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Louisiana Birds Act as Reservoirs for Antibiotic-resistant Bacteria

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LOUISIANA BIRDS ACT AS RESERVOIRS FOR ANTIBIOTIC-RESISTANT BACTERIA

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Table of Contents

List of Tables

List of Figures

Abstract

Wild birds carry diverse microbial communities, including antibiotic-resistant bacteria (ARB). With the ever-increasing use of antibiotics in agricultural and clinical settings, genes that code for antibiotic resistance in bacteria have been selected for. These bacteria persist in the environment in a culturable state, but little is understood about communities of antibiotic-resistant bacteria in the environment. Birds with predictable behaviors may serve as useful indicators of these communities, and provide insights into how bacterial communities spread and evolve in the environment. To understand the utility of birds as indicators of the presence of antibiotic-resistant bacteria in the environment, we collected bacterial samples from forest birds in a cypress-tupelo/bottomland hardwood forest fragment surrounded by urban Baton Rouge, Louisiana. Densities of total and antibiotic-resistant bacteria varied by bird sex, age group, and foraging guild. Specifically, female birds had a higher prevalence of antibiotic-resistant bacteria than males, juvenile birds carried higher densities of antibiotic-resistant bacteria than adult birds, and tree-foraging birds carried higher densities than did ground-foraging birds. These data suggest that specific behaviors from each group may be associated with higher colonization by antibiotic-resistant bacteria and that birds may be useful indicators of contamination by viable potential pathogens in the environment. In a separate analysis, we sequenced the 16S rRNA gene from almost 100 isolates and used BLASTn analysis to determine the lowest possible taxonomic level for each sequence. We found that there were four orders of bacteria present from all of our samples; Lactobacillales, Pseudomonadales, Bacillales, and Enterbacterales. The Louisiana birds sampled in this study yielded a diverse array of bacteria, and highlighted the importance of future studies of antibiotic-resistant bacteria in birds.

CHAPTER 1. GENERAL INTRODUCTION

1.1 History of Antibiotics and their Use

Antibiotics are defined as a chemical product that inhibits growth of a microorganism There are 3 ways of obtaining antibiotics; harvesting from another microorganism, creation of synthetic antibiotics, and semi-synthesis of antibiotics, which is a combination of the first two methods (27). Antibiotics operate in two distinct functions, either killing bacteria, called bactericidal, or inhibiting bacterial growth, called bacteriostatic (30). Both types of antibiotics may target any one of five specific areas in the bacterial cell to kill or inhibit bacteria. These five pathways include cell wall growth inhibition, protein synthesis inhibition, DNA/RNA inhibition, metabolic pathway blockage, and destruction of cell membrane (97). Antibiotics used to treat infections have been used throughout history, dating back to AD 350-550 (5). Despite this, antibiotics were first described in the early 1920s, when penicillin was discovered by Sir Alexander Fleming (23). It was not until the 1940s that penicillin, streptomycin, chloramphenicol, and tetracycline were thrust into the clinical spotlight and were first used to treat human bacterial infections (23). After the first clinical use of antibiotics, the evolutionary arms race began between functional antibiotics and the bacteria they worked to destroy.

1.2 Antibiotic Resistance Background

Antibiotic resistance occurs in bacteria that are treated with antibiotics (24). Normally, bacteria treated with an antibiotic are eliminated, and sicknesses within humans are easily treatable. Over time and with continued use of an antibiotic, random mutations and point mutations in the bacterial genome occur that enable the bacteria to survive antibiotic treatment (71). The genes of the antibiotic-resistant bacteria (ARB) are then selected for, as only bacteria with those genes survive, and the antibiotic is rendered useless for treatment of those bacteria. The evolution of antibiotic-resistant genes in bacteria not only occurs by spontaneous mutations, but by horizontal gene transfer, which involves the “jumping” of a gene coding for antibiotic resistance from one bacterial cell to another in close proximity (83). These genes that

can “jump” from one bacterial cell to another that encode for antibiotic resistance are called plasmids (17). Plasmids are capable of surviving long periods of time in the natural environment, such as in water (17). Similar segments of DNA, called pathogenicity islands, are genes that had been present in certain bacterial strains and code for antibiotic resistance. They also do not degrade for long periods of time in the environment and may be incorporated into the genome of a bacterial cell (18). Evolution of antibiotic-resistance genes in bacteria is a growing public health concern.

The continued use of antibiotics in medicine has caused a dramatic increase in genes, point mutations, or other antibiotic resistance anomalies inside the bacterial cell that code for resistance to these antibiotics (11). Human concern arises with antibiotic-resistance as once treatable infections are no longer treatable (6). This causes a rise in human sickness, which triggers a response in clinical research to develop new antibiotics, and the cycle begins anew as the bacteria evolves with new genes to combat the new antibiotic. The rise of antibiotic resistance genes in bacterial populations paired with the decline of innovative antibiotics has created a rapidly increasing threat to human society (14). The threat becomes more imposing when antibiotic-resistant bacteria are immune to the effects of multiple drugs (81). Widespread antibiotic resistance genes and multidrug-resistant bacteria pose the eventual threat of untreatable illness, and their ability to persist in a wide array of environments makes antibiotic resistance an area of major concern.

1.3 Antibiotic Resistance in the Environment

ARB and antibiotic resistance genes (ARG) are not only viable in a clinical setting, but in the environment as well (61). Some environmental settings where antibiotic resistance genes have been found include reservoirs that provide drinking water (75), soil on livestock farms (100), and wastewater from urban settings (62). Antibiotic resistance genes and ARB get into the environment through clinical settings, as most are treated as waste, enter the hospital sewage system, and eventually end up in the environment. Although the wastewater from

clinical settings is heavily treated before being recycled into the environment, some antibiotic resistance genes and antibiotic-resistant bacteria make it through this process (50). Receiving rivers of wastewater contaminated with antibiotic-resistant bacteria and the genes coding for antibiotic resistance are severely impacted by their presence, as concentrations of the genes can increase downstream from the wastewater disposal site (68).

Through horizontal transfer of genes between bacteria or deposition of the bacteria through wastewater, ARB are also present in soil (39). The genes have been found in a variety of ecosystems, including soil in urban areas (91). This presents a particular problem due to the variety of organisms present in urban environments. Farming operations contribute to antibiotic-resistance genes into the environment (85). The application of manure and sewage sludge to crop production contributes a host of antibiotic-resistance genes to the soils, which runoff into other, nearby ecosystems (21). Once into the ecosystem, animals such as birds may pick up bacteria containing antibiotic-resistance genes.

1.4 Birds and their Relationship with Antibiotic Resistance

Birds are unique in their near ubiquitous presence throughout our world, and their ability to occupy a wide variety of ecological niches. For example, birds can generally access smaller areas, such as nest cavities, and their ability to fly allows them to travel virtually anywhere in the environment (48). Birds also inhabit, forage in, and reside in a large variety of areas in their environment, including high in treetops, on the ground, near water sources, and in areas disturbed by human presence (53). Their ability to inhabit unique habitats can expose birds to antibiotic-resistant pathogens and antibiotic resistance genes (40). Particularly, avian use of wetlands with surrounding urban areas can cause exposure, as many ARB and antibiotic resistance genes (ARGs) end up in streams and rivers (7, 67). Birds also have a close relationship to livestock farms, where ARB and ARGs are known to be found (22), and studies have shown that birds pick up antibiotic-resistant pathogens from these livestock farming environments (35). Because birds exhibit a wide variety of behaviors, including habitat

selection, nesting behavior, sexual selection behaviors, and foraging behaviors, they can act as excellent indicators for possible sources of ARB and ARGs (74).

Fortunately, there has been no evidence of direct transfer of antibiotic-resistant pathogens from birds to humans. Despite this, the ability of birds to carry and maintain populations of ARB is troubling, as birds are able to migrate long distances. Birds are able to transfer numerous infectious diseases throughout all parts of the world during migration, including bacterial pathogens with antibiotic resistance (20). As the birds migrate and move throughout their environment, they can deposit ARB and ARGs into a variety of habitats, helping facilitate the spread of genes that cause antibiotic resistance. As ARB and ARGs spread, the need for development of more antibiotics continues to grow, presenting a worrying public health issue.

1.5 Sampling Protocol and Antibiotics Used

The Louisiana Bird Observatory (LABO), a program of the Baton Rouge Audubon Society (BRAS), operated two sets of 15 mist-nets (36-mm mesh, 12 x 2 m) at the Bluebonnet Swamp Nature Center in Baton Rouge, LA (30.369529° N, -91.105644° W), where birds were examined, banded, and released from early February 2016 to late December 2016. We collected bacterial samples from birds captured in the mist nets. We sampled for bacteria from the cloacae and fecal matter (when available) using sterile cotton-tipped swabs pre-moistened with lactated Ringer's solution (VWR, Radnor, PA). The tip of the swab was placed on the outside of the cloaca and spun for a standard time of 3 seconds to ensure a bacterial sample was collected on the swab. Swabs were placed into 5 mL phosphate buffered saline (PBS, 3.72 mM $NaH_2PO_4 \cdot 2H_2O$, 14.0 mM $Na_2HPO_4 \cdot 2H_2O$, 0.145 M NaCl, pH 7.4) and transported to the laboratory at ambient temperature for immediate processing. These samples were spread onto plates and cultured. After a 24-hour incubation period, the colony-forming units (CFUs) were counted using a hand counter.

Cefotaxime is a cephalosporin antibiotic that acts as a bactericidal agent. It disrupts cell wall function, killing the cell. Cefotaxime is a common clinical antibiotic for surgical use, and is commonly able to survive the wastewater treatment process when discarded in hospitals. Erythromycin is a macrolide antibiotic that is bacteriostatic in nature. It inhibits growth of bacterial cells by blocking protein translation. It can be prescribed by doctors and is a common clinical antibiotic, and has been found to last long periods of time in aquatic environments. These two antibiotics were used due to their completely separate mechanisms of action against bacteria, their widespread use, their availability in the lab, and their use in the preliminary trials of this study.

1.6 Louisiana Birds Captured at Bluebonnet Swamp and their Life Histories

1.6.1 Northern Cardinal (*Cardinalis cardinalis*). The Northern Cardinal (Figure 1), with its vibrant red plumage (in males), is one of the most widespread and well-known birds in North America from the family Cardinalidae.

Figure 1. Northern Cardinal (*C. cardinalis*). Photo courtesy of Cornell Lab of Ornithology (https://www.allaboutbirds.org, accessed March 21, 2017).

They inhabit the eastern portion of the United States from west Texas to the Atlantic coast. Northern Cardinals can inhabit a wide variety of ecosystems, including forested areas, forest

edges, wooded streams, and suburban areas. It is a common bird seen at bird feeders throughout the United States, and with its large, thick bill size, feeds mostly on seeds. It is one of the only birds in North America in which both males and females sing all year long. These are sedentary songbirds, with a large range that has constantly been expanding westward in the United States. The Northern Cardinal pairs with a mate for the breeding season and the vibrant red males mate-guard the duller buffy brown females. Mate-guarding is a phenomenon in monogamous relationships in birds where the male continuously follows the female to ensure that she does not mate with any other males. Due to the color variation between males and females, this species is classified as sexually dimorphic (101). In our study, we mist-netted and sampled 33 individuals, the most of any species in the study.

1.6.2 Carolina Wren (*Thryothorus ludovicianus*). The Carolina Wren (Figure 2) is a small, streaky brown member of the family Troglodytidae. They inhabit the southern half of the United States from Texas to the Atlantic Coast, where they have been spotted as year-round residents as far north as New England.

Figure 2. Carolina Wren (*T. ludovicianus*). Photo courtesy of Cornell Lab of Ornithology (https://www.allaboutbirds.org, accessed March 21, 2017).

The species can inhabit a wide variety of habitats, including urban backyards, but is most often found in swampy forests. It is secretive, foraging mostly near or on the ground, and is commonly found in underbrush of forested areas. Unlike most other birds, the Carolina Wren sings all year, claiming its territory with its familiar song. These birds are mostly sedentary, with some range differences occurring due to changes in the climate of the year. The Carolina Wren is monogamous and sexually monomorphic, showing no plumage differences between males and females in the population (101). In our study, we mist-netted and sampled 30 different individual Carolina Wrens, the second most of any species in the study.

1.6.3 Prothonotary Warbler (*Prothonotaria citrea*). The Protonotary Warbler (Figure 3) is a striking, golden bird of the family Parulidae. They reside in southeastern North America during the breeding season (roughly February-September), where they pair with a mate and form nests in secondary cavities, or holes that have already been created and used by another species.

Figure 3. Prothonotary Warbler (*P. citrea*). Photo courtesy of Cornell Lab of Ornithology (https://www.allaboutbirds.org, accessed March 21, 2017).

This migratory bird spends its winters in Central America and northern South America, and returns to breed in the United States from eastern Texas to the Atlantic coast and as far north

as southern Michigan and Wisconsin. They generally spend most of their time in swampy forested areas, where they find nest cavities near slowly moving or stagnant water. Its vibrant golden color bore resemblance to that of the robes of papal clerks, also named prothonotaries, in the Roman Catholic Church, hence its name. The females within this species are a duller yellowish color, with more olive green above the wings and near the head. Prothonotary Warblers generally exhibit breeding site fidelity, and many species that leave during migration return to the same breeding site in the next breeding season. Due to population conservation concerns over habitat loss, human nest-box provision is widespread throughout their breeding range, allowing the species to use these manmade fixtures as false secondary nesting cavities (101). In our study, we not only sampled adult birds that were captured using mist nets, but we also sampled the cloacae of recently hatched chicks in nest boxes set up in Bluebonnet Swamp. We included these in our study by classifying the chicks as juvenile birds, as they had just hatched during the breeding season. We sampled a total of 22 individual Prothonotary Warblers in this study, ranking third in our study.

1.6.4 Hermit Thrush (*Catharus guttatus*). The Hermit Thrush (Figure 4) is a secretive bird from the family Turdidae. They appear in all parts of the United States, but only appear in Louisiana during the winter. Of all of the thrushes, Hermit Thrushes are the most widespread.

Figure 4. Hermit Thrush (*C. guttatus faxoni*). Photo courtesy of Cornell Lab of Ornithology (https://www.allaboutbirds.org, accessed March 21, 2017).

Generally, they stay out of sight in the underbrush of wooded forests, foraging on small insects. Hermit Thrushes are known throughout ornithological circles as having one of the most beautiful songs of all songbirds. They are a migratory songbird that winter in the southern United States, and have a wide breeding range that spans from northern Canada and the northern United States, down the Rocky Mountains into Arizona and New Mexico. Hermit Thrushes are a sexually monomorphic species, with both males and females sporting a mostly buffy brown head and back, with a white underbelly marked with buffy brown spots (101). This species is composed of 12-13 subspecies, varying slightly in plumage color and geographic range. In our study, we mist-netted and sampled 9 individuals, the fourth most of all of our species.

1.6.5 Brown Thrasher (*Toxostoma rufum*). The Brown Thrasher (Figure 5) belongs to the family Mimidae.

Figure 5. Brown Thrasher (*T. rufum*). Photo courtesy of Cornell Lab of Ornithology (https://www.allaboutbirds.org, accessed March 21, 2017).

Mimids are known throughout North America as species who learn the songs of other birds throughout their life, and are capable of mimicking them in order to attract a mate or claim a territory. The Brown Thrasher, in particular, does not often mimic other birds, but uses a variety of different phrases when singing. They are found in year-round and migrant populations in Louisiana and other Gulf Coast states, but only in breeding populations in the Midwest and northeastern United States. They are common inhabitants of the edges of woodlands, and generally stay low to the ground, feeding on insects, seeds, and berries that are hidden on the ground. They use their long curved bill to unearth insects and other food from fallen leaves. Brown Thrashers are larger, streaky, brown-backed birds, with tan underbellies that have a varying degree of darker brown streaks. Most striking is their bright, yellow iris, which develops as they grow older. Females and males of this species exhibit the same plumage, making them a sexually monomorphic species (101). In our study, we mist-netted and sampled 7 individuals, the fifth most of all species sampled.

1.6.6 White-throated Sparrow (*Zonotrichia albicollis*). White-throated Sparrows (Figure 6), from the family Emberizidae, is a common, streaky sparrow found throughout the United States.

Figure 6. White-throated Sparrow (white morph) (*Z. albicollis*). Photo courtesy of Cornell Lab of Ornithology (https://www.allaboutbirds.org, accessed March 21, 2017).

In Louisiana in particular, it is only found in wooded or backyard areas during the winter. Its normal breeding range is in the northern United States and Canada. It is commonly seen at bird feeders throughout the United States, especially before migration and on colder days throughout its wintering range. The White-throated Sparrow has a stubby, thick bill used for small seed crushing, from which it obtains a large chunk of its diet. White-throated Sparrows exhibit polymorphism within the species, with one part of the population exhibiting a tan head stripe behind the eye, and another part of the population exhibiting a white stripe behind the eye. Due to the species being sexually monomorphic, both males and female populations are found to have both morph types. Morphs have been linked to differing behaviors within the species (101). Tan-morph males are less aggressive, spend less time inhabiting adjacent territories, and invest more energy into mate-guarding and parental care than white-morph males. Tan-morph females are also less aggressive and do not attempt to copulate with males

as frequently as white-morph males (84). White-throated Sparrows generally form flocks during the non-breeding season, foraging together. In our study, we mist-netted and sampled 6 individual White-throated Sparrows, the sixth most of any species in this study.

1.6.7 Red-winged Blackbird (*Agelaius phoeniceus*). Red-winged Blackbirds (Figure 7), from the family Icteridae, are one of the most abundant birds in the United States.

Figure 7. Red-winged Blackbird (*A. phoeniceus*). Photo courtesy of Cornell Lab of Ornithology (https://www.allaboutbirds.org, accessed March 21, 2017).

Their range spans across the entire United States from the Pacific to the Atlantic coast and from southern Texas to northern Canada. They generally forage in large, and often times mixed-species flocks on the ground, eating large seeds. They are a notorious pest to crop farmers across the United States, as they feed on common crops grown in North America during the wintertime. The males of this species have a mostly jet black body, with large red and yellow patches on the shoulder. Red-winged Blackbirds are sexually dimorphic, and females have mostly brown and white streaky plumage. They breed in marshland habitats, and are extremely territorial. Male Red-winged Blackbirds establish a territory, singing incessantly to keep other males away from the territory (101). Those males who inhabit the highest quality territories have the most females in their “harem”, or group of females, who mate with the male “territory

owner" and all contribute to the parental care of the offspring. This form of mating, called polygyny, can result in as many as 15 females caring for the offspring of one male. Red-winged Blackbirds are one of the few species in North America that exhibit this form of mating. In our study, we mist-netted and sampled 4 different individual Red-winged Blackbirds.

1.6.8 Tufted Titmouse (*Baeolophus bicolor*). The Tufted Titmouse (Figure 8) is a small bird in the Paridae family, and a close relative of chickadees.

Figure 8. Tufted Titmouse (*B. bicolor*). Photo courtesy of Cornell Lab of Ornithology (https://www.allaboutbirds.org, accessed March 21, 2017).

They are year-round sedentary residents of the eastern United States, from eastern Texas to the Atlantic coast, ranging as far north as southern Maine. This striking bird is a regular backyard feeder visitor, and is particularly admired for its tall crest on the crown of its head. It is an inhabitant of deciduous woodlands and urban areas, and is particularly active in its movement and calls. Tufted Titmice have a generally gray and white body, with a gray back, head and wings, with a black patch just above the bill. Its underbelly is white with a patch of brown underneath the wing on the flanks. They commonly hybridize with the closely related Black-crested Titmouse (*Baeolophus atricristatus*) in the hybrid zone of middle Texas. The birds are sexually monomorphic, and often form long lasting pair bonds with mates (101).

Tufted Titmice and chickadees often have a close relationship, in which they forage and mob, or attempt to scare away by calling and attacking, potential predators in mixed-species groups (8). In our study, we mist-netted and sampled 4 individual Tufted Titmice.

1.6.9 Brown-headed Cowbird (*Molothrus ater*). The Brown-headed Cowbird (Figure 9) is a species that occurs ubiquitously throughout North America, and belongs to the family Icteridae.

Figure 9. Brown-headed Cowbird (*M. ater*). Photo courtesy of Cornell Lab of Ornithology (https://www.allaboutbirds.org, accessed March 21, 2017).

They are found in year-round populations all across the southern United States, and have a breeding only range across the northern United States and into Canada. Brown-head Cowbirds are commonly found in woodlands, urban areas, and farmlands, where they forage in flocks on the ground, using their large, conical bills to crush seeds. Adult males have a jet-black body with a dark brown head, while females are a dull brown all over. Brown-headed Cowbirds exhibit an uncommon phenomenon called brood parasitism, in which the females deposit eggs in the nests of other species, and allow other parents to raise their young. They are one of few species that exhibit no parental care whatsoever. Birds such as the Blue-headed Vireo, (*Vireo solitarius*) and Yellow Warbler (*Setophaga petechia*) are common victims of brood parasitism,

and are tricked into raising the much larger Brown-headed Cowbird hatchling along with their own offspring (58, 38). As the name implies, Brown-headed Cowbirds are commonly seen in farm settings and open fields, foraging in flocks of thousands, sometimes even millions, of birds. They often forage in mixed-species flocks, along with the closely related Red-winged Blackbird and Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*) (101). In our study, we mist-netted and sampled 2 Brown-headed Cowbird individuals.

1.6.10 Red-bellied Woodpecker (*Melanerpes carolinus*). The Red-bellied Woodpecker (Figure 10) was one of three species in our study from the woodpecker family, Picidae.

Figure 10. Red-bellied Woodpecker (*M. carolinus*). Photo courtesy of Cornell Lab of Ornithology (https://www.allaboutbirds.org, accessed March 21, 2017).

This medium-sized woodpecker is common to the eastern United States, from eastern Texas to the Atlantic coast and as far north as the New England area. They inhabit wooded and suburban areas, and are almost always found scaling trees picking at the bark with the diagnostic symbol of the Picidae family, its sturdy bark-drilling bill. They use this bill to bore nest cavities, call using a drumming sound, and forage for small insects as a main part of their diet. They are also common backyard feeder inhabitants, choosing to pick at and eat suet blocks. The Red-bellied Woodpecker has conspicuous black and white barring on its back. Males having an all red crown and back of the neck, while females only have red on the back of the neck, making this a sexually dimorphic species. These birds are common in woodlands and in areas with human disturbance, and their characteristic call can be heard throughout the year (101). In our study, we mist-netted and sampled 2 Red-bellied Woodpecker individuals.

1.6.11 Downy Woodpecker (*Picoides pubescens*). The Downy Woodpecker (Figure 11) was one of three species in our study from the woodpecker family, Picidae.

Figure 11. Downy Woodpecker (*P. pubescens*). Photo courtesy of Cornell Lab of Ornithology (https://www.allaboutbirds.org, accessed March 21, 2017).

They are small woodpeckers that bare a striking resemblance to their close relative, the Hairy Woodpecker (*Picoides villosus*). Downy Woodpeckers have a smaller bill relative to their body

size when compared to the larger Hairy Woodpecker. These birds year-round residents to much of the United States and Canada, with the exception of certain areas in the southwest United States. They are often found in wooded habitats, but will inhabit urban habitats, and are a common backyard feeder visitor. Much like the Red-bellied Woodpecker, they choose to pick at suet in urban and suburban backyards. Downy Woodpeckers have white backs with an alternating black and white striped head. Their wings are mostly black, with white spots throughout. Males and females are sexually dimorphic, as males have a small red spot towards the back of the crown of their head that is absent in females (101). In our study, we mist-netted and sampled 2 Downy Woodpecker individuals, tied for eighth most of all species sampled.

1.6.12 Thirteen Species Represented by Only One Individual. There were a total of 13 species that were only represented by 1 individual in our study. Across these 13 species, there were 7 families represented. Four species of the family Parulidae (wood warblers) were represented; Kentucky Warbler (*Geothlypis formosa*), Wilson's Warbler (*Geothlypis pusilla*), Common Yellowthroat (*Geothlypis trichas*), and Orange-crowned Warbler (*Oreothlypis celata*). This family is characterized by a small, insect-eating bill, generally colorful plumage, and they are most often migratory (101). Two species of the family Turdidae (thrushes) were represented; Swainson's Thrush (*Catharus ustulatus*) and Gray-cheeked Thrush (*Catharus minimus*). Turdidae generally have beautiful songs, are tough to identify from one another, and feed mainly on insects and fruit (101). Two species of Vireonidae (vireos) were represented; Blue-headed Vireo (*Vireo solitarius*) and White-eyed Vireo (*Vireo griseus*). A short, hooked bill used for catching insects is diagnostic for the vireo family (101). One species of each family Paridae (chickadees and titmice), Mimidae (mimics), Picidae (woodpeckers), Tyrannidae (tyrant flycatchers), and Emberizidae (towhees, sparrows, and some buntings) was represented. These included Carolina Chickadee (*Poecile carolinensis*), Gray Catbird (*Dumetella carolinensis*), Northern Flicker (*Colaptes auratus*), Acadian Flycatcher (*Empidonax virescens*), Eastern

Towhee (*Pipilo erythrophthalmus*). The species sampled in this study represented a wide array of resident and non-resident birds from Louisiana.

Chapter 2. Louisiana Birds Act as Reservoirs of Antibiotic-Resistant Bacteria

2.1 Purpose and Hypotheses

Antibiotic-resistant bacteria (ARB) are a growing problem for public health, as they complicate the management of otherwise treatable infections. These bacteria are selected for as a result of antibiotic overuse and misuse. In clinical and agricultural settings, these bacteria can serve as potential pathogens untreatable by common antibiotics. Antibiotic resistance in bacteria may be evolving rapidly (69), so understanding temporal and spatial changes in prevalence is particularly important. Antibiotic-resistant bacteria have been described in environmental sources such as sediment, soil, surface water, and ground water (52). One common source for the deposition of antibiotic resistance into the environment is the treatment of livestock (22, 85, 100). With increased use of antibiotics and the associated development of resistance to more drugs, environmental contributions of antibiotic-resistant pathogens may increase as well (86).

The fate of these bacteria and their antibiotic resistance genes in the environment, however, is not well described. It is assumed that most die over time, but questions remain about their abundance, fitness, viability, virulence, and genetic factors (10, 22, 52, 85, 100). Intensive environmental monitoring can yield insight into ARB populations in the environment, but live indicators such as songbirds may also aid in our understanding of antibiotic-resistant bacteria in the natural environment because they naturally occupy (forage, roost, and nest in) specific habitats. Few studies have examined bacterial communities associated with bird species in the United States, and even fewer have investigated antibiotic-resistant bacteria (37, 41, 63). Antibiotic-resistant bacteria in birds have been described previously, with ARB found in as many as 16 species of songbirds (Passeriformes), two species of Galliformes, and two species of Charadriiformes (72). Bacterial pathogens such as *Salmonella* sp., *Campylobacter* sp., and *Escherichia coli* are often found in the intestinal tracts of broiler chickens, and these species have all been shown to carry antibiotic resistance factors (2, 3).

Examining environmental ARB cultured from living, motile hosts with predictable behaviors makes it possible to predict the likely sources of these bacteria, to examine bacterial populations on a large spatial scale, and to make inferences regarding the fitness of these bacteria. To determine the sources of the antibiotic-resistant bacteria colonizing birds, it is important to explore the bird behaviors that could potentially expose them to these bacteria. Birds are colonized by diverse bacterial communities on their feathers, skin, internally, and on their cloacae (87). Some of these bacteria have a direct effect on the fitness and health of the birds they inhabit (76, 90), but the connections between these behaviors and ARB in the environment are still largely unknown. Although bacterial communities can affect the fitness and health of birds, few studies have focused on understanding what behavioral characteristics of birds influence exposure and colonization by bacteria, and whether multiple attributes work in tandem and synergistically.

Total and antibiotic-resistant bacterial communities can vary based on bird behaviors. For example, specific bacterial communities are associated with certain feeding guilds and proximity to agricultural sites (36). Age-related differences in the microbiomes of birds can help predict whether or not parental rearing and investment, nest structure, and nestling interaction affect exposure of birds to bacteria (73, 87, 89). Cloacal communities of microbes also differ between sexes (51), providing evidence that sex-specific behaviors may be related to bacterial composition. Communities of the human enteric pathogen *Campylobacter jejuni* that have been cultured from Black-headed Gulls (*Chroicocephalus ridibundus*) suggest how this host's close relationship with human activity and their dependence on anthropogenic food sources may increase the likelihood of colonization by this pathogen (13).

In this study, we targeted the cloacal ARB communities of Passeriformes and Piciformes in a cypress-tupelo/bottomland hardwood forest fragment surrounded by urban Baton Rouge, Louisiana. We assessed loads of culturable, antibiotic-resistant bacteria in resident and migratory birds with predictable behavioral traits. We hypothesized that: (1) adult birds would

contain higher levels of antibiotic-resistant bacteria than young birds and (2) ground-foraging birds would contain higher loads of antibiotic-resistant bacteria than other foraging guilds. To our knowledge, this is the first study to quantify, analyze the antibiotic resistance profiles of, and determine the lowest taxonomic level of antibiotic-resistant bacteria from birds. This study is also unique in that it represents a rare glimpse into the use of songbirds as indicators of potential pathogens in an urban environment, with a specific focus on antibiotic-resistant bacteria.

2.2 Materials and Methods

2.2.1 Sample Collections. The Louisiana Bird Observatory (LABO), a program of the Baton Rouge Audubon Society (BRAS), operated two sets of 15 mist-nets (36-mm mesh, 12 x 2 m) at the Bluebonnet Swamp Nature Center in Baton Rouge, LA (30.369529° N, -91.105644° W), where birds were examined, banded, and released from early February to late July 2016. The age and sex of each bird was determined following Pyle (1997) (16). Juvenile birds were defined as birds that had been banded in the same calendar year in which they hatched, and adult birds were defined as birds that had been banded after the calendar year in which they hatched. Microbiological samples were collected from birds captured between sunrise and two hours after sunrise. We sampled for bacteria from the cloacae and fecal matter (when available) using sterile cotton-tipped swabs pre-moistened with lactated Ringer's solution (VWR, Radnor, PA). The tip of the swab was placed on the outside of the cloaca and spun for a standard time of 3 seconds to ensure a bacterial sample was collected on the swab. Swabs were placed into 5 mL phosphate buffered saline (PBS, 3.72 mM $NaH_2PO_4 \cdot 2H_2O$, 14.0 mM $Na_2HPO_4 \cdot 2H_2O$, 0.145 M NaCl, pH 7.4) and transported to the laboratory at ambient temperature for immediate processing.

2.2.2 Quantitation of Antibiotic-resistant Bacteria. Samples were homogenized by pipetting vigorously 10 times using sterile transfer pipets. Each suspension was spread onto Brain Heart Infusion agar (BHI; Becton, Dickinson, and Company, Franklin Lakes, NJ) using

sterile cell spreaders. BHI agar plates all contained 0.24 µg/mL cycloheximide (Sigma-Aldrich, St. Louis, MO) to minimize fungal contamination of plates. Serially increasing volumes, representing 0.001 - 500 µL of suspension, were spread onto plates containing cycloheximide plus (1) no antibacterial drugs, (2) 4 µg/mL of cefotaxime, or (3) 8 µg/mL erythromycin (Sigma-Aldrich, St. Louis, MO). Fecal and cloacal samples were spread onto plates in volumes representing 0.1 - 100 µL and 50 - 400 µL of sample suspension, respectively. The plates were incubated at 37°C for 22 - 24 hours, as described previously (33). Colonies were counted within a range of 1 - 300 colony forming units (CFU) using a hand counter, and weighted averages were used to determine the final bacterial densities in CFU/mL from plates yielding countable colonies, as described previously (Table S1) (46).

2.2.3 Statistical Analyses. Statistical analyses were carried out using RGui 3.3.0 (66). To analyze differences in bacterial loads, we used a Generalized Linear Mixed Modeling approach to analyze log-transformed CFU count values from cloacal and fecal samples. When the data was log-transformed, we added a small constant to each value in order to account for all zero values collected in our dataset, as log-transformed datasets cannot account for zeroes normally. Weighted values over 5000 CFUs/mL were not included in the analysis, as these weighted values often included raw counts of over 300 CFUs, which is classified as too numerous to count by the FDA. We modeled fecal samples and cloacal samples separately, as they showed large differences in overall means. Our predictor variables were age, foraging guild, and sex. Our response variable was the log-transformed CFUs/mL. Due to a large number of species for which the sex was unknown, we excluded sex from our model and focused primarily on age and foraging guild. Our model also included a random effect that accounted for species-level variability. Each model included calculation of an intraclass correlation coefficient (ICC) that described how much of the variability was due to the species-level factor. When performing statistical analysis on the foraging guilds, the tree-foragers category included both leaf- and bark-foragers, and this included Gray Catbird (*Dumetella*

carolinensis), Northern Cardinal (*Cardinalis cardinalis*), Prothonotary Warbler (*Prothonotaria citrea*), Red-bellied Woodpecker (*Melanerpes carolinus)*, Swainson's Thrush (*Catharus ustulatus*), Tufted Titmouse (*Baeolophus bicolor*), Blue-headed Vireo (*Vireo solitarius*), Wilson's Warbler (*Cardellina pusilla*), Kentucky Warbler (*Geothlypis formosa*), Common Yellowthroat (*Geothlypis trichas*), Orange-crowned Warbler (*Oreothlypis celata*), Acadian Flycatcher (*Empidonax virescens*), Northern Flicker (*Colaptes auratus*), Downy Woodpecker (*Picoides pubescens*), White-eyed Vireo (*Vireo griseus*), and Carolina Chickadee (*Poecile carolinensis*). The ground-foragers included Brown-headed Cowbird (*Molothrus ater*), Brown Thrasher (*Toxostoma rufum*), Carolina Wren (*Thryothorus ludovicianus*), Gray-cheeked Thrush (*Catharus minimus*), Hermit Thrush (*Catharus guttatus*), White-throated Sparrow (*Zonotrichia albicollis*), Eastern Towhee (*Pipilo erythrophthalmus*), and Red-winged Blackbird (*Agelaius phoeniceus*).

2.3 Results

We collected bacterial samples from 135 individual birds representing 24 species. All but 2 individuals had cloacal swabs taken and 75 fecal samples were collected from birds that defecated while held in bleached cotton bags (Table 1).

Table 1. Common name (species name), number of individuals of each species, total number of samples per species, and numbers of cloacal and fecal samples per species sampled during the study period.

Species	Four Letter Code	Number of Individuals	Number of Samples	Cloacal Samples	Fecal Samples
Northern Cardinal (*Cardinalis cardinalis*)	NOCA	33	54	33	21
Carolina Wren (*Thryothorus ludovicianus*)	CARW	30	50	30	20
Prothonotary Warbler (*Prothonotaria citrea*)	PROW	22	28	22	6

Table 1 Continued

Species	Four Letter Code	Number of Individuals	Number of Samples	Cloacal Samples	Fecal Samples
Brown Thrasher (*Toxostoma rufum*)	BRTH	7	13	7	6
Hermit Thrush (*Catharus guttatus*)	HETH	9	11	8	3
White-throated Sparrow (*Zonotrichia albicollis*)	WTSP	6	10	5	5
Red-winged Blackbird (*Agelaius phoeniceus*)	RWBL	4	7	4	3
Brown-headed Cowbird (*Molothrus ater*)	BHCO	2	4	2	2
Tufted Titmouse (*Baeolophus bicolor*)	TUTI	4	4	4	0
Red-bellied Woodpecker (*Melanerpes carolinus*)	RBWO	2	3	2	1
Gray Catbird (*Dumetella carolinensis*)	GRCA	2	3	2	1
Downy Woodpecker (*Picoides pubescens*)	DOWO	2	2	2	0
White-eyed Vireo (*Vireo griseus*)	WEVI	1	2	1	1
Swainson's Thrush (*Catharus ustulatus*)	SWTH	1	2	1	1
Kentucky Warbler (*Geothlypis formosa*)	KEWA	1	2	1	1
Wilson's Warbler (*Cardellina pusilla*)	WIWA	1	2	1	1
Eastern Towhee (*Pipilo erythrophthalmus*)	EATO	1	2	1	1
Common Yellowthroat (*Geothlypis trichas*)	COYE	1	2	1	1
Orange-crowned Warbler (*Oreothylpus celata*)	OCWA	1	2	1	1
Carolina Chickadee (*Poecile carolinensis*)	CACH	1	1	1	0

Table 1 Continued

Species	Four Letter Code	Number of Individuals	Number of Samples	Cloacal Samples	Fecal Samples
Gray-cheeked Thrush (*Catharus minimus*)	GCTH	1	1	1	0
Northern Flicker (*Colaptes auratus*)	NOFL	1	1	1	0
Acadian Flycatcher (*Empidonax virescens*)	ACFL	1	1	1	0
Blue-headed Vireo (*Vireo solitaries*)	BHVI	1	1	1	0
Totals		135	208	133	75

Determining differences in sample type (fecal or cloacal) allowed us to effectively model differences in bacterial levels using our predictor variables. Sample types (fecal and cloacal) for all species sampled had different total averages and showed different patterns across species and were modeled separately (Figure 12).

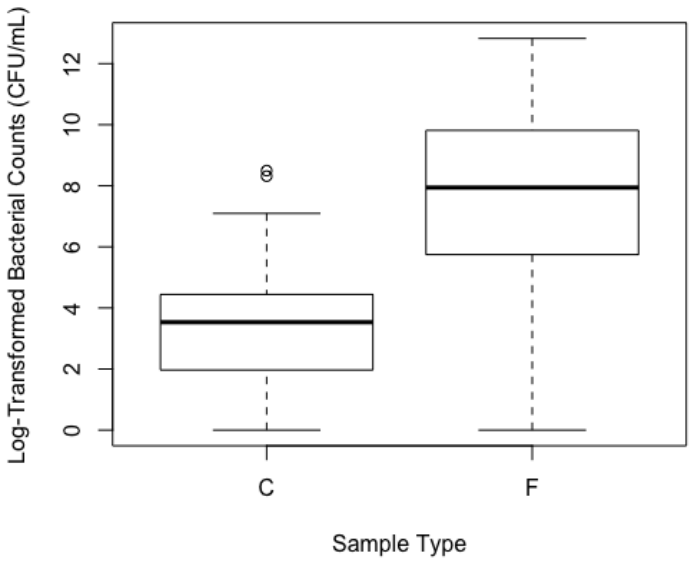

Figure 12. Log_{10}-transformed CFU/mL bacterial counts grouped by sample type (C=cloacal (n=126), F=fecal (n=69)) for all samples.

We used log-transformed CFU counts to determine whether or not there were species-level differences in the bacterial levels of each bird. For cloacal samples, there was an effect of species on bacterial levels within our samples shown in (Figure 13) (ICC=0.23).

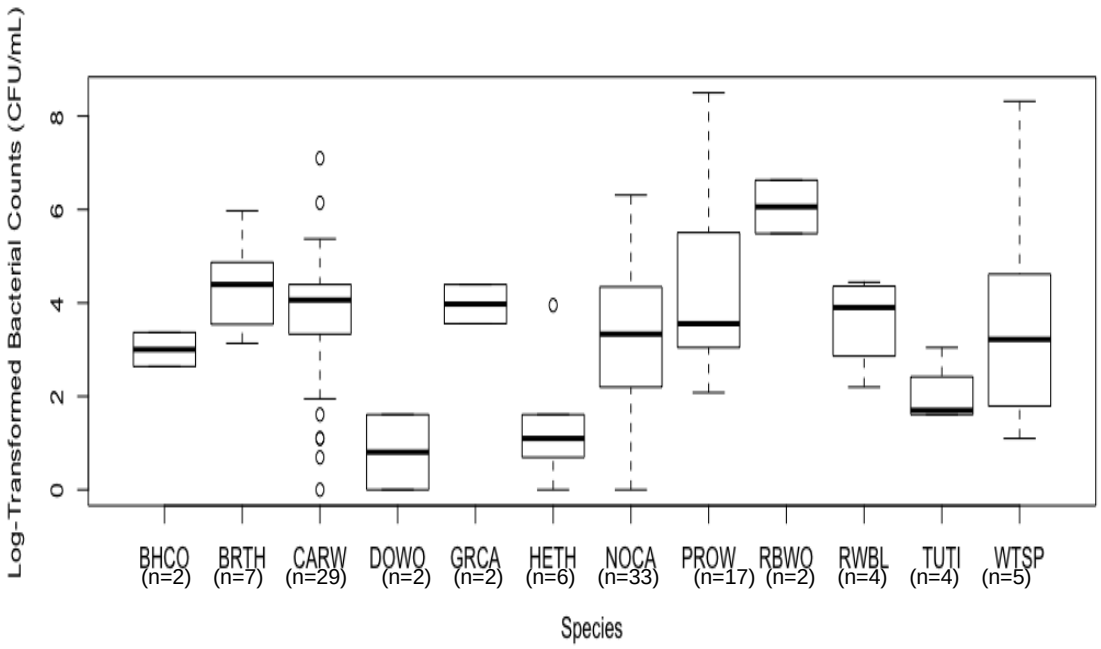

Figure 13. Log_{10}-transformed CFU/mL bacterial levels grouped by species for all samples, showing different means for each sample and a random effect of species.

The same log-transformed CFU counts were used to determine whether or not there was an age effect on levels of bacteria in our samples. There was an age-related effect on bacterial levels in the cloacal samples, showing the juvenile birds contained higher levels of bacteria than adult birds (Figure 14). Species shown in Figure 14 included the 3 bird species contributing the most variability in our dataset.

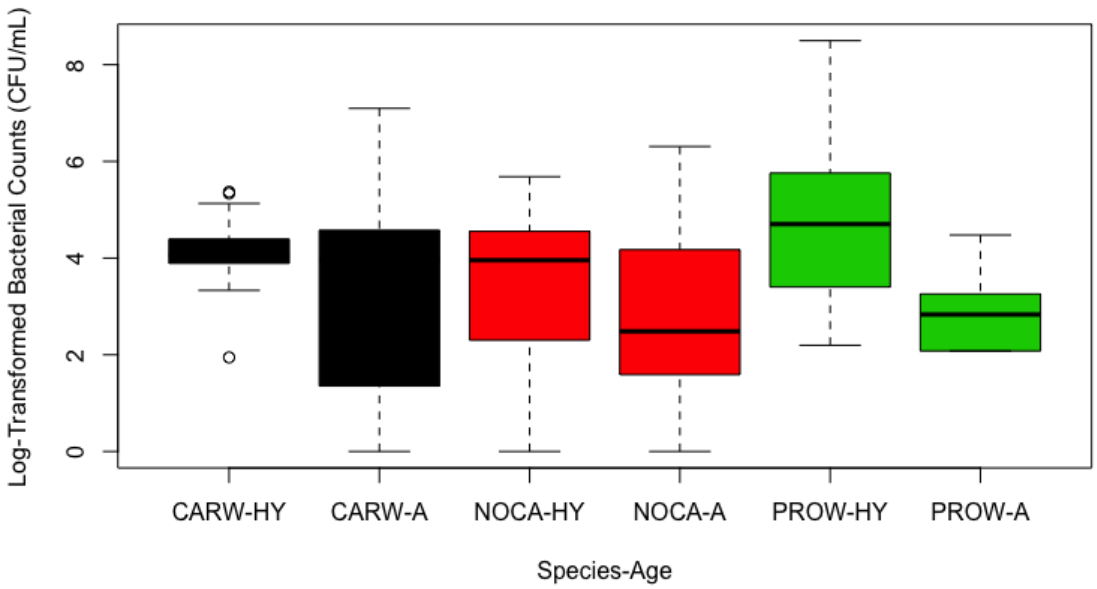

Figure 14. Log_{10}-transformed CFU/mL bacterial counts grouped by age and by species. The three bird species that contributed the most variability to our dataset were included in this graph.

There was no effect of foraging guild or sex on bacterial levels in our samples. Also, the fecal samples did not yield any statistically significant relationships, as the number of factor levels was low, and variability between those factor levels were very high.

2.4 Discussion and Future Research

In this study, we examined the bacterial communities in Louisiana birds and provided insight into colonization by viable antibiotic-resistant bacteria based on species, age, and foraging strategies. We found evidence that there were differences in bacterial assemblages in cloacal and fecal samples. Fecal samples had a much higher total average density of bacteria than cloacal samples. We also describe a species-level effect and an age-related effect, but foraging guild was not a good predictor of bacterial levels. This study is the first of its kind to find differences in antibiotic-resistant bacterial communities in different species and birds of different ages. Sex, although originally thought to be a contributor to levels of antibiotic-

resistant bacteria, did not provide a large enough sample size to be included in our model, and more work must be done to provide evidence for a sex effect on bacterial levels.

Fecal samples showed a higher total average density of antibiotic-resistant bacteria than cloacal samples. This was likely because more starting maerial was provided from swabbed fecal matter than from cloacal swabs, and both swab collections were placed into the same volumes of diluent PBS. This result suggests that antibiotic-resistant bacteria may be acquired via the diet of bird and that bacteria survive digestion through the bird's digestive tract. As the food enters the digestive tract, if it already contains ARB, it can lead to high numbers within fecal material. However, as the food travels to the cloaca to be defecated, some ARB may shed off onto cloacal tissue, leaving viable ARB within the bird cloaca.

Bacterial levels were higher in juvenile birds than in adult birds. Nestlings and their parents can share similar bacterial community structure (54), suggesting that parents may transfer microbes to the nestlings via nestling feeding. Bacteria present in the cloacae of nestlings may ultimately impact the success of fledglings (60). Juveniles are consistently exposed to ARB, as distinct bacterial communities vary by nest component, including eggs, nest material, and nestling fecal material throughout the full nesting cycle (12). Because juvenile birds generally spend 2 - 3 weeks in the nest between hatching and fledging, it is possible that total and antibiotic-resistant bacteria on nest material could transfer to nestlings. The possibility of exposure to antibiotic-resistant bacteria likely increases when nest material is more anthropogenic than natural, as seen previously (79), where used cigarette butts were incorporated into nests. Once the juvenile birds have been exposed to bacteria, their lack of fully developed immune systems (70) may leave them unable to defend against bacterial colonization. This could also explain the higher densities in juvenile birds.

There was a species-level effect on bacterial levels in our samples. Each bird species has a distinct ARB community from one another. There was high variability of bacterial levels between all species in this study, but the within-species variability was lower, pointing to a

species-level effect. Species-level differences in ARB communities may be due to high behavioral variability between species. Larger sample size for all birds in this study may point to a specific behavior that differs between all species included in this study. This provides evidence that this study should be continued with a host of target species, but larger sample sizes for those species that were underrepresented in this study must be obtained. If a few species with shared characteristics stand out as having higher or lower antibiotic-resistant bacteria densities than the others, conjectures could be made about why these birds are carrying higher loads of ARB.

There was no effect of foraging guild on the bacterial levels in the birds sampled, contradicting our hypothesis. With a larger sample size, and birds from each guild having a larger representation of bacterial isolates, it is possible that an effect of foraging guild may be shown. Despite no effect occurring within our study, foraging strategies remain as a viable predictor of ARB communities in birds. In future studies, focusing on one bird from each foraging guild and obtaining large sample sizes for each may reveal an effect.

Colonization of the avian cloaca by bacteria can occur in numerous ways, including sexual transmission (51), contact with feathers through preening (55), and direct ingestion (49). DNA analysis of microbial community structure can link two mates to each other (54). Female birds may have higher loads of bacteria present in their cloacae post-copulation than males due to bacterial presence in male ejaculate, which is deposited in the female cloaca (51), but more sampling and modeling must be done to provide more evidence for this hypothesis.

This culture-based study provided insight into the antibiotic-resistant bacteria associated with Louisiana birds and therefore the bacteria in their natural habitats. This study showed that songbirds may be excellent indicators of the presence of viable and active antibiotic-resistant bacteria. Their behavior may serve as excellent indicators of the ecology of these potentially pathogenic populations. It will be important to further characterize these bacterial communities to determine their genetic structures, multidrug resistance, antibiograms, resistance

mechanisms, and phylogenetic relationships with clinical isolates we have done in the past (43, 44, 46, 47). This study has generated over 300 antibiotic-resistant isolates of avian origin, and their deeper characterization is under way. Future studies will also include avian microbiome, resistome, and phylogenetic analyses of migratory and non-migratory passerines outfitted with geolocators (94, 95) and assessment of the phylogenetic relationships between the bacterial species carried by these birds and human pathogens previously shown to carry resistance factors (42, 47, 96). Future studies will also include analysis of soil and water samples, as well as brood patches of birds during the mating/nesting season to discover the links from the urban development surrounding the swamp, to the habitat within the swamp, and the microcosms within the birds.

Chapter 3. An Analysis of the Microbial Community of Louisiana Birds using 16s rRNA Gene Sequencing

3.1 Purpose and Hypothesis

Antibiotic-resistant bacteria (ARB) present a broad-scale public health concern. The need for new antibiotics to treat human pathogen infection is constant, as many pathogens have evolved the ability to defend themselves against multiple drugs (25). Numerous clinical studies have shed light on the evolution of different mechanisms that evolve in bacteria that provide antibiotic resistance (11). The defense mechanisms undoubtedly evolved first in a clinical setting, but due to horizontal gene transfer and clinical wastewater deposition into the environment, the resistome of the natural environment emerged (15, 28).

Antibiotic resistance in bacterial populations continues to challenge not just the clinical realm, but the environmental realm as well (34). Wastewater from clinical settings is deposited into municipal wastewater plants, which act as reservoirs for antibiotic-resistant bacteria and the genes that code for resistance (31). From this point, ARB and antibiotic resistance genes persist after treatment, and are subsequently released into the environment, most often in urban settings (67). Once in the environment, ARB have the ability to acquire antibiotic resistance genes in three ways; horizontal gene transfer via a mobile genetic element, random mutations, and point mutations (71). Due to the ability of pathogenic bacteria to acquire these genes and the antibiotic resistance genes' ability to persist in the environment, many environmental settings can act as reservoirs for pathogenic, antibiotic-resistant bacteria.

Antibiotic-resistant bacteria (ARB) have consistently been found in multiple environmental realms, including soils, urban wastewater, aquatic ecosystems, and animals that inhabit different environments (57, 65, 68). Birds, reptiles, and mammals alike have been shown to host a broad spectrum of antibiotic-resistant bacteria (19). Evidence for birds carrying antibiotic-resistant bacteria is mounting, and due to their high variability in behavior and their tendency to migrate, they represent a serious public health concern. Unfortunately, few studies

have emphasized the importance of birds as potential vectors for antibiotic-resistant bacteria. In one study alone, 16 different species of birds were consistent carriers of cephalosporin-resistant *Escherichia coli*, a common human pathogen (4). As most of the bacteria found in birds thrive in their gut, they are easily deposited back into the environment through fecal matter and by contact with the cloaca (99). Through the pick up of antibiotic-resistant bacteria by birds via distinct bird behavior, movement through migratory and other movement patterns, and the deposition of these gut bacteria back into the environment, birds have become an important area of concern regarding potentially pathogenic antibiotic-resistant bacteria.

In the present study, using 16S rRNA sequencing performed at LSU School of Veterinary Medicine, we aimed to analyze and identify the microbial communities and resistance profiles of birds from a cypress-tupelo/bottomland hardwood forest surrounded by the urban area of Baton Rouge, Louisiana. We assessed microbial composition from the different species of bird collected, and aimed to identify bacteria to the lowest possible taxonomic classification. This study provided insight into the numerous different potentially pathogenic antibiotic-resistant bacteria that birds can host, and provided the scientific community with evidence for birds as vectors of these bacteria.

3.2 Materials and Methods

3.2.1 Sample Collections. The Louisiana Bird Observatory (LABO), a program of the Baton Rouge Audubon Society (BRAS), operated two sets of 15 mist-nets (36-mm mesh, 12 x 2 m) at the Bluebonnet Swamp Nature Center in Baton Rouge, LA (30.369529° N, -91.105644° W), where birds were examined, banded, and released from early February, 2016 to late December, 2016. During this processing, microbiological samples were collected from birds captured during a two-hour period starting when the first bird was captured after 6am. We sampled for bacteria from the cloacae and fecal matter (when available) using sterile cotton-tipped swabs pre-moistened with lactated Ringer's solution (VWR, Radnor, PA). Swabs were placed into 5 mL phosphate buffered saline (PBS, 3.72 mM NaH2PO4•2H2O, 14.0 mM Na2HPO4•2H2O,

0.145 M NaCl, pH 7.4) and transported to the laboratory at ambient temperature for immediate processing.

3.2.2 Quantitation of Antibiotic-resistant Bacteria. Samples were homogenized by pipetting vigorously 10 times using sterile transfer pipets. Each suspension was spread onto Brain Heart Infusion agar (BHI; Becton, Dickinson, and Company, Franklin Lakes, NJ) using sterile cell spreaders. In the preliminary stages of this study, other agars were tested for their ability to culture antibiotic-resistant bacteria more effectively. Originally, the study was designed in a way where only enteric, or bacteria from the gut of the bird, would be grown. MacConkey agar (Sigma-Aldrich, St. Louis, MO) was used in the preliminary trial, due to its ability to selectively isolate, or allow only bacteria from the family Enterobacteriacae to grow. When little to no growth occurred using MacConkey agar, a similar agar was used as a substitute; Violet-Red Bile Glucose (VRBD) agar (Thermo Fisher Scientific, Waltham, MA). This agar also selectively isolates Enterobacteriacae, and it was thought that it would allow for culturable colony forming units to grow from our samples. Both agars used in the preliminary trials did not grow any culturable bacteria on them from our cloacal and fecal samples, so a less selective agar, Brain Heart Infusion agar, was used. BHI agar plates all contained 0.24 µg/mL cycloheximide (Sigma-Aldrich, St. Louis, MO) to minimize fungal contamination of plates. Serially increasing volumes, representing 0.001 - 400 µL of suspension, were spread onto plates containing no antibacterial drugs, 4 µg/mL cefotaxime, or 8 µg/mL erythromycin (Sigma-Aldrich, St. Louis, MO). Fecal samples and cloacal samples were spread onto plates in volumes representing 0.1 - 100 µL and 50 - 500 µL of bacterial suspension, respectively. The plates were incubated at 37°C for 22-24 hours, as described previously (33). Colonies were counted within a range of 1 - 300 colony forming units (CFUs) using a hand counter, and weighted averages were used to determine the final concentrations from plates yielding countable colonies, as described previously (45).

3.2.3 Isolation and Bacterial Identification. Individual morphologically diverse colonies were chosen from plates after enumeration, and these colonies were isolated using a three-phase

streak onto BHI agar containing no antimicrobials. Isolated colonies were incubated at room temperature overnight. Once incubated, a specific, isolated colony was chosen on the plate and spread onto a BHI agar slant using a small sterile inoculating loop. The BHI agar slants were incubated overnight at room temperature. Using a sterile inoculating loop, a subsample was taken from the lawn of bacterial colonies on the slant and placed into 200 µL of DNA suspension buffer (10mM Tris, 0.1mM EDTA, pH 8.0, Teknova, Hollister, CA). To extract genomic DNA, the suspension was boiled for 10 minutes, flash-chilled for another 10 minutes, and stored at -20°C. Genes coding for 16S rRNA (Forward- 5'-GTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAG -3', Reverse- 5'-AACAGCTATGACCATGATTACCGCGGGTGCTGG -3') were amplified from this genomic DNA using the forward primer P338F and the reverse primer P518R. Each PCR reaction was a 25-µL mix of 1 unit 5X Phusion HF buffer, 0.20 mM of dNTPs, 0.20 µM of each primer, 0.25 unit/µL of Phusion High-Fidelity polymerase, and 2.00 µL of the genomic DNA sample. PCR amplification was carried out using the Applied Biosystems Veriti 96-Well Thermal Cycler (Thermo Fisher Scientific, Waltham, MA). The PCR reaction included: initial denaturation at 98°C for 5 minutes, 35 cycles of denaturation at 98°C for 10 seconds, annealing at 66° for 50 seconds, and extension at 72°C for 60 seconds, and a final extension at 72°C for 7 minutes.

3.2.4 Gel Electrophoresis and Sequence Analysis. Each PCR product was dispersed into the wells of a 1% agarose gel for gel electrophoresis. Each sample was run for 40 minutes at 300 volts. A UVP High Performance UV Transmitter (UVP, LLC, Upland, CA) was used to view samples on the agarose gels. Using Doc-ItLS software (UVP, LLC, Upland, CA), we viewed our bands under the UV light and captured an image (Figure 15).

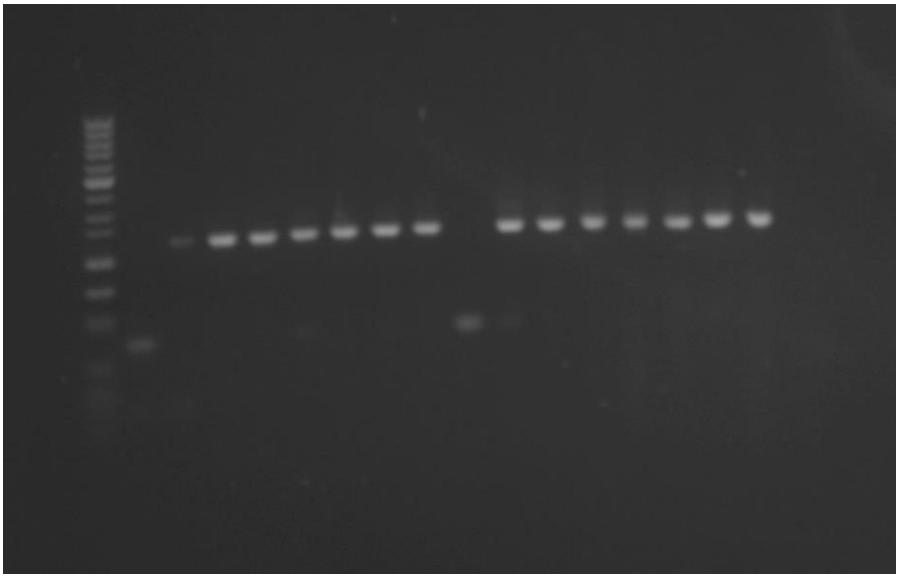

Figure 15. Bands on a 1% agarose gel representing samples of 16S rRNA gene enumerated through PCR. In lane 1 is the 50 base pair ladder used to determine size of the sequence fragment. Lanes 2 and 10 represent the negative control.

Once it was determined that rRNA was present in the samples after viewing the bands, the samples were sent to the Louisiana State University School of Veterinary Medicine for sequencing. Using the free, online DNA/RNA sequence analysis software, SeqTrace, we viewed chromatograms, edited low quality-score bases, and exported the edited sequences (Figure 16) (78).

Figure 16. Screenshot of sample AM-2016-001 in the sequence editing program from the SeqTrace software program.

We then used the BLASTn program on the National Center for Biotechnology (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch, Accessed March 10, 2017) to obtain an accurate match at the Order taxonomic level for each bacterial sequence. BioEdit software was used to create an alignment of the 90 sequences. A neighbor-joining phylogenetic tree was created using the EMBL-EBI Simple Phylogeny website (http://www.ebi.ac.uk/Tools/phylogeny/simple_phylogeny/, accessed March 22, 2017).

3.3 Results

Out of 90 complete 16S rRNA gene sequences, order Enterobacterales were the most numerous, followed by Pseudomonadales, Lactobacillales, and Bacillales, respectively (Table 2, Figure 17).

Table 2. Breakdown of each bird species sampled for microbial communities (n=11) and the number of each order of bacteria found in each bird species (n=4 orders). Total numbers of bacteria in each species grouping are in the right most column, while total numbers of each order of bacteria are in the bottom row.

	Bacillales	Enterobacterales	Lactobacillales	Pseudomonadales	TOTAL
BHCO	1	0	0	0	1
CARW	3	8	5	6	22
GRCA	0	1	0	0	1
HETH	0	2	0	0	2
NOCA	2	13	4	9	28
PROW	4	1	1	1	7
RBWO	0	1	0	1	2
RWBL	2	4	2	3	11
SWTH	0	3	1	3	7
WEVI	0	1	0	0	1
WTSP	0	1	0	7	8
TOTAL	12	35	13	30	

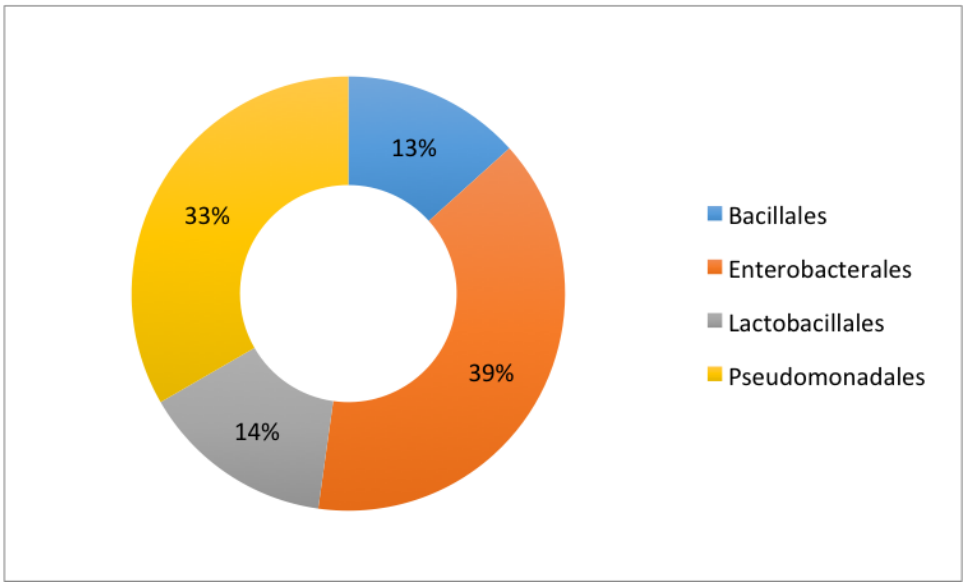

Figure 17. Percentage of each order of bacteria of all total bacteria from the analysis (n=90). These ninety sequences were collected from 11 species of birds (Table 2). Five species of birds (Brown-headed Cowbird, Gray Catbird, Hermit Thrush, Red-winged Blackbird, and White-eyed Vireo) only carried one order of bacteria in their cloacae, 2 bird species (Red-bellied Woodpecker and White-throated Sparrow) carried two orders of bacteria, 1 bird species (Swainson's Thrush) carried 3 orders of bacteria, and 3 bird species (Carolina Wren, Northern Cardinal, and Prothonotary Warbler) carried all 4 orders of bacteria found from these sequences (Figure 18).

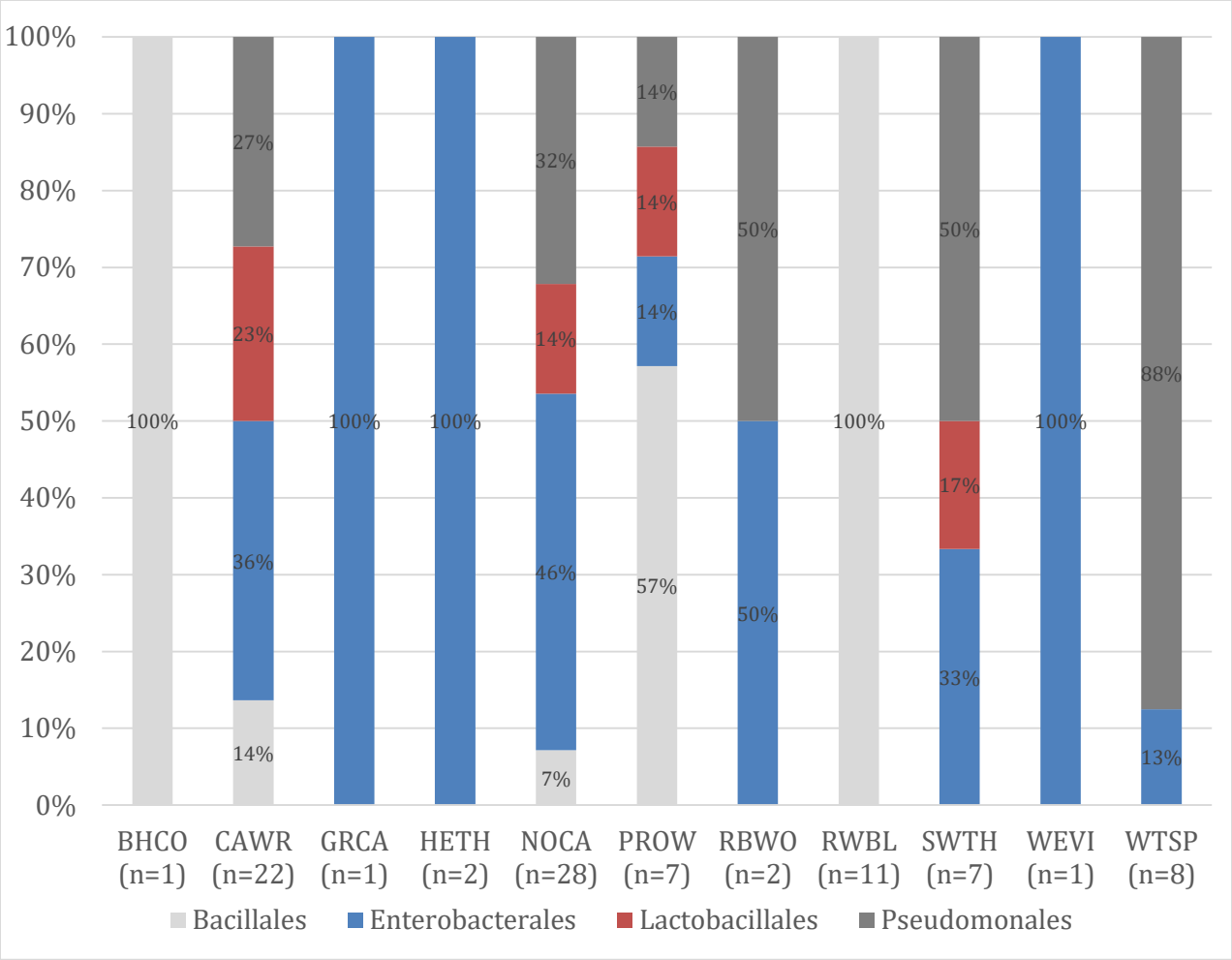

Figure 18. Percentage of each order of bacteria in each of the 11 different bird species captured and sampled. The numbers of each species that were captured are listed with the 4-letter abbreviation for each of their names.

Of the total 90 samples of bacteria isolated, half grew on plates that contained cefotaxime, while the other half grew on plates that contained erythromycin (Table 3).

Table 3. Numbers and totals of each order of bacteria, and number of samples that grew on plates with either cefotaxime or erythromycin.

Order	N Samples	Cef-Resistant	Ery-Resistant
Bacillales	12	11	1
Enterobacterales	35	0	35
Lactobacillales	13	13	0
Pseudomonadales	30	21	9
TOTAL	90	45	45

All samples from two orders of bacteria in this study were resistant to one antibiotic; all bacteria in order Enterobacterales were resistant to erythromycin only and all bacteria in Order Lactobacillales were resistant to cefotaxime only. Both Bacillales and Pseudomonadales had samples that grew on both cefotaxime agar and erythromycin agar, with a higher percentage of these samples growing on cefotaxime agar than erythromycin (Figure 19).

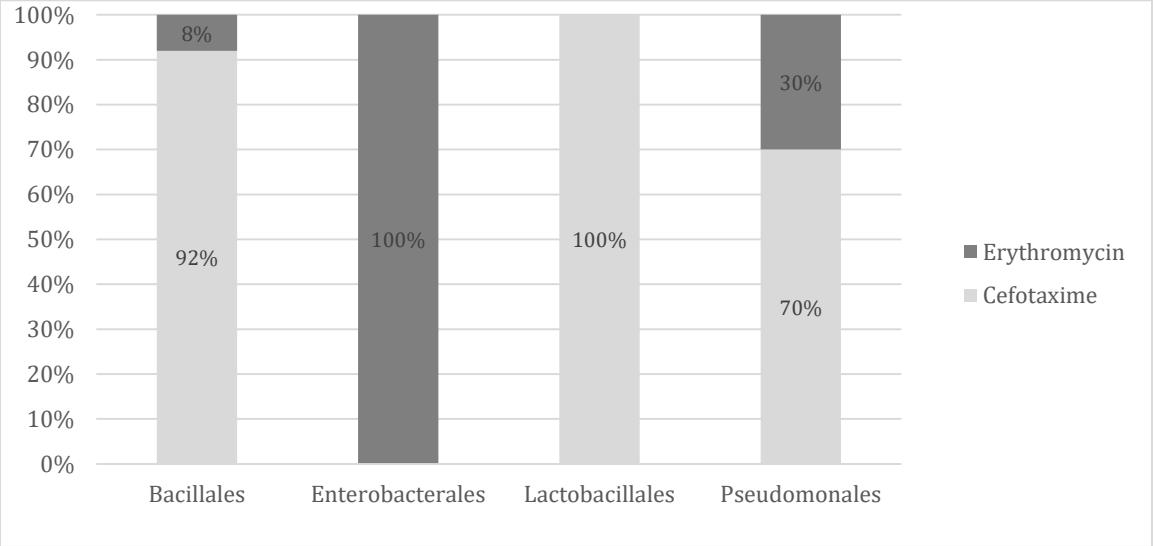

Figure 19. Resistance profiles for each order of bacteria found in the microbial communities of the birds sampled. Percentages indicate the percent of bacteria in that order resistant to the corresponding antibiotic (light gray=cefotaxime resistant, dark grayerythromycin resistant).

Phylogenetic relationships of the bacterial sequences from each bird were analyzed using a neighbor-joining phylogenetic tree analysis (Figure 20). There were 3 main branches in the tree.

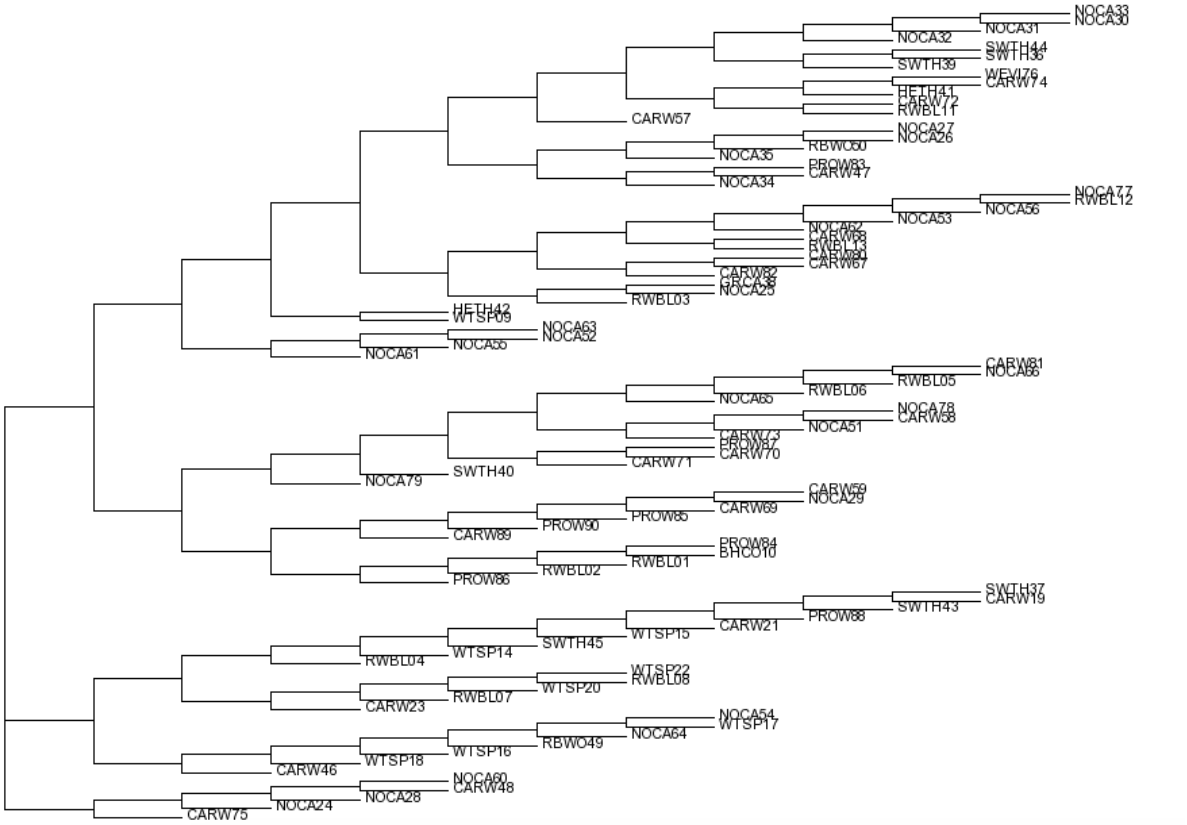

Figure 20. Relatedness of each bacterial sample sequenced. Each sample is labeled with the species name of the bird from which it was taken and a sample number (n=90).

Branch 3 (topmost main branch) contained the highest number of isolates from different bird species, followed by Branch 2, and then Branch 1.

Table 4. The 3 main branches of lowest taxonomic split from the neighbor-joining phylogenetic tree in Figure 20, and the number of sequences from each bird species represented in each branch.

	Migratory vs. Sedentary (M/S)	Number of individual Birds	Branch 1	Branch 2	Branch 3
BHCO	S	1	0	0	1
CARW	S	11	2	4	16
GRCA	M	1	0	0	1
HETH	M	1	0	0	2
NOCA	S	5	3	2	23
PROW	M	4	0	1	6
RBWO	S	1	0	1	1
RWBL	M	2	0	3	8
SWTH	M	1	0	3	4
WEVI	M	1	0	0	1
WTSP	M	3	0	7	1
TOTALS			5	21	64

3.4 Discussion and Future Research

In this analysis, we aimed to provide a description of the microbial community and antibiotic resistance profile in a sample of cypress/tupelo, lowland forest birds. We found a total

of 4 different bacterial orders (Bacillales, Enterobacterales, Lactobacillales, and Pseudomonadales) that were resistant to either cefotaxime, erythromycin, or both. Overall, the total numbers of bacteria resistant to each antibiotic were exactly even. To the best of our knowledge, this is the first study that has reported multiple orders of antibiotic-resistant bacteria in bird cloaca samples to date.

Enterobacterales are a common inhabitant of soils and other environmental settings (26, 9). It is very likely that birds closely associated with foraging on or near the ground, such as the Carolina Wren and White-throated Sparrow in our study, picked up these bacteria while foraging near or in close association with the soil. Thirty-six percent of the Carolina Wren's microbial community was comprised of bacteria from the order Enterobacterales, while 13% of the White-throated Sparrow's microbial community was from Enterobacterales (Figure 18). Nine out of our 11 bird species had Enterobacterales present in their microbial communities, the most of any bacterial order analyzed. This could be due to the orders close association with hospital settings, and its persistence in water and soil. Enterobacterales includes the widespread human pathogens such as *Escherichia coli* and *Salmonella sp*. Evidence has shown that these bacterial pathogens move from hospital waste into the environment (50). As these common human pathogens move from hospitals to wastewater, they survive the wastewater treatment process and are commonly found in soils and freshwater ecosystems. From this point, birds closely linked to foraging strategies near the soil and freshwater streams or ponds are likely to pick up Enterobacterales.

The next most frequent bacterial order found in the analysis was the Pseudomonadales, found in 6 different bird species (Figure 17). This order includes the genera *Acinetobacter* and *Pseudomonas*, two bacterial pathogens also commonly found in hospital or clinical settings (64, 93). In particular, *Pseudomonas aeruginosa* is a highly pathogenic bacterium to humans that has been shown to have high survivability in environmental settings and intrinsic resistance to multiple antibiotics (77). This bacterium occurs naturally in the environment, and is able to

effectively cling to animal tissues. Due to these properties, *P. aeruginosa* may be a main contributor to the microbial communities of the birds in this study. Also, *P. aeruginosa,* a bacterium with naturally occurring genes that cause resistance to antibiotics, may transfers these genes in the form of MGEs to similar bacteria in the same order, leading to a higher number of Pseudomonadales in our samples.

Bacterium from the Bacillales order was included in the microbial community of 5 different birds (Figure 18). Commonly, some species from this order such as *Bacillus thuringiensis* have been used on crops due to their ability to act as an insecticide (98). There is significant evidence that antibiotic-resistant bacteria are present in the soils of farming operations, and runoff from those farms leads to ARB in the environment, and thus inhabiting the other organisms in that environment (59). It is possible that antibiotic-resistant Bacillales used as pesticides on farms ended up in Bluebonnet Swamp through the runoff of this soil, and the birds in this environment picked them up either from water or soil sources.

Lactobacillales were found in the microbial community of only 4 birds, the fewest of all bacterial orders studied in this project (Figure 18). Lactobacillales span a wide range of environments, including insect intestinal tracts and aquatic environments contaminated with crude oil (1). Lactobacillales are also commonly found in the human gut, but the order also includes highly pathogenic species such as *Streptococcus pneumoniae*. This species is known to cause pneumonia in humans and has been shown to be resistant to multiple different antibiotics, including being one of the earliest human pathogens to be studied for resistance to penicillin (82). *Steptococcus pnemoniae* has also been found to be extremely adaptable to changes in its environment, which would facilitate its growth in a setting such as Bluebonnet swamp (82). Pneumonia is still common in hospital patients today, and with the pathogenicity and multi-drug resistance profile of the bacterium that causes the infection, it is likely that *S. pneumoniae* is deposited into the environment from a clinical setting. This includes transfer from a hospital into soils and water in Bluebonnet swamp. From this point, birds using fresh

water or soil resources from Bluebonnet would be contaminated with antibiotic-resistant Lactobacillales.

Two orders of bacteria analyzed in this study (Enterobacterales and Lactobacillales) grew on only one type of antibiotic-infused agar plate when isolated. Bacteria from the Enterobacterales order only grew on plates with erythromycin, while all Lactobacillales only grew on plates with cefotaxime. Enterobacterales were treated with erythromycin in the past, which could lead to an overwhelming resistance profile within this order for the drug (32). Conversely, Lactobacillales have been treated with cefotaxime frequently in the past, which could have led to a spike in genes related to cefotaxime resistance (80). The majority of both Bacillales and Pseudomonadales were able to grow on cefotaxime plates, with some samples able to grow on erythromycin plates (Figure 19).

There were isolates from 4 separate bird species (BHCO, GRCA, HETH, WEVI) that were only located within Branch 3 of Figure 20 (Table 4). All 4 of these bird species consume insects as a sizeable portion of their diet, and could possibly have picked up ARB in this fashion. Branch 3 also held the overwhelming majority of isolates, as every bird species was represented. It is quite likely that the genetically related isolates in Branch 3 came from a common source. It is possible that the bacterial samples genetically related in Branch 3 came from a water source within Bluebonnet swamp that is universally used by all birds in the swamp. Phylogenetic relatedness of antibiotic-resistant bacterial samples from birds has yet to be quantified, and this study pioneers the understudied field of antibiotic-resistance in birds.

This analysis was the first of its kind for characterizing the microbial community and resistance profile in Louisiana birds. The impact of antibiotic resistance in many scientific fields is well known, and more work must be done to fully understand the dynamics behind ARB and their presence in the environment. This study was limited by few samples for certain bird species and the use of a resolving technique that does not identify bacteria to the species level. Future studies could include a wider variety of birds from the Baton Rouge, LA area, and next-

generation sequence analysis to determine bacterial communities at the species level. Some of the cheaper methods include gram staining identification, API strips, and the use of a Biolog System (29, 88, 92). This could also be achieved by using whole-genome shotgun sequencing and comparing the whole-genome sequence to a database to determine exactly which species are in your study (56). Unfortunately, this technique is one of the most expensive. The next step would be to identify exactly which genes are causing resistance and the mechanisms by which they cause resistance. Despite this, this analysis revealed that a variety of orders of bacteria are present in bird populations with variable resistance profiles, helping to provide a clearer picture of the growing field of antibiotic resistance in the environment.

Chapter 4. Conclusions

4.1 Significance

Antibiotic resistance has recently come into the scientific spotlight due to its ubiquity in modern society, and the tremendous negative effect it can have on human populations. The presence of antibiotic-resistant bacteria and the genes that code for resistance in the environment is well studied, however studies concerning avian roles in the antibiotic resistance realm are limited. Some research has shown that birds carry antibiotic-resistant bacteria as they move through their environment, but little is known regarding how these birds obtain ARB in the first place. Gaining insight into the behaviors that facilitate transfer of antibiotic-resistant bacteria from the environment to birds is key to understanding antibiotic resistance as a whole, and has significant impacts for understanding how these potentially pathogenic microbes move throughout our society. Considering bird behavior in relationship to the antibiotic-resistant microbes they carry is an unstudied field, and in this study, we were successful in finding meaningful relationships between the ARB Louisiana birds carry, and the behaviors that allow them to obtain ARB. This study laid the groundwork for numerous research projects to come.

4.2 Future Directions

Understanding which families of antibiotic-resistant bacteria are present in avian populations is another key to understanding the field of antibiotic-resistance as a whole. Differences in genetic and morphological characteristics of differing orders of bacteria make it a necessity to study the resistance profiles of as many orders as possible. The use of 16S rRNA gene sequence analysis is a fairly recent approach to identifying antibiotic-resistant bacteria at the family taxonomic level, and employing these methods with a more accurate sequencing method may help to identify exact genes coding for resistance to a whole host of antibiotics. For now, determining resistance profiles of 4 different families of bacteria in birds has helped to lay the groundwork for future studies. Both of the studies conducted throughout this project have delved into a territory largely undocumented in the scientific community, and could provide

a base for the field of antibiotic-resistant bacteria in birds. With the rise of antibiotic resistance throughout the world, pubic health is facing a serious issue. Mostly thought of as coming from clinical systems, the role of birds in the spread and perpetuation of antibiotic resistance is largely unknown. These studies could lay the foundation for a new, exciting avenue of pursuit for the public health domain. This analysis will allow future research to heighten our understanding of the exact genetic makeup that codes for these dangerous pathogenic bacteria.

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Appendix

Table A1. Final concentrations of bacteria, percentage of resistant bacteria, and characteristics of the bird sampled throughout the study.

Bird species	IsFemale	IsAdult	IsGroundForager	BHIA CFU/mL	BHICef CFU/mL	BHIEry CFU/mL	%cefR	%eryR
CARW	U	A	G	0.00	0.00	0		
CARW	U	HY	G	0.00	0.00	0		
NOCA	M	HY	G	0.00	0.00	0		
CARW	U	A	G	0.77	0.00	0	0.00	0.00
SWTH	U	A	T	0.77	0.00	0	0.00	0.00
NOCA	M	A	G	1.54	0.00	0	0.00	0.00
WTSP	U	A	G	2.00	0.00	0	0.00	0.00
HETH	U	HY	G	2.31	0.00	0	0.00	0.00
HETH	U	A	G	2.31	0.00	0	0.00	0.00
NOCA	M	A	G	2.31	0.00	0	0.00	0.00
NOCA	F	HY	G	3.08	0.00	0	0.00	0.00
TUTI	U	A	T	3.85	0.00	0	0.00	0.00
DOWO	F	A	T	4.00	0.00	0	0.00	0.00
BHVI	U	HY	T	5.56	0.00	0	0.00	0.00
CARW	U	HY	G	6.15	0.00	0	0.00	0.00
NOCA	M	HY	G	8.89	0.00	0	0.00	0.00
NOCA	M	A	G	10.77	0.00	0	0.00	0.00
NOCA	F	HY	G	12.31	0.00	0	0.00	0.00
NOCA	F	A	G	13.85	0.00	0	0.00	0.00
TUTI	U	HY	T	20.00	0.00	0	0.00	0.00
WTSP	U	A	G	24.00	0.00	0	0.00	0.00
WEVI	M	A	T	26.15	0.00	0	0.00	0.00
NOCA	F	A	G	27.27	0.00	0	0.00	0.00
OCWA	M	HY	T	32.31	0.00	0	0.00	0.00
PROW	U	HY	T	33.85	0.00	0	0.00	0.00
NOCA	F	HY	G	40.77	0.00	0	0.00	0.00
PROW	U	HY	T	53.08	0.00	0	0.00	0.00
CARW	U	HY	G	206.92	0.00	0	0.00	0.00
NOCA	F	A	G	396.15	0.00	0	0.00	0.00
BHCO	F	A	G	440.00	0.00	0	0.00	0.00
CARW	U	A	G	464.00	0.00	0	0.00	0.00

NOCA	F	A	G	548.00	0.00	0	0.00	0.00
CARW	U	A	G	625.00	0.00	0	0.00	0.00
WTSP	U	A	G	896.88	0.00	0	0.00	0.00
NOCA	F	HY	G	81363.64	0.00	0	0.00	0.00
NOCA	M	A	G	3.00	0.67	0	22.22	0.00
NOCA	M	A	G	0.00	0.77	0		
HETH	U	A	G	0.91	0.77	0	84.62	0.00
CARW	U	A	G	1.54	0.77	0	50.00	0.00
CARW	U	A	G	3.85	0.77	0	20.00	0.00
BRTH	U	A	G	22.31	0.77	0	3.45	0.00
CARW	U	HY	G	47.78	0.77	0	1.61	0.00
CARW	U	HY	G	49.23	0.77	0	1.56	0.00
CARW	U	HY	G	56.92	0.77	0	1.35	0.00
NOCA	M	HY	G	76.15	0.77	0	1.01	0.00
CARW	U	A	G	76.92	0.77	0	1.00	0.00
WIWA	F	HY	T	77.69	0.77	0	0.99	0.00
CARW	U	A	G	128.46	0.77	0	0.60	0.00
BHCO	F	A	G	28.00	1.33	0	4.76	0.00
NOCA	M	A	G	0.77	1.54	0		0.00
GCTH	U	A	T	3.08	1.54	0	50.00	0.00
CARW	U	A	G	53.85	1.54	0	2.86	0.00
NOCA	F	A	G	74.55	1.54	0	2.06	0.00
TUTI	U	A	T	5.45	2.31	0	42.31	0.00
PROW	M	A	T	16.36	2.31	0	14.10	0.00
NOCA	F	HY	G	63.85	2.31	0	3.61	0.00
COYE	F	A	T	156.15	2.31	0	1.48	0.00
GRCA	F	HY	T	33.85	3.08	0	9.09	0.00
PROW	U	HY	T	0.00	3.13	0		
PROW	U	HY	T	3437.50	3.13	0	0.09	0.00
CARW	U	HY	G	213.85	4.62	0	2.16	0.00
BHCO	F	A	G	13.33	5.33	0	40.00	0.00
CARW	U	HY	G	168.46	6.15	0	3.65	0.00
CARW	U	HY	G	80.00	6.92	0	8.65	0.00
RBWO	U	A	T	756.00	6.92	0	0.92	0.00
CARW	U	A	G	73.08	7.69	0	10.53	0.00
CARW	U	HY	G	56.67	10.77	0	19.00	0.00
BRTH	U	A	G	0.00	12.50	0		
BHCO	F	A	G	550.00	18.75	0	3.41	0.00
NOCA	F	HY	G	141.54	46.15	0	32.61	0.00
CARW	U	U	G	2812.50	78.13	0	2.78	0.00
CARW	U	A	G	2.31	92.31	0		0.00

HETH	U	U	G	3.85	170.00	0		0.00
TUTI	U	A	T	3.85	218.46	0		0.00
WTSP	U	A	G	4.62	0.00	0.769230769	0.00	16.67
PROW	U	HY	T	29.23	0.00	0.769230769	0.00	2.63
NOCA	M	HY	G	93.85	0.77	0.769230769	0.82	0.82
ACFL	U	A	T	100.77	0.77	0.769230769	0.76	0.76
CARW	U	A	G		0.77	0.769230769		
RBWO	U	A	T	240.00	2.31	0.769230769	0.96	0.32
NOCA	F	HY	G	36.15	3.08	0.769230769	8.51	2.13
RWBL	M	A	G	70.77	3.08	0.769230769	4.35	1.09
NOCA	M	HY	G	114.62	8.46	0.769230769	7.38	0.67
PROW	F	A	T	6.92	0.00	1.538461538	0.00	22.22
NOCA	M	A	G	27.14	0.00	1.538461538	0.00	5.67
PROW	U	HY	T	29.09	0.00	1.538461538	0.00	5.29
CARW	M	A	G	120.00	0.77	1.538461538	0.64	1.28
PROW	F	A	T	6.67	1.54	1.538461538	23.08	23.08
NOCA	F	A	G	10.00	1.54	1.538461538	15.38	15.38
PROW	M	HY	T	20.00	3.85	1.538461538	19.23	7.69
PROW	U	HY	T	7.69	5.38	1.538461538	70.00	20.00
CARW	U	HY	G	69.09	12.31	1.538461538	17.81	2.23
DOWO	F	A	T	0.00	53.33	1.538461538		
BRTH	U	HY	G	33.08	5.79	1.652892562	17.49	5.00
HETH	U	HY	G	50.77	0.00	2.307692308	0.00	4.55
NOCA	M	A	G	10.77	0.77	2.307692308	7.14	21.43
PROW	F	A	T	25.38	0.77	2.30769230	3.03	9.09

						8		
NOCA	F	A	G	63.85	0.77	2.307692308	1.20	3.61
HETH	U	A	G	3.85	1.54	2.307692308	40.00	60.00
CARW	U	HY	G	74.62	3.08	2.307692308	4.12	3.09
NOCA	F	HY	G	90.77	3.85	2.307692308	4.24	2.54
PROW	M	A	T	86.92	4.62	2.307692308	5.31	2.65
BRTH	U	A	G	80.00	6.92	2.307692308	8.65	2.88
HETH	U	U	G	0.00	0.00	3.125		
WEVI	M	A	T	0.00	0.00	3.125		
NOCA	M	A	G	554.55	0.00	3.125	0.00	0.56
CARW	U	A	G		0.00	3.125		
NOCA	M	A	G	947.06	6.25	3.125	0.66	0.33
CARW	U	A	G	0.00	25.00	3.125		
OCWA	M	HY	T	156250.00	2228.13	3.125	1.43	0.00
PROW	U	HY	T	2903.23		3.125	0.00	0.11
CARW	U	A	G	16.00	0.00	3.333333333	0.00	20.83
GRCA	U	A	T	80.00	73.33	3.333333333	91.67	4.17
BRTH	U	A	G	390.77	2.31	3.846153846	0.59	0.98
CARW	U	HY	G	26.92	3.85	3.846153846	14.29	14.29
BRTH	U	A	G	83.08	3.85	3.846153846	4.63	4.63
PROW	U	HY	T	4900.00	1346.00	3.846153846	27.47	0.08
KEWA	M	A	T	103.08	0.77	4.615384615	0.75	4.48
PROW	U	HY	T	223.85	0.77	4.615384615	0.34	2.06
EATO	M	HY	G	39.23	1.54	4.615384615	3.92	11.76
NOCA	F	HY	G	0.00	0.00	5.384615385		
CARW	U	HY	G	33.08	1.11	5.384615385	3.36	16.28
BRTH	U	A	G	33.85	2.40	6.153846154	7.09	18.18

NOCA	F	A	G	8.46	12.22	6.153846154		72.73
NOCA	M	A	G	625.00	0.00	6.25	0.00	1.00
NOCA	F	HY	G	13636.36	0.00	6.25	0.00	0.05
NOCA	M	A	G	937.50	215.63	6.25	23.00	0.67
NOCA	M	HY	G	9.23	0.00	7.692307692	0.00	83.33
CARW	U	A	G	26.67		7.692307692		28.85
PROW	U	HY	T	303.85	1.54	10	0.51	3.29
GRCA	F	HY	T	61904.76	0.00	12.5	0.00	0.02
BRTH	U	A	G	312.50	6.25	12.5	2.00	4.00
CARW	U	A	G	48.46	26.92	13.07692308	55.56	26.98
CARW	U	HY	G	312.50	329.63	15.625		5.00
RWBL	F	A	G	33.33	29.33	16	88.00	48.00
CARW	U	A	G	63.33	17.69	17.69230769	27.94	27.94
NOCA	F	A	G	818.75	6.25	18.75	0.76	2.29
NOCA	F	HY	G	937.50	43.75	18.75	4.67	2.00
CARW	U	A	G	0.00	146.88	21.875		
CARW	U	HY	G	12857.14	921.88	21.875	7.17	0.17
NOCA	F	A	G	63.75	5.38	26.15384615	8.45	41.03
PROW	F	HY	T	245.38	2.31	29.23076923	0.94	11.91
CARW	U	A	G	18.75	0.00	31.25	0.00	
RWBL	M	A	G	84.00	8.00	32	9.52	38.10
NOCA	F	A	G	625.00	56.25	40.625	9.00	6.50
WIWA	F	HY	T	17187.50	3.13	43.75	0.02	0.25
BRTH	U	A	G	0.00	156.25	43.75		
PROW	U	HY	T		784.29	45.38461538		
PROW	U	HY	T	327.69	10.77	48.46153846	3.29	14.79
NOCA	M	A	G	0.00	0.00	56.25		
NOCA	F	HY	G	312.50	28.13	56.25	9.00	18.00
NOCA	M	HY	G	21875.00	53.13	65.625	0.24	0.30
CARW	U	A	G	6875.00	2640.63	71.875	38.41	1.05
CARW	U	HY	G	5625.00	540.63	81.25	9.61	1.44
PROW	M	HY	T	22187.50	6.25	121.875	0.03	0.55
PROW	U	HY	T	380.00	20.00	126.9230769	5.26	33.40

WTSP	U	A	G	3616.67	18.75	137.5	0.52	3.80
NOCA	F	HY	G	293.08	0.77	143.0769231	0.26	48.82
RWBL	M	A	G	2131.25	18.75	156.25	0.88	7.33
CARW	U	A	G	1206.67	50.00	253.8461538	4.14	21.04
CARW	U	HY	G	67500.00	14800.00	343.75	21.93	0.51
CARW	U	A	G	937.50	109.38	356.25	11.67	38.00
HETH	U	A	G	1885.71	31.25	390.625	1.66	20.71
WTSP	U	A	G	128.13	46.88	396.875	36.59	
NOCA	F	HY	G	23437.50	2141.67	450	9.14	1.92
BRTH	U	A	G	371875.00	1156.25	493.75	0.31	0.13
WTSP	U	A	G	5275.00	159.38	528.125	3.02	10.01
COYE	F	A	T	312.50	3.13	593.75	1.00	
PROW	U	HY	T			647.6923077		
WTSP	U	A	G	4090.00	144.00	1018	3.52	24.89
NOCA	F	A	G	2500.00	21.88	1293.75	0.88	51.75
PROW	F	A	T	3750.00	1625.00	1446.875	43.33	38.58
SWTH	U	A	T	23100.00	1003.13	1450	4.34	6.28
RWBL	F	A	G		137.50	1556.25		
CARW	U	A	G		25000.00	1631.25		
RBWO	U	A	T	30200.00	2463.64	2484.375	8.16	8.23
BRTH	U	HY	G	84062.50	3300.00	4600	3.93	5.47
PROW	U	HY	T			4960		
CARW	U	HY	G	4062.50	5016.67	5100		
KEWA	M	A	T	11250.00	1484.38	5391.666667	13.19	47.93
CARW	U	A	G	11850.00	756.25	5700	6.38	48.10
NOCA	M	HY	G	15937.50	9.38	6857.142857	0.06	43.03
CARW	U	A	G	18300.00	6285.71	7350	34.35	40.16
NOCA	F	A	G	9566.67	0.00	8400	0.00	87.80
EATO	M	HY	G	5312.50	1037.50	11600	19.53	
NOCA	F	A	G	30937.50	1012.50	21700	3.27	70.14
NOCA	M	A	G	33125.00	325.00	27050	0.98	81.66
CARW	U	A	G	24062.50	193.75	28100	0.81	
CARW	U	A	G	0.00	0.00			
HETH	U	A	G	0.00	0.00			
NOFL	U	A	T	0.00	0.00			

WTSP	U	A	G	100.00	0.00	0.00
NOCA	M	A	G	4.62	0.77	16.67
CACH	U	A	G	0.00	7.69	
RWBL	F	A	G	7.69	7.69	100.00
BRTH	U	A	G	200.00	7.69	3.85
BRTH	U	A	G	72.07	9.01	12.50
NOCA	F	A	G	1756.76	225.23	12.82
RWBL	M	A	G		318.75	
WTSP	U	A	G	19727.27	1639.64	8.31
HETH	U	A	G	30000.00	1666.67	5.56
PROW	U	HY	T		4850.00	
NOCA	F	A	G	107.69		
CARW	U	HY	G	82272.73		
NOCA	F	A	G			
PROW	U	HY	T			
PROW	U	HY	T			

Table A2. Isolate data from each sample sequenced, including the edited sequence and the most likely order that the sample belongs to.

Sample	Bird Species	Antibiotic	Order	Edited 16S rRNA Gene Sequence
AM-2016-001	RWBL	BC	Bacillales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG CCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTAACTGCTTGCACCT TGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-002	RWBL	BC	Bacillales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG CCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTAACTGCTTGCACCT TGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-003	RWBL	BE	Enterobacteriales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGGACGAGGTTAATAACCNCGTTCAT TGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA

AM-2016-004	RWBL	BE	Pseudomonadales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATTAACCTAATACGTTAGTGTCT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTN
AM-2016-005	RWBL	BC	Lactobacillales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACG CCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAAAATGTTCATCCC TTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-006	RWBL	BC	Lactobacillales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACG CCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAAAATGTTCATCCC TTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-007	RWBL	BC	Pseudomonadales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCCATTGCCTAATACGTGATGGTTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCT
AM-2016-008	RWBL	BC	Pseudomonadales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCCGTTACCTAATACGTGATGGTTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-009	WTSP	BE	Enterobacterales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTGTGAAGAAGGCCTTAGGGTTGTAAAGCACTTTCAGCGAGGAGGAAGGGTTCAGTGTTAATAGCACTGTGCAT TGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-015	BHCO	BC	Bacillales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG CCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTAACTGCTTGCACCT TGACGGTACCTAACCAGAAAGCCACGGCTAACNACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-016	RWBL	BE	Enterobacterales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGCATTGTGGTTAATAACCNCAGTGAT TGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA

AM-2016-017	RWBL	BE	Enterobacterales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCAT TGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTGTAAAACGAC NGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATG AAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCC GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGNCATAGCTN
AM-2016-019	RWBL	BE	Enterobacterales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCAT TGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-020	WTSP	BC	Pseudomonadales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATTAACCTAATACGTTAGTGTTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-023	WTSP	BE	Pseudomonadales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATTAACCTAATACGTTAGTGTTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-024	WTSP	BE	Pseudomonadales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGCGAATACCTTGCTGTTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-025	WTSP	BC	Pseudomonadales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGCGAATACCTTGCTGTTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-026	WTSP	BC	Pseudomonadales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGCGAATACCTTGCTGTTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-028	CARW	BE	Pseudomonadales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATTAACCTAATACGTTAGTGTTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGNCATAGCTGTT

AM-2016-029	WTSP	BC	Pseudomonadales	TGTAAAACGATCGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCAT GCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCCGTTACCTAATACGTGATGGTT TTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-030	CARW	BC	Pseudomonadales	GTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGC CGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATTAACCTAATACGTTAGTGTTTT GACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-032	WTSP	BE	Pseudomonadales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCCGTTACCTAATACGTGATGGTTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-034	CARW	BC	Pseudomonadales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTTACCTAATACGTGATTGTTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-058	NOCA	BE	Pseudomonadales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTTGTAGATTAATACTCTGCAATTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-059	NOCA	BE	Enterobacterales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGGAAATGGTTAATAACCATTTTCAT TGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-060	NOCA	BE	Enterobacterales	GTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGC CGCGTGTATGAAGAAGGCCTTAGGGTTGTAAAGTACTTTCAGTCGGGAGGAAGGCGTTGATGCTAATATCATCAACGATT GACGTTACCGACAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-061	NOCA	BE	Enterobacterales	TTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGC CGCGTGTATGAAGAAGGCCTTAGGGTTGTAAAGTACTTTCAGTCGGGAGGAAGGCGTTGATGCTAATATCATCAACGATT GACGTTACCGACAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA

AM-2016-066	NOCA	BC	Pseudomonadales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCC GCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTTGTAGATTAATACTCTGCAATTTTG ACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-067	NOCA	BC	Bacillales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCC GCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTT GACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-068	NOCA	BE	Enterobacterales	AAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG CGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGTTGTGGTTAATAACCACAGCAATTGA CGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-070	NOCA	BE	Enterobacterales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC GCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGTTGTGGTTAATAACCACAGCAATTG ACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-071	NOCA	BE	Enterobacterales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC GCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGTTGTGGTTAATAACCACAGCAATTG ACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-072	NOCA	BE	Enterobacterales	AAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG CGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGTTGTGGTTAATAACCACAGCAATTGA CGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-073	NOCA	BE	Enterobacterales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC GCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATGCGGTTAATAACCGCGTCGATTG ACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-074	NOCA	BE	Enterobacterales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC GCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGTGGGGAGGAAGGCGAAGAGGTTAATAACCTTTTCGATTG ACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT

AM-2016-077	SWTH	BE	Enterobacteriales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGTGTTGAGGTTAATAACCTCAGCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-079	SWTH	BC	Pseudomonadales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATTAACCTAATACGTTAGTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-082	GRCA	BE	Enterobacteriales	AAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGGAAGTGGTTAATAACCATTTTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-088	SWTH	BE	Enterobacteriales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-090	SWTH	BC	Lactobacillales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGTAGGAGAGTAACTGCTCTTACCTTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTG
AM-2016-094	HETH	BE	Enterobacteriales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGCGTTAAGGTTAATAACCTTAGCGATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-095	HETH	BE	Enterobacteriales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGGAAGGGTTCGGTGTTAATAGCACCGTGCATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCNTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-102	SWTH	BC	Pseudomonadales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATTAACCTAATACGTTAGTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA

AM-2016-104	SWTH	BE	Enterobacterales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC GCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGTGTTGAGGTTAATAACCTCAGCAATTG ACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-105	SWTH	BE	Pseudomonadales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATTAACCTAATACGTTAGTGTTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-107	CARW	BC	Pseudomonadales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGATAATACCTTGCTGTTT TGACGTTACCGACAGAATAAGCACCCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-108	CARW	BE	Enterobacterales	TGTAAAACGACGGCCAGTACTCCTANGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGGTGAGGTTAATAACCTCGCCGAT TGACGTTACCCGCAGAAGAAGCCCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-109	CARW	BC	Pseudomonadales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTTGTAGATTAATACTCTGCAATTT TGACGTTACCGACAGAATAAGCACCGCGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-115	RBWO	BC	Pseudomonadales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGCGAATACCTTGCTGTTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-118	RBWO	BE	Enterobacterales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGTCGGGAGGAAGGTGNNAAGGTTAATAACCTTNNCAAT TGACGTTACCGACAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-126	NOCA	BC	Lactobacillales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACG CCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAGAACGTTCATCCC TTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTN

AM-2016-127	NOCA	BC	Pseudomonadales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGGAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTACTTTAGTTAATACCTAGAGATAG TGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-128	NOCA	BE	Enterobacterales	GTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGC CGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATT GACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-129	NOCA	BC	Pseudomonadales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGCGAATACCTTGCTGTTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-130	NOCA	BC	Pseudomonadales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGGAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTACTTTAGTTAATACCTAGAGATAG TGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-131	NOCA	BE	Enterobacterales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC GCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTG ACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-132	CARW	BE	Enterobacterales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCNTTNGAGGTTAATAACCTNNNNGN GATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-133	CARW	BC	Lactobacillales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACG CCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAGAACGTTCATCCC TTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-134	CARW	BC	Bacillales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCC GCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTT GACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT

AM-2016-135	NOCA	BE	Pseudomonadales	GTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGC CGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTTGTAGATTAATACTCTGCAATTTT GACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-136	NOCA	BC	Pseudomonadales	GTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGGAAGCCTGATCCAGCCATGC CGCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTACTTTAGTTAATACCTAGAGATAGT GGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-138	NOCA	BE	Enterobacterales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCAT TGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-139	NOCA	BC	Pseudomonadales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGGAAGCCTGATCCAGCCATGCC GCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTACTTTAGTTAATACCTAGAGATAGTG GACGTTACTCGCAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-140	NOCA	BC	Pseudomonadales	GTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGC CGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGCGAATACCTTGCTGTTTT GACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-141	NOCA	BC	Lactobacillales	GTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGC CGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAACTGTTCATCCCTT GACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-145	NOCA	BC	Lactobacillales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACG CCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAAAATGTTCATCCC TTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTN
AM-2016-146	CARW	BE	Enterobacterales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTGAGGTTAATAACCTTATTCAT TGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCANGGTCATAGCTGTTA

AM-2016-147	CARW	BE	Enterobacterales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC GCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTG ACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-149	CARW	BC	Bacillales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCC GCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTT GACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-150	CARW	BC	Lactobacillales	GTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGC CGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGACGTTAGTAACTGAACGTCCCCT GACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-151	CARW	BC	Lactobacillales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACG CCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGACGTTAGTAACTGAACGTCCCC TGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-152	CARW	BE	Enterobacterales	TGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGCATTGTGGTTAATAACCACAGTGAT TGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGG
AM-2016-153	CARW	BC	Lactobacillales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACG CCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTANAACGTTCATCCC TTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-154	CARW	BE	Enterobacterales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGCATNAAGGTTAATAACCTTGGTGAT TGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-155	CARW	BE	Pseudomonadales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTTGTACGTTAATACCGTGCAATTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA

AM-2016-156	WEVI	BE	Enterobacterales	GTAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC GCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGCATTAAGGTTAATAACCTTGGTGATTG ACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTT
AM-2016-157	NOCA	BE	Enterobacterales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCC GCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTG ACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTG
AM-2016-158	NOCA	BC	Lactobacillales	GTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGC CGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAGAACGTTCATCCCT TGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-161	NOCA	BC	Bacillales	GTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGC CGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTAAGGGAAGAACAAGTACGTTAGGAAATGAACGTACCTT GACGGTACCTTATTAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-162	CARW	BE	Enterobacterales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTGAGGTTAATAACCTTATTCAT TGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-164	CARW	BC	Lactobacillales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACG CCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAAAATGTTCATCCC TTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCANGGTCATAGCTGTTA
AM-2016-165	CARW	BE	Enterobacterales	GTAAAACGACGGCCANTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGC CGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTGAGGTTAATAACCTCATTCATT GACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-167	PROW	BE	Enterobacterales	NTGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCAT GCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTNCAGCGGGGAGGAAGGCGGTGAGGTTAANAACCTCACCGA TGACGTACCCGCAGAAGAAGCACCGGCA

AM-2016-169	PROW	BC	Bacillales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG CCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTAACTGCTTGCACCT TGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-170	PROW	BC	Bacillales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG CCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACC TTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-171	PROW	BE	Bacillales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG CCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACGAGAGTAACTGCTCGTACCT TGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT
AM-2016-172	PROW	BC	Lactobacillales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACG CCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGACGTTAGTAACTGAACGTCCCC TGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-173	PROW	BC	Pseudomonadales	TAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCC GCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATTAACCTAATACGTTAGTGTTTTG ACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-176	CARW	BC	Bacillales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG CCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACC TTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGTTA
AM-2016-179	PROW	BC	Bacillales	NGTAAAACGACGGCCAGTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG CCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACC TTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATCATGGTCATAGCTGT

Vita

Collin Thomas Brown was born on Long Island, New York, and raised in Wernersville, Pennsylvania. He received his Bachelor of Arts degree in Biology from St. Mary's College of Maryland in 2015. As a result of a growing interest in birds and bird behavior and the genetic techniques he honed in his undergraduate education, he made the decision to attend graduate school to pursue a Master of Science degree in the Department of Environmental Sciences at Louisiana State University. The topic of his research would revolve around antibiotic-resistant bacteria in birds. He hopes to receive his Master of Science in May 2017 and plans to pursue a career in the clinical health realm, continuing his research on antibiotic resistance.