

INTERNATIONAL INFECTIOUS DISEASE MANAGEMENT AND ITS ROLE IN
THE 'ONE WORLD, ONE HEALTH, ONE MEDICINE' CONCEPT

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ABSTRACT

The knowledge that almost 75% of all new human pathogens have animal origins, requires health professionals from all fields, (i.e. human medicine, veterinary medicine, and public health professionals), to work on solving the major problems associated with infectious disease threats by utilizing the ‘One World, One Health, One Medicine’ approach. It is clear that the lack of surveillance systems, proper training, and communication presents the biggest obstacles when dealing with pathogens. In accordance with the goals of the Master’s of International Infectious Disease Management and Biosecurity program at North Dakota State University, the objectives of this paper are to examine two small projects completed by the student to understand the contribution to the growth and enrichment of the student’s career development path. These projects, though different in form, clearly demonstrate the importance of surveillance, education, and multi-sectoral approach to infectious disease.

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INTRODUCTION

‘One World, One Health, One Medicine’ was coined by Dr. Calvin Schwabe, DVM during the twentieth century and supported by prominent physicians Dr. William Osler, MD and Dr. Rudolph Virchow, MD, but the concept is not new. Dr. Schwabe called for a contemporary understanding that humans, animals, and the environment live synergistically and that a “trans-disciplinary” approach to health should be applied to improve the lives of humans and animals alike (9). In 2004, the inaugural workshop dedicated to the ‘One World, One Health, One Medicine’ principle was held at Rockefeller University in New York City in conjunction with the Wildlife Conservation Society to address the decline in public health services and lack of collaboration among the various disciplines of medicine (9). From here, a mission statement evolved stating: “Recognizing that human and animal health are inextricably linked, One Health seeks to promote, improve, and defend the health and well-being of all species by enhancing cooperation and collaboration between physicians, veterinarians, and other scientific health professionals by promoting strengths in leadership and management to achieve these goals.” (9).

At the core of these projects are the central ideas that infectious agents affect people and animals, regardless of geographic location, and that a multi-dimensional approach is necessary to combat and control these infectious agents. With respect to Human Immunodeficiency Virus (HIV), global travel of the host and the ability of the pathogen to lay dormant within the body for years have allowed HIV to infect millions of people, impacting populations on all continents. Of the 33.3 million adults and children infected with HIV worldwide, 22.5 million live in sub-Saharan Africa (32). HIV has

reached epidemic proportions in many parts of the world and a great deal of work is being done to combat its spread and disease progression. My project at The AIDS Support Organization (TASO) in Kampala, Uganda allowed me to observe regional efforts to combat this epidemic and to witness groundbreaking research being conducted to stop the spread of the virus. My second project, involving *Salmonella enterica* and its antimicrobial susceptibility, has allowed me the opportunity to work with an ubiquitous, zoonotic pathogen that has serious economic impacts in the developed world and is the leading cause of food-borne illness in the United States (20). An appreciation of the ‘One World, One Health, One Medicine’ concept becomes obvious when examining the use of antimicrobials in domestic animals and the potential impacts on human health.

CHAPTER 1. CONTINUAL HIV CARE, TREATMENT, AND COUNSELING AT TASO CLINIC, MULAGO, KAMPALA, UGANDA: AN AUTOETHNOGRAPHY

Abstract

The AIDS Support Organization, TASO, was formed in 1987 by Noerine Kaleeba and others who were directly affected by the HIV/AIDS epidemic in Uganda. They provided psychosocial support for others and themselves by listening and instilling hope and trust through their philosophy; “Living positively with AIDS”. The goal of this comprehensive study is to examine TASO’s practices and to understand how it directly impacts the community and the HIV epidemic in Uganda. TASO is a shining example of what can be accomplished when respect for human dignity, equal rights, and opportunities are shared.

Materials and Methods

Through TASO’s TEACH (TASO Experiential Attachment to Combat HIV) attaché program, I learned and experienced four different activities via retrospective analysis of project summaries and conversations with other volunteers. These included learning about TASO’s counseling programs, medical services, community outreach clinics, and sensitization and community trainings. By understanding these activities, I have had the opportunity to learn more about TASO’s best practices like their HIV/AIDS integrated service delivery model, counseling model (especially couples and family), “positive living” model, home-based HIV counseling and testing, and community service delivery.

Introduction

In 1982, the first AIDS case was diagnosed in Uganda and the link between AIDS and “slimming” disease was first realized (28). In 1986, civil war finally ended and HIV prevention campaigns began. But efforts were already too late to prevent widespread infection; HIV prevalence was as high as 29% in some urban areas (27). In 1987, the ABC approach was introduced to the people of Uganda – Abstain, Be faithful, and use Condoms. This method proved to be quite successful, particularly when combined with community efforts educate people about HIV and its associated problems. By 1995, 90% of all Ugandans knew at least one person who had died of HIV/AIDS so fear was also a determining factor in behavioral changes (25). People thought that the diagnosis of HIV was a death sentence and that they needed to change their lifestyles (18). This is when The AIDS Support Organization (TASO) became such an influential part of the HIV/AIDS epidemic in Uganda (7). During the time between 1991 and 2000, there was a marked reduction in HIV prevalence within the country from 15% to 5% (7, 25). In 1998, the government began plans to distribute antiretroviral therapy to the people of Uganda. During 2001-2005, HIV prevalence stabilized at about 5.5%. However, in 2006, there was a significant increase in HIV prevalence to about 6.5%. It has been suggested that this rise in prevalence is due to the increased availability of antiretroviral therapy (ART), reliance on US-funding, and the non-adherence to the ABC method which may lead to riskier behavior (18, 16). Currently, there are more new infections (approximately 120,000 per year), than HIV/AIDS related deaths (approximately 64,000 per year) suggesting that prevalence is again rising in Uganda (18). There is certainly no question that HIV/AIDS has made a lasting impact in Uganda – it has killed more than

one million people and left more than one million orphans since its appearance in 1982 (31).

TASO – The AIDS Support Organization

Arguably, TASO's biggest achievements have been their ability to focus on so many different communities at a "ground level". Not only have they trained over 1500 community volunteers throughout the country to assist with HIV awareness and education (known as sensitization) and adherence, but they have also been able to reach over 65,000 individuals through their home-based HIV testing and counseling programs. These are truly amazing numbers and credit also needs to be given to the communities themselves. It is important to understand that TASO has embraced the ideology that persons living with HIV/AIDS are a valuable resource because they provide important knowledge and insight about the issues and problems associated with HIV/AIDS. They use GIPA, Greater Involvement of People with AIDS, to sensitize and motivate community members to create and implement their own projects within their communities. With some basic skills training, communities are able to see improvements in the quality of life of those living with HIV/AIDS and gain an immense sense of community empowerment and the importance of HIV/AIDS education.

Community AIDS Support Agents (CASA) are a group of HIV-positive community volunteers who facilitate counseling and sensitization about the importance of adhering to the prescribed antiretroviral therapy (ART). They are extremely important instruments of local change, and TASO has reported that CASA members are responsible for over 80% adherence to ART throughout its outreach communities (29).

TASO is commendable in their understanding that the HIV/AIDS epidemic is a global problem and for sustainable change, they cannot do it alone. In order to help the people of Uganda, they need support from the government as well as other national and international organizations. The initial funder and continued major contributor of TASO's free antiretroviral therapy is PEPFAR, the United States President's Emergency Plan for AIDS Relief. Started in 2004 by President George W. Bush, PEPFAR contributes \$280 million USD annually to Uganda's AIDS relief efforts. In the 2010 fiscal year alone, 200,000 Ugandan's received ART, 845,000 Ugandan's received HIV care and support, 350,000 orphans and vulnerable children received support, and 33,000 HIV-positive pregnant women received prophylactic ART to help prevent transmission to their unborn children from (10). In 2010, PEPFAR also increased their efforts to secure better and more substantial pediatric ART coverage and to reduce the number of mother-to-child-transmissions of HIV. More than 70% of AIDS relief funding for Uganda, comes from PEPFAR. The rest of the funding comes from other international and national NGO's, such as the Clinton Foundation and DANIDA (the Danish government), as well as the Ugandan Ministry of Health. This funding has played a vital role in the advancements of HIV/AIDS treatment and therapy.

TASO is one of the leading advocates for people infected or affected by HIV/AIDS. It has stuck firmly to the institutions main principles: equal rights, shared responsibilities, and equal opportunities to advocate for the protection and empowerment of persons living with HIV/AIDS and their families (29). TASO's mission statement simply and rationally encompasses the ideologies of the organization: "To contribute to a process of preventing HIV infection, restoring hope and improving the quality of life of

persons, families, and communities affected by HIV infection and disease.” By being an effective advocate, TASO has improved the lives of millions in Uganda by showing the world that HIV/AIDS issues should not be cast into the shadows, but rather brought to the forefront of the global health initiative.

Outreach

My experiences with TASO’s outreach program have been extremely enlightening. I was fortunate to experience one of TASO’s eighteen outreach communities and see first-hand the battle against the HIV/AIDS epidemic. I was so intrigued with their ability to efficiently manage 160 patients in a rural setting with no formal clinic. The patient needs only to remember to bring her-/himself and the exercise book that documents his/her progress when they come to the clinic every month.

TASO is comprised of eleven official clinics throughout the entire country of Uganda. TASO Mulago, located in Kampala, is the institution’s headquarters and one of the largest clinics, currently serving about 6800 clients receiving various forms of care, with 3900 of them receiving ART. The Mulago clinic sees on average about 2400 clients per month from the surrounding area. TASO Mulago also has eighteen outreach communities that it services every four to five weeks. This enables clients who can’t afford to travel to Mulago or who are too sick, to receive care and counseling as well as laboratory services in order to check their health status. TASO Mulago also has 56 Community Drug Distribution Points (CDDP’s) throughout Kampala in which they distribute ARTs and cotrimexazole, as well as disseminate information about HIV/AIDS prevention and education.

During the outreach process, I observed operations in the mobile clinic. For efficiency, the patients were split into smaller groups in correspondence to their individual needs. After their medical consultations, and depending on their needs, they either moved to different counseling groups or to the laboratory for blood draws in order to check CD4⁺ cell counts. Some were also sent to receive more ART and cotrimexazole, and others were there for the first time so they had to go through the registration process. All of these activities were accomplished in one localized area with great organization and patience from all involved. Within six hours, everyone had been seen, assessed, and provided with treatment, care, and counseling.

I was very impressed with the amount of time that was spent with each patient. In western medicine, we talk so much about “patient centered” health care and how we can improve the quality of physician visits by simply taking time to listen carefully to each patient and addressing all of their concerns. The social worker whom I was assigned to work with admitted that it can be exhausting to continually explain the same thing to patients, but she was very adamant that it is, really, the only way to successfully change behaviors and perceptions surrounding HIV/AIDS. If the community remains uneducated about the ways to stay healthy and prevent the spread of HIV, then TASO, as an organization, feels that they have failed at a fundamental level.

Clinic Setting

TASO Clinic Mulago is a bustling clinic that sees patients three days per week and provides counseling, care, and treatment to persons infected or affected by HIV/AIDS. They also have a diagnostic laboratory to test for HIV, monitor CD4⁺ cell counts, as well as the ability to test for some other opportunistic infections.

The setting in the clinic is very similar to that in the community outreach programs. Patients start arriving at around 8:30AM from various parts of the city and by various forms of transportation. All age groups were present; but there is a huge gender disparity with three females to every one male receiving care. They wait patiently outside for counselors to organize their records. Once their name has been called, they enter the main building and get an accurate weight measurement. This is a critical measurement as often weight loss can be the first sign of change in the disease process. After they have been weighed, they move to the medical consultation area where they wait to meet with a physician to discuss any problems or questions. From there, they proceed to either the laboratory or the counseling area. Group counseling is usually offered at this time, or depending on the needs of the individual, personal, family, or couple counseling is provided.

The whole process is very organized and it appeared that a patient was around the clinic for about two hours, depending on if they need to utilize the pharmacy. A technician who has been trained to interpret the physician's orders and the past history of the patients handles the distribution of ARTs and cotrimexazole. It is reiterated time and again to each patient how important it is to take the ARTs everyday and to make sure that their family and friends witness them take the drugs. Chronic treatment and care of HIV/AIDS really requires family support and community involvement to ensure adherence and prevention of the spread of the virus (29).

CD4⁺ cell counts are done routinely for every patient every six months unless the physician dictates otherwise. The counts are monitored closely and ART is initiated if their CD4⁺ cell count drops below 250 cells/ μ L. This guideline changes for pregnant

women; they will begin ART when their CD4⁺ cell count is below 350 cells/ μ L. This earlier intervention decreases the probability that the fetus will acquire HIV from the mother. Over recent years, TASO has reduced the mother to child transmission rate in their clients by 70% simply by initiating ART earlier in pregnant women.

Discussion

Riding on the wave of success from the recently released Pre-Exposure Prophylaxis (PrEP) study, TASO has made great strides in the prevention of the transmission of HIV in discordant couples in parts of the country. In Uganda, the PrEP study is taking place in Tororo and Mbale and was started in November of 2008 and is expected to be complete in December 2012. In conjunction with the University of Washington, TASO and its researchers are comparing two different antiretroviral drug combinations in HIV-negative partners of discordant couples (33). The study has found that not only does early intervention of ART among HIV-positive patients greatly reduce the risk of transmission, but that the initiation of ART among high risk HIV-negative clients, e.g. patients in a discordant relationship, decreases the rate of HIV transmission and infection by about 70% (33). This finding has serious implications in the potential to disrupt the infection cycle and thus would substantially impact the reduction of the HIV/AIDS epidemic. There are still many obstacles to conquer in order to incorporate PrEP into the standards of HIV/AIDS care and treatment. In this study, the counseling burden was very high and it is thought that perhaps if this level of counseling isn't maintained, then the transmission rates may be higher than reported in the study (33). The long-term effects of ART use have not been comprehensively studied in Uganda, although I expect that this data will be forthcoming soon. Drug levels in the blood, cell

and tissue culture, and viral genotyping to see resistance will all need to be analyzed for further implementation of similar studies.

The timing of this study has thrust Uganda into the forefront of HIV prevention. Other countries around the world are challenging Uganda to become the leader in pre-exposure prophylaxis. At the UN global HIV convention held this year in Rome, there is one phrase that is said over and over again; “Treatment is prevention” (32). Uganda has a unique opportunity to contribute significant advances in the ending of an epidemic that has ravaged the globe for thirty years.

**CHAPTER 2. ANTIMICROBIAL RESISTANCE SCREENING OF 101
SALMONELLA ENTERICA ISOLATES FROM THE NORTH DAKOTA STATE
UNIVERSITY VETERINARY DIAGNOSTIC LAB: 2009-2011**

Abstract

Salmonella enterica is a food-borne pathogen that infects animals and humans alike. Salmonellosis is the leading cause of food-borne illness in the United States and costs approximately \$14 billion dollars annually (20). Antimicrobial resistance among these organisms has increased over the past years due to the high use of antimicrobials in both veterinary and human medicine. Of most concern is the development of resistance to 3rd generation cephalosporins and fluoroquinolones because these are used exclusively to treat severe salmonellosis in humans. The objective of this study was to examine antimicrobial resistance patterns in *Salmonella* isolates obtained from the North Dakota State University Veterinary Diagnostic Lab (NDSU VDL) located in Fargo, ND. Susceptibility testing using Sensititre antimicrobial plates showed an increase from 25%(10/40) in 2009, to 31.3%(10/32) in 2010, and finally to 58.6% (17/29) in 2011 of isolates exhibiting resistance to 6 or more antimicrobials. Antimicrobial resistance profiles to amoxicillin/clavulanic acid, ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline were the most commonly observed. It is important to understand these trends in antimicrobial resistance to ensure that veterinary and human drugs are used appropriately in order to prevent the persistence of antimicrobial resistant *Salmonella* serotypes in the environment.

Introduction

Salmonellosis, caused by the gram-negative bacteria *Salmonella enterica*, is the leading cause of food-borne illness in the United States (20). *S. enterica* includes more than 2500 serotypes, although only five serotypes are commonly associated with human disease. *S. Typhimurium*, *S. Enteritidis*, *S. Newport*, *S. Heidelberg*, and *S. Javiana* encompass the most common human pathogenic serotypes (12, 20). However, it is important to note that the most common pathogenic serotypes found in production animals are also among the most common found in humans, indicating the possibility of contamination of the food supply (20).

With the emergence of multi-drug resistant (MDR) *S. enterica* serotypes, efforts have been made to understand the implications of increased antimicrobial use in production animals for growth promotion, veterinary medicine, and human medicine (35). In 1996, The National Antimicrobial Resistance Monitoring System (NARMS) was established for the submission of enteric bacteria isolated from humans, including *Salmonella*, from state and local public health facilities to monitor antimicrobial resistance in real time (12). The program has expanded to include research into mechanisms of resistance and its impact on public health, public health education, education to promote proper use of antimicrobials in veterinary and human medicine, and development of commercially available antimicrobial susceptibility screening products (12).

The greatest concern from the clinical aspect is the development of resistance among *Salmonella* to fluoroquinolones and third generation cephalosporins as they are used to treat severe, complicated human salmonellosis (12). The use and misuse of these

antimicrobials in both human and veterinary medicine has provided an environment in which selective pressure has dictated and directed resistance patterns for decades (8). Therefore, it is critically important that monitoring systems and policies be put in place to control antimicrobial resistance to these clinically important antimicrobial drug classes.

Objectives

The objective of this study is to characterize *Salmonella* isolates obtained from the North Dakota State University Veterinary Diagnostic Laboratory in the context of multi-drug resistance and decreased 3rd generation cephalosporin and fluoroquinolone susceptibility. Distribution of *Salmonella* serotypes among animal species is imperative in understanding the risk posed to human health through food borne illness.

Literature Review

Salmonella spp

Salmonella enterica is a gram-negative, facultative intracellular bacterium that infects many species including humans (1). Globally, infections with non-typhoidal *Salmonella* account for up to 1 billion cases of diarrheal disease and 3 million deaths per year (5). It is therefore easy to understand why non-typhoidal *Salmonella* are considered to have serious public health and economic impact. Humans usually acquire this infection through ingestion of contaminated food or water where acute symptoms develop in 6-72 hours resulting in severe abdominal pain, nausea, vomiting, and diarrhea – with or without blood (5). Salmonellosis is a self-limiting disease and symptoms usually resolve in 5-7 days. Rehydration therapy is indicated in individuals with serious dehydration and electrolyte imbalances, and in cases where invasive disease is evident, antimicrobial therapy is prescribed, but doesn't shorten the duration of illness (5).

Antimicrobial Resistance

The first documented multi-drug resistant (MDR) *Salmonella* was identified in 1964 in the United Kingdom (30). Since then, there have been waves of increasing resistance among *Salmonella*. From 1975 to 1985, there was increased incidence of MDR *Salmonella* found in food animals, also coinciding with increased cases of MDR salmonellosis in humans (30). The discovery of chromosomally-encoded phage-type DT104 (resistance to ampicillin, chloramphenicol, streptomycin, sulphamethoxazole, and tetracycline) in the early 1980's was integral to understanding the epidemic spread among cattle in 1990 and the subsequent finding of decreased ciprofloxacin sensitivity worldwide (30). This decreased susceptibility to fluoroquinolones is of great public health concern because this class of drugs is used in treatment for severe salmonellosis in humans (34).

Antimicrobial Resistance Mechanisms Utilized by *Salmonella*

Salmonella utilize several different mechanisms to achieve antimicrobial resistance. These include, but are not limited to, production of enzymes that render antimicrobial agents ineffective (β -lactamases), reduction of bacterial cell wall permeability, utilization of efflux pumps, and modification of drug targets (12). These changes are especially effective against β -lactam antimicrobials such as penicillins and cephalosporins.

β -lactamases can be grouped by their affinity for particular structures; those with limited diversity target only a few antimicrobials, whereas those with extended or a broad spectrum effect degrade many types of antimicrobials (12). There are over 700 β -lactamases currently classified, and *Salmonella* serotypes utilize variations within

families of extended-spectrum β -lactamases(ESBLs) (23). Recently, Arlet *et. al.* (2006) described a new family of β -lactamases that have higher resistance to 3rd and 4th generation cephalosporins and are resistant to clavulanic acid(an anti- β -lactamase).

Salmonella resistance to fluoroquinolones and their elementary precursors, quinolones, is mediated by metabolic DNA mutations that affect bacterial DNA gyrase (12). These mutations effectively change the antimicrobial drug targets (bacterial DNA gyrase) and facilitate prevention of antimicrobial binding. Quinolone and fluoroquinolone resistance is also attributed to efflux pump mechanisms (12). Baucheron *et. al.* (2004) demonstrated that inactivation of multi-drug membrane transporter pump genes significantly reduced resistance levels to ciprofloxacin (a fluoroquinolone). Therefore, it is important to understand that resistance to quinolones and fluoroquinolones is mediated by several mechanisms including two major resistance pathways via target gene mutations and efflux pumps and they are both plasmid-mediated (12).

Materials and Methods

***Salmonella* Isolates**

Isolates were obtained from the NDSU VDL. Standard operating procedures were followed by NDSU VDL to isolate *Salmonella* and samples were kept in -80°C freezer for preservation. All *Salmonella* isolates were sent to the National Veterinary Services Laboratories (NVSL) in Ames, Iowa for serotyping.

Antimicrobial Screening

A total of 101 *Salmonella* isolates were tested for antimicrobial susceptibility at the Department of Veterinary and Microbiological Sciences, NDSU. Antimicrobial susceptibility of *Salmonella* isolates was determined using the National Antimicrobial

Resistance Monitoring System (NARMS) antimicrobial panel according to the Food and Drug Administration and National Committee for Clinical Laboratory Standards (NCCLS) recommendations. Each isolate was screened using Sensititre[®] (Trek Diagnostics System, Inc., Westlake, OH, USA) resistance plates using full-range minimum inhibitory concentrations according to the manufacturer's instructions. The 15 antimicrobials used included amoxicillin/clavulanic acid (1/0.5-32/16 µg/mL), ampicillin (2-32 µg/mL), azithromycin (0.12-16 µg/mL), cefoxitin (0.5-32 µg/mL), ceftiofur (0.12-8 µg/mL), ceftriaxone (0.25-64 µg/mL), chloramphenicol (2-32 µg/mL), ciprofloxacin (0.015-2 µg/mL), gentamicin (0.25-16 µg/mL), kanamycin (6-64 µg/mL), naladixic acid (0.5-32 µg/mL), streptomycin (32-64 µg/mL), sulfizoxazole (16-512 µg/mL), tetracycline (4-32 µg/mL), and trimethoprim-sulfamethoxazole (0.12/2.4-4/76 µg/mL). NARMS panels were used to assess the extent to which the *Salmonella* isolates would respond to antimicrobial agents used in human and veterinary medicine. SWIN[®] software (Trek Diagnostics System, Inc., Westlake, OH, USA) was used in conjunction with Sensititre[®] (Trek Diagnostics System, Inc., Westlake, OH, USA) optical density reader to determine degree of growth. Isolates were termed resistant according the U.S. FDA recommended break points. Breakpoints are defined as minimum drug concentration above which growth of the tested isolate does not occur (17, 22).

Data Analysis

Descriptive statistics of *Salmonella* serotypes and resistance were calculated using Epi Info version 7.1 software (Epi Info[™], U.S. Centers for Disease Control and Prevention (CDC), Atlanta, GA). Tabular and graphical representations were created in

Excel (Microsoft Office 2011) to demonstrate resistance trends by year. For statistical analysis, isolates determined to be of intermediate resistance were considered resistant.

Results

Serotype Distribution

This study included 101 domestic animal samples submitted to the NDSU VDL between 2009 and 2011. Animal species sampled included cattle, turkey, sheep, horse, dog, bobcat, turtle, water dragon (reptile), hedgehog, buffalo, mink, and pig. Of the samples taken, cattle were most often infected (45.5%), followed by turkeys (27.7%) and pigs (6.93%). The most predominant serotype was *Salmonella* Dublin (28, 27.7%) and the most species specific serotype was *Salmonella* Dublin (28/46, 60.9%) in cattle, *Salmonella* Senftenberg (16/28, 57.1%) in turkeys, and *Salmonella* Arizonae (4/5, 80.0%) in sheep. Species distribution by serotype is depicted in Table 1 and Figure 1.

MDR Patterns

Antimicrobial susceptibility patterns are described in Table 2 and depicted in Figure 2. The most common antimicrobials shown to have resistance were amoxicillin/clavulanic acid, ampicillin, cefoxitin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline. Amoxicillin/clavulanic acid ($p=0.01$) and ceftriaxone ($p=0.05$) showed a statistically significant decrease in susceptibility from 2009 to 2011 as depicted in Figure 5. There was also a statistically significant increase in the number of multi-drug resistant isolates ($p=0.04$) per year. Figure 3 demonstrates this trend.

Two well-defined multiple-drug resistant phenotypes also emerged during this study. They include resistance to ampicillin, chloramphenicol, streptomycin,

sulfisoxazole, and tetracycline (ACSSuT) and ACSSuT plus amoxicillin/clavulanic acid (ACSSuTAu). This is depicted in Figure 4.

Table 1 – *Salmonella* serotype distribution by species, NDSU VDL, 2009-2011.

<i>Salmonella</i> Serotype	Cattle	Avian	Sheep	Horse	Dog	Pig	Reptile*
4, 12 : 1:-	1 (0.99)	7 (6.93)		2 (1.98)			
4, 5, 12; i							
4, 5, 12: 1:-							
6, 7; nonmotile						1 (0.99)	
Agona						1 (0.99)	
Arizonae		1 (0.99)	4 (3.96)				
Choleraesuis						1 (0.99)	
Derby						4 (3.96)	
Dublin	28 (27.7)						
Enteritidis							1 (0.99)
Give							
Havanna	2 (1.98)						
Heidelberg		1 (0.99)					
IV_44: Z4, Z32							1 (0.99)
Kentucky		1 (0.99)					
Kiamba					1 (0.99)		
Kisarawe							1 (0.99)
Meleagridis	1 (0.99)						
Montevideo	1 (0.99)						
Muenster	1 (0.99)						
Mult. Serotypes	1 (0.99)						
NA	1 (0.99)	1 (0.99)	1 (0.99)				
Newport	5 (4.95)	1 (0.99)		1 (0.99)	1 (0.99)		
Oranienburg							
Rough O: r: 1,2							
Senftenberg	1 (0.99)	16 (15.8)		1 (0.99)			
Typhimurium	4 (3.96)				1 (0.99)		
Total	46 (45.5)	28 (27.7)	5 (4.95)	4 (3.96)	3 (2.97)	7 (6.93)	3 (2.97)

*Reptile includes water dragon, turtle, and bearded dragon. Mustelid, buffalo, feline, and hedgehog were not included and accounted for 5 samples.

Figure 1 – *Salmonella* serotype distribution by year, 2009-2011.

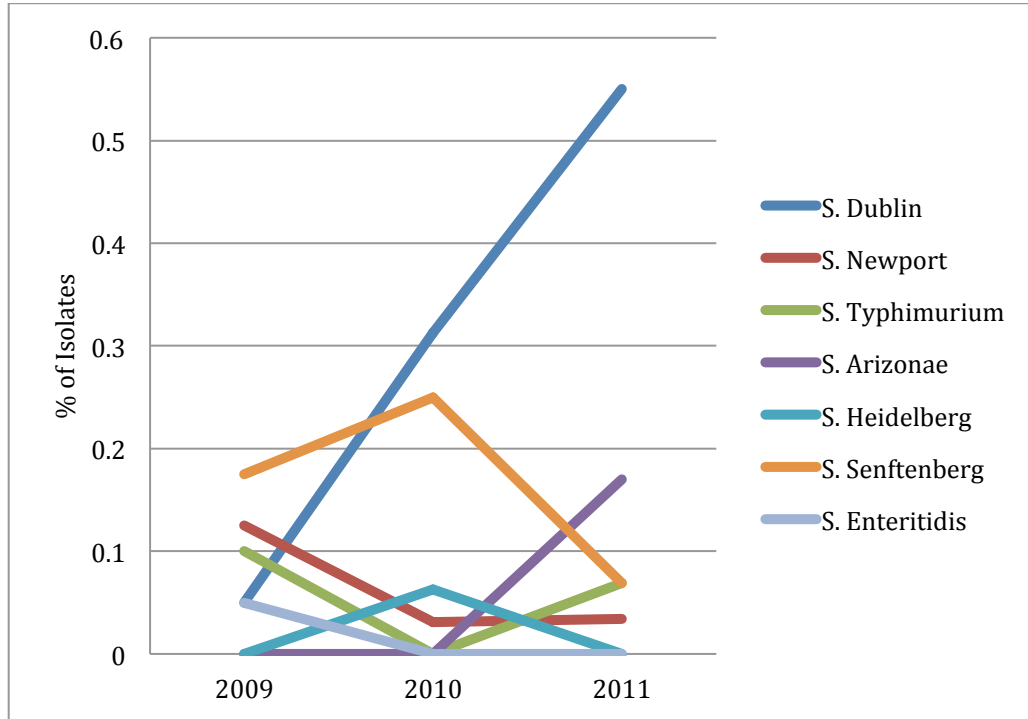


Table 2 – *Salmonella* antimicrobial resistance patterns from NDSU VDL, 2009-2011.

Antimicrobial Agent	Breakpoint (µg/mL)	2009 Resistant (%)	2010 Resistant (%)	2011 Resistant (%)
Amoxicillin/Clavulanic Acid	≥32/16	10 (25)	9 (28.1)	17 (58.6)
Ampicillin	≥32	14 (35)	10 (31.3)	17 (58.6)
Azithromycin	≥16	0 (0.0)	0 (0.0)	0 (0.0)
Cefoxitin	≥32	11 (27.5)	9 (28.1)	14 (48.2)
Ceftiofur	≥8	0 (0.0)	0 (0.0)	0 (0.0)
Ceftriaxone	≥64	6 (15)	8 (25)	12 (41.4)
Chloramphenicol	≥32	13 (32.5)	12 (37.5)	15 (51.7)
Ciprofloxacin	≥1	1 (2.5)	1 (3.1)	1 (3.4)
Gentamicin	≥16	1 (2.5)	1 (3.1)	1 (3.4)
Kanamycin	≥64	7 (17.5)	6 (18.8)	10 (34.5)
Naladixic Acid	≥32	1 (2.5)	1 (3.1)	1 (3.4)
Streptomycin	≥64	13 (32.5)	18 (56.2)	15 (51.7)
Sulfisoxazole	≥256	15 (37.5)	18 (56.2)	17 (58.6)
Tetracycline	≥32	15 (37.5)	19 (59.4)	16 (55.2)
Trimethoprim/Sulphamethoxazole	≥4/76	5 (12.5)	0 (0.0)	1 (3.4)

Antimicrobial (**bold**) – statistically significant decrease in susceptibility.

Figure 2 – *Salmonella* resistance to antimicrobials by year, 2009-2011.

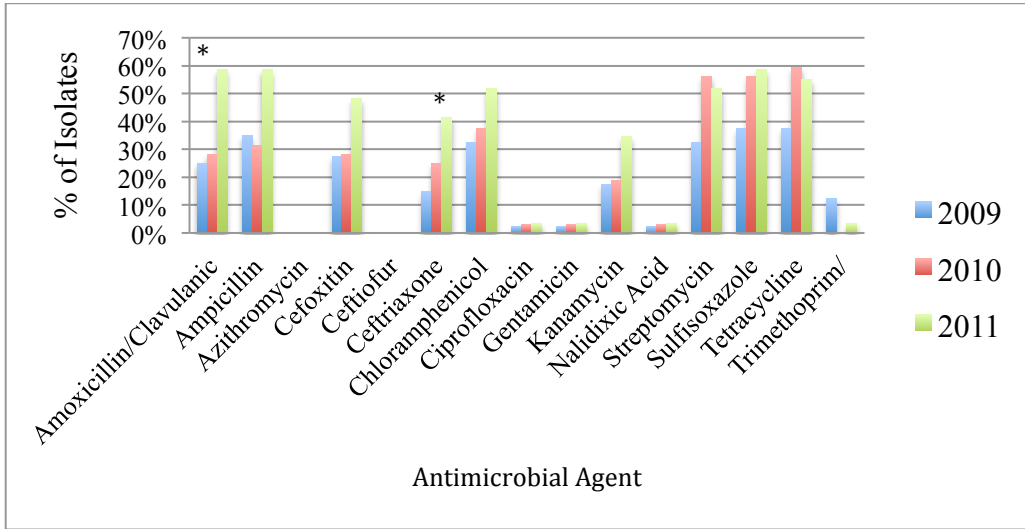


Figure 3 – Multi-drug resistant *Salmonella* isolates by year, 2009-2011.

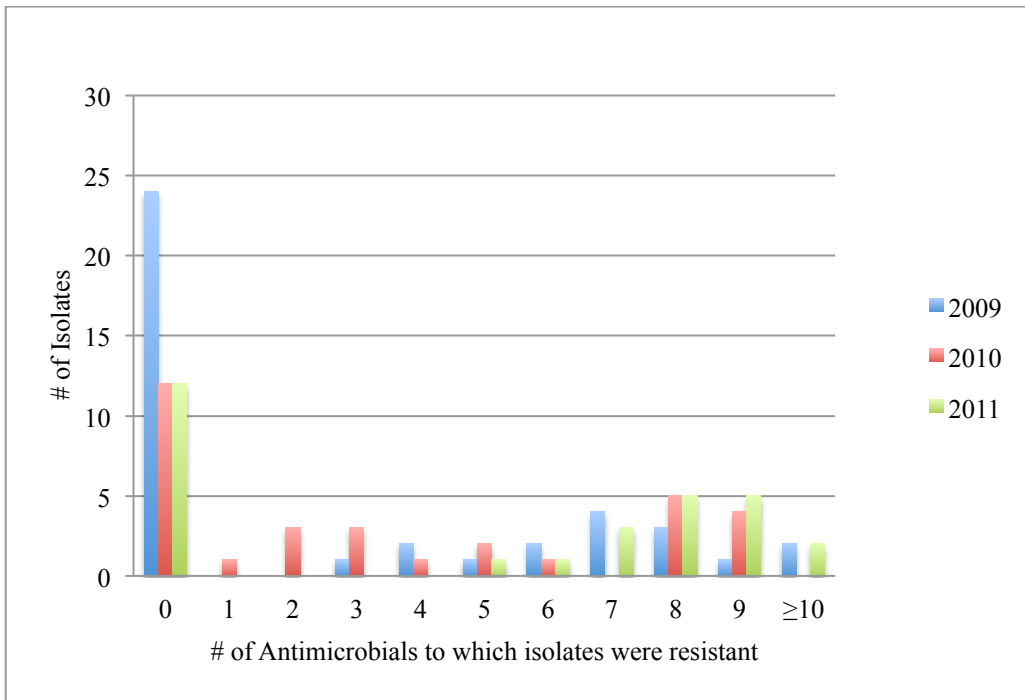


Figure 4 – MDR *Salmonella* phenotypes by year, 2009-2011.

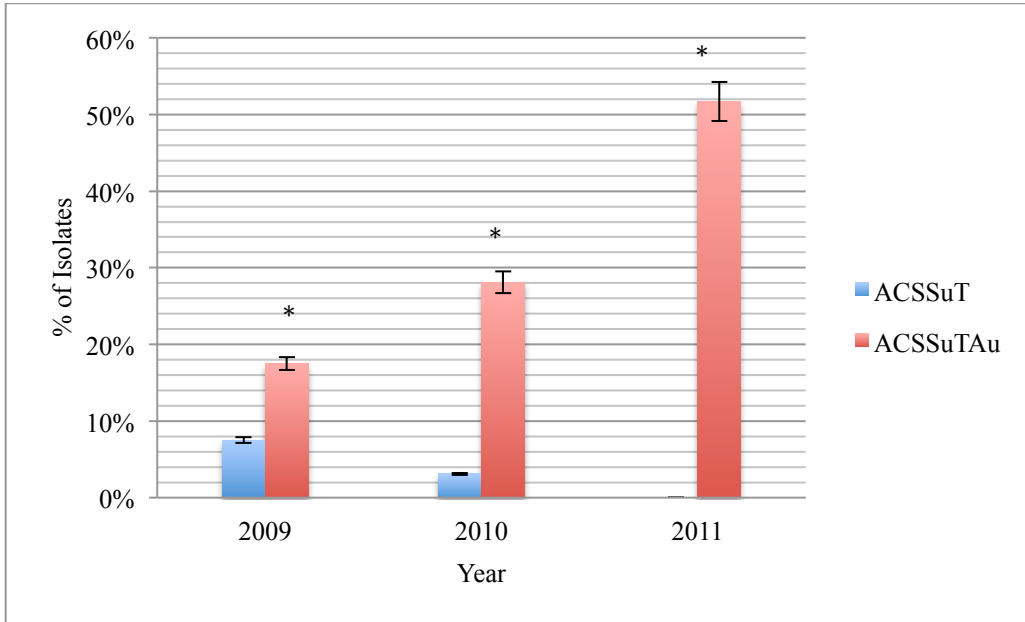
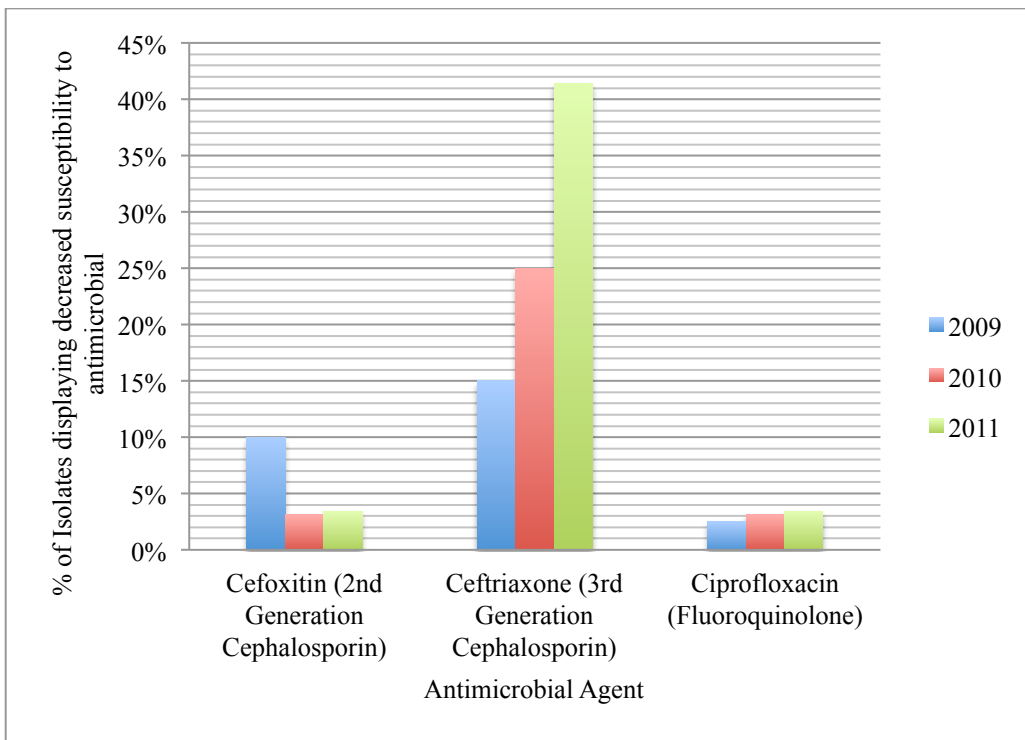


Figure 5 – Decreasing susceptibility of *Salmonella* isolates to cephalosporins and fluoroquinolones, 2009-2011.



Discussion

Serotype Distribution

Salmonella serotypes are genetically similar, but are very distinct in the types of disease they cause (26). As stated previously, this study found that *S. enterica* serotype Dublin was most common and exclusively found in cattle. It is known that *S. Dublin* is mainly associated with cattle and these animals serve as a reservoir for this particular serotype (14, 26). Interestingly, *S. Dublin* is considered an uncommon serotype and has been found to cause more severe disease than other non-typhoidal *Salmonella* serotypes (14). Jones *et. al.* (2008) found that *S. Dublin* infections in humans caused a 67.0% and 64.0% hospitalization and invasive disease rate, respectively. They also reported a 3.0% death rate; significantly higher than *S. Typhimurium* (0.5%).

In this study, we found that 85.7% (24/28) of *S. Dublin* isolates were resistant to amoxicillin/clavulanic acid, 89.3% (25/28) were resistant to ampicillin, and 92.9% (26/28) were resistant to streptomycin, sulfisoxazole, and tetracycline. Every year, the proportion of *S. Dublin* isolates displaying intermediate resistance to ceftriaxone (3rd generation cephalosporin) increased from 0% (0/2) in 2009, to 70% (7/10) in 2010, and to 68.8% (11/16) in 2011. This decrease in susceptibility to ceftriaxone may indicate an increasing resistance pattern to 3rd generation cephalosporins and fluoroquinolones (4). Also, two *S. Dublin* isolates, one from 2010 and one from 2011, displayed resistance to nalidixic acid, an elementary quinolone. This correlates with decreased susceptibility to ciprofloxacin (fluoroquinolone) and therefore possible fluoroquinolone treatment failure (12, 24). As discussed earlier, this has serious public health implications, and outbreaks due to a serotype with greater potential to be invasive should alert local public health

officials of the possible higher risk of bacteremia (6). Increased exposure for populations working and living in close proximity to these animals may put them at an increased risk for more serious disease.

Salmonella Senftenberg was the second most common serotype reported in this study. *S. Senftenberg* also showed high species specificity (16/28, 57.1%) for avian species. Jones *et. al.* (2008) reports that *S. Senftenberg* also has a statistically significant decreased hospitalization rate of 15.5% as compared to *S. Typhimurium* (24.2%).

It is also interesting to note that the *Salmonella* isolates submitted from domestic animals used as pets (hedge hog, turtle, and dog) were in the top five *Salmonella* serotypes that cause human salmonellosis (*S. Typhimurium* [1], *S. Enteritidis* [2], and *S. Newport* [1])(12). Pets can transmit *Salmonella* to humans so it is important to educate patients about proper hand hygiene and disposal of animal waste to ensure that the risk of transmission is ultimately decreased.

Multiple Drug Resistant Isolates

According to NARMS and the FDA (12), common multiple drug resistant (MDR) phenotypes have emerged over the past ten years. The most common MDR phenotype reported by NARMS in 2007 was ACSSuT – exhibiting resistance to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (12). In the same report, higher incidences of phenotype ACCSuTAu, characterized by ACSSuT phenotype plus resistance to amoxicillin/clavulanic acid (Augmentin), were detected nationally. In this study, it was found that the percentage of isolates exhibiting the ACSSuT phenotype declined from 7.5% (3/40) in 2009, to 3.1% (1/32) in 2010, and 0% (0/29) in 2011 and that the percentage of isolates exhibiting the ACCSuTAu phenotype increased from

17.5% (7/40) in 2009, to 28.1% (9/32) in 2010, to 51.7% (15/29) in 2011 [p=0.01] as seen in Figure 4.

Decreasing Susceptibility to 3rd Generation Cephalosporins and Fluoroquinolones

Decreasing susceptibility to 3rd generation cephalosporins like ceftriaxone was also demonstrated during this study. As Crump *et. al.* (2010) indicates in a recent study, ceftriaxone resistance still remains low, but public health officials and medical professionals should be aware that resistance to this 3rd generation cephalosporin may occur in invasive Salmonellosis so local epidemiology of *Salmonella enterica* serotypes and close examination of susceptibility patterns are very important (11). In this study, there was a statistically significant decrease in susceptibility to ceftriaxone from 15.0%(6/40) in 2009, to 25.0%(8/32) in 2010, to 41.4%(12/29) in 2011 [p=0.05]. This decrease in susceptibility was extrapolated from isolates demonstrating “intermediate resistance” – those isolates that did not cross the breakpoint ($\geq 64 \mu\text{g/mL}$) for resistance, but were defined by NCCLS as being concentrated at 16-32 $\mu\text{g/mL}$. As Crump *et. al.*(2010) also explains, resistance to elementary quinolones like nalidixic acid usually arise from single point mutations in the DNA gyrase gene, and therefore can be considered one of the initial steps in developing fluoroquinolone resistance.

Conclusion

Multi-drug resistance has been shown by various studies to be associated with large plasmids (15, 19). Even more importantly, these plasmids have been shown to move amongst different enteric pathogens e.g. *Escherichia coli*, *Campylobacter*, and *Shigella* (13) via conjugation. This ability to transfer resistance genes across various species of enteric bacteria is another reason why antimicrobial resistance monitoring is

crucial to our understanding of drug resistance and subsequent chemotherapy. Gay *et. al.* (2006) indicates that quinolone resistance genes on plasmids promote the rapid spread of fluoroquinolone resistance and that these genes are co-transmitted with β -lactamase genes, effectively rendering two major antimicrobial drug classes useless. Genetic analysis of these plasmids is the next step to genotypically identifying these resistant isolates to characterize antimicrobial resistance and its spread.

The information obtained from this study is important in understanding and quantifying antimicrobial resistance in *Salmonella enterica* serotypes locally. The approach to this study is from a resistance standpoint and can only be applied locally. An important component to this type of study would be to include an antimicrobial dosing and usage study to effectively complement and justify the current information (21). Continued surveillance and prudent use of antimicrobials in both human and veterinary medicine on an international scale are extremely vital in monitoring and controlling the spread of antimicrobial resistant enteric bacteria.

CONCLUSION

With my experiences at The AIDS Support Organization (TASO), I was able to comprehend the nature of such an epidemic, devastating disease and appreciate the successes and failures of one country's attempt to stop the spread of HIV. The realization that HIV/AIDS has a different perception in Western culture has given me the ability to critically examine how important patient-centered care is and that social support is just as important as medicine alone.

The antimicrobial screening project had a translational effect in that I gained knowledge about antimicrobial resistance in animals and how that may have serious impacts on human health and visa versa. This experience has instilled a conviction that the prudent use of antimicrobials in both human and veterinary medicine and the constant surveillance of antimicrobial resistance are the only ways to limit the acquisition of such antimicrobial resistance genes among pathogenic organisms. Communication between veterinarians, public health officials, and physicians plays an important role in protecting the health and wellbeing of humans and animals alike.

By participating in an international program dedicated to the integration of the One Health principle and working with infectious pathogens that are trans-boundary in nature, I have gained experience and knowledge that will propel me through my training as a physician and allow me to be the independent, forward thinking member of the scientific community who is able to make positive contributions to my local, national, and international communities. To appreciate this reality so early in my training allows me to constantly seek new knowledge and grow as a life long learner, with a constant reminder that our lives are all inextricably linked together.

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