ISOLATION OF BACTERIA AND FUNGI FROM THE DROPPINGS OF WESTERN HOUSE MARTIN (*Delichon urbicum*) IN ELELE, NIGERIA.

Abstract

The Western House Martin, scientifically known as *Delichon urbicum* is a small migratory passerine bird in the swallow family. It is the most prevalent house bird found in Elele Rivers State Nigeria. Sparrow is differentiated from *Delichon urbicum* by the type of nest they build as the former builds its own with grass whereas the latter builds with mud. The droppings of Western house martin (*Delichon urbicum*) present a unique habitat potentially rich in microbial diversity yet remains understudied in microbiological research. This study aimed to identify bacteria and fungi inhabiting Western house martin droppings Elele to bridge the gap in our understanding of their microbial ecology. Droppings were collected from birds whose nesting sites are in Madonna University which include: Edeani hostel, Emmanuel hostel, mother of Jesus the Saviour hostel, Marian podium, medical auditorium, special canteen and Barnabas hostel. Samples are collected by easy-to-build box which is made up of a plastic box, a plastic tray and a Sterile gauze. for a noninvasive fecal collection method, droppings were collected into a sterile universal container and was analyzed using culture-based method. Bacteria were isolated and identified through colonial morphology, gram staining, biochemical tests such as catalase, indole, urease, citrate test etc. Fungal isolates were identified based on morphology, microscopic examination. A total of twenty-one (21) sample was collected and cultured yielding nine 9(42.9%) positive results for both bacteria and fungi. Results revealed a diverse microbial community in Western house martin dungs, encompassing bacterial genera such as *Salmonella* spp 5(55.5%)*, Escherichia spp* 2(22.2%)*, Campylobacter* spp 2(22.2%) and fungal species including *Aspergillus* spp 3(33.3%)*, Candida* spp 3(33.3%), *Mucor* spp 2(22.2%), *Chrysosporium* spp 1(11.1) with a *P* value <0.05 which was considered not significant, meaning deviation from the null hypothesis is not statistically significant, and the null hypothesis is not rejected. The sites with the highest number of bacteria identified are Emmanuel hostel and special canteen while of fungi: Edeani hostel, Mother of Jesus the saviour and medical auditorium. The study identified potential pathogenic and beneficial microorganisms as well as contributing to ecological knowledge by highlighting the role of Western house martins as dispersers of microorganisms and their potential impacts on local ecosystems. It also underscores the importance of understanding microbial interactions within avian dung for ecological conservation and public health implications. These health implications include possible transmissions of pathogens to human especially food handlers. Recommendation includes proper food handling among students and pilgrims, disinfection of area concentrated with the bird droppings to avoid salmonella, campylobacter and other possible zoonotic infections, periodic removal of bird nest, proper sanitary measures and health check for proper diagnosis of infection.

Key Words: Bacterial, fungi, *Delichon urbicum* and Elele

INTRODUCTION

The Western House Martin, scientifically known as *Delichon urbicum* is a small migratory passerine bird in the swallow family. It is also commonly referred to simply as the House Martin. This bird is notable for its distinctive appearance and behavior. It has a glossy blue-black upperparts and pure white underparts. Their short tail is slightly forked, and they have a white rump, which is quite visible in flight. The legs and feet are feathered, which is unique among swallows. House Martins breed across Europe and parts of Asia, particularly favoring open country with access to mud for nest-building and proximity to water sources. During the winter, they migrate to sub-Saharan Africa (1).
These birds are known for their aerial acrobatics as they catch insects' mid-flight. They build their nests out of mud, typically under the eaves of buildings where humans reside, which provides them with some protection from predators and weather. The close proximity of their nest to humans further increases the possibility of zoonotic infection on human. House Martins are social birds and often nest in colonies. Their nests are closed structures with a small entrance hole, made from pellets of mud mixed with grass and other plant fibers (2).

Their diet primarily consists of flying insects, which they capture in the air. This makes them beneficial for controlling insect populations in their habitats. The House Martin is currently not considered to be under threat and has a status of Least Concern, although their populations can be locally affected by changes in their environment, such as the availability of suitable nesting sites and food sources (3).

These small, agile birds are known for their distinctive white rumps and habit of building mud nests under eaves and cliffs. They play a crucial role in controlling insect populations, making them valuable to ecosystems and human interests, particularly in agricultural settings. The dungs of this house bird could also be a source of viral, parasitic, bacterial or fungi infection because of the close proximity of these dungs to humans (4). It is important to note that there could be limited knowledge on the possible pathogens in the dungs of a *Delichon urbicum* which is an important factor in public health. This research hope to address major public health concerns arising from the co-habitation of birds and humans in other to improve human safety and also understand ecological diversity.

The adult western house martin is 13 cm (5 in) long, with a wingspan of 26–29 cm and a weight averaging 18.3 g. It is steel-blue above with a white rump, and white underparts, including the underwings; even its short legs have white downy feathering. It has brown eyes and a small black bill, and its toes and exposed parts of the legs are pink. The sexes are similar, but the juvenile bird is sooty black, and some of its wing coverts and quills have white tips and edgings. *Delichon urbicum lagopodum* differs from the nominate race in that its white rump extends much further onto the tail, and the fork of its tail is intermediate in depth between that of *Delichon urbicum urbicum* and that of the Asian house martin (5).

The study of bacteria and fungi from the droppings of Western house martin is essential for several reasons. Firstly, it provides insights into potential pathogenic or beneficial microorganisms associated with these birds, impacting both animal and human health. Secondly, understanding the microbial diversity in these dungs contributes to broader ecological knowledge, aiding in the comprehension of the possibility of animal to human transmission of infection. Lastly, this study contributes to the conservation of Western house martin populations by assessing their impact on local ecosystems.

MATERIAL AND METHODS

This research work was carried out in Madonna University Elele, Rivers state between January to June 2024. The university was established in 1999 as the first private university in Nigeria and the first catholic university in West Africa. Madonna University is located in Elele located in Ikwerre local government area situated in River's state, Nigeria. It is located at the south-south geographical zone in Nigeria with a population of five million, one hundred and eighty-five thousand four hundred (5,185,400) and with and average temperature of about $37^{O}C$ during the day and about $28^{O}C$ during harmattan. Madonna University comprises of a university and a pilgrimage centre and it is graciously surrounded by dense forest which gives home to natural livestock.

Sample collection

Bird droppings were collected from various nesting site in Madonna hostel auditorium, which includes Edeani hostel, Emmanuel hostel, mother of Jesus the saviour, Marian podium, Medical Auditorium, special canteen and Barnabas hostel. The easy-to-build box for a noninvasive fecal collection method was adopted for sample collection.

Identifications of bacteria and fungi isolates

The in-vitro isolation media used for this study were: MacConkey Agar, Blood Agar, Nutrient Agar, Salmonella-Shigella agar and Sabouraud dextrose agar for fungi. The plates inoculated for bacterial identification were incubated at $37^{O}C$ for 24hrs while for fungi identification at room temperature $(22-25^{o}C)$ and $37^{O}C$ for 7- 14days with daily check for growth. The incubated plate media were observed for colonial growth, gram stained and biochemically tested, direct microscopy and slide culture with staining technique for fungi. Uninoculated plates was used as controls and incubated during culturing procedures to check for contamination.

The different media used for the study include Sabouraud dextrose agar (SDA), MacConkey agar (MA), Nutrient agar (NA), Blood agar (BA), Salmonella-Shigella agar (SSA), Eosin methylene blue agar, Simmon's citrate agar, Christensen's urea agar, Kligler iron agar (KIA) and peptone water. A given amount of each media was measured according to the manufacturers' instruction and dissolved in distilled water in a sterile conical flask. The conical flask was covered with aluminum foil and autoclaved for sterilization at $121^{0}\mathrm{C}$ for 15 minutes and allowed to cool at which agar remain molten. Salmonella-Shigella agar was sterilized by heating for 30 minutes and not autoclaving. Blood agar and chocolate agar were not autoclaved but prepared after autoclaving nutrient agar (6).

2grams of each dropping was weighed into sterile tubes. Sterile physiological saline was added to the tubes and mix thoroughly to create dropping slurry. 0.1ml of the dropping slurry was pipetted and inoculated onto appropriate agar media (Nutrient agar, MacConkey agar, Blood agar, Salmonella-Shigella agar, Sabouraud dextrose agar) and evenly distributed using a sterile spreader. The plates were incubated at appropriate temperatures (37°C) for 24-48 hours and weeks for fungi. After incubation, the isolates were aseptically sub cultured to fresh agar plate to obtain pure culture and incubated. The colony morphology of isolates was observed and recorded. Representative colonies were selected for further biochemical testing and sub cultured to selective medium. Following aseptic procedures, the identification of isolates after incubation was done by using some characteristics which includes colonial morphology, staining technique and biochemical test.

Results and discussion

Table 1. Represent the results of bacteria identification from the dung of *Delichon urbicum* conducted at different site in Madonna University. The table lists the number of samples collected which altogether summed 21 and the number of isolates for three types of bacteria identified: *Salmonella* spp*, Escherichia coli, Campylobacter* spp.

The dung of *Delichon urbicum* has been studied for bacteria and fungi that can be isolated from it. Table 1 shows the predominate bacteria isolated which varies across different site. A total of 21 samples was collected from 7 different sites and analyzed bacteriologically by culture method. The sites include: Edeani hostel, Emmanuel hostel, mother of Jesus the Savior (MJS) hostel, Marian podium, special canteen, medical auditorium and Saint Barnabas hostel. These sites had varying number of samples collected which was as a result of the number of bird nesting in the location, periodic removal of the nest by cleaners of this site. Edeani, Mother of Jesus the saviour, special canteen had a total of 3 samples collected from the sites. Emmanuel and medical auditorium had a number of 4 samples collected whereas Marian podium, Barnabas hostel had 2 sample collected from the site. Out of the 21 samples obtained, 9 isolates were

obtained from these sites. The bacteria isolated from the dungs are *Salmonella* spp 5(55.5%), *Campylobacter* spp 2(22.2%) and *Escherichia coli* 2(22.2%) having a prevalence of 9(42.9%).

Table 1: Bacteria identified from different sites in Madonna University having 21 samples collected from 7 sites

SITE	Number of samples collected	NUMBER OF ISOLATE		
		Salmonella spp	Escherichia coli	Campylobacter spp
Edeani hostel	3	1	0	0
Emmanuel hostel	4	1	1	1
MJS hostel	3	0	0	0
Marian podium	2	1	0	0
Special canteen	3	0	1	1
Medical auditorium	4	1	0	0
Barnabas hostel	2	1	0	0
Total	21	5	2	2

Fischer exact test statistic value is 1.

($X^2 = 25.667,$ 0.370)

P<0.05……..not significant.

Salmonella spp have higher predominance in the dung of this specie of bird. It is followed by *Campylobacter* spp and *Escherichia coli* (an Enterobacteriaceae). This has similarities with the work done on other avian specie conducted by various researchers referenced in this study as most bacteria isolated from them included *Salmonella* spp, *Escherichia coli* and *Campylobacter* spp, although other bacteria species were identified. *Salmonella* spp was isolated at multiple locations which include Edeani hostel, Emmanuel hostel, Marian podium, and medical auditorium and Barnabas hostel. *Escherichia coli* were isolated from Emmanuel hostel and special canteen. *Campylobacter* spp was isolated from Emmanuel and special canteen. Emmanuel hostel and special canteen showed presence of all three bacteria tested indicating potentially high contamination levels or possibly a high diverse bacterial presence in these areas. The study shows the P value <0.05 is considered not significant.

The specie of these bacteria was not confirmed because molecular based technique using polymerase chain reaction was not studied. Hence, the study of the strain of these bacteria is subject to further analysis. Reasons for the isolation of these bacterium could be as a result of environmental factor which include temperature, humidity as the school is richly surrounded by forest with cool temperature yearly, level of sanitary practices, specific activities such as food handling in canteens. Dwellers in this sites which include student and pilgrims coming for church activities are exposed to these pathogens which could lead to bacterial infection. For instance, *Salmonella* spp which affect human and animals and *Escherichia coli* pose

a serious health risk such as food poisoning and gastroenteritis. Therefore, proper health hygiene such as proper food handling, regular disinfection and health attention is required in the site of higher prevalence of the bacterium.

The study conducted is not similar to the bacteriological study by Margaret *et al*. (1988) (7) which showed that *Escherichia coli* isolated was 1% and *Salmonella* spp was 0% from the cloacal swabs from 364 passerines and wood-peckers bird, indicating *Escherichia coli* as a possible cause of bacterial infection from bird. Similar findings were observed in the study by Rashed *et al*. (2022) (8) which showed that *Campylobacter* spp, *Escherichia coli*, *Salmonella* spp was isolated from the pigeons. This shows that these bacteria are predominant in pigeons and hence transmitted to humans.The study also agrees with research conducted by Asma Afshari *et al*. (2018) (9) which shows a high prevalence of *Salmonella* spp (14%) out of 100 samples collected having 43% as *Salmonella enteritidis* and 36% as *Salmonella typhimuruim* from poultry carcasses.

Table 2: Fungi identified from 7 sites in Madonna University having 21 samples collected.

		NUMBER OF ISOLATES				
SITE	samples collected (N)	*Aspergillus* spp	*Candida* spp	Mucor spp	*Chrysosporium* spp	P value
Edeani hostel	3	0	0	1	1	0.30
Emmanuel hostel	4	1	0	0	0	0.60
MJS hostel	3	0	2	0	0	0.06
Marian podium	2	1	0	0	0	0.13
Special canteen	3	0	0	1	0	0.38
Medical auditorium	4	1	1	0	0	0.18
Barnabas hostel	2	0	0	0	0	0.14
Total	21	3	3	2	1	

($X^2 = 24.545$)

P<0.05……not significant.

Out of 21 samples collected from 7 different site, a total of 9 (42.9) fungi was isolated. The fungi isolated from the dungs are *Aspergillus* spp 3(33.3%), *Candida* spp 3(33.3%), Mucor spp 2(22.2%), *Chrysosporium* spp 1(11.1%) having a p value <0.05 which is considered not significant. This has similarities with the work done on other avian specie conducted by various researchers referenced in this study as most fungi isolated from them included *Aspergillus* spp*, Candida* spp*, Mucor* spp. Although other fungus species were identified by former researchers, *Chrysosporium* spp was not identified in any of their work. *Aspergillus* spp. 3(33.3%) and *Candida* spp. 3(33.3%) were predominantly isolated. The prevalent fungus *Aspergillus* spp causes aspergillosis which present clinical manifestation of pneumonia-like symptom and is easily misdiagnosed whereas *Candida* spp. causes candidiasis which affect immunocompromised individual. Mucor spp. 2(22.2%) and *Chrysosporium* spp. 1(11.1%) were less prevalent but still present could further proliferate and cause infection.

Aspergillus spp. was found in multiple locations which include Marian podium, Emmanuel hostel, medical auditorium. *Candida* spp. was found in different locations which include Mother of Jesus the saviour hostel, special canteen, medical auditorium. Mucor spp. was isolated from Edeani hostel while *Chrysosporium* spp. was isolated from Edeani hostel. The location with the highest prevalence of fungi isolated is Edeani hostel, special canteen and medical auditorium. Hence, dwellers in this location are prone to have this fungi infection if personal hygiene and sanitary precaution are not duly practiced. Although, other locations had fungi isolated from their site, proper sanitary practices and personal hygiene must be duly observed. Barnabas hostel is free from fungi isolate. These isolates could be as a result of environmental factors such as warm and humid temperature, human activity such as moisture, cooking or cleaning, poor air ventilation. The specie of this fungus was not identified because molecular based technique involving polymerase chain reaction was not studied. Hence the study of the strain of this fungus is subject to further analysis. Reasons for the isolation of these fungus could be as a result of environmental factor which include temperature, humidity as the school is richly surrounded by forest with cool temperature yearly, level of sanitary practices. Dwellers in this sites which include student and pilgrims coming for church activities are exposed to these pathogens which could lead to fungi infection.

Fungi studies done by various researchers in the past two decade shows *Aspergillus* spp, *Candida* spp, *Cryptococcus neoformans* were predominately isolated (10). Similar findings were observed by Mahdis *et al*. (2023) (11) which showed prevalence of *Aspergillus* spp as 6.66% from fecal sample of pigeon.Similarly, a similar study conducted by Mirhosseni and Khorsravi (2023) (12) shows a high prevalence of *Aspergillus* spp isolated accompanied by *Mucor* and *Candida* spp from the fecal samples of ornamental birds of the passerines, psittacine and Columbiformes family in Iran. Their study was similar to a study conducted in Brazil by Arne *et al.* (2021) (13). The study also agrees with the observation conducted by Josiara *et al*. (2014) which shows a prevalence of *Candida* spp accompanied by *Aspergillus* spp and *Mucor* from the excreta of wild birds in Brazil. A fungus, *Chrysosporium* spp 1(4.8%) was isolated from the dung of *Delichon urbicum* and was not recorded in any study as mentioned in this context. It is said to be a ubiquitous mold fungus as it is seen on soil, feather, hair and skin of mammals, reptiles and birds. It is a keratophillic fungus having the ability to digest keratin (14).

CONCLUSION

The evidence from this study showed that the rate of fungal and bacteria contamination in western house martin dung was not significant. Although, it has ecological, health and environmental implications posing threat to human and avian health and contributing to soil fertility and decomposition of organic matter. Keeping the birds in close contact with humans can double the importance of these contaminations and provide the basis for the transmission of contamination to humans. Therefore, the accumulations of western house martin faeces for long periods suggest a potential threat to public health. This diversity could result from its diet, exposure, and physiological state of the bird. It has also helped to bridge the gap in the current understanding of the specific interactions between *Delichon urbicum* and associated micro biota. Further research should also be conducted on most animal dungs like dogs, pigeons, cat and other mammals whose habitats are within human settlements in other to reduce zoonotic infections.

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