Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Chiamaka Uzuegbunam Date

Approval Sheet

The approval sheet should be designed according to the plan below. This form should be turned in to your ADAP with requisite signatures on hard copy. Electronically submitted form requires no signature. *Due the same date as Thesis Submission deadline.*

Multidrug Resistant Enterobacteriaceae in Healthy Pet Owners and their Companion Animals in the Greater-Atlanta Area – a Pilot Study

By

Chiamaka Ujuaku Uzuegbunam

MPH

Global Health

_____ [Chair's signature]

Renuka Kapoor

Committee Chair

_____ [Member's signature]

Nadine Rouphael

Committee Member

_____ [Member's signature]

Alex Edwards

Committee Member

Abstract: Multidrug Resistant Enterobacteriaceae in Healthy Pet Owners and their Companion Animals in the Greater-Atlanta Area – a Pilot Study

Chiamaka Uzuegbunam

Emory University – Rollins School of Public Health

July 31st, 2020

Abstract

Objectives: To determine the prevalence and association between multi-drug resistant (MDR) Enterobacteriaceae colonization in healthy pets and their owners in the greater Atlanta area from July 2018- December 2019.

Methods: A convenience sample of participants was gathered to conduct this study. All participants chosen for the study met both the inclusion and exclusion criteria required to join the study. Clinical information and stool samples were collected from participants at baseline, 2, and 6 months later. The stool samples were analyzed using extended- spectrum beta-lactamase (ESBL) agar plates and MacConkey agar plates. MDR Enterobacteriaceae colonization was defined as positive results on the ESBL and MacConkey plates. Human-pet pair specimens who both had MDR isolates would undergo PCR for MDR gene identification. A Fisher's Exact Test was conducted to determine statistical significance.

Results: 26 participants and 43 pets were enrolled. MDR Enterobacteriaceae were present in the stool of 31% (8/26) of participants and 28% (12/43) of pets at any timepoint during the study. The fisher's exact test was not statistically significant when looking at the association between human and pet MDR colonization ($p=0.38$). Five human-pet pairs were both colonized at some point during the study. One of the human-pet pairs shared an identical genetic MDR strain.

Conclusions: Colonization with MDR Enterobacteriaceae is prevalent in healthy humans and pets. There is reason to believe there can be transmission of MDR Enterobacteriaceae between humans and pets. More studies with larger sample sizes need to be done to explore these results further.

Thesis: Multidrug Resistant Enterobacteriaceae in Healthy Pet Owners and their Companion Animals in the Greater-Atlanta Area – a Pilot Study

Chiamaka Uzuegbunam

Emory University – Rollins School of Public Health

July 31st, 2020

Acknowledgements

First and foremost, I want to thank God for guiding, comforting, and strengthening me through this journey. I would like to express my deepest gratitude to Nadine Rouphael, MD, who is the Principal Investigator of this study. I wish to thank her for taking me under her wing and allowing me to work with her on this study. She has provided much guidance to me over the year and a half while taking on my practicum and thesis. Additionally, I would like to thank Renuka Kapoor, PhD, MPH for guiding me on through the process of writing a thesis. I would also like to thank Alex Edwards, MSPH, who provided feedback and help whenever I hit a rough patch in the road. Additionally, I would like to thank Alexandra Dretler, MD, and Taé Stallworth, RN, whom I collaborated and worked with for the data collection and analysis of the study. They gave me both the instruction to grow and the freedom to thrive while working on this study. Last but certainly not least I would like to thank my family. Thank you, Mom and Dad, for supporting me throughout my program. Thank you to my brothers and sister-in-law, who have provided me with both emotional and spiritual support during this long journey. Finally, thanks to my new baby Niece who's smile lights up my being.

Table of Contents

Chapter 1: Introduction *1*

I. Introduction and Rationale 1

II. Problem Statement 2

III. Purpose Statement 2

IV. Research Questions 3

V. Significance Statement 3

VI. Definition of Terms 4

Chapter 2: Literature Review *6*

Thesis References *15*

Chapter 3: Manuscript: *22*

Title Page 1

Abstract 2

1. Introduction 3

2. Materials and Methods 4

3. Results 8

4. Discussion 10

5. Conclusions 12

Key Words 13

Funding 13

Tables 14

References 19

Chapter 1: Introduction

I. Introduction and Rationale

Antimicrobial resistance (AMR) is a well-recognized and rapidly growing concern in the field of public health. According to the United States Centers for Disease Control and Prevention (CDC), it is difficult to understand the full burden and impact of AMR globally due to lack of a system to track AMR. However, they have concluded that AMR causes more than 2 million illnesses and more than 23,000 deaths per year [1]. AMR leads to increased treatment cost with more expensive antibiotics and longer hospital stay and loss of productivity [2]. The CDC reports $55 billion a year as the cost of AMR [1]. The studies that will be discussed in Chapter 2.2, have identified identical AMR genes in humans and their companion animals. The sharing of these genes points to AMR transmission within the households. This gives reason to believe that companion animals colonized with microbes with AMR are able to participate in some method of transmission from companion animal to human. The studies also do not rule out the possibility of transmission of MDR bacteria from humans to pets. The social ramifications for this possibility can be devastating. Having household pets is a cornerstone to traditional American life. The thought of human to pet or pet to human transmission of MDR bacteria can drastically change the way our society views having a pet. The theoretical significance for this problem would be that if the problem of transmission of AMR microbes from household pets to humans or vice versa is in fact occurring, more research is needed to understand the mechanisms of transmission. The practical significance would be to actively have veterinarians promote getting the household pets screened for colonization of AMR microbes. Additionally, it will be key to limit the overuse of prescription antibiotics in both humans and animals.

II. Problem Statement

There is a growing problem in relation to the number of bacteria gaining antimicrobial resistance (AMR) to multiple antimicrobials. There is a current and recognized issue in the overuse of antibiotics in veterinary and human medicine. This contributes to the growing problem of AMR in both humans and animals. There is now reason to suspect the possibility of interspecies transmission of AMR genes within households. However, there is a gap in knowledge when looking at the relationship between AMR in household pets and in pet owners. While some previous studies have explored the relationship between colonization of AMR bacteria in household pets and pet owners, including in the US, no such study has been done in Georgia, USA. The present study conducted in Atlanta, GA will begin to fill this gap in knowledge.

III. Purpose Statement

For this thesis, the purpose is to look at the prevalence and association between MDR Enterobacteriaceae colonization in pets with MDR Enterobacteriaceae colonization in healthy pet owners in Atlanta, Georgia. Enterobacteriaceae (Gram-negative bacteria) was chosen for their well-known characteristics of being intrinsically more resistant to antibiotics compared to Gram-positive bacteria. Humans and animals can be colonized with MDR bacteria and they can still present as asymptomatic. I will be looking at healthy individuals because the literature that will be reviewed in Chapter 2 has shown that colonization of MDR bacteria is a risk factor for developing more serious symptomatic diseases. Additionally, it is important to investigate the prevalence of asymptomatic MDR bacterial colonization in healthy companion animals due to evidence in the literature that suggests the transmission of MDR bacteria between humans and pets.

IV. Research Questions

Question 1: What is the prevalence of MDR Enterobacteriaceae colonization in healthy humans and pets?

Question 2: Is there an association between MDR Enterobacteriaceae colonization in healthy humans and their pets?

Null Hypothesis: There is no association between MDR Enterobacteriaceae colonization in healthy humans and their pets.

Alternative Hypothesis: There is an association between MDR Enterobacteriaceae colonization in healthy humans and their pets.

V. Significance Statement

This study aims to further validate the hypothesis that there is an association between MDR Enterobacteriaceae colonization in healthy humans and their pets. More scientific support for this hypothesis can bring more awareness to the problem of AMR in humans and companion animals. The findings from the study could trigger new public health policies and practices that restricts the unnecessary use of antibiotics in the veterinary field and the medical field. It is important to establish preventative measures to stop the growing issue that is AMR. The results could also launch a public health campaign that urges people to screen their pets for MDR bacteria colonization. The increase in screening can lead to an improvement in quick diagnostic tools for AMR in general. This could bring the pressure needed in the public health field to attack AMR in a more aggressive manner.

VI. Definition of Terms

Amplified fragment length polymorphism (AFLP): a DNA fingerprinting method that utilizes restriction enzymes digestion of DNA, and then uses selective amplification of a subset of fragments and separation by electrophoresis on polyacrylamide gel.

Antibiotics: An antimicrobial used to treat bacterial infections

Antimicrobial agent: A drug used to treat infection in the body caused by microbes by inhibiting the growth of microorganisms.

Antimicrobial resistance: Microbes that have developed an immunity to antimicrobials.

Antivirals: An antimicrobial used to treat viral infections.

Asymptomatic: showing no evidence of disease

DNA Microarray: a collection of microscopic DNA spots attached to a solid surface. Scientists use DNA microarrays to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome.

Metagenomics: the study of genetic material recovered directly from environmental samples

Microbes: a microorganism, especially a pathogenic bacterium.

Plasmid: a segment of DNA independent of the chromosomes and capable of replication, occurring in bacteria and yeast: used in recombinant DNA procedures to transfer genetic material from one cell to another.

Polymerase Chain Reaction (PCR): a method used widely in molecular biology to make millions to billions of copies of a specific DNA sample rapidly, allowing scientists to take a very small sample of DNA and amplify it to a large enough amount to study in detail.

Pulsed-Field Gel Electrophoresis (PFGE): used to produce a DNA fingerprint for a bacterial isolate.

Symptomatic: pertaining to a symptom or symptoms.

Whole-genome sequencing (WGS): is a comprehensive method for analyzing entire genomes

Chapter 2: Literature Review

Antimicrobial resistance is a broad subject area with many subtopics. The main focus of this review is bacterial AMR. AMR is addressed broadly, followed by an introduction to the One Health perspective. Asymptomatic cases of MDR bacterial colonization in individuals and companion animals is discussed along with common methods for AMR detection and surveillance. Finally, the prevalence of AMR in companion animals, and different cases of transmission of MDR bacteria between pets and humans is highlighted, with a focus on Enterobacteriaceae (Gram-negative bacteria) that are known for being intrinsically more resistant to antibiotics compared to Gram-positive bacteria.

Antimicrobial Resistance

Antimicrobial resistance (AMR) is a well-recognized and rapidly growing concern in the field of public health. According to the US Centers for Disease Control and Prevention (CDC) AMR causes more than 2 million illnesses and more than 23,000 deaths per year, although full burden and impact cannot be determined due to lack of a system to track AMR globally [1]. AMR leads to increased treatment cost with more expensive antibiotics and longer hospital stay and loss of productivity [2]. The CDC reports $55 billion a year as the cost of AMR [1].

One Health

Antimicrobial resistance is a problem in both humans and animals. The concept of "One Health" can help us understand this connection better. The premise of One health is based on the historical concept of comparative medicine [3]. Historically, the purpose of comparative medicine was studying animal medicine in an effort to learn more about human medicine. Vicq d'Azyr, a pioneer

for comparative medicine in the 18th century, made the connection between animal and human epidemics to climatic and geographical conditions [4]. Vicq d'Azyr wanted to highlight the fact that animal health is not the only thing to be compared to human health. He saw the connection between environmental health, animal health, and human health; Three centuries later the world would understand these three things cannot be separated as well [3]. One Health has evolved into a collaborative effort across multiple health science professions in an effort to learn about and achieve the best health outcomes for humans, animals, plants, and our environment [5].

One Health and Antimicrobial Resistance

When looking at the globally recognized problem of antimicrobial resistance, it is important to look at it from multiple health sectors [6,7]. The overuse of antimicrobials is a common concern in human medicine, animal medicine, and agriculture [8, 9]. Antifungals are utilized in large quantities on broad acre crops, like wheat [10]. The majority of antimicrobial agents are used in humans as well as animals, including farmed fish, pigs and chicken, where antimicrobial agents are used for treatment and prevention of infection, and as supplements in feed to promote growth [7, 11, 12].

When microbes with resistant genes begin to thrive, they can then pass the genes on to other microbes, and this is when the spread can become uncontainable [13, 14]. In a meta-analysis, researchers reported a prevalence of MDR *E. coli* in human, environmental, and animal isolates to be 22%, 31.3% and 5.7% respectively [15]. Factors that significantly contribute to the spread of AMR include geographical movement of infected humans and animals, environmental contamination, run off from intensive agriculture, poverty, poor housing, poor sanitation, poor water supplies, and poor infection control [16, 17, 18].

Symptomatic vs Asymptomatic Cases

Bacterial AMR is present in both humans and animals. However, it is important to distinguish the difference between symptomatic and asymptomatic cases of AMR. The human body is colonized with millions of bacterial cells as a part of the normal microflora [19]. Many of these bacteria may become resistant to antibiotics. However, they usually cause no symptoms, and the individuals carrying these bacteria remain asymptomatic carriers [20]. Some of these bacteria include Enterobacteriaceae (which include *Klebsiella pneumoniae, Escherichia coli, Enterobacter spp., Serratia spp., and Proteus spp.*), *Providencia spp., Morganella spp*., *Enterococcus faecium, Clostridium difficile, Staphylococcus aureus, Streptococcus pneumonia*, and *Haemophilus influenzae*. These bacteria are present anywhere from the digestive tract to the respiratory tract, and even on the skin [20].

There is a far larger number of asymptomatic carriers of bacteria with AMR compared to symptomatic cases [21, 22, 23]. Asymptomatic carriers of AMR bacteria may unknowingly carry these bacteria for years; this poses a risk of disease in a carrier, as well as a risk of transmission to others [24, 25]. Symptomatic cases of bacterial AMR, like carbapenem-resistant Enterobacteriaceae (CRE), can prove challenging to treat as they are often extensively drug resistant and are associated with high mortality [26]. Studies suggest an overall 16.5% risk of infection with CRE among patients colonized with CRE [21]. Since colonization of antimicrobial-resistant bacteria is a prerequisite for symptomatic infection, it is imperative to understand the process of colonization and transmission from individual to individual.

AMR Surveillance

Surveillance of AMR is an essential tool that allows for the monitoring of the spread and trends of AMR prevalence globally. When dealing with surveillance of complex health issues, such as AMR, it's important to shift from an isolated, linear, and sectoral approach to a systematic and transdisciplinary approach [27]. This approach aligns well with the One Health framework. Good surveillance systems should encourage close collaboration between health systems, especially when dealing with health issues that affect humans, animals, and their environment [28]. AMR surveillance is meant to be an ongoing and systematic way of collecting, analyzing, and interpreting data related to AMR for the purpose of prevention and for identifying patterns and out-of-the-ordinary health events that may relate to AMR. This helps to inform the development of targeted approaches for control of antimicrobial resistance [29]. Routine AMR surveillance is a great tool for countries and local governments which inform and establish treatment guidelines and aids in appropriate empirical antimicrobial therapy [29]. Additionally, it allows public health officials to analyze and interpret data on antimicrobial use and compare that to the patterns of AMR seen in the data collected [29].

AMR Detection

Detection of AMR genes in bacteria is important to determine potential threats in veterinary medicine and public health. Detection is usually performed by using standardized phenotypic methods [30]. Some of the most common molecular methods used for AMR gene detection are Polymerase chain reaction (PCR), DNA microarray, whole-genome sequencing and metagenomics (WGS). PCR, which is the most common method, uses heat and PCR primers to amplify targeted sections of DNA. Then, that amplified DNA product can be visualized with

agarose gels and by staining DNA with chemicals such as ethidium bromide [31]. This allows scientists to identify genes that are known for causing antimicrobial resistance.

AMR Prevalence in Companion Animals

Antimicrobial resistance in companion animals, specifically household pets, is regarded as a complex area that is relevant in both medicine and public health [32]. It is necessary to look at AMR in household pets because of the close proximity pets share with their pet owners, which allows for the chance of interspecies transmission of MDRO [32]. The research done in this area has been limited, and warrants studies to better understand the relationship and mechanism of MDRO colonization in humans and in household pets [32].

Staphylococcus aureus and Staphylococcus pseudintermedius

Staphylococci are a group of opportunistic pathogens, that are known to be particularly resistant to penicillin and methicillin. Penicillin resistant staphylococci isolates were reported from companion animals at rates of up to 74% in a study from Canada [33]. Clinical methicillin-resistant *S. aureus* (MRSA) isolates has been found in various companion animals including dogs, cats, rabbits, and horses [32, 37]. In addition to MRSA, methicillin-resistant *S. pseudintermedius* (MRSP) is of significance in human medicine and in the potential for zoonotic transmission [32]. MRSP has been reported to be found in cats and dogs, and the MRSP infections have increased globally over the past years [35, 36]. MRSA and MRSP strains can also be resistant to other antimicrobials, including macrolides, aminoglycosides and fluoroquinolones, at rates varying at different geographic locations and between animal species [32]. MRSP is not as common as

MRSA, however more research needs to be done on the prevalence of MRSP in companion animals [32].

Enterococci

Enterococci is a group of opportunistic pathogens that are found in the gastrointestinal tract of many animals. Enterococci are inherently resistant to cephalosporins, penicillin, clindamycin, and trimethoprim [32]. Although vancomycin resistant enterococci (VRE) are rare in companion animals, there have been reports of VRE colonization in household pets such as dogs and in horses [38, 39, 40]. MDR enterococci have been reported at varying rates in dogs in Denmark, Finland, Portugal, and Belgium [41-44]. With varying rates of resistance across the world, considering the target population when analyzing AMR data is important.

Escherichia coli

E. coli is a common and important pathogen that is found in the intestinal tract. In regards to AMR, the main area of focus is extended spectrum beta-lactamase (ESBL) production due to the heavy use of beta-lactam antimicrobials in animals for the treatment of infections [32]. ESBLs are known to hydrolyze a broad range of beta-lactam antimicrobials, making this class of antimicrobials ineffective when it comes to treating infections. Multiple studies have shown the presence of MDR *E. coli* in stool isolates of both healthy and sick companion animals, indicating colonization with MDR *E. coli* [32]. A systematic review and meta-analysis of prevalence of antibiotic resistance in *E. coli* strains in humans and animals, showed a prevalence of 53.4% (95% CI 22% - 82.3%) for amoxicillin resistant and 60% (95% CI 50% - 72.5%) for tetracycline resistant in *E. coli* strains in humans and animals respectively [15].

Pseudomonas

Multidrug resistance is commonplace in *Pseudomonas* species. MDR *Pseudomonas* has been found in companion animals in the community, as well as in veterinary hospitals [32]. A study on AMR in 106 strains of *P. aeruginosa* isolated from canine ear and skin infections in the United States showed 90-100% resistance to beta-lactams (ampicillin, cefazolin, cefoxitin, cephalothin, cefpodoxime, ceftiofur, amoxicillin/clavulanic acid), quinolones (nalidixic acid), aminoglycosides (kanamycin), tetracycline, and chloramphenicol [45].

Transmission of MDR Bacteria between Pets and Humans

Some studies have investigated the link between AMR in humans and companion animals. One such study recognized patterns in the presence of genetically identical *E. coli* that cause diarrhea in dogs and urinary tract infections in humans; this suggests domestic animals can serve as a reservoir for pathogenic bacteria in humans [47].

In Tennessee, USA, a cross-sectional study to determine the prevalence of within-household sharing of fecal *E. coli* between dogs and their owners was conducted with 61 healthy dog-owner pairs and a control group (n = 30) [48]. Pulsed-field gel electrophoresis (PFGE) was used to compare antimicrobial susceptibility and determine relatedness among the bacterial isolates. This study found a 9.8% prevalence of within-household sharing of fecal *E. coli* [48]. A similar study conducted in Minneapolis, Indiana on 152 individuals and 76 pets in 63 households showed within-household sharing of *E. coli* in 68% of households [49]. Another study in Knoxville, Tennessee to characterize the fecal colonization and sharing of *Klebsiella pneumoniae* between healthy humans and their companion animals that are living in close contact, analyzed stool samples from 24

humans, 18 dogs, and 8 cats belonging to 18 households. Seven dogs and 9 humans from 12 households had *K. pneumoniae* colonization, and 1/12 (8%) of positive households had within-household human-animal sharing of *K. pneumoniae* strains [50]. In Philadelphia, a study was conducted to determine the potential for pet animals to be colonized with MRSA when living with patients who have been diagnosed with MRSA [51]. A total of 99 pets from 66 households were screened, and MRSA were detected in 11/99 pets and 6/11 households had human and animal strains that were genetically concordant [51].

A longitudinal study in Finland followed two family members and two of their dogs for 2 years to screen for the presence of carbapenemase/ESBL-producing Gram-negative rods from ear and rectal specimens. Twenty-eight percent of specimens were positive for ESBL-producing *E. coli,* and identical strains were found in both the dogs and humans. At the end of the study, there was a strong evidence for the transmission of ST167 NDM-5 and ST69 CTX-M group 9 *E. coli* between two dogs and humans in the same family likely from human to dogs [52]. A similar study in Copenhagen, Denmark followed 8 dog-owning families to gain insight on *E. coli* shedding patterns in humans and dogs [54]. Ten fecal swabs were collected from 18 humans and 13 dogs over 6 months. *E. coli* was isolated from 89% (264/295) of all specimens, and 9% (12/154) of distinct *E. coli* clones were shared within-households. There was a statistical significance ($p < 0.001$) in the frequency of *E. coli* clone sharing within household pairs at a rate of 24% (12/51 household pairs) [54]. Another study in Sweden collected rectal samples from 22 dog owners who had tested positive for Extended-spectrum cephalosporin-resistant *Enterobacteriaceae* (ESCRE). Two out of the 22 ESCRE positive households had pets and pet owners with identical strains of ESCRE, suggesting possible transmission of the ESCRE between the two [53]. A study in Japan that

analyzed fecal samples from 34 healthy dog-owner pairs showed 8.8% of participating households involved in within-household fecal *E. coli* clone sharing between dogs and owners [55].

All of these studies shed light on transmission of MDR bacteria between humans and companion animals. Research is needed to address the mechanisms of transmission of AMR pathogens between humans and their companion animals.

Summary

To summarize, AMR is an emerging global health problem. A One Health approach, involving close collaboration between all sectors, including human medicine, veterinary medicine, public health, and environmental health, should be utilized to tackle the surveillance of AMR. When it comes to detection, although standardized phenotypic methods like disk diffusion are commonplace, PCR is the most common method used for identifying the genes that are responsible for AMR. Studies have shown carriage of multiple species of bacteria and a high prevalence of AMR in companion animals. There is evidence of transmission of AMR bacteria between humans and pets. More research needs to be done to further understand this potential relationship.

Thesis References

1. Centres for Disease Control and Prevention, US Department of Health and Human Services. Antibiotic resistance threats in the United States. Atlanta: CDC; 2013. Available from: http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf
2. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015;109(7):309-318. doi:10.1179/2047773215Y.0000000030
3. Ryu S, Kim BI, Lim JS, Tan CS, Chun BC. One Health Perspectives on Emerging Public Health Threats. J Prev Med Public Health. 2017;50(6):411-4.
4. Parent A. Felix Vicq d'Azyr: anatomy, medicine and revolution. Can J Neurol Sci. 2007;34(1):30-7.
5. McEwen SA, Collignon PJ. Antimicrobial Resistance: a One Health Perspective. Microbiol Spectr. 2018;6(2).
6. World Health Organization (WHO) Antimicrobial Resistance: Global Report on Surveillance. WHO; Geneva, Switzerland: 2014.
7. Collignon PJ, McEwen SA. One Health-Its Importance in Helping to Better Control Antimicrobial Resistance. Trop Med Infect Dis. 2019;4(1).
8. O'Neill J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations the Review On Antimicrobial Resistance. [(accessed on 32 March2020)];2016 Availableonline: https://amr-review.org/sites/default/files/160525_Final paper_with cover.pdf
9. Aarestrup FM, Wegener HC, Collignon P. Resistance in bacteria of the food chain: epidemiology and control strategies. Expert Rev Anti Infect Ther. 2008;6(5):733-50.

10. Collignon P. Use of Critically Important Antimicrobials in Food Production. In: Grayson L., editor. Kucers' The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic and Antivral Drugs. 7th ed. American Society for Microbiology and CRC Press; Boca Raton, FL, USA: 2018. pp. 9–18.
11. McEwen SA, Fedorka-Cray PJ. Antimicrobial use and resistance in animals. Clin Infect Dis. 2002;34 Suppl 3:S93-s106.
12. National Research Council . The Use of Drugs in Food Animals: Benefits and Risks. The National Academies Press; Washington, DC, USA: 1999.
13. Collignon P, Beggs JJ, Walsh TR, Gandra S, Laxminarayan R. Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. Lancet Planet Health. 2018;2(9):e398-e405.
14. Holmes AH, Moore LS, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, et al. Understanding the mechanisms and drivers of antimicrobial resistance. Lancet (London, England). 2016;387(10014):176-87.
15. Pormohammad A, Nasiri MJ, Azimi T. Prevalence of antibiotic resistance in Escherichia coli strains simultaneously isolated from humans, animals, food, and the environment: a systematic review and meta-analysis. Infect Drug Resist. 2019;12:1181-1197. Published 2019 May 8. doi:10.2147/IDR.S201324
16. Burow E, Kasbohrer A. Risk Factors for Antimicrobial Resistance in Escherichia coli in Pigs Receiving Oral Antimicrobial Treatment: A Systematic Review. Microb Drug Resist. 2017;23(2):194-205.
17. Marti E, Variatza E, Balcazar JL. The role of aquatic ecosystems as reservoirs of antibiotic resistance. Trends Microbiol. 2014;22(1):36-41.

18. Burgmann H, Frigon D, W HG, C MM, Pruden A, Singer AC, et al. Water and sanitation: an essential battlefront in the war on antimicrobial resistance. FEMS Microbiol Ecol. 2018;94(9).

19. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016;14(8):e1002533. Published 2016 Aug 19. doi:10.1371/journal.pbio.1002533

20. Jamrozik E, Selgelid MJ. Surveillance and control of asymptomatic carriers of drug-resistant bacteria. Bioethics. 2019;33(7):766-75.

21. Tischendorf J, de Avila RA, Safdar N. Risk of infection following colonization with carbapenem-resistant Enterobactericeae: A systematic review. Am J Infect Control. 2016;44(5):539-543. doi:10.1016/j.ajic.2015.12.005

22. Smith DL, Dushoff J, Perencevich EN, Harris AD, Levin SA. Persistent colonization and the spread of antibiotic resistance in nosocomial pathogens: Resistance is a regional problem. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(10):3709-14.

23. Safdar N, Bradley EA. The risk of infection after nasal colonization with Staphylococcus aureus. Am J Med. 2008;121(4):310-315. doi:10.1016/j.amjmed.2007.07.034

24. Marchaim D, Navon-Venezia S, Schwartz D, et al. Surveillance cultures and duration of carriage of multidrug-resistant Acinetobacter baumannii. J Clin Microbiol. 2007;45(5):1551-1555. doi:10.1128/JCM.02424-06

25. Giske CG, Monnet DL, Cars O, Carmeli Y; ReAct-Action on Antibiotic Resistance. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrob Agents Chemother. 2008;52(3):813-821. doi:10.1128/AAC.01169-07

26. Borer A, Saidel-Odes L, Riesenberg K, Eskira S, Peled N, Nativ R, et al. Attributable mortality rate for carbapenem-resistant Klebsiella pneumoniae bacteremia. Infect Control Hosp Epidemiol. 2009;30(10):972-6.
27. Bordier M, Uea-Anuwong T, Binot A, Hendrikx P, Goutard FL. Characteristics of One Health surveillance systems: A systematic literature review. Prev Vet Med. 2018.
28. FAO O. The FAO-OIE-WHO Collaboration: Sharing responsibilities and coordinating global activities to address health risks at the anima-human-ecosystems interfaces: A Tripartite Concept Note. O. FAO, WHO Rome, Paris, Geneva; 2010.
29. Masterton R. The importance and future of antimicrobial surveillance studies. Clin Infect Dis. 2008;47 Suppl 1:S21-31.
30. Khan ZA, Siddiqui MF, Park S. Current and Emerging Methods of Antibiotic Susceptibility Testing. Diagnostics (Basel). 2019;9(2):49.
31. Anjum MF, Zankari E, Hasman H. Molecular Methods for Detection of Antimicrobial Resistance. Microbiol Spectr. 2017;5(6).
32. Scott Weese J. Antimicrobial resistance in companion animals. Anim Health Res Rev. 2008;9(2):169-76.
33. Hoekstra KA, Paulton RJ. Clinical prevalence and antimicrobial susceptibility of Staphylococcus aureus and Staph. intermedius in dogs. J Appl Microbiol. 2002;93(3):406-13.
34. Faires MC, Gard S, Aucoin D, Weese JS. Inducible clindamycin-resistance in methicillin-resistant Staphylococcus aureus and methicillin-resistant Staphylococcus pseudintermedius isolates from dogs and cats. Veterinary Microbiology. 2009;139(3-4):419–20.

35. Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. Methicillin-resistant Staphylococcus pseudintermedius in a veterinary teaching hospital. J Clin Microbiol. 2007;45(4):1118–1125.

36. Schwarz S, Kadlec K, Strommenger B. Methicillin-resistant Staphylococcus aureus and Staphylococcus pseudintermedius detected in the BfT-GermVet monitoring programme 2004-2006 in Germany. J Antimicrob Chemother. 2008;61(2):282-5.

37. Bierowiec K, Płoneczka-Janeczko K, Rypuła K. Is the Colonisation of Staphylococcus aureus in Pets Associated with Their Close Contact with Owners?. PLoS One. 2016;11(5):e0156052. Published 2016 May 26. doi:10.1371/journal.pone.0156052

38. Ossiprandi MC, Bottarelli E, Cattabiani F, Bianchi E. Susceptibility to vancomycin and other antibiotics of 165 Enterococcus strains isolated from dogs in Italy. Comp Immunol Microbiol Infect Dis. 2008;31(1):1-9.

39. Devriese LA, Ieven M, Goossens H, Vandamme P, Pot B, Hommez J, et al. Presence of vancomycin-resistant enterococci in farm and pet animals. Antimicrob Agents Chemother. 1996;40(10):2285-7.

40. de Niederhausern S, Sabia C, Messi P, Guerrieri E, Manicardi G, Bondi M. VanA-type vancomycin-resistant enterococci in equine and swine rectal swabs and in human clinical samples. Curr Microbiol. 2007;55(3):240-6.

41. Damborg P, Sorensen AH, Guardabassi L. Monitoring of antimicrobial resistance in healthy dogs: first report of canine ampicillin-resistant Enterococcus faecium clonal complex 17. Vet Microbiol. 2008;132(1-2):190-6.

42. Rantala M, Lahti E, Kuhalampil J, Pesonen S, Jarvinen AK, Saijonmaa K, et al. Antimicrobial resistance in Staphylococcus spp., Escherichia coli and Enterococcus spp.

in dogs given antibiotics for chronic dermatological disorders, compared with non-treated control dogs. Acta Vet Scand. 2004;45(1-2):37-45.

43. Poeta P, Costa D, Rodrigues J, Torres C. Antimicrobial resistance and the mechanisms implicated in faecal enterococci from healthy humans, poultry and pets in Portugal. Int J Antimicrob Agents. 2006;27(2):131-7.
44. De Graef EM, Decostere A, Devriese LA, Haesebrouck F. Antibiotic resistance among fecal indicator bacteria from healthy individually owned and kennel dogs. Microb Drug Resist. 2004;10(1):65-9.
45. Rubin J, Walker R, Blickenstaff K, Bodeis-Jones S, Zhao S. Antimicrobial resistance and genetic characterization of fluoroquinolone resistance of Pseudomonas aeruginosa isolated from canine infections. Veterinary Microbiology. 2008;131(1-2):164–72.
46. Wildermuth BE, Griffin CE, Rosenkrantz WS, Boord MJ. Susceptibility of Pseudomonas isolates from the ears and skin of dogs to enrofloxacin, marbofloxacin, and ciprofloxacin. J Am Anim Hosp Assoc. 2007;43(6):337-41.
47. Starcic M, Johnson JR, Stell AL, van der Goot J, Hendriks HG, van Vorstenbosch C, et al. Haemolytic Escherichia coli isolated from dogs with diarrhea have characteristics of both uropathogenic and necrotoxigenic strains. Vet Microbiol. 2002;85(4):361-77.
48. Stenske KA, Bemis DA, Gillespie BE, D'Souza DH, Oliver SP, Draughon FA, et al. Comparison of clonal relatedness and antimicrobial susceptibility of fecal Escherichia coli from healthy dogs and their owners. Am J Vet Res. 2009;70(9):1108-16.
49. Johnson JR, Owens K, Gajewski A, Clabots C. Escherichia coli colonization patterns among human household members and pets, with attention to acute urinary tract infection. J Infect Dis. 2008;197(2):218-24.

50. Marques C, Belas A, Aboim C, et al. Evidence of Sharing of Klebsiella pneumoniae Strains between Healthy Companion Animals and Cohabiting Humans. J Clin Microbiol. 2019;57(6):e01537-18. Published 2019 May 24. doi:10.1128/JCM.01537-18
51. Morris DO, Lautenbach E, Zaoutis T, Leckerman K, Edelstein PH, Rankin SC. Potential for pet animals to harbour methicillin-resistant Staphylococcus aureus when residing with human MRSA patients. Zoonoses Public Health. 2012;59(4):286-293. doi:10.1111/j.1863-2378.2011.01448.x
52. Gronthal T, Osterblad M, Eklund M, Jalava J, Nykasenoja S, Pekkanen K, et al. Sharing more than friendship - transmission of NDM-5 ST167 and CTX-M-9 ST69 Escherichia coli between dogs and humans in a family, Finland, 2015. Euro Surveill. 2018;23(27).
53. Ljungquist O, Ljungquist D, Myrenas M, Ryden C, Finn M, Bengtsson B. Evidence of household transfer of ESBL-/pAmpC-producing Enterobacteriaceae between humans and dogs - a pilot study. Infect Ecol Epidemiol. 2016;6:31514
54. Damborg P, Nielsen SS, Guardabassi L. Escherichia coli shedding patterns in humans and dogs: insights into within-household transmission of phylotypes associated with urinary tract infections. Epidemiol Infect. 2009;137(10):1457-64.
55. Harada K, Okada E, Shimizu T, Kataoka Y, Sawada T, Takahashi T. Antimicrobial resistance, virulence profiles, and phylogenetic groups of fecal Escherichia coli isolates: a comparative analysis between dogs and their owners in Japan. Comp Immunol Microbiol Infect Dis. 2012;35(2):139-144. doi:10.1016/j.cimid.2011.12.005

Chapter 3: Manuscript: Multidrug Resistant Enterobacteriaceae in Healthy Pet Owners and their Companion Animals in the Greater-Atlanta Area – a Pilot Study

Contribution of the Student

I did not participate in the study design or initiation of this study. The study was designed by Emory Hope Clinic. I participated with the study team in collection of data for the study, including demographics, medical history, social history, and stool specimens from the participants and their pet(s). Information about antibiotic use, household members, and additional pets other than cats or dogs were also collected. I analyzed the data, developed tables to accurately demonstrate the results and wrote the manuscript.

Intended Journal for Submission: Journal of Global Antimicrobial Resistance (JGAR)

Title Page

Manuscript Title: Multidrug Resistant Enterobacteriaceae in Healthy Pet Owners and their Companion Animals in the Greater-Atlanta Area – a Pilot Study

Uzuegbunam, Chiamaka[a], Rouphael, Nadine[a], Dretler, Alexandra[a]

Affiliations:

[a]Emory University- Emory Hope Clinic,

500 Irvin Court

Suite 200

Decatur GA, USA, 30030

Author Emails:

Chiamaka.uzuegbunam@emory.edu

nroupha@emory.edu

Alexandra.wolcott.dretler@emory.edu

Corresponding Author: Alexandra Dretler (Alexandra.wolcott.dretler@emory.edu)

Abstract

Objectives: To determine the prevalence and association between multi-drug resistant (MDR) Enterobacteriaceae colonization in healthy pets and their owners in the greater Atlanta area from July 2018- December 2019.

Methods: A convenience sample of participants was gathered to conduct this study. All participants chosen for the study met both the inclusion and exclusion criteria required to join the study. Clinical information and stool samples were collected from participants at enrollment, and at 2, and 6 months follow up. The stool samples were analyzed using extended- spectrum beta-lactamase (ESBL) agar plates and MacConkey agar plates. MDR Enterobacteriaceae colonization was defined as positive results on the ESBL and MacConkey plates. Human-pet pair specimens who both had MDR isolates were tested for PCR for MDR gene identification. A Fisher's Exact Test was conducted to determine statistical significance.

Results: 26 participants and 43 pets were enrolled. MDR Enterobacteriaceae were present in the stool of 31% (8/26) of participants and 28% (12/43) of pets at any timepoint during the study. The fisher's exact test was not statistically significant when looking at the association between human and pet MDR colonization ($p=0.38$). Five human-pet pairs were both colonized at some point during the study. One of the human-pet pairs shared an identical genetic MDR strain.

Conclusions: Colonization with MDR Enterobacteriaceae is prevalent in healthy humans and pets. There is reason to believe there can be transmission of MDR Enterobacteriaceae between humans and pets. More studies with larger sample sizes need to be done to explore these results further.

1. Introduction

Antimicrobial resistance (AMR) is a widely recognized global health crisis that threatens the ability to effectively treat infections [1]. A unique outlook on the growing issue of AMR is the "One Health" perspective. One Health is defined as "the collaborative effort of multiple health science professions, together with their related disciplines and institutions—working locally, nationally, and globally—to attain optimal health for people, domestic animals, wildlife, plants, and our environment" by the One Health Commission [2]. Taking into consideration the impact of antimicrobial use in animals and the environment, there are studies that show evidence that antimicrobial use in animals is a contributor to AMR in human pathogens [3]. Additionally, the presence of antimicrobial-resistant bacteria in companion animals been reported in several studies. A study on AMR in 106 strains of *P. aeruginosa* isolated from canine ear and skin infections in the United States showed 90-100% resistance to beta-lactams (ampicillin, cefazolin, cefoxitin, cephalothin, cefpodoxime, ceftiofur, amoxicillin/clavulanic acid), quinolones (nalidixic acid), aminoglycosides (kanamycin), tetracycline, and chloramphenicol [4]. Multiple studies have also shown the presence of MDR *E. coli* in stool isolates of both healthy and sick companion animals [5]. Recently, in Japan, the presence of cephalosporin-resistant Enterobacteriaceae colonization in fecal samples were found in domesticated cats and dogs in animal shelters [6]. Additionally, varying rates of resistance in *Enterococci* to most antimicrobials was reported in dogs in Denmark, Finland, Portugal, and Belgium [7-10]. With varying rates of resistance across the world, considering the target population when analyzing AMR data is important.

Furthermore, there have been studies that report a possibility of MDR bacteria transmission between humans and pets. A study in Finland followed two family members and their two dogs

for 2 years. Both the family members and pets were found to be colonized with MDR *E. coli* at some point during the study, and there was a strong evidence for transmission of ST167 NDM-5 and ST69 CTX-M group 9 *E. coli* between dogs and human at the end of the study. While the authors were not certain on the mechanism of transmission, they believed it was most likely from humans to dog because carbapenemase-producing *E. coli* (CPE) is mainly found in humans, and up until the end of this study in 2018, CPE had not been reported in companion animals in Finland [11]. Another study in Sweden collected stool specimens from 22 participants who previously tested positive for Extended-spectrum cephalosporin-resistant Enterobacteriaceae (ESCRE); stool specimens from the participants' dogs were also collected. Two of the 22 ESCRE positive households had pets and pet owners with identical strains of ESCRE, suggesting possible interspecies transmission of the ESCRE [12]. These studies suggest that humans colonized with AMR bacteria are able to participate in some method of transmission to companion animals. However, there is a gap in knowledge when determining transmission of MDR bacteria from healthy companion animals to healthy humans. This study aims to address this gap.

The objective for this study is to determine the prevalence of MDR Enterobacteriaceae colonization in healthy humans and pets in the Greater-Atlanta area. Additionally, this study aims to determine if there is an association between MDR Enterobacteriaceae colonization in healthy pets with MDR Enterobacteriaceae colonization in healthy humans. This is the first such study to be reported from Georgia, USA.

2. Materials and Methods

2.1 Study Design and Participant Enrollment

This was a prospective case control cohort study designed and conducted by the investigators at the Emory Hope Clinic at Emory University in Atlanta, Georgia. The data was collected from July 2018 to January 2020. The data was managed using REDCap (Research Electronic Data Capture), a secure, web-based software platform designed to support data capture for research studies and hosted by Emory University [13]. The study was approved by the Emory University Institutional Review Board.

The study included three visits, one at enrollment, and two follow-up visits at 2- and 6-month. The enrollment visit was an in-person consent interview, and the follow-up visits were either in-person or phone communication. Flyers were placed in the Greater Atlanta area for healthy participants to contact coordinators for study and enrollment details. The participants that met the inclusion and exclusion criteria were enrolled in the study.

2.2 Inclusion criteria:

1. Male or female 18 years of age or older at the time of study entry
2. Written informed consent obtained from the subject/legal representative prior to performing any protocol-related procedures.
3. Expected life expectancy of at least 1 year. (Note: This was included because the study was supposed to last for one year, and in other arms of this study, hospitalized patients were recruited. If the patients had a diagnosis that suggested they would pass before the study was complete, they were not enrolled)

4. At least one companion animal living in the same household with the subject for at least 1 month prior to enrollment.

5. Subject must be willing to collect stool samples from self and companion animal(s) and agree to submit these samples for processing.

2.3 Exclusion criteria:

1. Prior history in subject or in any household members of any documented or suspected infection including (but not limited to) bacteremia, urinary tract infection, intra-abdominal abscess and/or pneumonia due to a MDR-GNB
2. Prior history in companion animal of any documented or suspected infection including (but not limited to) bacteremia, urinary tract infection, intra-abdominal abscess and/or pneumonia due to a MDR-GNB
3. Travel of subject or any household members within the last year to the following endemic ESBL regions: Southeast Asia, Eastern Mediterranean, or Western Pacific.
4. Hospitalization of subject or any household member for >24 hours within the last year. Short stays for labor and delivery or planned elective procedures were allowed per investigator discretion.
5. Any veterinary hospitalization of companion animal for >24 hours within the last year.
6. Any antibiotic use in either human or companion animal within the last 6 months.
7. Pregnant female
8. Current residence in a nursing home or long-term care facility

2.4 Data Collection

During the first in-person enrollment visit, data was collected for demographics, medical history, and social history for both the participant and their pet(s), including information about antibiotic use, household members, and additional pets other than cats or dogs. A stool sample was also collected from both the participant and the pet(s). At the follow-up visits, participants were asked for updates regarding themselves and their enrolled pets pertaining to medical history, antibiotic use, household members, and additional pets. Stool samples were collected at both follow-up visits. All participants who were lost to follow-up or early termination were excluded from the analysis.

2.5 Microbiological analysis of stool samples

All stool samples were collected at home by the participant and provided to study coordinators at the Emory University Health Sciences and Research Building. The samples were labeled with subject IDs and preserved at 2-8 degrees Celsius until processing. At the beginning of stool processing, the specimens were allowed to reach room temperature. MacConkey plates (Becton, Dickinson and Company, Sparks, MD) and ESBL agar plates (Hardy Diagnostics, Santa Maria, CA) and were utilized to obtain and interpret data on the bacterial isolates according to manufacturers' instructions [14,15].

2.6. Molecular analysis of ESBL positive isolates

If a pet and owner were both found to have ESBL-producing *E. coli,* ESBL-producing *K. pneumoniae* or ESBL-producing *K. oxytoca* in their stool specimens at any point in the study, qPCR (Illumina Inc., San Diego, CA) was performed to determine the genes present using the protocol referenced [16]. The preparation of primers, probes, and characterization of beta-

lactamase and ampC genes were conducted as per manufacturer instructions using the beta lactamase and ampC Streck ARM-D Kits (Streck, La Vista, NE) [17,18].

2.7 Data Analysis

The data was analyzed in a cross-sectional manner at the end of all 3 visits. Fisher's Exact test of independence was used to determine an association between the outcome (colonization of MDR Enterobacteriaceae in subjects) and the exposure (colonization of MDR Enterobacteriaceae in pets) at the end of the study. Specifically, we were looking to determine if there is an association between pets that had ever tested positive for MDR Enterobacteriaceae and pet owners who had ever tested positive for MDR Enterobacteriaceae across all three visits. For all tests p-value < 0.05 indicated statistical significance. Data management and statistical analyses were performed using R version 3.6.2 and RStudio version 1.2.5033 software.

3. Results

3.1 Descriptive data and prevalence of MDR colonization

Although the data was collected in a longitudinal manner, for this study the data was analyzed in a cross-sectional manner at the end of the three time points. A total of 36 participants (pet owners) were enrolled for this study, of which 26 ($n_{women} = 22$, $n_{men} = 4$) completed the study and provided a stool sample at all three time points. Out of this sample size, 88% (n = 23) of the participants were white, 7.6% (n = 2) were African American, and 3.8% (n = 1) were American Indian. The age of the participants ranged from 25 to 69 years (median 37 years, mean 43 years). At the end of the study, 8 (31%) out of 26 participants tested positive for MDR Enterobacteriaceae colonization at any point in the study, and all of these were women.

Of the 54 pets screened for the study (along with the pet owners), stool samples were available at all three time points from 43 pets (n_{dogs} =28, n_{cats} = 15). Out of these, 28 (65%) pets were male. Overall 12 (28%) pets tested positive for MDR Enterobacteriaceae at any point in time during the study, including 3 cats and 9 dogs, and 4 female and 8 male pets. Table 1 shows the descriptive data and prevalence of MDR Enterobacteriaceae colonization among study participants and their pets.

Due to the small sample size of this study, Fisher's exact test was used to analyze a one-way association between MDR Enterobacteriaceae colonization among healthy pets and MDR Enterobacteriaceae colonization in healthy pet owners. The test did not show a significant association between pets that had ever tested positive for MDR Enterobacteriaceae and subjects that had ever tested positive for MDR Enterobacteriaceae ($p = 0.38$) (Table 2).

Odds ratios were calculated to interpret the risk of MDR Enterobacteriaceae colonization by the descriptive characteristics laid out in Table 1. Table 3 shows the odds of MDR Enterobacteriaceae colonization in male pet owners (0.19 (95% CI: 0.01, 3.97)), female owners (5.28 (95% CI: 0.25, 110.51)), cats (0.53 (95% CI: 0.12, 2.35)), dogs (1.89 (95% CI: 0.43, 8.43)), male pets (1.10 (95% CI: 0.27, 4.50)), and female pets (0.91 (95% CI: 0.22, 3.72)). None of the odds ratios calculated were significant at the $p = 0.05$ level.

3.2 Bacterial Isolates and Distribution of Phenotypic Resistance

Of the 8 pet owners found positive for MDR Enterobacteriaceae colonization at the end of the study, 4 were colonized with MDR ESBL- producing *E. coli*, 2 with MDR *Klebsiella sp.*, and 1 each with MDR *Citrobacter sp*., MDR *Pseudomonas sp*. Of the 12 pets found positive for MDR colonization, 9 were colonized with MDR ESBL-producing *E. coli*, 1 each with MDR *Klebsiella sp.* and MDR *Enterobacter cloacae/asburiae*, and 1 with both MDR ESBL-producing *E. coli* and MDR *P. aeruginosa*. Four of the human-pet pairs were colonized with MDR ESBL-producing *E. coli* at any point during the study.

3.3 Molecular analyses of MDR bacteria isolates

During the course of the study, 5 (12%) out of 43 unique human-pet pairs were colonized with MDR Enterobacteriaceae at the same time. PCR results of the bacterial isolates revealed identical strains of MDR (CTX-M-14 from MDR ESBL-producing *E. coli*) in one of the human-pet pairs. PCR results for bacterial isolates from other pairs were either not identical or inconclusive (Table 4). The other MDR bacterial strains isolated included EBC and CMY-2 (Table 4).

4. Discussion

The MDR Enterobacteriaceae colonization was detected in 31% of healthy humans and 28% of healthy pets enrolled in the study. A similar study conducted in Tennessee reported fecal colonization of *K. pneumoniae* in 37.5% (9/24) of human participants and 38.9% (7/18) of dogs [19]. In another study conducted in Tennessee the prevalence of MDR *E. coli* in pet owners was 13.7% and in pets 4.4% [20]. The MDR ESBL-producing *E. coli* were the most common colonizers detected in 50% and 75% of humans and pets positive for MDR Enterobacteriaceae colonization, respectively. This is consistent with an earlier study that found 20 out of 22 participants carrying ESBL-producing *E. coli*, making it the predominant species [12].

One of the five human-pet pair colonized with MDR Enterobacteriaceae in this study showed identical strains of MDR ESBL-producing *E. coli* (CTX-M-14), leading to sharing of MDR strains in 4% (1/26) of the study households. Previous studies conducted with similar or slightly higher number of participants reported a 9-10% sharing rate for *E. coli* [12, 20, 21]. However, a study with a larger sample size of 228 participants ($n_{humans} = 152$, $n_{pets} = 76$) reported within household strain sharing in 68% of households [22].

Our findings do not suggest a statistically significant association between pet who ever tested positive for colonization of MDR Enterobacteriaceae and pet owners who ever tested positive for colonization of MDR Enterobacteriaceae. Furthermore, only 1/26 households in this study had human-pet pairs that were both colonized with identical MDR ESBL-producing *E. coli* clone. These findings are in contrast to the study from Denmark, that showed statistical significance ($p < 0.001$) in the frequency of *E. coli* clone sharing at a rate of 24% (12/51) in within- household pairs compared to 3% (4/414) across-household pairs. [21].

4.1 Limitations

The small sample size did not provide enough power to conduct other, more complicated methods of analysis. Ideally a logistic regression would be the preferred method of analysis with a larger sample size. Additionally, the convenience sampling method and lack of diversity in age, race and sex hinders generalizability of these results. To overcome this, intentional screening needs to be done when enrolling participants.

4.2 Public Health Implications

It will be hard to influence policy or implement programs to target the growing problem of antimicrobial resistance in public health without strong and significant evidence that is rigorous and peer reviewed. Effort should be placed in replicating this study and addressing the limitations laid out. More research should be conducted to study the factors that influence AMR in both humans and companion animals, and transmission of MDR Enterobacteriaceae between humans and their pets.

5. Conclusions

Antimicrobial resistance is a growing concern that has become a global health problem. Due to the alarming rate in which microbes are developing resistance to many antibiotics, it is important to study all aspects of the issue, including the methods and rate of acquisition, as well as modes of transmission. These factors should be studied in both animals and humans, and a One Health approach should be used. In an effort to learn about modes of transmission between humans and their pets, pet owners and pets were studied to determine the prevalence of MDR Enterobacteriaceae colonization in humans and pets, and association between pet MDR Enterobacteriaceae colonization and subject (pet owner) MDR Enterobacteriaceae colonization. In conclusion, there is a prevalence of MDR Enterobacteriaceae colonization in healthy humans and pets. Pets are capable of carrying multiple types of MDR bacteria. There is evidence to believe transmission is occurring between humans and pets. Additionally, there is not a statistically significant association between pet MDR Enterobacteriaceae colonization and subject MDR Enterobacteriaceae colonization in this study. Further research with a larger sample size, and more diversity in race, age, and sex, should be conducted to learn about the relationship between pets and pet owners when it comes to the transmission of MDR organisms.

Key Words
antimicrobial resistance, companion animals, Enterobacteriaceae, prevalence

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Tables

Table 1

Descriptive data for study participants and their Pets

Variables	Total	Prevalence of MDR Colonization
Age Group (Owners)		
25-34	12	25%
35-44	4	25%
45-54	1	100%
55-64	8	38%
>64	1	0%
Sex		
Male	4	0%
Female	22	36%
Race		
White	23	35%
African American	2	0%
American Indian/ Alaska Native	1	0%
Pet Type		
Cat	15	20%
Dog	28	32%

Pet Sex		
Male	28	29%
Female	15	27%

Table 2

Fisher's Exact Test to determine relationship between MDR Enterobacteriaceae colonization in pets (exposure) and MDR Enterobacteriaceae colonization in their owners (outcome) at the end of the study[1]

	Pet Colonization (+) (%)	**Pet Colonization (-) (%)**	**Total (%)**
Subject Colonization (+) (%)	4	4	8 (31%)
Subject Colonization (-) (%)	5	13	18 (69%)
Total (%)	9 (35%)	17 (65%)	26[2]

[1] This 2x2 table has 3 out of 4 cells with values less than 10. This may lead to misrepresentation of significance due to the small sample size.
[2] These data points are breakdowns by human subject data.

Table 3

Odds ratios based on descriptive characteristics of study participants

Characteristics	**Colonization** **OR (95% CI)**	**p-value** **(p < 0.05)**
Male Subject	0.19 (0.01, 3.97)	0.28
Female Subject	5.28 (0.25, 110.51)	0.28
Cat	0.53 (0.12, 2.35)	0.40
Dog	1.89 (0.43, 8.43)	0.40
Male Pet	1.10 (0.27, 4.50)	0.89
Female Pet	0.91 (0.22, 3.72)	0.89

Table 4

MDR genes detected in bacterial strains isolated from humans and their pets

Participants	**Individual**	**MDR genes detected**	**Bacteria Species**
302	302	N/A[3]	*K. pneumoniae*
	302A[4]	EBC[5], CMY-2[6]	*Enterobacter sp.*
304	304	N/A	*E. coli*
	304A	N/A	*E. coli*
	304B	N/A	*E. coli*
311	311	CTX-M-14[7]	*E. coli*
	311A	CTX-M-14, CMY-2	*E. coli*
313	313	CTX-M-14	*E. coli*
	313C	CMY-2	*E. coli*

[3] N/A = inconclusive
[4] All individuals with numbers and letters (ex: 302A) are pets.
[5] EBC also known as blaEBC is an AmpC plasmid-mediated beta-lactamase that codes for the beta-lactamase which aids in the antibiotic catabolic process in *Enterobacter cloacae* complexes. [23]
[6] CMY-2 also known as blaCMY-2 (beta-lactamase cephamycinase) is a plasmid-mediated gene that encodes for beta-lactamase production which allows for extended broad spectrum resistance to cephamycin [24].
[7] CTX-M-14 is the widest spread ESBL in class A beta-lactamases. It codes for the beta-lactamase protein which aids in the antibiotic catabolic process. They are named after their great resistance to cefotaxime [25].

References

1. Ferri M, Ranucci E, Romagnoli P, Giaccone V. Antimicrobial resistance: A global emerging threat to public health systems. Crit Rev Food Sci Nutr. 2017;57(13):2857-76.
2. Commission OH. What is One Health? 2018 [Available from: https://www.onehealthcommission.org/en/why_one_health/what_is_one_health/.
3. McEwen SA, Collignon PJ. Antimicrobial Resistance: a One Health Perspective. Microbiol Spectr. 2018;6(2).
4. Rubin J, Walker R, Blickenstaff K, Bodeis-Jones S, Zhao S. Antimicrobial resistance and genetic characterization of fluoroquinolone resistance of Pseudomonas aeruginosa isolated from canine infections. Veterinary Microbiology. 2008;131(1-2):164–72.
5. Scott Weese J. Antimicrobial resistance in companion animals. Anim Health Res Rev. 2008;9(2):169-76.
6. Umeda K, Hase A, Matsuo M, Horimoto T, Ogasawara J. Prevalence and genetic characterization of cephalosporin-resistant Enterobacteriaceae among dogs and cats in an animal shelter. J Med Microbiol. 2019;68(3):339-45.
7. Damborg P, Sorensen AH, Guardabassi L. Monitoring of antimicrobial resistance in healthy dogs: first report of canine ampicillin-resistant Enterococcus faecium clonal complex 17. Vet Microbiol. 2008;132(1-2):190-6.
8. Rantala M, Lahti E, Kuhalampil J, Pesonen S, Jarvinen AK, Saijonmaa K, et al. Antimicrobial resistance in Staphylococcus spp., Escherichia coli and Enterococcus spp. in dogs given antibiotics for chronic dermatological disorders, compared with non-treated control dogs. Acta Vet Scand. 2004;45(1-2):37-45.

9. Poeta P, Costa D, Rodrigues J, Torres C. Antimicrobial resistance and the mechanisms implicated in faecal enterococci from healthy humans, poultry and pets in Portugal. Int J Antimicrob Agents. 2006;27(2):131-7.
10. De Graef EM, Decostere A, Devriese LA, Haesebrouck F. Antibiotic resistance among fecal indicator bacteria from healthy individually owned and kennel dogs. Microb Drug Resist. 2004;10(1):65-9.
11. Gronthal T, Osterblad M, Eklund M, Jalava J, Nykasenoja S, Pekkanen K, et al. Sharing more than friendship - transmission of NDM-5 ST167 and CTX-M-9 ST69 Escherichia coli between dogs and humans in a family, Finland, 2015. Euro Surveill. 2018;23(27).
12. Ljungquist O, Ljungquist D, Myrenas M, Ryden C, Finn M, Bengtsson B. Evidence of household transfer of ESBL-/pAmpC-producing Enterobacteriaceae between humans and dogs - a pilot study. Infect Ecol Epidemiol. 2016;6:31514.
13. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—A metadata-driven methodology and workflow process for providing translational research informatics support. Journal of Biomedical Informatics. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010
14. Instruction For Use: HardyCHROMtm ESBL. Hardy Diagnostics; 1996. https://catalog.hardydiagnostics.com/cp_prod/content/hugo/HardyCHROMESBL.pdf. Accessed July 17, 2020.
15. BBLTM Macconkey II Agar. Becton, Dickinson and Company; 2015. https://legacy.bd.com/ds/technicalCenter/inserts/L007388(10).pdf. Accessed July 17, 2020.

16. Qpcr Quantification Protocol Guide FOR RESEARCH USE ONLY. Illumina, Inc; 2009. https://www.bu.edu/iscf/files/2010/07/qPCR_Quant_Protocol_Guide_11322363_A.pdf. Accessed July 14, 2020.
17. Streck ARM-D® Kit, B-Lactamase (RUO). La Vista: STRECK https://www.streck.com/wp-content/uploads/sync/Molecular/ARM-D_Kit_B-Lactamase/01_Instructions_(IFU)/01_Streck_ARM-D_Kit_β-Lactamase_(RUO)_IFU.pdf. Accessed July 20, 2020.
18. 5. Streck ARM-D® Kit, Ampc (RUO). La Vista: STRECK https://www.streck.com/wp-content/uploads/sync/Molecular/ARM-D_Kit_ampC/01_Instructions_(IFU)/02_Streck_ARM-D_Kit_ampC_(RUO)_IFU.pdf. Accessed July 20, 2020.
19. Sears HJ, Janes H, Saloum R, Brownlee I, Lamoreaux LF. Persistence of individual strains of Escherichia coli in man and dog under varying conditions. J Bacteriol. 1956;71(3):370-
20. Damborg P, Nielsen SS, Guardabassi L. Escherichia coli shedding patterns in humans and dogs: insights into within-household transmission of phylotypes associated with urinary tract infections. Epidemiol Infect. 2009;137(10):1457-64.
21. Stenske KA, Bemis DA, Gillespie BE, D'Souza DH, Oliver SP, Draughon FA, et al. Comparison of clonal relatedness and antimicrobial susceptibility of fecal Escherichia coli from healthy dogs and their owners. Am J Vet Res. 2009;70(9):1108-16.
22. Johnson JR, Owens K, Gajewski A, Clabots C. Escherichia coli colonization patterns among human household members and pets, with attention to acute urinary tract infection. J Infect Dis. 2008;197(2):218-24.

23. Esteban-Cuesta I, Dorn-In S, Drees N, et al. Antimicrobial resistance of Enterobacter cloacae complex isolates from the surface of muskmelons. Int J Food Microbiol. 2019;301:19-26. doi:10.1016/j.ijfoodmicro.2019.04.010

24. Livermore DM. beta-Lactamases in laboratory and clinical resistance. Clin Microbiol Rev. 1995;8(4):557-584.

25. Cantón R, González-Alba JM, Galán JC. CTX-M Enzymes: Origin and Diffusion. Front Microbiol. 2012;3:110. Published 2012 Apr 2. doi:10.3389/fmicb.2012.00110