

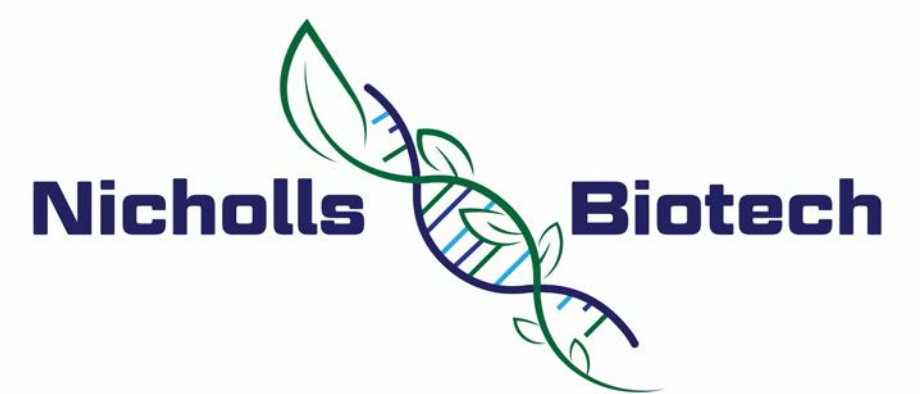
Presence of Antibiotic Resistant Bacteria and Antibiotic Resistant Genes in the Migratory Birds of Louisiana

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Abstract

Since the dawn of the commercialization of antibiotics, antibiotic resistant bacteria (ARB's) and antibiotic resistance genes (ARG's) have been a rapidly growing problem. As international travel is popularized, these ARB's and ARG's are able to move to new places using humans as a vector in a somewhat anthropogenic way. However, these potentially harmful bacteria and genes could be spreading in another way less reliant on human involvement. The Nicholls State University Biotechnology lab has been studying the increasing presence of ARB's and ARG's in local waterways for a number of years now. These ARB's and ARG's have been correlated to the consumer, commercial, and hospital related improper disposal of antibiotics and their presence in the waters of Southeast Louisiana is well studied. Also present in these waterways are several species of migratory birds which use Louisiana either as a stopover point or endpoint for migration along the Mississippi Flyway route. It is possible that these migratory birds could serve as a vector for the aforementioned ARB's and ARG's, allowing these antibiotic resistant bacteria and genes to travel all along the migratory route, from the southern coast of the United States to central Canada and everywhere in between. In this study, fecal and cloacal bacterial samples were taken from Louisiana migratory birds. These samples were enriched in TSB and streaked onto TSA to collect isolates. These isolates were then tested for the presence of ARB's and ARG's using the Kirby Bauer Assay and PCR techniques respectively. Identification of bacteria was achieved using 16sRNA sequencing techniques.

Introduction

Antibiotic resistance in bacteria is a natural phenomenon arising from the selective pressure of exposure to clinical antibiotics and is also caused by misuse and overuse of antibiotics. This can cause considerable public health problems. Within the past decade alone, the Center for Disease Control has identified various bacteria as posing serious, urgent, and concerning threats to the US healthcare system due to its resistance to antibiotics (CDC, 2013). Improper usage and disposal of antibiotics by consumers, hospitals, industries, and sewage treatment plants has furthered the emergence of antibiotic resistance in the waterways of Louisiana. Furthermore, antibiotic resistant bacteria (ARB) has been found in raw sewage and treated sewage which is released into the environment in the waterways. Ultimately, the ARB and antibiotic resistance genes (ARGs) in the treatment plants affect wildlife, where the treated water is being released (Naquin et al., 2015). These water sources are home to much of Louisiana's wildlife including migratory birds. Because Louisiana is part of the Mississippi Flyway migratory route, it is possible that birds may be serving as a vector for transportation of antibiotic resistant and antibiotic resistant bacterial genes from our waterways all throughout North America leading to an overall increase in antibiotic resistance on the continent.

Objectives:

Isolate and identify various antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARG) in the fecal matter and cloacae of migratory birds in Louisiana. Study the ecological relevance of the ARB and ARGs in our waterways. Create public awareness on the presence of ARB and ARGs in the local water samples and seafood.

Methods:

- Pure cultures of bacteria were isolated and identified from various migratory bird samples. Colonies were isolated as pure cultures in Tryptic Soy Agar (TSA) using quadrant streak method from various local water and seafood samples listed in the result section.
- Antibiotic resistance was observed using Kirby-Bauer Assay: Pure Cultures were streaked as a bacterial lawn onto Mueller Hinton (MH) Agar and antibiotic discs were stamped onto the agar using an antibiotic disc dispenser.

Methods cont.:

The following six antibiotics were used: Clindamycin (CC), Vancomycin (VA), Streptomycin (S), Amoxicillin (AMC), Bacitracin (B), and Penicillin (P). Plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured. Bacteria were classified as Susceptible (no color), Intermediate (yellow), or Resistant (red).



Image 1. Antibiotic resistant bacteria plated on Mueller Hinton Agar (Kirby Bauer assay)

Antibiotic	Susceptible	Intermediate	Resistant
Clindamycin (CC)	≤ 14	> 14 & ≤ 20	> 20
Vancomycin (VA)	≤ 9	> 9 & ≤ 11	> 11
Streptomycin (S)	≤ 11	> 11 & ≤ 14	> 14
Amoxicillin (AMC)	≤ 19	> 19 & ≤ 17	> 17
Bacitracin (B)	≤ 8	> 8 & ≤ 12	> 12
Penicillin (P)	≤ 19	> 19 & ≤ 27	> 27

Table 1. Antibiotic susceptibility standards used for assessing Kirby Bauer assay

Results:



Image 2. Ruby-crowned Kinglet (Regulus calendula). Photograph obtained by Delaina LeBlanc

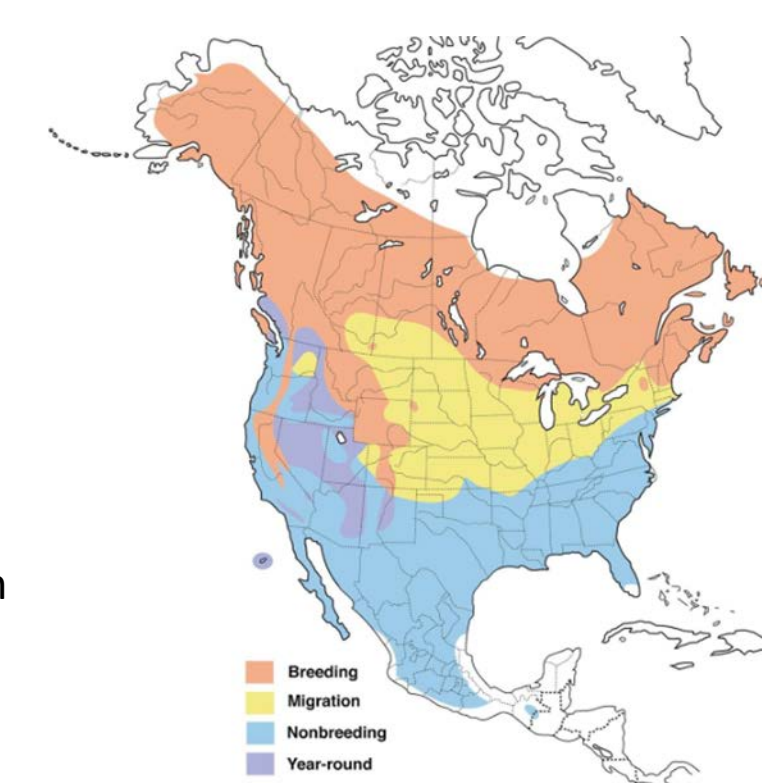


Image 3. Ruby-crowned Kinglet range map taken from the Cornell Lab's website "All About Birds"

Antibiotic	Consortium	Isolate a	Isolate b
AMC	11.97	0	0
B	0	0	0
VA	0	0	0
P	0	0	0
CC	0	0	0
S	11.76	14.24	11.91

Table 2. Kirby Bauer results for Ruby-crowned Kinglet #1

Antibiotic	Consortium	Isolate a	Isolate b
AMC	0	0	0
B	0	0	0
VA	0	0	0
P	0	0	0
CC	0	0	0
S	12.78	12.78	12.78

Table 3. Kirby Bauer results for Ruby-crowned Kinglet #2

Antibiotic	Consortium	Isolate a	Isolate b
AMC	9.99	0	0
B	0	0	0
VA	0	0	0
P	0	0	0
CC	12.14	12.14	12.14
S	11.76	11.76	11.76

Table 4. Kirby Bauer results for Ruby-crowned Kinglet #3



Image 4. White-eyed Vireo (Vireo griseus). Photograph obtained by Delaina LeBlanc

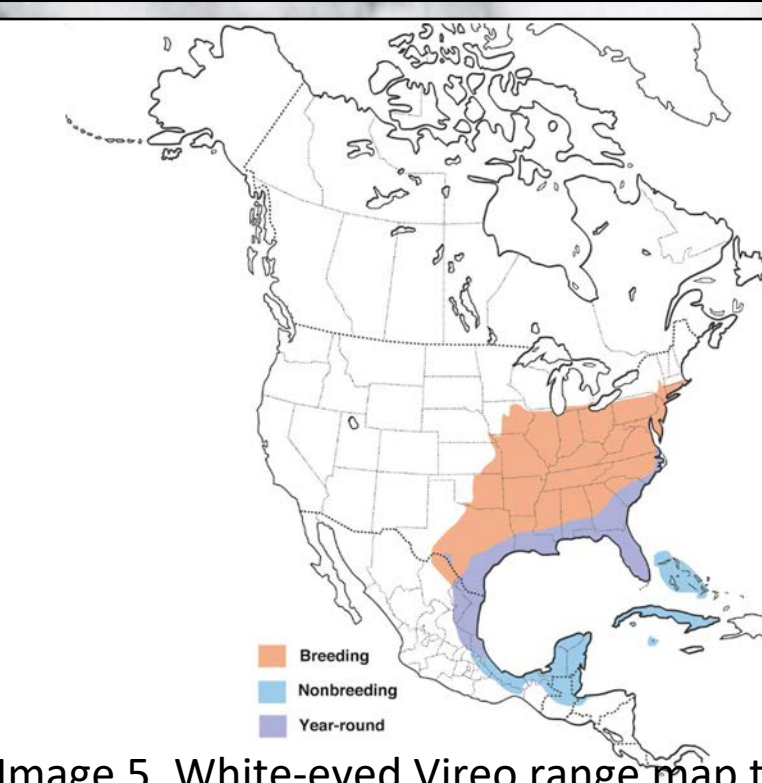


Image 5. White-eyed Vireo range map taken from the Cornell Lab's website "All About Birds"

Antibiotic	Consortium	Isolate a	Isolate b
AMC	26.67	0	0
B	0	0	0
VA	0	0	0
P	0	0	0
CC	14.07	14.07	14.07
S	11.91	11.91	11.91

Table 5. Kirby Bauer results for White-eyed Vireo



Image 6. Orange-crowned Warbler (Vermivora celata). Photograph obtained by Delaina LeBlanc

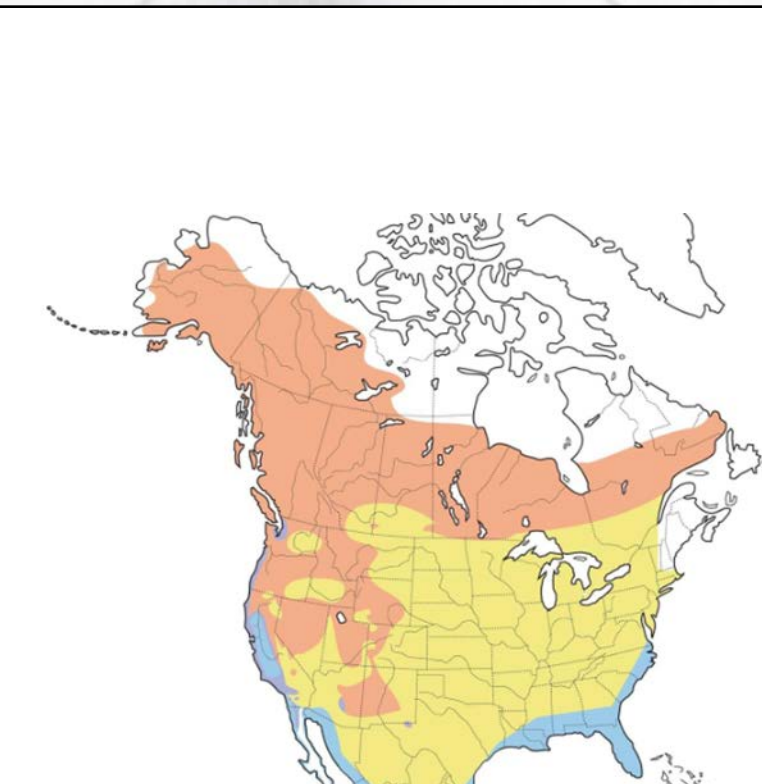


Image 7. Orange-crowned Warbler range map taken from the Cornell Lab's website "All About Birds"

Antibiotic	Consortium	Isolate a	Isolate b
AMC	37.58	14	16.16
B	11	16.64	24.7
VA	18.72	26.66	23.74
P	19.36	17.66	17.66
CC	26.6	26	19.03
S	10.14	22.68	12.25

Table 6. Kirby Bauer results for Orange-crowned Warbler #1

Antibiotic	Consortium	Isolate a	Isolate b
AMC	14.57	0	0
B	0	0	0
VA	0	0	0
P	0	0	0
CC	14.51	14.51	14.51
S	14.71	14.71	14.71

Table 7. Kirby Bauer results for Orange-crowned Warbler #2



Image 8. Swamp Sparrow (Melospiza georgiana). Image taken from the Cornell Lab's website "All About Birds"

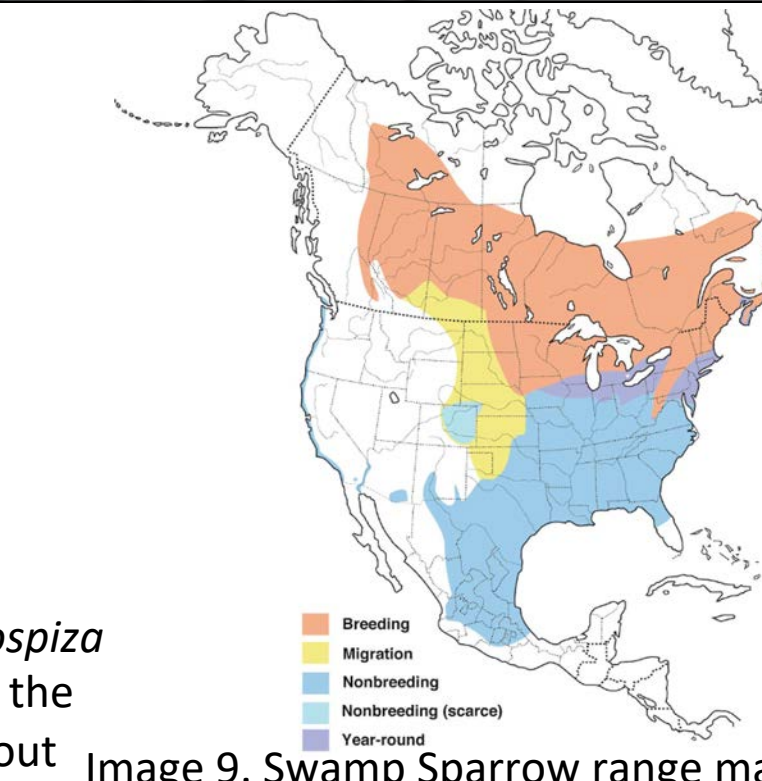


Image 9. Swamp Sparrow range map taken from the Cornell Lab's website "All About Birds"

Antibiotic	Consortium	Isolate a	Isolate b
AMC	0	0	0
B	0	0	0
VA	0	0	0
P	0	0	0
CC	14.69	14.69	14.69
S	17.7	17.7	17.7

Table 8. Kirby Bauer results for Swamp Sparrow



Image 10. House Sparrow (Passer domesticus). Image taken from the Cornell Lab's website "All About Birds"

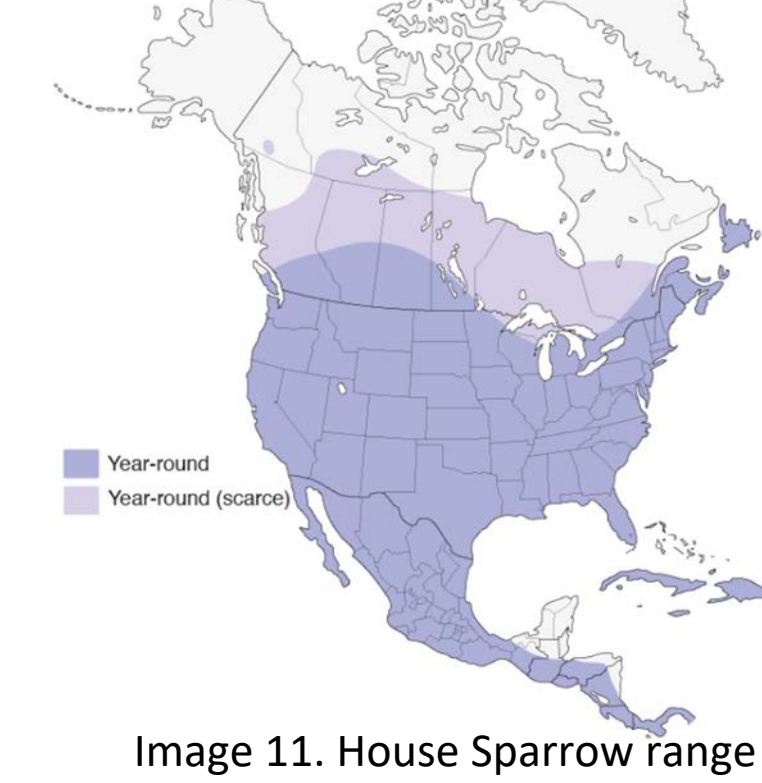


Image 11. House Sparrow range map taken from the Cornell Lab's website "All About Birds"

Antibiotic	Consortium	Isolate a	Isolate b
AMC	22.5	24.5	14.03
B	20.36	10.76	15.24
VA	0	0	0
P	0	0	0
CC	18.06	18.06	18.06
S	14.7	14.7	14.7

Table 9. Kirby Bauer results for House Sparrow

Results cont.:



Image 12. Northern Cardinal (Cardinalis cardinalis). Photograph obtained by Delaina LeBlanc

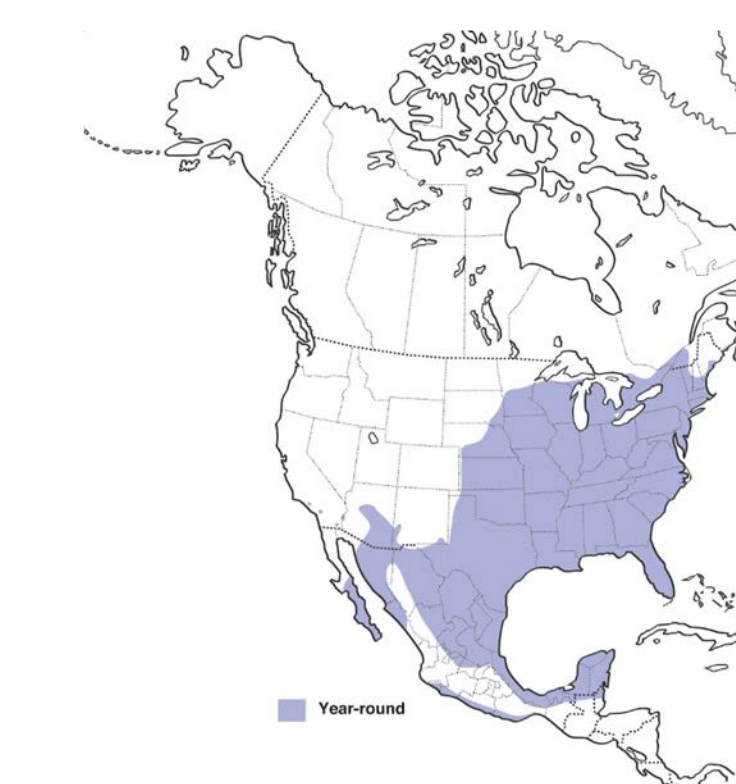


Image 13. Ruby-crowned Kinglet range map taken from the Cornell Lab's website "All About Birds"

Antibiotic	Consortium	Isolate a	Isolate b
AMC	23.87	0	0
B	0	0	0
VA	0	0	0
P	0	0	0
CC	14.23	14.23	14.23
S	15.39	15.39	15.39

Table 10. Kirby Bauer results for Northern Cardinal #1

Antibiotic	Consortium	Isolate a	Isolate b
AMC	21.14	0	0
B	19.09	0	0
VA	0	0	0
P	0	0	0
CC	15.71	15.71	15.71
S	15.69	15.69	15.69

Table 11. Kirby Bauer results for Northern Cardinal #2

Antibiotic	Consortium	Isolate a	Isolate b
AMC	20.14	0	0
B	21.24	0	0
VA	14.11	12.24	26.7
P	26.7	26.7	26.7
CC	15.23	15.23	15.23
S	15.23	15.23	15.23

Table 12. Kirby Bauer results for Northern Cardinal #3

Discussion and Conclusions:

Bird fecal and cloacae samples were collected from several birds at a few different sites in Louisiana and both the consortiums and isolates taken from the consortiums were tested for the presence of antibiotic resistant bacteria. All the bird fecal samples and most of the bird cloacae samples tested showed the presence of various antibiotic resistant bacteria. The presence of antibiotics in our waterways will exert selection pressure on bacteria to develop antibiotic resistance which will inevitably lead to migratory birds spreading these antibiotic resistant bacteria throughout North America. Horizontal transfer of antibiotic resistance genes will allow non-native bacteria introduced by birds to spread their antibiotic resistance to native bacteria.

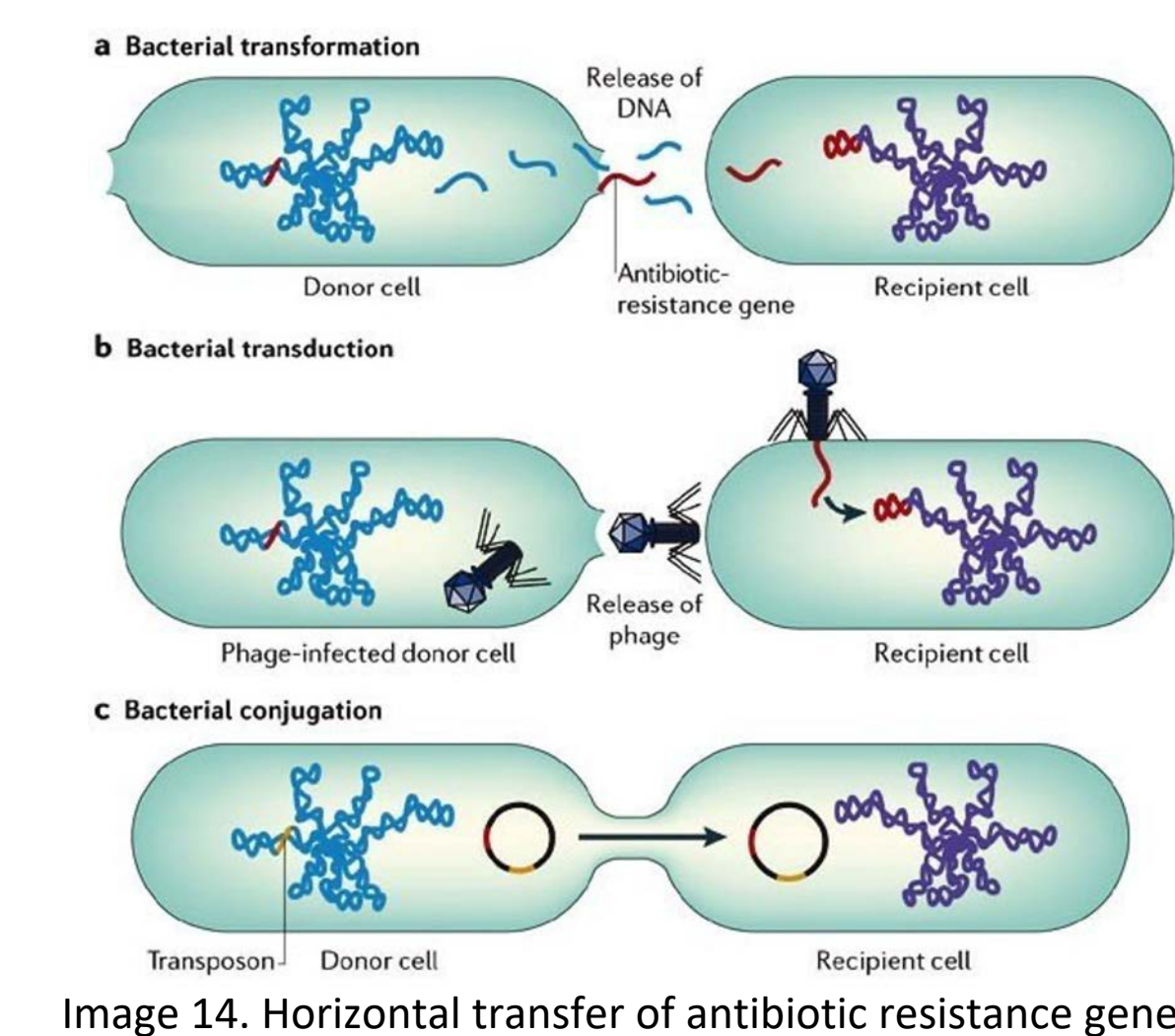


Image 14. Horizontal transfer of antibiotic resistance genes

Future Work:

Future work will focus on identifying the isolates obtained from the bird sample consortiums and identifying the presence of antibiotic resistant genes within these isolates. Future work will also focus on obtaining water samples from the sites where the bird samples were collected, and testing these sites for the presence of antibiotics, antibiotic resistant bacteria, and antibiotic resistant genes as well as performing water quality tests on the areas.

Citations:

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