An Assessment of Nontuberculous Mycobacteria in Nebraska

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An Assessment of Nontuberculous Mycobacteria in Nebraska

Molly Hoffman BS/BA
UNIVERSITY OF NEBRASKA MEDICAL CENTER | NEBRASKA DEPARTMENT OF HEALTH AND HUMAN SERVICES
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Abstract

Background. Nontuberculous mycobacteria (NTM) are an important cause of morbidity and mortality in the U.S., yet not much is known about the prevalence and risk factors. It is thought to be increasing and most commonly found in women and older adults. The objectives of this project were to estimate the burden of NTM in Nebraska and to describe the patterns of NTM infection including location, species, specimen source, and patient demographic characteristics.

Methods. The National Electronic Disease Surveillance System was searched for all NTM infections reported from 2012-2017. Cases were classified as either pulmonary or nonpulmonary and available information was assessed including age, gender, and species type.

Results. 759 cases of NTM infection in Nebraska were identified over the six-year period. The annualized isolation rate was 6.16 cases per 100,000 population. The overall isolation rate decreased over the study period, however, there was an increase in nonpulmonary isolates. Nonpulmonary cases predominated (54%) and Mycobacterium Avium Intracellulare was the most common species identified.

Conclusions. Nonpulmonary NTM may be a more important cause of disease than previously recognized. The differing trends in pulmonary and nonpulmonary NTM isolation indicate a difference in disease and associated risk factors. Further efforts to monitor disease trends and differences in risk factors should be undertaken.
**Introduction**

Nontuberculous mycobacteria (NTM) cause illness most frequently in the form of lung disease, but can also infect other areas of the body including skin and soft tissue, bones, joints, and lymph nodes. Recently nontuberculous mycobacteria have been identified as an important cause of healthcare associated infections. Over 140 NTM species have been identified and include all mycobacteria other than *Mycobacterium tuberculosis* and *Mycobacterium leprae*. They are traditionally classified by their rate of growth, either rapid or slow growers (Griffith, 2017; Li, Abebe, Cronk, & Bartram, 2017). These mycobacteria are free-living organisms found in the environment, and have been recovered from tap water, surface water, soil, animals, and food products (Baker et al., 2017; Griffith et al., 2007; Griffith, 2017). Biofilms that often form in water systems provide a suitable, nutrient rich-environment for mycobacteria (Griffith et al., 2007; Li et al., 2017). Commonly found in household and hospital plumbing such as water taps and shower heads, these opportunistic pathogens can also be readily aerosolized (Baker et al., 2015; Falkinham, 2016).

Although these organisms can inhabit body secretions or surfaces without causing disease, there are four distinct manifestations of clinical illness caused by NTM. These clinical syndromes include progressive pulmonary disease, superficial lymphadenitis, disseminated disease in immunocompromised persons, and skin and soft tissue infection (Cassidy, Hedberg, Saulson, McNelly, & Winthrop, 2009; Griffith, 2017). Chronic pulmonary disease has been documented as the most common clinical presence of NTM (Griffith et al., 2007). The incubation period for NTM disease varies, but on average, NTM disease presents symptoms less than four weeks after
exposure, however, it can be indolent for as long as nine months (Li et al., 2017). Symptoms are dependent upon the site of infection and present in a variety of ways (Bancroft et al., 2017).

**Epidemiology of NTM**

The burden of disease due to NTM in the United States is unknown, however, experts believe that it is increasing (Cassidy et al., 2009; Griffith, 2017; Griffith et al., 2007; Henkle, Hedberg, Schafer, Novosad, & Winthrop, 2015; Prevots & Marras, 2015). As the prevalence is increasing, it is suggested that mortality due to nontuberculous mycobacterial disease will also increase (Mirsaeidi, Machado, Garcia, & Schraufnagel, 2014). Annual rates of pulmonary NTM disease in North America are estimated to range from 5 to 10 per 100,000 persons, but this varies by geographic region (Prevots & Marras, 2015; Prevots et al., 2010). Population-based data to confirm this are limited due to the lack of a mandate for reporting of laboratory isolation of, or disease due to, nontuberculous mycobacteria in the U.S. (Cassidy et al., 2009). Historically, men comprised a majority of those who were infected with NTM, however, the majority of NTM infected individuals are currently women (Cassidy et al., 2009; Falkinham, 2016; Griffith, 2017). Pulmonary NTM disease has shown increased incidence and mortality rates with increasing age in both males and females (Henkle et al., 2015; Mirsaeidi et al., 2014). Without population-based data, the burden of disease due to different NTM species is currently unknown, but the most commonly identified NTM species causing disease in humans in the U.S. are the species of the *M. avium complex (MAC)* and *M. kansasii* (Griffith et al., 2007; Griffith, 2017; Prevots & Marras, 2015). Other medically important species include *M. chimaera, M. abscessus, M. chelonae*, and *M. fortuitum* (Falkinham, 2016).
As the prevalence of NTM disease has appeared to increase with no obvious environmental source, experts believe that host factors are of increasing importance in the pathogenesis of NTM infections. Host factors have also been thought to be related to the severity of the disease experienced (Mirsaeidi et al., 2014). Two major categories of NTM disease are pulmonary and nonpulmonary. There are a few groups of individuals that have been found to be at a particularly heightened risk for pulmonary NTM disease (Falkinham, 2016; Griffith, 2017). The first high-risk group includes older men who are smokers and heavy alcohol drinkers. This group is at increased risk of developing NTM pulmonary infection (Falkinham, 2016; Griffith, 2017). The second group also involves older men who suffer extensive lung damage from occupational exposures to dusts. These individuals can include, but are not limited to, miners and farmers (Falkinham, 2016). A third population group with higher risk of NTM pulmonary disease are thin (BMI < 20), older women above the age of 50 years (Falkinham, 2016; Griffith, 2017; Prevots & Marras, 2015). These women are generally nonsmokers with no underlying lung disease (Griffith, 2017). Immunodeficiency has been found to be a risk factor of nonpulmonary disease, most often resulting in disseminated disease (Falkinham, 2016; Griffith, 2017). Comorbidities are commonly found in patients with pulmonary nontuberculous mycobacteria disease. Among these comorbidities include Human Immunodeficiency Virus (HIV), Chronic Obstructive Pulmonary Disease (COPD), bronchiectasis, and cystic fibrosis (Mirsaeidi et al., 2014; Prevots et al., 2010).

Nonpulmonary NTM infections often occur in normally sterile body sites and are a cause of both sporadic and healthcare associated infections (Bancroft et al., 2017). Nonpulmonary NTM infections typically present clinically as superficial lymphadenitis, disseminated disease, and skin and soft tissue infections (Bancroft et al., 2017; Griffith, 2017). Cases of superficial lymphadenitis
primarily occur in children (ages 1 to 5 years) and most often present as cervical lymphadenitis (Piersimoni & Scarparo, 2009). These cases are most frequently caused by *M. avium complex* and *M. scrofulaceum* in the U.S. (Bancroft et al., 2017; Griffith 2017; Piersimoni & Scarparo, 2009). Patients impacted by lymphadenitis are generally otherwise healthy and clinically present with a chronic neck mass (Piersimoni & Scarparo, 2009). Disseminated disease primarily affects severely immunocompromised patients and is believed to arise from infection of a mucosal surface and subsequent local multiplication and entry into the bloodstream (Bancroft et al., 2017; Griffith 2017). Cases of NTM impacting the musculoskeletal system are rare, however, they do occur from both rapidly and slow growing species in the bones and joints (Piersimoni & Scarparo, 2009). Lastly, skin and soft tissue infection can also occur, most often as a result of direct inoculation (Griffith, 2017). Cutaneous NTM infections have also been found in immunocompromised patients, as well as in immunocompetent persons resulting from a traumatic injury, surgery, or cosmetic procedures (Piersimoni & Scarparo, 2009; Wentworth, Wengenack, Wilson, & Lohse, 2013).

**Healthcare Associated Infections**

There is evidence of healthcare associated NTM infections involving hematopoietic stem cell transplant recipients, cardiac surgery, dialysis, injections, plastic surgery, liposuction, LASIK, long-term central intravenous catheters, and a variety of surgical procedures (Baker et al., 2017; Bancroft et al., 2017; Griffith et al., 2007; Li et al., 2017). Previously documented sources of NTM in healthcare settings include tap water, ice made from tap water, distilled water used for preparing solutions, and processed tap water used for dialysis (Griffith et al., 2007). From a systematic review of NTM infections from healthcare facility water systems, Li et al. found that the median attack rate of patients infected per patients exposed to NTM in health care settings was 12.1% and that the
most affected populations include the immunocompromised, post-surgical and hemodialysis patients (Li et al., 2017).

Recently, a notable outbreak of nonpulmonary invasive NTM infection occurred among hospital patients who underwent cardiopulmonary bypass during cardiac surgery. This was hypothesized to be due to aerosols generated from colonized heater-cooler units (Baker et al., 2017). The primary organism responsible for NTM contamination in heater-cooler devices is the slow-growing *M. chimaera* (Ninh, Weiner, & Goldberg, 2017). Positive *M. chimaera* cultures have been drawn from a range of specimen sources including cardiac tissue, bone, blood, cardiac prostheses, and urine (Ninh et al., 2017). Individuals in hospital settings are a vulnerable population and if exposed to NTM, are more likely to become ill than the general population (Li et al., 2017). A majority of exposed patients are initially asymptomatic, although, when symptoms are present, patients often exhibit cardiac complications that can be fatal (Ninh et al., 2017). A median of 21 months from the time of cardiac surgery to the onset of symptoms has been described, however, latency periods have been reported anywhere from three months to five years (Ninh et al., 2017; Sommerstein et al., 2017). With a limited number of reported cases, the impact of *M. chimaera* has not yet been thoroughly measured and accordingly, a specific treatment course has not been defined (Ninh et al., 2017; Sommerstein et al., 2017). The American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) guidelines recommend that surgical wounds, injection sites, and intravenous catheters are not exposed to tap water to tap water-derived fluids to prevent healthcare related NTM infections (Griffith et al., 2007).

Another outbreak, this time of *M. abscessus* occurred in association with a pediatric dental clinic (Hatzenbuehler et al., 2017). Facial and neck swelling occurred among 24 children who
underwent a pulpotomy where municipal water was used for the drilling and irrigation (Hatzenbuehler et al., 2017). Among 24 children, the median age was 7.3 years and clinical diagnoses included cervical lymphadenitis, mandibular or maxillary osteomyelitis, and disseminated infection with pulmonary nodules (Hatzenbuehler et al., 2017). All of the children were hospitalized at least once and there was a median of two surgeries per child (Hatzenbuehler et al., 2017). 79% of the children experienced complications including vascular access malfunction, high-frequency hearing loss, permanent tooth loss, facial nerve palsy, urticarial rash, elevated liver enzyme levels acute kidney injury, neutropenia, and incision dehiscence/fibrosis (Hatzenbuehler et al., 2017).

Outbreaks of extrapulmonary NTM infection have also occurred in patients undergoing dialysis (Phillips & Von Reyn, 2001). Typically caused by contaminated water solutions or the use of colonized potable water in the processing of reusable hemodialysis filters, \textit{M. fortuitum} infection has caused clusters of peritonitis cases in individuals on continuous ambulatory peritoneal dialysis (Phillips & Von Reyn, 2001). \textit{M. chelonae} and \textit{M. abscessus} have also been associated with outbreaks among patients undergoing dialysis (Phillips & Von Reyn, 2001).

**Diagnosis of NTM**

When diagnosing nontuberculous lung disease, microbiologic, clinical, and radiographic criteria are all equally important. According to the ATS/IDSA, to meet the criteria for the diagnosis of NTM lung disease, the minimum evaluation of a patient must include “chest radiograph or, in the absence of cavitation, chest high-resolution computed tomography (HRCT) scan; three or more sputum specimens for acid-fast bacilli (AFB) analysis; and exclusion of other disorders, such as tuberculosis” (Griffith et al., 2007, p. 367). These 2007 ATS/IDSA guidelines are in agreement with the British Thoracic Society Guidelines published in 2017 (Haworth et al., 2017). Many studies rely
on microbiologic evidence alone to identify NTM lung disease, with the ATS/IDSA defining microbiologic evidence of pulmonary disease as positive culture results from at least two separate sputum samples, positive culture results from one or more bronchial wash or lavage, or transbronchial or other lung biopsy with mycobacterial histopathologic features in addition to a positive culture for NTM or biopsy showing mycobacterial histopathologic features and a positive culture from one or more sputum or bronchial washings (Griffith et al., 2007).

The diagnosis of extrapulmonary disease varies based on clinical manifestation and species identified (Griffith et al., 2007). Guidelines for nonpulmonary NTM case ascertainment have recently been established by the Council of State and Territorial Epidemiologists (CSTE). Suggested laboratory criteria for a confirmatory case of nonpulmonary NTM are as follows: “a positive culture and identification to the species or complex level of nontuberculous mycobacteria, or positive nucleic acid test specific for a given species or complex of nontuberculous mycobacteria” excluding specimens that indicate *M. tuberculosis* complex organisms, *M. gordonae*, or *M. leprae*, and excluding cultures from lower respiratory samples (Bancroft et al., 2017, p. 5). Guidelines for treatment vary based on the species identified and the severity of disease, but typically consists of 3 to 6 months of multi-drug antimicrobial therapy (Griffith et al., 2007; Haworth et al., 2017; Henkle, Hedberg, Schafer, & Winthrop, 2017). An effective treatment regimen has not been identified for some species and disease combinations such as treatment of *M. abscessus* pulmonary disease (Griffith et al., 2007). Guidelines for susceptibility testing also vary by species identified and should only be carried out when there is clinical suspicion of disease (Griffith et al., 2007; Haworth et al., 2017). Although the antimicrobial susceptibility varies, high rates of resistance have been seen for some species (Florita, Zangeneh, & Georgescu, 2017).
**Project Significance**

The public health and economic burdens of NTM disease in the U.S. are unknown, however, there are estimates of the disease burden and the costs associated with it. Strollo et al. estimated that there were 86,244 cases of NTM disease in the U.S. in 2010, equating to about $815 million in costs (Strollo, Adjemian, J., Adjemian, M., & Prevots, 2015). The state estimates came out to a median of 1,208 annual cases with an estimated range of 48 to 12,544 cases per year in each state. This associated cost ranges from $503,000 to $111 million per year in each U.S. state (Strollo et al., 2015).

Further research is needed to quantify the complete burden of disease on public health, to identify the risk factors associated with NTM disease, to estimate the cost of NTM disease, and to further inform public health interventions (Cassidy et al., 2009; Henkle et al., 2015; Prevots & Marras, 2015; Strollo et al., 2015). And while reporting of nontuberculous mycobacterial infection is not mandated for the United States as a whole, it is mandated in the state of Nebraska. Using reports of NTM infection in the state of Nebraska from 2012 until 2017, the objectives of this project were to estimate the population-based prevalence of NTM disease in Nebraska and to describe the patterns of NTM infection including location, species, specimen source, and patient demographic characteristics. This project serves to better estimate the burden of NTM in the United States through population-based prevalence and the associated risk factors of NTM infection within the state of Nebraska. By understanding the scope of the disease, populations affected, and species involved, public health professionals and health care providers can better understand the need for additional surveillance and which populations are at risk of NTM infection, in order to improve surveillance methods and develop strategies for prevention.
This project was conducted in collaboration with the Nebraska Department of Health and Human Services (DHHS) whose mission serves to help people live better lives (Nebraska Department of Health and Human Services [DHHS], 2017). At every level of DHHS, their goal is to be honest, trustworthy, competent and loyal. DHHS strives to be transparent and accountable as they protect the health of and serve the residents of Nebraska (DHHS, 2017). Within the Epidemiology Department, I worked with the Healthcare Associated Infections (HAI) group.

Methods

Study design & data collection. A retrospective review of surveillance reports of nontuberculous mycobacteria was conducted to provide estimates of the burden of infection in Nebraska. Nontuberculous mycobacteria (NTM) infections have been reportable in the state of Nebraska beginning in 2010, but this reporting was not considered reliable until 2012 due to the time required to establish this reporting with providers and laboratories. Nebraska was one of the first states to adopt the Center for Disease Control and Prevention’s electronic platform for disease reporting. The system is called the Nebraska Electronic Disease Surveillance System (NEDSS) in Nebraska, and there are an additional 23 jurisdictions that utilize this platform to extract relevant information from the HL7 messages that are sent via Public Health Information Network (PHIN) secure messages to the NEDSS system (CDC, 2017). NEDSS was searched for all NTM infections reported from 01/01/2012 to 12/31/2017 to examine the burden of NTM infections in the state of Nebraska over this six-year period. All of the data that was used for analysis was extracted from the NEDSS database into Microsoft Excel and cleaned using SAS Software version 9.4 (SAS Institute, Inc., Cary, North Carolina). The University of Nebraska Medical Center Institutional Review Board approved of this investigation.
Variables. Patient state and/or local health department jurisdiction were used to determine residency in the state of Nebraska. The date the isolation was first reported was used to determine the year reported and was used in annual rate calculations.

Specimen source. To identify the specimen source from which the sample was collected, all of the information from four available specimen source fields was examined and manually reviewed for each of the individual reports. The specimen source was then classified by body site or source of the specimen depending on the data available. Specimen source was primarily defined by the body site it was obtained from as it provided more information pertaining to pulmonary and nonpulmonary cases. If information conveying the body site was not available, specimen source was defined by the type of source it was from (i.e. body fluid, aspirate, tissue, abscess, etc.). The specimen sources were then categorized into pulmonary and nonpulmonary based on medical expertise and current literature. All specimen source categorizations were verified by Nebraska DHHS supervisors. If no information was provided in any of the fields for specimen source, it was designated as missing. Information on specimen source was not available for nearly half of the samples, therefore the American Thoracic Society/Infectious Diseases Society of America definition of clinical disease could not be applied. Additionally, none of the patients had more than one report and so ATS/IDSA recommendations of at least 2 consecutive sputum samples could not be applied. Therefore, the sample that was analyzed here represents only reports of positive isolates and not confirmed clinical cases since the necessary supporting information was not reported to public health. Specimens from environmental sources that were not connected to an individual were noted and subsequently removed before analysis.
**Pulmonary NTM.** Isolation of pulmonary NTM was defined as one positive laboratory test from sputum, bronchial wash, lavage or culture, specimens from the chest wall, sinus or pleural fluid, or lung tissue or biopsy.

**Nonpulmonary NTM.** Nonpulmonary NTM was categorized into skin/soft tissue, bone/joint, disseminated, lymphadenitis, and other. A case of skin/soft tissue was defined as specimens from wounds, mass, lesions, muscle, or bodily tissue. Isolates from tissue or drainage from bone or joints were defined as bone/joint. A disseminated case was defined as isolation from cerebrospinal fluid, blood, or heart. Lymphadenitis is defined as isolation from lymph node tissue or aspirate. The category of other includes all other nonpulmonary isolates including those from swabs, glands, urine, stool, peritoneal fluid, abdominal fluid, other unidentified body fluid, eye, ear, and neck. *Mycobacterium Gordonae* isolates were considered as contaminants and excluded from analysis.

**Device.** Specimens identified as isolated from a catheter tip were defined as a device, and categorized as neither pulmonary nor nonpulmonary isolation.

**Species type.** Species type was derived from two fields indicating the test performed and the result from that test. As this data was inconsistently entered, a new variable for species type was created. A manual review of the two fields was conducted and the identified species was input into the new field using Microsoft Excel. All results, including species identified as “Group IV Rapid Growers”, that did not specify a species type were assigned as missing.

**Age.** Age in years was computed by subtracting the patient’s date of birth from the specimen collection date.

**Exclusion Criteria.** Laboratory reports from individuals who reside outside of the state of Nebraska, those from an environmental source that is not connected to an individual, and those reported
before January 1st, 2012 or after December 31st, 2017 were excluded. Negative laboratory reports, or those for tests other than nontuberculous mycobacteria were also excluded.

**Data Analysis.** Laboratory data was imported and managed using SAS Software version 9.4 (SAS Institute, Inc., Cary, North Carolina). Frequencies, percentages, means, standard deviations, were computed using SAS Software version 9.4. Isolation rates were computed using Microsoft Excel. Population estimates used in rates were determined using the U.S. Census Bureau’s annual population projections (U.S. Census Bureau, 2017).

**Data quality:** An examination of data quality was conducted in order to recommend improvements for future surveillance. Data quality was assessed through the identification of missing data and the evaluation of the timeliness and consistency of reporting. Timeliness of reporting was assessed through a measure of the time from sample collection to case reporting. This was computed by subtracting the laboratory report date from the specimen collection date reported and measured in days. An examination of outliers in the data was conducted to identify possible data misentry. Laboratory reports identified as test reports from varying facilities were removed before analysis. A qualitative examination of the consistency of reporting was conducted by comparing consistency of data entry throughout reports from different reporting facilities and ordering organizations.

**Results**

During 2012 to 2017, there were 759 reports of nontuberculous mycobacteria in the state of Nebraska for a period isolation rate equal to 40.21 cases per 100,000 population over the 6-year period. The annualized isolation rate was 6.70 cases per 100,000 population and 6.16 cases per 100,000 population after exclusion of *Mycobacterium Gordonae*. From the original 934 lab reports from this time period, 111 were excluded due to residency outside of Nebraska or an unknown
location, 57 were excluded due to negative results or because results reported detected organisms other than NTM including *Mycobacterium Tuberculosis*, *Mycobacterium Leprae*, Histoplasma Capsulatum, and Corynebacterium Amycolatum, and 7 results from environmental sources were excluded because they were not connected to an identifiable patient (Figure 1). Environmental sources reported include scrub sink aerators, PMS water, turnout tap PMS, and counter tap H₂O PMS. Species identified from environmental sources include *Mycobacterium Mucogenicum*, *Mycobacterium Avium Intracellulare*, *Mycobacterium Chimaera*, *Mycobacterium Kansasi*, and *Mycobacterium Arupense*. 
**Data quality.** 388 reports had complete data for patient age, patient gender, specimen source, species type, specimen collection date, and lab report date. Missing data were observed for specimen source, gender, species type, ordering organization, and patient address. Information regarding the specimen source was missing for nearly half of the cases (46.42%). The quality of this
measure improved over time with a large decrease in missing data for specimen source from 68% of cases in 2012 to 15% of cases in 2017 missing data for specimen source. Nearly half (46.47%) of the cases were also missing the patient’s address. Less than 5% of cases did not identify a species type isolated (n=32, including 6 cases with the species reported as Group IV Rapid Grower) and less than 1% of cases were missing information for the patient’s gender (n=5). 3.29% of the ordering organizations were missing (n=25). 10 test laboratory reports all from the same reporting facility were identified and therefore excluded. 47 reports were excluded due to negative results or due to results identifying organisms other than NTM. Over the 6-year period 14 different facilities reported at least one NTM isolate.

Ages ranged from 0 to 97 years with a mean of 57 years (STD=22.57 years). The mean age for patients with pulmonary isolates was nearly twenty years older than the mean age of patients with nonpulmonary isolates. A majority of the overall sample were males (53%). The time from sample collection to laboratory reporting ranged from 0 to 393 days with an overall mean of 35.5 days. This overall mean greatly differs from that of reports with complete data for the specimen source as seen in Table 1a.

Of the cases with an identified specimen source, 180 (46%) were pulmonary and 209 (54%) were nonpulmonary cases. The specimen source from 368 cases was not identified. It should also be noted that the specimen source for two cases was documented as being obtained from a device and therefore was not categorized into either pulmonary and nonpulmonary. The species identified in both cases was *Mycobacterium Mucogenicum*, and both were from a catheter tip. They do not appear to be connected as they are from different facilities during different time periods.
Table 1a. Characteristics of Individuals With Positive Lab Reports of Nontuberculous Mycobacteria
By Specimen Source, Nebraska, 2012-2017 (n=388)

<table>
<thead>
<tr>
<th>Specimen Source&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>NonPulmonary (n=208)</th>
<th>Pulmonary (n=180)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td><strong>Sex</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>105</td>
<td>50.48</td>
</tr>
<tr>
<td>Female</td>
<td>103</td>
<td>49.52</td>
</tr>
<tr>
<td><strong>Specimen Collection Year</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>9</td>
<td>4.33</td>
</tr>
<tr>
<td>2013</td>
<td>9</td>
<td>4.33</td>
</tr>
<tr>
<td>2014</td>
<td>31</td>
<td>14.90</td>
</tr>
<tr>
<td>2015</td>
<td>44</td>
<td>21.15</td>
</tr>
<tr>
<td>2016</td>
<td>62</td>
<td>29.81</td>
</tr>
<tr>
<td>2017</td>
<td>53</td>
<td>25.48</td>
</tr>
<tr>
<td><strong>Age, years&lt;sup&gt;d&lt;/sup&gt;</strong></td>
<td>46.98(26.16)</td>
<td>65.50(18.23)</td>
</tr>
<tr>
<td><strong>Time from Sample Collection to Lab Report, days&lt;sup&gt;d&lt;/sup&gt;</strong></td>
<td>25.68(20.94)</td>
<td>25.14(24.28)</td>
</tr>
</tbody>
</table>

<sup>a</sup> 368 observations with missing specimen data were excluded from descriptive statistics
<sup>b</sup> 2 observations with device as the specimen source were excluded from descriptive statistics
<sup>c</sup> 1 observation with missing gender was excluded from descriptive statistics
<sup>d</sup> Values expressed as mean (standard deviation)

24 species types including the contaminant, *Mycobacterium Gordonae*, and *Mycobacterium Chimaera*, from an environmental source, were identified (Table 1b). A majority of isolates (58%) were identified as *Mycobacterium Avium Intracellulare* with an annualized isolation rate of 3.71 cases per 100,000 population. The second most commonly isolated species was *Mycobacterium Chelonae* with an annualized isolation rate of 0.81 cases per 100,000 population.

Table 1b. Reported Isolates by Species Type, Nebraska NTM Infections 2012-2017 (n=727)<sup>a</sup>

<table>
<thead>
<tr>
<th>Species&lt;sup&gt;b,c,d&lt;/sup&gt;</th>
<th>No. (%) of reported isolates&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Annualized Isolation Rate&lt;sup&gt;b,f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. Abscessus</em></td>
<td>33 (4.54)</td>
<td>0.29</td>
</tr>
<tr>
<td><em>M. Arupense</em></td>
<td>12 (1.65)</td>
<td>0.11</td>
</tr>
<tr>
<td><em>M. Aurum</em></td>
<td>1 (0.14)</td>
<td>...</td>
</tr>
<tr>
<td><em>M. Avium Intracellulare</em></td>
<td>420 (57.77)</td>
<td>3.71</td>
</tr>
<tr>
<td>Species</td>
<td>Isolates</td>
<td>Rate (per 100,000 persons)</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>M. Chelonae</td>
<td>92 (12.65)</td>
<td>0.81</td>
</tr>
<tr>
<td>M. Chelonae/Abscessus Complex</td>
<td>1 (0.14)</td>
<td>...</td>
</tr>
<tr>
<td>M. Fortuitum</td>
<td>41 (5.64)</td>
<td>0.35</td>
</tr>
<tr>
<td>M. Goodii</td>
<td>1 (0.14)</td>
<td>...</td>
</tr>
<tr>
<td>M. Gordonae</td>
<td>61 (8.39)</td>
<td>...</td>
</tr>
<tr>
<td>M. Immunogenium</td>
<td>1 (0.14)</td>
<td>...</td>
</tr>
<tr>
<td>M. Kansasii</td>
<td>15 (2.06)</td>
<td>0.13</td>
</tr>
<tr>
<td>M. Lentiflavum</td>
<td>1 (0.14)</td>
<td>...</td>
</tr>
<tr>
<td>M. Mageritense</td>
<td>1 (0.14)</td>
<td>...</td>
</tr>
<tr>
<td>M. Marinum</td>
<td>6 (0.83)</td>
<td>0.05</td>
</tr>
<tr>
<td>M. Massilense</td>
<td>1 (0.14)</td>
<td>...</td>
</tr>
<tr>
<td>M. Monacense</td>
<td>1 (0.14)</td>
<td>...</td>
</tr>
<tr>
<td>M. Mucogenicum</td>
<td>28 (3.85)</td>
<td>0.25</td>
</tr>
<tr>
<td>M. Neoaurum</td>
<td>3 (0.41)</td>
<td>0.03</td>
</tr>
<tr>
<td>M. Peregrinum</td>
<td>2 (0.28)</td>
<td>0.02</td>
</tr>
<tr>
<td>M. Phocaicum</td>
<td>1 (0.14)</td>
<td>...</td>
</tr>
<tr>
<td>M. Scrofulaceum</td>
<td>1 (0.14)</td>
<td>...</td>
</tr>
<tr>
<td>M. Smegmatis</td>
<td>1 (0.14)</td>
<td>...</td>
</tr>
<tr>
<td>M. Terra</td>
<td>3 (0.41)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

a 28 observations with missing species data were excluded  
b Isolates from environmental sources are not included  
c Mycobacterium Chimaera is excluded due to isolation from an environmental source  
d 4 species identified as Group IV Rapid Grower were excluded  
e Results from a former reporting system unable to differentiate between M. Chelonae and M. Abscessus  
f Rates ≤0.01 were excluded  
g Considered a contaminant so rate was not calculated  

The overall rate of isolation, excluding Mycobacterium Gordonae, decreased throughout the study period from 9.33 isolates per 100,000 persons in 2012 down to 3.75 isolates per 100,000 persons in 2017 (Figure 2). The isolation trends differed for pulmonary and nonpulmonary isolates (Figures 3a & 3b). Although the overall rate of isolation decreased throughout the 6-year period, however, the rate of isolation in nonpulmonary cases increased from 0.48 to 2.60 isolates per 100,000 persons after exclusion of Mycobacterium Gordonae. This is contrary to the decrease in pulmonary cases from 2.32 to 0.57 isolates per 100,000 persons after exclusion of Mycobacterium Gordonae.
Figure 2. Annual NTM Isolation Rate per 100,000 population in Nebraska from 2012 to 2017\textsuperscript{a,b}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Annual NTM Isolation Rate per 100,000 population in Nebraska from 2012 to 2017\textsuperscript{a,b}}
\end{figure}

\textsuperscript{a} Isolates identified as \textit{M. Gordonae} were excluded due to identification of \textit{M. Gordonae} as a contaminant
\textsuperscript{b} Population estimates used in rates were determined using the U.S. Census Bureau’s annual population projections

Figure 3a. Annual Nonpulmonary NTM Isolation Rate per 100,000 population in Nebraska\textsuperscript{a,b}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3a.png}
\caption{Annual Nonpulmonary NTM Isolation Rate per 100,000 population in Nebraska\textsuperscript{a,b}}
\end{figure}

\textsuperscript{a} Isolates identified as \textit{M. Gordonae} were excluded due to identification of \textit{M. Gordonae} as a contaminant
Population estimates used in rates were determined using the U.S. Census Bureau’s annual population projections.

Figure 3b. Annual Pulmonary NTM Isolation Rate per 100,000 population in Nebraska\(^{a,b}\)

![Graph showing annual pulmonary NTM isolation rate per 100,000 population in Nebraska]

\(^{a}\) Isolates identified as *M. Gordonaee* were excluded due to identification of *M. Gordonaee* as a contaminant.

\(^{b}\) Population estimates used in rates were determined using the U.S. Census Bureau’s annual population projections.

The species identified from a majority of the nonpulmonary isolates, and within each category, was *Mycobacterium Avium Intracellulare* (Table 2). All of the isolates categorized as lymphadenitis (n=5) were identified as *Mycobacterium Avium Intracellulare*. The most frequently isolated specimens were from skin/soft tissue (31.5%), followed by isolates from bone/joints (19%).

Table 2. Species Distribution Characterized by Nonpulmonary Specimen Source, Nebraska NTM Infections 2012-2017\(^{a}\) (n=200)

<table>
<thead>
<tr>
<th>Species Type(^{a,b})</th>
<th>Bone/Joint (n=38)</th>
<th>Disseminated(^{c}) (n=20)</th>
<th>Lymphadenitis(^{d}) (n=5)</th>
<th>Skin/Soft Tissue(^{e}) (n=63)</th>
<th>Other(^{f}) (n=74)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td><em>M. Abscessus</em></td>
<td>2 (5.26%)</td>
<td>2 (10.0%)</td>
<td>0</td>
<td>4 (6.35%)</td>
<td>5 (6.76%)</td>
</tr>
<tr>
<td><em>M. Avium Intracellulare</em></td>
<td>18 (47.37%)</td>
<td>10 (50.0%)</td>
<td>5 (100%)</td>
<td>30 (47.62%)</td>
<td>49 (66.22%)</td>
</tr>
<tr>
<td><em>M. Chelonae</em></td>
<td>11 (28.95%)</td>
<td>0</td>
<td>0</td>
<td>8 (12.70%)</td>
<td>7 (9.46%)</td>
</tr>
<tr>
<td>Species</td>
<td>Count</td>
<td>Percent</td>
<td>Count</td>
<td>Percent</td>
<td>Count</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-------</td>
<td>---------</td>
<td>-------</td>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td><em>M. Chelonae/Abscessus</em> Complex</td>
<td>0</td>
<td>1 (5.0%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. Fortuitum</em></td>
<td>3</td>
<td>7.89%</td>
<td>3</td>
<td>15.0%</td>
<td>0</td>
</tr>
<tr>
<td><em>M. Goodii</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. Gordonae</em></td>
<td>2</td>
<td>5.26%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. Mageritense</em></td>
<td>1</td>
<td>2.63%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. Massiliense</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. Marinum</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><em>M. Monacense</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>M. Mucogenicum</em></td>
<td>1</td>
<td>2.63%</td>
<td>3</td>
<td>15.0%</td>
<td>0</td>
</tr>
<tr>
<td><em>M. Neoaurum</em></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5.0%</td>
<td>0</td>
</tr>
<tr>
<td><em>M. Smegmatis</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

a 7 observations with missing species data were excluded
b 2 species identified as Group IV Rapid Grower were excluded
c Blood, cerebrospinal fluid, & heart included in ‘Disseminated’
d Lymph node included in ‘Lymphadenitis’
e Wound, mass, abscess, & lesion included in ‘Skin/Soft Tissue’
f Urine, stool, peritoneal fluid, neck, ear, eye, body fluid, aspirate, biopsy, gland, swab, & abdominal fluid/abdomen included in ‘Other’
g Results from a former reporting system unable to differentiate between *M. Chelonae* and *M. Abscessus*

**Discussion**

A population-based assessment of patients with both pulmonary and nonpulmonary NTM isolates reported in the state of Nebraska was conducted over a six-year period from 2012 to 2017. This assessment estimates an existing annualized population-based isolation rate of NTM in Nebraska of 6.16 cases per 100,000 population (excluding *Mycobacterium Gordonae*). However, in contrast with other documented North American studies reporting an increase in NTM isolation and disease, there was an observed overall decrease in rates of NTM isolation in Nebraska from 9.33 isolates in 2012 to 3.75 isolates per 100,000 population in 2017 (Adjemian et al., 2017; Brode et al., 2017; Henkle et al., 2015). While rates of pulmonary NTM isolation and disease have been cited as increasing in other studies, estimated rates of pulmonary NTM isolation in Nebraska decreased from 2.32 isolates in 2012 to 0.57 isolates per 100,000 population in 2017 (Adjemian et al., 2017; Henkle et al., 2015). Conversely, rates of nonpulmonary NTM isolation had an overall
increase during this study period from 0.48 isolates in 2012 to 2.6 isolates per 100,000 population in 2017. Similar to other U.S. studies, the majority of cases were due to *Mycobacterium Avium* (Adjemian et al., 2017; Cassidy et al., 2009; Henkle et al., 2015; Smith et al., 2016; Winthrop et al., 2010). Recent studies have found a predominance of female cases, however, the majority of Nebraska cases were male.

A similar evaluation in Ontario, Canada by Brode and colleagues found an increase in NTM isolation rates from 11.4 isolates per 100,000 population in 1998 to 22.2 NTM isolates per 100,000 population in 2010. This equated to a prevalence of NTM disease of 4.65 cases per 100,000 population in 1998 which increased to 9.08 cases per 100,000 population in 2010. The annual prevalence of pulmonary cases was found to steadily increase, while the annual prevalence of nonpulmonary cases did not change substantially over time (Brode et al., 2017). Population-based studies of NTM disease have also been conducted in Oregon. Contrary to our findings, Henkle and colleagues estimated the incidence of pulmonary NTM disease in Oregon increased from 4.8 cases per 100,000 population in 2007 to 5.6 cases per 100,000 population in 2012 and that the annual incidence of extrapulmonary NTM disease was stable from 2007 to 2012 averaging at 1.5 cases per 100,000 population (Henkle et al., 2015; Henkle et al., 2017). Other North American studies have also found a predominance of pulmonary cases contrasting to the majority of nonpulmonary cases (54%) in Nebraska. A study of three counties in North Carolina from 2006 to 2010 found a predominance of pulmonary NTM isolations (79.4% of all isolates with an identified source) compared to nonpulmonary isolates (20.6%) (Smith et al., 2016). A majority of cases in Oregon from 2005 to 2006 (77.2%) and Onatrio, Canada from 1998 to 2010 (96%) were also pulmonary (Brode et al., 2017; Cassidy et al., 2009). The difference in rates of pulmonary isolates in Nebraska...
as compared to in other regions may be indicative of a decrease in either testing or reporting of pulmonary NTM disease in Nebraska.

Over a six-year period from 2007 to 2012, Henkle et al. found 334 nonpulmonary NTM isolates in the state of Oregon compared to 200 nonpulmonary NTM isolates found in Nebraska over the six-year period from 2012 to 2017. In Oregon, a majority of these isolates were identified as skin/soft tissue (58.9%). Comparably, nonpulmonary infections in Nebraska classified as ‘other’ predominated (37%), followed by skin/soft tissue infections (31.5%). In Nebraska, infections of the bones or joints made up 19% of nonpulmonary infections while infections of the joints made up only 4% of nonpulmonary infections in Oregon (Henkle et al., 2017). These differences may result from differences in the categorization of infections, true differences in infections, and the differences in the reporting time period. Because infection site classifications vary between studies, it is difficult to directly compare results by site. However, it is notable that at least one NTM infection of the eye was reported in both Oregon and Nebraska (Henkle et al., 2017).

Over a thirteen-year period (1998 – 2010) there was an average of 103 annual isolates of nonpulmonary NTM reported in Ontario, Canada making up 4% of all NTM isolates (Brode et al., 2017). Gastrointestinal system/genitourinary system infections (included in the ‘other’ category in our analysis) made up 40% of these infections, followed by skin and soft tissue (22%) and blood/bone marrow (22%) infections. Only 6% of nonpulmonary infections were of the musculoskeletal system and 3% of the lymph nodes (Brode et al., 2017). This is comparable to Nebraska in which only 3% of nonplumonary infections were of the lymph nodes.

A study of medical records from Banner University Medical Center in Tucson, Arizona identified 33 nonpulmonary NTM isolates in patients older than 18 years from 2012 to 2016 in
which 27% were skin and soft tissue, 27% were bone and joint, and 15% were from stool. One case was also identified in brain tissue (Florita et al., 2017). Skin and soft tissue and gastrointestinal system/genitourinary system infections have been found to make up a significant amount of nonpulmonary NTM infections in North America, however, there have been reports of infections in other notable places including the heart (Nebraska), eye (Nebraska & Oregon), and brain tissue (Tucson, Arizona) (Florita et al., 2017; Henkle et al., 2017).

This evaluation is the first of its kind to estimate both pulmonary and nonpulmonary isolation rates in the state of Nebraska. A strength of this study is that it provides true population-based estimates due to the mandatory reporting of NTM infections in the state of Nebraska. Few other U.S. states mandate the reporting of NTM infection and therefore rely on collaborations with individual laboratories for disease estimation.

A notable limitation is the inability to accurately estimate specific clinical manifestations and measures of NTM disease due to the large quantity of cases missing information regarding the specimen source (46%). Additionally, in many cases, pulmonary disease was not able to be established according to the ATS/IDSA guidelines for sputum samples (2+ positive cultures from consecutive samples) because no patient had more than one report within the six-year period. Consequently, isolation rates were computed to provide a better capture of the burden of NTM in Nebraska, as disease estimates from the current data would greatly underestimate that burden in Nebraska.

Winthrop et al. found that the microbiologic criteria alone was highly predictive of pulmonary disease with 86% of patients meeting the ATS/ISDA microbiologic criteria also meeting the full ATS/IDSA disease criteria (Winthrop et al., 2010). Other studies have successfully used the
guidelines for 2+ consecutive positive sputum samples to determine pulmonary NTM disease rates, however, further research should examine typical practices for and timeliness of retesting individuals with positive sputum samples to determine if this definition of disease is practical (Brode et al., 2017; Cassidy et al., 2009; Henkle et al., 2015; Winthrop et al., 2010).

Additional limitations include a likely underestimation of the isolation rates due to the indolent nature of NTM disease, the possibility of some laboratories failing to report cases of NTM infection to DHHS, and underreporting of Nebraska residents who seek care in bordering states that are not included in the sample.

Less than 5% of cases were missing information regarding the species type isolated, however, the implementation of a requirement for the reporting of species type identified in all laboratory reports is recommended. It is recommended by the ATS/IDSA that species-level identification be established due to differences in antimicrobial susceptibility that determine treatment options (Griffith et al., 2007). The species type contains valuable information for the identification of outbreaks as well as for antimicrobial susceptibility.

Along with this, it is recommended that laboratories be encouraged to submit complete reports which should include specimen source. The specimen source is required to establish disease and it is suggested that pulmonary and nonpulmonary NTM represent different diseases with different risk factors (Brode et al., 2017). Therefore, information regarding the specimen source is required not only to estimate the burden of disease, but also, to identify outbreaks and risk factors. Although there is a place for clinical information in the laboratory reporting, it was missing for 100% of the sample. When tracking possible healthcare associated outbreaks, correlation of positive cultures with clinical symptoms is imperative to avoid pseudo-outbreaks and
unnecessary treatment. Contamination of samples is common and positive samples tend to be more likely to indicate contamination than disease (Li. et al, 2017). It is therefore also recommended that methods to obtain this kind of information with laboratory reports should be considered.

**Conclusion**

This assessment provides the first population-based estimates of both pulmonary and nonpulmonary NTM isolation rates in the state of Nebraska. Contrary to other studies, there was an overall decrease in NTM isolation over the study period from 2012 to 2017, however, there was an increase in nonpulmonary NTM isolates. These results, along with findings from other studies, may suggest that the difference in trends for pulmonary and nonpulmonary NTM isolates indicate a difference in disease and different associated risk factors. This may also be indicative of a decrease in reporting or testing of pulmonary isolates in Nebraska. Recommendations include improvements to the NTM surveillance system in Nebraska to produce higher quality data for future disease estimation and outbreak identification. Further evaluation should be conducted to monitor disease trends and risk factors to inform public health management of infections with these organisms.
References

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Service Learning/Capstone Experience Reflection

The Nebraska Department of Health and Human Services was the placement site for my service learning and capstone experience. Before this project, I had never been to the state health department, and didn’t know much about how it functioned. It was a good experience for me to learn about the organizational structure and how it differed from local health departments. Nebraska DHHS is uniquely placed because it is not only a health department, but it also provides social services, which is not true of all state health departments. My preceptor, Dr. Pedati, did a good job of introducing me to the organizational structure of DHHS when I first started my project there. I did not expect approval to be needed from so many levels of leadership to complete various tasks and achieve goals. I learned about the importance of this in ensuring that everyone in the health department is on the same page and working together to achieve similar goals.

I learned how DHHS collaborates with local health departments, as well as nationally with the Centers for Disease Control and Prevention. Dr. Pedati and Dr. Tierney are members of the Council of State and Territorial’s healthcare associated infections subgroup, and they invited me to listen in on a couple of the monthly calls. I enjoyed learning about how health departments and epidemiology professionals from across the country collaborate together and communicate about what is going on in their health departments and areas for improvement.

Other service learning activities included participation in the healthcare associated infections (HAI) group at Nebraska DHHS. I attended weekly meetings where I learned about the projects that everyone was working on, as well as updated them on my progress assessing the atypical mycobacteria data. While working with the HAI group, I assisted with various projects as needed including conducting research on various topics regarding healthcare associated infections.
I also helped with the preparation of events such as Antibiotic awareness week. November 13-19 is US Antibiotic Awareness Week, so I worked with the HAI group for the promotion of Antibiotic Awareness Week in Nebraska. Dr. Tierney headed an effort for the official proclamation of Antibiotic Awareness Week in Nebraska by Gov. Pete Ricketts and I worked with the rest of the HAI group in the set up and promotion of Antibiotic Awareness Week. We set up a display in the state capitol and answered questions about Antibiotic Awareness Week from visitors viewing our display.

A large majority of my service learning involved data clean up. Before beginning my project, I did not realize how large of an investment of time was needed for proper data clean up. My project involved the assessment of a new surveillance system and required a large effort to clean the data for analysis. My skills using SAS software aided me in my data clean up and allowed for me to do it effectively. From this I also created a data dictionary describing the variables associated with the NTM surveillance data for DHHS staff members to use in the future.

A major challenge I encountered during the data clean up portion of my service learning was the messiness of the data. Most of the data I have worked with for class projects in the past has been pretty structured and cleaned before I analyzed it. I did not anticipate how messy the data was going to be, and therefore the time and effort it took to clean the data. An important variable in the data set, specimen source, was very difficult to clean because it was located in four different fields and was not systematically entered. Although I tried to use SAS to clean this field, I could not find a way to do it without losing quality of the data. Ultimately, I manually went through each of the observations to identify the correct specimen source. This became very useful in my analysis as well as for DHHS. There was recently a healthcare associated case of NTM in which my
data was needed to identify if any more cases had occurred. Because I had already spent time cleaning the data, I was able to quickly send it to my preceptor for use in outbreak detection.

My service learning and capstone project have given me experience in applied epidemiology and public health. From this experience I have learned that not everything is always as clean and clear cut as it appears to be in school, and there often is not a clearly defined best way of doing things. This has taught me to think critically and quickly on my feet to adapt to the various situations I encountered. Although sometimes it was challenging, it ultimately made me excited to dive into a career in epidemiology and public health.
Acknowledgements

I would primarily like to thank my capstone committee members, Dr. Caitlin Pedati, Dr. Minhas, and Dr. Wichman, for their dedication in assisting me compete this project. I would also like to thank staff members, Dr. Maureen Tierney and Margaret Drake, from the Nebraska Department of Health and Human Services for working with me on the healthcare associated infections group and on my service learning and capstone project. I would also like to thank Dr. Tom Safranek from the Nebraska Department of Health and Human Services for his assistance with my project. Lastly, I would like to thank Karis Bowen from the Nebraska Department of Health and Human Services for her assistance in helping me with my GIS analysis.