are a potential health hazard to the community.

MATERIALS AND METHODS

Study site

The study site was the southern edge of the Kandy Lake in the Central Province, Sri Lanka. The area was rich with large trees such as *Ficus* *benghalensis, Delonix regia, Tabebuia rosea* that provide habitats for feeding, resting, and roosting to different bird species. The surrounded area is highly populated and urbanized with schools, hospitals, preschools, tourist hotels and human residences. The sampling sites were along the walkway and the adjacent roads are highly traffic congested (Figure 1).

Sample processing and isolation of bacteria

One gram of fresh droppings was thoroughly mixed in 10.0 ml of 0.85% w/v saline solution. The material was homogenized by shaking and allowed to stand for 30 min. 1.0 mL was transferred into the next test tube and diluted serially in one-tenth stepwise to 10^{-5} dilution (Wambura, 2014). From the dilution of 10^{-4} of dropping sample, 50 μL aliquot was spread on LB (Luria-Bertani, H26760, Alfa Aesar, England) and M17 agar (VK474508, Merck, Germany) media. Plates were incubated at 37 ºC for 24 hours. Colony counts were taken and different morphotypes were subcultured to be used in subsequent gram staining, morphological identifications and molecular analysis.

FIGURE 1: Map indicating the locations where the sample collection carried out along the southern edge of the Kandy Lake (Source: www.google.com)

Sample collection

A bird survey was carried out from February to June 2018 in the morning (0630 – 0800 h), in the afternoon (1330 – 1500 h) and in the evening (1700 – 1800 h) for the identification of most common bird species, their nesting and roosting sites around the Kandy Lake. Fifty samples of bird droppings from House Crow (15), Little Cormorant (20), Rock Pigeon (05), Little Egret (05) and Black – crowned Night Heron (05) were collected at five different sample collection points using sterile swabs and transported to the laboratory as soon as possible for the analysis. The sample were collected freshly and the collection, transportation and the processing were done under the sterile conditions.

DNA extraction and amplification

DNA was extracted from the isolated cultures using the standard CTAB method (Somerville *et al.*, 2005) and quantified using the Fluorometer (QuantiFluor® Single-Tube Fluorometer, Promega, USA). A PCR was performed using universal bacterial primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-GGY TAC CTT GTT ACG ACT T-3'; Macrogen Inc, Seoul, Republic of Korea) to amplify the 16S rRNA gene (Lan *et al.*, 2002). Amplification reactions were performed in a 25 μL reaction volume containing 5 μL of dissolved DNA, 0.2 μL of 1 U Taq (Promega, USA), 5 μL of 5X green GoTaq ® Flexi buffer (Promega, USA), 2.5 μL of dNTP mixture (1 mM each), and 40 pmol of each primer.

Reaction mixtures were amplified using conventional PCR in Rotor-Gene Q (QIAGEN, USA). PCR conditions consisted of 35 cycles of: 60 s at 94°C, 60 s at 50°C and 60 s at 72°C, with an initial denaturing step of 2 min at 94°C, and followed by a final extension step of 20 min at 72°C. Reactions were verified to contain visible amplification using 2% agarose gel electrophoresis. Residual primers were removed from the PCR product using the Wizard® SV Gel and PCR Clean-Up System (Promega, USA) following manufacturer's instructions and stored at – 20 ° C until sequenced.

16S rDNA sequencing and sequence analysis

PCR products were sequenced commercially using the Sanger sequencing (Macrogen Inc., Seoul, Republic of Korea; Unno, 2015). Obtained sequences were trimmed and aligned using BioEdit software (Ibis Theraputics, CA). Any sequences with uncertainties (chimeric sequences) were removed from the data set. The taxonomic affiliation was done by BLAST using GenBank of the National Center for Biotechnology Information (NCBI) and the gene sequences were (accession numbers from MK000881 to MK000895) deposited in the NCBI.

Data analysis

Microbial richness and the diversity of the bird fecal samples were calculated using diversity indices. The relationship between the host and the microbial community was statistically analyzed to see whether there is a significant correlation between microbial richness and the host species.

RESULTS

Twenty-one bird species belong into 13 families were identified in the vicinity. The most common bird species observed during the study were House Crow (23.73%), Little Cormorant (32.62%), Rock Pigeon (16.42%), Little Egret (3.53%) and Black – crowned Night Heron (14.16%) in which the droppings were collected. Bacterial counts were calculated and highest colony count was obtained from the House Crow (5.5×10^3 CFU/ml) and the lowest colony counts were obtained from Rock Pigeon (3.6×10^3 CFU/ml). Total of 20 different bacterial isolates were isolated from collected bird droppings and according to the Gram's staining results, there were a smaller number of Gram-positive isolates in comparison to the Gram-negative isolates of 88%.

Fecal microbial composition and the diversity

Different isolates obtained from the bird dropping samples were analyzed by sequencing the V3 and V4 variable regions of the bacterial ribosomal RNA (rRNA) gene. The isolated strains were classified into 3 distinct phyla i.e. Proteobacteria, Actinobacteria, and Firmicutes (Figure 2).

Out of them Firmicutes were the most common phyla in all bird species and Actinobacteria were only observed in Little Egret. When considering the α-diversity, Shannon diversity index was given the value of 2.357 and the Margalef's Richness Index was 2.934 for the isolated bacterial strains. Highest number of organisms were isolated from House Crows' droppings (85.71%). *Enterobacter soli, Enterobacter cloacae, Escherichia coli, Staphylococcus saprophyticus, Sporosarcina koreensis, Staphylococcus sciuri, Sporosarcina aquimarina* were observed in dropping samples of all birds. However, *Enterobacter hormaechei* and *Klebsiella quasipneumoniae* were only observed in House Crow (Figure 3).

The relationship between the host and the microbial community

When considering the host species, the highest number of bacteria were residing on House Crow while the lowest recorded in Black-

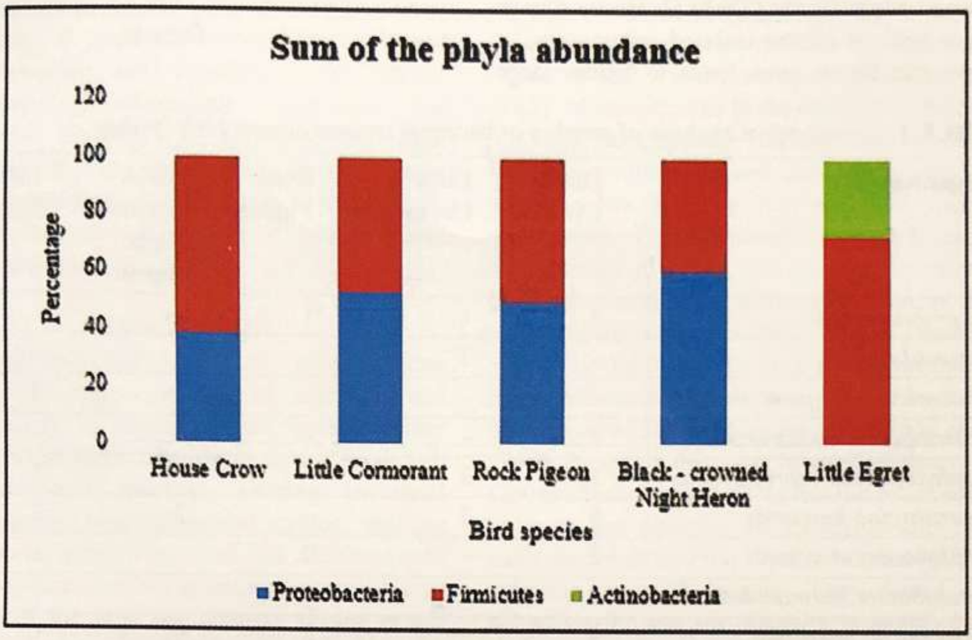

FIGURE 2: Summary of the abundance of the microflora in bird dropping samples

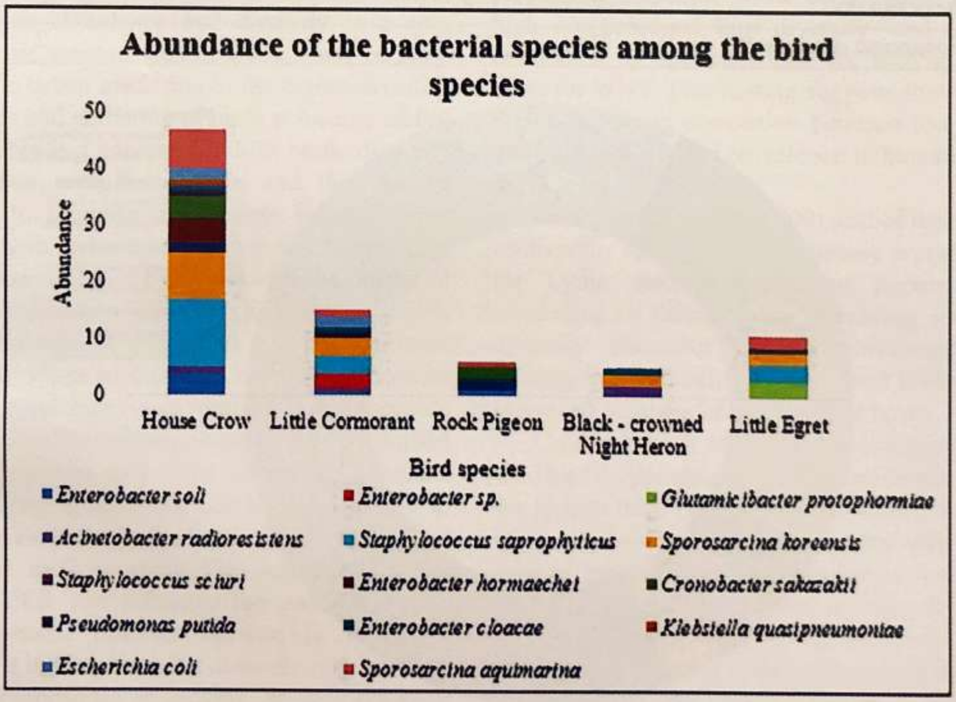

FIGURE 3: Abundance of the different microbial species with the bird species

crowned Night Heron (Table 1). House Crows harbor 85% of all the isolated cultures and it shows that house crow tends to harbor large number of microbes and can act as a vector for several human related diseases.

TABLE 1: Comparative analysis of number of bacterial isolates among bird species

Organism	**House Crow**	**Little Cormorant**	**Rock Pigeon**	**Black - crowned Night Heron**	**Little Egret**
Enterobacter soli	4	1	1	-	-
Enterobacter sp.	-	3	-	-	-
Glutamicibacter protophormiae	-	-	-	-	3
Acinetobacter radioresistens	1	-	-	2	-
Staphylococcus saprophyticus	12	3	-	-	3
Sporosarcina koreensis	8	3	-	2	2
Staphylococcus sciuri	2	-	2		1
Enterobacter hormaechei	4	-	-	-	-
Cronobacter sakazakii	4	-	2	-	-
Pseudomonas putida	1	1	-	-	-
Enterobacter cloacae	1	1	-	1	
Klebsiella quasipneumoniae	1	-	-	-	-
Escherichia coli	2	2	-	-	-
Sporosarcina aquimarina	7	1	1	-	2

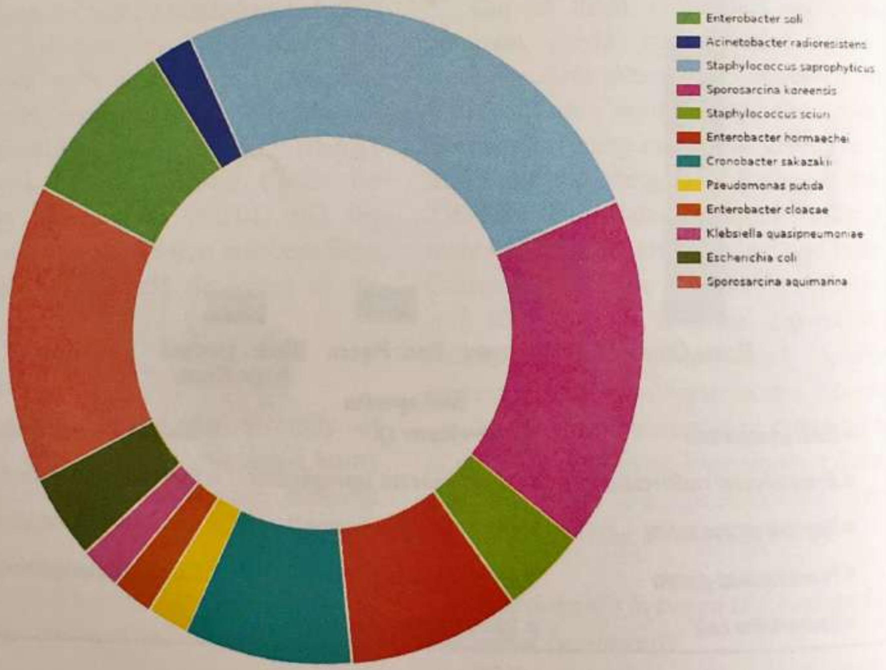

FIGURE 4: The percentage distribution of observed microorganisms in crow's dropping samples

According to the results, in the crows' droppings, *S. saprophyticus, S. koreensis and S. aquimarina* were recorded as the highest prevalent microorganisms. However, the microbial diversity is comparatively high and most of them have the potential to cause health hazards to human such as *E. coli, K. quasipneumoniae, E. cloacae, C. sakazakii* and *S. sciuri* (Figure 4).

DISCUSSION

Anthropogenic land use changes can negatively impact ecological integrity and biodiversity by disrupting the usual stability of an ecosystem. Disturbing to the food web structure and function, altering terrestrial and aquatic biogeochemical cycles, shifting ecosystem properties, and introducing non-native species, including pathogens are adverse effects of the land use changes (Foley *et al.*, 2005). Pathogen abundance and diversity show also great geographic variation and even variation across cities from the same region (Tietze, 2018). Climate and presence of water bodies are the two main factors that affect pathogen abundance and diversity in a city. However, transmission of disease may be more rapid in urban areas due to the higher densities of birds and gathering of birds at feeding tables.

There is a general trend for biotic diversity in urban areas to decline and this decline tends to increase as habitats become more and more urbanized. However, the patterns of biotic diversity can vary with the degree of the urbanization (Bradley and Altizer, 2006; Soulsbury and White, 2015). There are different complications in this kind of habitats. Species homogenization is a current issue because species that are willing to survive in a disturbed condition can only exist in urban landscape. Therefore, species that are highly sensitive to the environment or feed on a special diet cannot survive in these areas. This reduced diversity of wildlife can influence the transmission of vector-borne diseases because in urbanized areas, a low host species diversity may increase the proportional abundance of key reservoir hosts (Keesing *et al.*, 2006).

It is estimated that nearly 80% of emerging infections are zoonoses and thus are dependent on an animal for the survival (Hassel *et al.*, 2017). Meantime, due to the changes in the host diversity, host density, phylogenetic structure, immune competence and immune history, an interface has built up between host species and human. The relationship between animal host species diversity and disease prevalence has been tested using different animal models including birds (Grond, 2017). As an example, West Nile Encephalitis, is a recent mosquito-borne infectious disease where several species of birds act as intermediate hosts (Keesing *et al.*, 2010). Based on this association, Ezenwa *et al.* (2006) conducted a study to test the relationship between the diversity of passerine and non-passerine birds and the West Nile virus (WNV) infection rates in human. They found that non-passerine species diversity is significantly correlated with human infection rates i.e. communities with low non-passerine bird diversity tend to be dominated by species that act as hosts for WNV, inducing high infection prevalence in people while communities with high non-passerine bird diversity tend to be dominated by species which are incompetent hosts for WNV. This finding suggests that there may be a strong correlation between low bird diversity and disease prevalence in humans for WNV.

Ostfeld and Keesing (2000) studied the effect of diversity of tick hosts on disease prevalence for Lyme disease in human populations. According to their results, increasing species diversity decreases disease prevalence by diluting the availability of competent hosts with increased numbers of incompetent hosts.

Contamination of urban environments by wild bird droppings and the resultant health risks for human have been known for a long period of time (Ebani *et al.*, 2016). Several wild bird species inhabiting urban areas act as a source for several human and animal diseases through direct or indirect transmission, i.e.by acting as vectors for zoonotic pathogens (Kozdruń and Czekaj, 2015). In addition, behavioral habits of the wild birds in assembling and resting on trees, bring them even closer to human. According to the Ebani, 2016 at least 110 organisms can be found among birds that are pathogenic to humans, including viruses, bacteria, fungi, and protozoa.

In the current study, microbial diversity in bird droppings of House Crow, Rock Pigeon, Little cormorant, Little Egret, and Black – crowned Night Heron were assessed by cultivation on LB media and by 16S rDNA sequencing technique. Using the molecular method, 15 different species belonging to six different families were found. Similar to other vertebrate hosts, the birds' gut microbiome is dominated by members of the phylum Firmicutes, with other members being from phyla such as Actinobacteria, Bacteroidetes, and Proteobacteria (Hird *et al.*, 2015; Waite and Taylor, 2015). According to our results, it also showed that the all the collected bird fecal samples were predominantly represented by the phylum Firmicutes. However, this can vary with both extrinsic and intrinsic factors (Bradley and Altizer, 2007). Extrinsic factors such as diet, environment, migratory behavior, social interactions, and intrinsic factors such as phylogenetic history, age, and sex, reproduction, physiology: GI tract morphology, health and fitness are the most common determinants of the birds' gut and fecal microbial communities (Santos *et al.*, 2012). According to our observations, as the study area is highly urbanized, these birds have limited resources for food and habitats which may lead to the low diversity seen in the gut microflora (Teyssier *et al.*, 2018). Many of the bacterial species identified in this study are common in human and birds but also can be found in soil and water from the habitats where they usually use (Santos *et al.*, 2012).

This study was conducted along the southern edge of the Kandy Lake, Central Sri Lanka. Birds have been using large trees along the edge for different purposes including feeding (House Crow, Rock Pigeon and Little Egret), nesting (House Crow, Little Cormorant, Little Egret, and Black – crowned Night Heron) and for roosting (Little Cormorant). Therefore, these birds use both aquatic and terrestrial ecosystems for the survival which might be the reason for the obtained results.

According to 16S rDNA sequencing results, we have observed that there are some potentially pathogenic bacteria in some of the bird droppings. *K. quasipneumoniae* which was isolated from House Crow can cause pneumonia or inflammation in human lung (Brisse *et al.*, 2014). *A. radioresistens* was isolated from both House Crow and the Black-crowned Night Heron, responsible for a range of systemic infections in critically ill and immunocompromised patients but rarely colonize in healthy people (Visca *et al.*, 2001). *S. sciuri* was isolated from House Crow, Rock Pigeon and Little Egret, has been associated with serious infections in human, such as endocarditis, peritonitis, septic shock, urine infections and wound infections (Jez *et al.*, 2003). We have isolated *C. sakazakii* from bird droppings of House Cow and Rock Pigeon. *C. sakazakii* is a rare opportunistic pathogen but can cause fatal infection in bloodstream and central nervous system. Infants with weakened immune systems, are most likely to contact *Cronobacter* infection, although the bacteria have caused illnesses in all age groups. *E. hormaechei* was isolated from House Crow's droppings and it can cause blood stream infections among immunosuppressed infants and adults (Wenger *et al.*, 1997). Most strains of *E. coli* are not harmful but are part of the healthy bacterial flora in the human gut. However, some types can cause illness in humans, including diarrhea, abdominal pain, fever, and sometimes vomiting (Harrington *et al.*, 2006). In our study, we have identified *E. coli* in both House Crow and Little Cormorants droppings. We have isolated *P. putida* that can cause a wide range of conditions such as pneumonia, catheter-related blood stream infections, acute cholecystitis, and cholangitis, tonsillitis, thrombophlebitis, and skin and soft tissue infections from both House Crow and Little Cormorants' droppings (Cowling *et al.*, 2013). *E. cloacae* was also isolated from House Crow, Little Cormorant and Black-crowned Night Herons' droppings which can cause nosocomial urinary tract or pulmonary infections among burn victims, immunocompromised patients, and patients with malignancy (Annawajhala *et al.*, 2019).

This result suggests that the composition of the bacterial community associated with the bird droppings might cause serious illnesses among humans including school children, general public, tourists and also the local municipal council workers who are engaged in city cleaning as they use this area for a long period of time. Also, as these birds can harbor those pathogenic bacteria, probably reflecting the presence of such isolates in their sources of food and/or water in the environment. On the other hand, all the large trees were occupied by birds for their nesting and roosting sites. Therefore, the whole area is totally covered with bird fecal matter and the air is highly polluted with the fecal dust which may lead to difficulties in respiratory system such as Asthma and allergic conditions in the body (Haag-Wackernagel and Moch, 2004).

The Shannon Wiener diversity for the microbial community gives a low value and the reason might be the urbanization which lead to alterations in the taxonomic composition of gut microbiome of birds (Teyssier *et al.*, 2018). These birds are not migratory birds, but they are often change their habitats within the city, according to their behaviors. This may lead to transmission of pathogenic bacteria between these birds.

According to our results, we observed that there is a significant increase in the number of bacterial species in House Crow. Crows are highly adaptable group of animals to urban landscapes and can survive on a wide range of food sources. Considering most of the urbanized areas in the country the most common bird species that could be found everywhere is the House Crow. They are known to roost well in urbanized areas but they cause public health hazard and can be a threat to human health as studies have shown that crows carry *Salmonella, Plesiomonas,* enteropathogenic *E. coli, Shigella, Aeromonas hydrophila* and many other microorganisms that can cause mild to severe infection on human (Yong *et al.*, 2008). This is the first study in which culturable and molecular approach was used to characterize of the birds' intestinal microbiota around the Kandy Lake area. However, an idea about the total picture of the microflora of the wild birds can be obtained using advance molecular techniques such as Next Generation Sequencing.

CONCLUSION

In conclusion, wild birds, which live in urban areas are considered a hazard for the human health, due to the presence of pathogens in their feces. The result of the present study confirmed that quite a number of potentially pathogenic bacteria are present in the droppings of the birds surrounding the Kandy Lake. Further studies are necessary to understand the correlation of the pathogenic agents and the human exposure by bird droppings in urban cities.

REFERENCES

Ahoyo, T.A., E. Yehouenou Pazou, L .Baba-Moussa, A. Attolou Gbohou, M. Boco, K.L. Dramane and T. Aminou (2013) Staphylococcus sciuri outbreak at Tertiary Hospital in Benin. *Journal of Medical Microbiology and Diagnosis* **2:126;** DOI:10.4172/2161-0703.1000126

Annavajhala M.K., A. Gomez-Simmonds, and A. Uhlemann (2019) Multidrug-Resistant Enterobacter cloacae Complex Emerging as a Global, Diversifying Threat. *Frontiers in Microbiology.* **10:44;** DOI:10.3389/fmicb.2019.00044

Anzai, Y., H. Kim, J. Park, H. Wakabayashi, and H. Oyaizu (2000) Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *International Journal of Systematic and Evolutionary Microbiology* **50:** 1563 – 1589

Aruji, Y., K. Tamura, S. Sugita and Y. Adachi (2004) Intestinal microflora in 45 crows in Ueno Zoo and the in vitro susceptibilities of 29 Escherichia coli isolates to 14 antimicrobial agents. *Journal of Veterinary Medical Science* **66(10):**1283-1286; DOI:10.1292/jvms.66.1283

Bradley, C.A. and S. Altizer (2007) Urbanization and the ecology of wildlife diseases. *Trends in Ecology and Evolution,* **22(2):** 95-102; DOI: 10.1016/j.tree.2006.11.001

Brisse, S., V. Passet and P.A.D. Grimont (2014) Description of *Klebsiella quasipneumoniae* sp. nov., Isolated from human infections, With two subspecies, *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* subsp. nov. and *Klebsiella quasipneumoniae* subsp. *similipneumoniae* subsp. nov., and demonstration that *Klebsiella singaporensis* is a junior heterotypic synonym of *Klebsiella variicola. International Journal of Systematic and Evolutionary Microbiology* **64(2014):** 3146-3152; DOI:10.1099/ijs.0.062737-0

Cowling, B.J., L. Jin, E.H.Y. Lau, Q. Liao, P. Wu, H. Jiang (2013) Comparative epidemiology of human infections with avian influenza A H7N9 and H5N1 viruses in China: A population-based study of laboratory-confirmed cases. *Lancet* **382(9887):** 129-137; DOI: 10.1016/S0140-6736(13)61171-X

Ebani, V.V., F. Bertelloni and P. Mani (2016) Molecular survey on zoonotic tick-borne bacteria and chlamydiae in feral pigeons (Columba livia domestica). *Asian Pacific Journal of Tropical Medicine.* **9(4):** 324-327; DOI: 10.1016/j.apjtm.2016.03.005

Er, K.B.H., J.L. Innes, K. Martin, and B. Klinkenberg (2005) Forest loss with urbanization predicts bird extirpations in Vancouver. *Biological Conservation* **126:** 410-419

Foley, J.A., R. DeFries, G.P. Asner, C. Barford, G. Bonan, S.R. Carpenter, F.S. Chapin and P.K. Snyder (2005) Global Consequences of Land Use. *Science* **309 (5734):** pp. 570-574

Ezenwa, V.O., M.S. Godsey, R.J. King and S.C. Guptill (2006) Avian diversity and West Nile virus : testing associations between biodiversity and infectious disease risk. Proceedings of the Royal Society B 109–117; DOI:10.1098/rspb.2005.3284

Gottdenker, N.L., D.G. Streicker, C.L. Faust, an C.R. Carroll (2014) Anthropogenic Lan Use Change and Infectious Diseases: Review of the Evidence. *EcoHealth* 11 **619;** DOI:10.1007/s10393-014-0941-z

Grond, K (2017) Development and dynamics o gut microbial communities of migrator shorebirds in the Western Hemispher A Dissertation submitted in partia fulfillment of the requirements for th degree Doctor of Philosophy Division of Biology College of Art and Sciences Kansas State University Manhattan, Kansas

Haag-Wackernagel, D and H. Moch (2004) Health hazards posed by feral pigeons. *Journal of Infection* **48(4):**307-313; DOI: 10.1016/j.jinf.2003.11.001

Hassan, M.M., A. Hoque, N.C. Debnath, M. Yamage and M. Klaassen (2017) Are Poultry or Wild Birds the Main Reservoirs for Avian Influenza in Bangladesh? *EcoHealth* **14:**490–500. DOI: 10.1007/s10393-017-1257-6

Hassell, J.M., M. Bagon, M.J. Ward and E.M. Fevre (2017) Urbanization and Disease Emergence: Dynamics at the Wildlife–Livestock–Human Interface. *Trends in Ecology and Evolution* **27(1):** 55-67; DOI: 10.1016/j.tree.2016.09.012

Harrington, S.M., E.G. Dudley and J.P. Nataro (2006) Pathogenesis of enteroaggregative Escherichia coli infection. *FEMS Microbiology Letters* **254:**12-18. DOI:10.1111/j.1574-6968.2005.00005.x

Hird, S.M., C. Sánchez, B.C. Carstens, R.T. Brumfield and D.W. Waite (2015) Comparative Gut Microbiota of 59 Neotropical Bird Species. Evolutionary and Genomic Microbiology 6 (December). doi:10.3389/fmicb.2015.01403

Jez, P., D. Vukovic, I. Dakic and P. Petra (2003) Isolation of Members of the Staphylococcus sciuri Group from Urine and Their Relationship to Urinary Tract Infections. *Journal of Clinical*

Microbiology **41(11):** 5262-5264; DOI:10.1128/JCM.41.11.5262

Keesing F, L.K. Belden and P. Daszak (2010) Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* **468(7324):** 647-652; DOI: 10.1038/nature09575

Kozdruń, W., H. Czekaj and N. Styš (2015) Avian zoonoses - A review. *Bulletin of Veterinary Institute in Pulawy* **59(2):**171-178; DOI: 10.1515/bvip-2015-0026

Lan, P.T.N., H. Hayashi, M. Sakamoto and Y. Benno (2002) Phylogenetic analysis of cecal microbiota in chicken by the use of 16S rDNA clone libraries. *Microbiology and Immunology* **46(6):** 371-382; DOI:10.1111/j.1348-0421.2002.tb02709.x

Madamarandawala, P., Y. Weerasinghe, D. Pathiraja, A. Ekanayake, D. Madegedara and D.N. Magana-Arachchi (2019) Impact of microbial air quality in preschools on paediatric respiratory health. *SN Applied Sciences* **1(1280);** DOI: 10.1007/s42452-019-1306-6

Nyakundi, W.O., and M. Wambura (2014) Isolation and Characterization of Pathogenic Bacteria and Fungi from Leptoptilos crumeni. *Journal of Applied Technology and Environmental Sanitation* **1(1):** 93 – 106

Ostfeld, R.S., and F. Keesing (2001) Biodiversity and Disease Risk: The Case of Lyme Disease. Conservation *Biology* **14(3):** 722-728; DOI:10.1046/j.1523-1739.2000.99014.x

Paauw, A., J. Verhoef and A.C. Fluit (2007) Failure to Control an Outbreak of qnrA1 -Positive Multidrug-Resistant Enterobacter cloacae Infection despite Adequate Implementation of Recommended Infection Control Measures **45(5):**1420-1425.

Perry, E.K., A. Digby and M.W. Taylor (2017) The Low-Diversity Fecal Microbiota of the Critically Endangered Kākāpō Is Robust to Anthropogenic Dietary and Geographic Influences. *Frontiers Microbiology* **8:2033;** DOI:10.3389/fmicb.2017.02033

Premasiri, H.D.S., I.H.K. Samarasinhge and K.M.N. Lakmini, (2012) Population exposure risk assessment to air pollution in Kandy city area. NBRO Symposium, Colombo

Rouffaer, L.O., D. Strubbe and A. Teyssier (2017) Effects of urbanization on host-pathogen interactions, using Yersinia in house sparrows as a model. *PLoS One* **12(12):**1-15; DOI: 10.1371/journal.pone.0189509

Santos, S.S., S. Pardal, D.N. Proença (2012) Diversity of cloacal microbial community in migratory shorebirds that use the Tagus estuary as stopover habitat and their potential to harbor and disperse pathogenic microorganisms. *FEMS Microbiology Ecology* **82(1):** 63-74; DOI:10.1111/j.1574-6941.2012.01407.x

Somerville, W., L. Thibert, K. Schwartzman, and M.A. Behr (2005) Extraction of Mycobacterium tuberculosis DNA: a Question of Containment. *Journal of Clinical Microbiology* **43(6):** 2996-2997; DOI:10.1128/JCM.43.6.2996

Soulsbury, C.D., and P.C.L. White. (2015) Human-wildlife interactions in urban areas: a review of conflicts, benefits, and opportunities. *Wildlife Research*, **42 (7):** pp. 541-553

Teyssier A, Oscar, L and N. Saleh, (2018) Inside the guts of the city: Urban-induced alterations of the gut microbiota in a wild passerine. *Science of the Total Environment* **612:**1276-1286; DOI: 10.1016/j.scitotenv.2017.09.035

Tietze, D.T., (2018) Bird Species: How They Arise, Modify and Vanish. Springer Nature Switzerland AG pp 235 – 266

Tsiodras, S., T. Kelesidis, I. Kelesidis, U. Bauchinger and M.E. Falagas (2007) Human infections associated with wild birds. *The Journal of Infection* **56(2):**83-98; DOI: 10.1016/j.jinf.2007.11.001

Unno, T. (2015) Bioinformatic suggestions on MiSeq-based microbial community analysis. *Journal of Microbiology and Biotechnology* **25(6):**765-770; DOI:10.4014/jmb.1409.09057

Visca, P.I., A. Petrucca, P. De Mori, A. Festa, E. Boumis, A. Antinori and N. Petrosillo (2001) Community-acquired Acinetobacter radioresistens bacteremia in an HIV-positive patient **7(6):**1032-1035

Videvall, E., M. Strandh, A. Engelbrecht, S. Cloete and C.K. Cornwallis (2017) Measuring the gut microbiome in birds : comparison of faecal and cloacal sampling. *bioRxiv* **2017:**1-22. DOI: 10.1101/160564

Vlahovi, K., E. Prukner-radov and D. Horvatek (2010) Bacterial and fungal flora in fecal samples from rooks (*Corvus frugilegus*) in the City of Zagreb, Croatia. *Veterinarski Arhiv* **80 (1):**81-92

Waite, D.W., and M.W. Taylor (2015) Exploring the avian gut microbiota: Current trends and future directions. *Frontiers in Microbiology* **6(JUL):**1-12; DOI:10.3389/fmicb.2015.00673

Weerasundara, L., R.W.K. Amarasekera, D.N. Magana-Arachchi, A.M. Ziyath, D.G.G.P. Karunaratna, A. Goonetilleke, and M. Vithanage (2017) Microorganisms and heavy metals associated with atmospheric deposition in a congested urban environment of a developing country: Sri Lanka. *Science of the Total Environment* **584-585:** 803-812; DOI: 10.1016/j.scitotenv.2017.01.121

Wenger, .PN., J.I. Tokars, P. Brennan, C. Samel, L. Bland, M. Miller, L. Carson, M. Arduino, P. Edelstein, S. Aguero, C. Riddle, C. O'Hara and W. Jarvis (1997) An Outbreak of Enterobacter hormaechei Infection and Colonization in an Intensive Care Nursery. *Clinical Infectious Diseases* **24(6):**1243-1244

Yong, L.H., S. Ambu, S. Devi and M. Maung (2008) Detection of protozoan and bacterial pathogens of public health importance in faeces of Corv spp. (large-billed crow). *Tropic* *Biomedicine* **25(2):**134-139

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