



Microbial Diversity in Gut of Cattle Egrets (*Bubulcus Ibis*) On Exposure to Different Environment

Roheela Yasmeen^{1*}, Hifsa Ali Muhammad¹, Bushra Nisar Khan², Syeda Shazia Bokhari¹,
Uzma Rafi¹, Aisha Waheed Qurashi¹

1. Department of Biology, Lahore Garrison University, Lahore, Pakistan.
2. Undergraduate Department, University of the Punjab, Lahore, Pakistan.

*Corresponding author's email: roheelayasmeen@lgu.edu.pk

ABSTRACT: *Cattle egrets (Bubulcus ibis) normally nest near water and normally feed in grass or wetlands. But now, they are changing their habitat and are also seen feeding on crop fields and garbage dumping sites. The study was conducted to assess gut flora of locally matured cattle egret exposed to different environments. The sampling was carried out in winter season of 2017 at two sites of Lahore, Havalian Karbath (rural, site 1) and Mehmood Booti (urban, site 2). The microbial analysis was carried out to find out diversity in bacterial fauna present in gut (stomach and intestine) of cattle egrets. The total of eight bacterial isolates was collected from stomach and intestine of site 1 and site 2. The characteristics study of bacterial isolates was based on morphological and biochemical tests. The bacterial isolates from site 1 were noticed as Bacillus spp. and Staphylococcus spp. However, from site 2, Staphylococcus spp., Listeria spp. and Streptococcus spp. were isolated. It was concluded on morphological characterization of isolates that diverse morphology of bacteria associated with gut flora of cattle egret on exposure to different environmental sites. However, a detailed study at species level is required to identify disease causing ability of microbes.*

Key words: *Cattle egrets, environment, morphology, microbes*

INTRODUCTION

Cattle egrets (*Bubulcus ibis*) are little, extensively dispersed waders that normally nest in large reliance, near water and normally feed in grass or wetlands (Seedikkoya et al., 2005). They depend on insects like house fly, other invertebrates and on fish for food, particularly those that fly after being scared by related large mammals (Zhang et al., 2006; Kour and Sahi, 2012; Jayaratne et al., 2015). The world population of crows and White Ibis (*Nipponia Nippon*) has increased significantly since 1983 and these birds are frequently observed in close contact with people and in urban areas (Shaw, 2000; Aruji et al., 2004). The egret species are considered important as they are found feeding on garbage sites of Sri Lanka and result in controlling number of house flies (Jayaratne et al., 2015). On the other hand, Ni et al., (2011) reported little egrets as mechanical carrier of *Laribacter hongkongensis* that contaminate the water bodies via feces. The bacterial contaminated water sources potentially infect the human via eating contaminated fish or drinking contaminated water.

The avian species contain thousands of microbial species (Waite and Taylor, 2015; Fink et al., 2018). The birds microbial gut flora seems to be associated with number of functions like synthesis of vitamins, improvement of digestion and improving immunity (Xu, and Gordon, 2003; Heijtz et al., 2011; Al-Asmakh et al., 2014). However, birds such as water fowls (geese, swans) have great potential for disease transfer in poultry, livestock and humans (Kang et al., 2015 Elmberg et al., 2017; Silva et al., 2018). Ibis transmits pathogen not only in poultry but is

also responsible for public health concerns. The occurrence of different bacterial isolates in *White ibis* was documented by the separation of *Pseudomonas*, *Escherichia coli*, *Salmonella spp*, *Proteus* and *Pasteurella haemolytica* (Soad and Wafaa, 2003; Silva et al., 2018). In a different study, the same pathogens were isolated in addition to *Streptococcus faecalis*, *Arizona hydrophila* and *Staphylococcus aureus* (El-Sheshtawy and Moursi, 2005). In six septicaemic cases of young Crested Ibis, *E. coli* were isolated from different internal organs (*Bubulcus ibis*) (Xi et al., 2007). Moreover, *E. coli* and *Salmonella spp.* were isolated from free-living passerines (Morishita et al., 1999) and *Salmonella spp* and *Enterococcus spp.* has also been reported in free-living, wild birds (Craven et al., 2000; Yahia et al., 2018). The intestines of wild birds appear to be relatively opposing to *Salmonellosis* but may serve as effective carriers of *Salmonella* by shedding the organism in their feces and could be a source of infection for domestic poultry (Tizard, 2004). The *Klebsiella* micro-organism which acts as an opportunistic pathogen causes localized or systemic infection in poultry. It can also harbor upper respiratory tract of healthy birds (Abd-El Gwad and Hebat-Allah Mohamed, 2004).

There is scarce of data regarding microbial fauna in cattle egret species as these species are shifting their niche due to habitat loss and now mostly found, feeding on composting sites in urban areas and on crop fields in rural areas. They are exposed to all sorts of chemicals in the form of pesticides and industrial effluents in crop fields and all types of household and municipal wastes on composting sites. The microbial fauna has ability to cope with changes in the environment so aim of the present study is to

assess microbial diversity on exposure to different environment such as rural and urban sites.

MATERIALS AND METHODS

Sampling Site

The samples of Cattle egret were collected from two study sites; rural (site 1) and urban (site 2). Havelian Karbath on Bedian Road was a rural site situated 3km away from Nawaz Sharif Interchange and the urban site was at Mehmood Booti, a dumping site. Cattle egret of site 1 feeds on field that received chemicals from the use of fertilizers and water from canals that might get polluted by the waste coming from nearby industrial areas. However, egret of site 2 feeds on sewage and municipal waste and these were exposed to the toxic metals through food intake and dust ingestion.

Collection of samples and Bacterial isolation from gut of cattle egrets

Cattle egrets of both sites were dissected with the help of sterile dissecting tools. The stomach and intestine of each cattle egret were exposed and one gram of food sample was weighed and added in already labelled and autoclaved test tubes. 10 ml of normal saline solution was present in the test tubes. The Petri plates containing N- agar media were prepared and 50 μ L solution from each test tube was used for spreading on plates separately. Plates were incubated for 24 hours at 37 $^{\circ}$ C, isolates were purified and results were recorded (Cappuccino and Sherman, 2007). Different stainings such as simple, gram, spore and capsule staining were performed along with biochemical tests such

as Urease, Citrate, methyl red and voges Proskauer test (Table 2).

RESULTS AND DISCUSSION

The total of eight microbial isolates were collected, four from each site and morphological characteristics of all eight bacterial isolates were noticed and categorized on the basis of their morphological distinctiveness (Table 1), biochemical tests and gram staining as done by Tshikhudo et al., (2013). The *Bacillus spp.* and *Staphylococcus spp.* were isolated from gut of rural site while *Staphylococcus spp.*, *Listeria spp.* and *Streptococcus spp.* were separated from urban site. The study is in line for bacillus and staphylococcus spp. as these were also reported in oral, cloacal and fecal samples of water birds; however, were found absent in Cattle egret (Sarker, et al., 2012). In another study, the presence of pathogens such as *Streptococcus faecalis* and *Staphylococcus aureus* were reported along with some other species in water Ibis (Effat and Moursi, 2005). The existence of *Staphylococcus spp.* in White ibis like present study is also reported by Abdien et al., (2010). However, presence of various members of *Enterobacteriaceae* family was reported in healthy Ibis at Sharkia province of Egypt (Awad-Alla et al., 2010). Isolation of members of *Enterobacteriaceae spp.* along with *Staphylococcus spp.* were reported in different organs such as lungs, heart, blood and liver of cattle egrets (Awad-Alla et al., 2010). Cattle egrets act as mechanical carrier and are responsible for spread of *Laribacter hongkongensis*, a bacterium that contaminate the water bodies. The bacteria spread by fecal material of egrets and can cause gastroenteritis and diarrhea in humans as reported by Ni et al., (2010). *Listeria spp.* are present in gut of cattle egrets

collected from urban sites. These are gram positive strains that usually spread by feces of animals and have ability to cause infections in men and ruminants (Vazquez-Boland et al., 2001; Nightingale et al., 2004; Guillet et al., 2010; Ferreira et al., 2014). Brittingham et al., (1988) surveyed 364 wild birds (passerines and wood peckers) and gave an account of six most commonly occurring genera found in birds including *Staphylococcus spp.* and *Streptococcus spp.* with prevalence of 15% and 18% respectively. Moreover, according to Benskin et al., (2009) the gut flora represents the habitat in which birds usually live. The *Staphylococcus spp.* is responsible

for food poisoning and various other intestinal diseases in birds (Le Loir et al., 2003). *Streptococcus spp.* results in many diseases like sore throat, contagious skin infections and scarlet fever. Similarly, bacillus spp. is also responsible for various problems like meningitis, cerebero-spinal fluid shunt infections, food borne diseases and wound infections (Barson, 1989; Patterson, 1996; Sarker et al., 2012). All the isolated genera from two sites are different for *Bacillus spp.*, *Listeria spp.* and *Streptococcus spp.* but similar to *Staphylococcus spp.* However, a more detailed study is required to see microbial diversity up to species levels.

Table 1: Morphological characters of different isolates collected from site 1 and site 2.

Bacterial isolates	Form	Pigmentation	Margin	Elevation
Isolate 1	Circular	Off white	Entire	Raised
Isolate 2	Circular	Off white	Entire	Raised
Isolate 3	Circular	Off white	Entire	Raised
Isolate 4	Circular	Off white	Entire	Raised
Isolate 5	Circular	Yellow	Entire	Raised
Isolate 6	Circular	White	Entire	Raised
Isolate 7	Circular	Off white	Entire	Raised
Isolate 8	Circular	Off white	Entire	Raised

Table 2: Biochemical tests of different isolates collected from site 1 and site 2.

Biochemical Tests	Isolate1	Isolate2	Isolate3	Isolate4	Isolate5	Isolate6	Isolate7	Isolate8
Urease test	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve
MR- test	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
VP-test	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve
Simmon citrate test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

CONCLUSION

This study was set out to estimate microbial characterization of gut flora in intestine of cattle egrets collected from Havelian Karbath (Site 1) and Mehmood Booti (Site 2) of Lahore areas. The microbial fauna was morphologically and biochemically characterized. Microbial diversity was noticed in bacterial isolates of cattle egret on exposure to different sites. However, more efforts are required for identification of species, to compare microbial diversity with changing environment and to know the disease transferring capability of isolated genera.

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