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Factors Associated with Testing Methods Used to Diagnose Non-typhoidal *Salmonella*

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Bachelor of Science

Emory University

2015

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
In Epidemiology
2017

Abstract

Factors Associated with Testing Methods Used to Diagnose Non-typhoidal *Salmonella*

By Matthew Cole

Background: Since 2012, culture-independent diagnostic testing (CIDT) for non-typhoidal *Salmonella* has increased in Georgia (GA). The number of salmonellosis cases diagnosed by only CIDT has doubled from 186 in 2014 to 392 in 2016. CIDTs are cheap, fast, and help create better estimates for disease burden, but also carry the risk of creating false-positives and rarely send isolates for analysis by public health laboratories. Data on demographics, clinical, and exposure is collected by active surveillance of the GA Department of Public Health (GDPH) Foodborne Active Surveillance Network (FoodNet). The impact of testing method on hospitalization status and timely reporting to public health has not been assessed.

Methods: Demographic, clinical, and exposure characteristics of non-typhoidal *Salmonella* cases tested only by culture and CIDT were compared form 2014 – 2016. Two multivariate logistic regression models were fitted to assess the relationship between testing methods on hospitalization status and timely reporting.

Results: From 2014 – 2016, 6,470 cases of salmonellosis with complete demographic information were reported to GDPH. 5,765 (89%) were diagnosed by culture or reflex culture; and 705 (11%) were diagnosed only with CIDT. 3,051 (53%) patients diagnosed with culture and 162 (23%) patients diagnosed with CIDT only were hospitalized. 3,175 (55%) salmonellosis cases diagnosed with culture and 461 (65%) cases diagnosed with CIDT only were reported to Public Health within 7 days of symptom onset.

CIDT diagnosed patients tended to be < 5 years old (67%) while culture-tested cases tended to be whiter and residing in areas outside of metro Atlanta. There were no significant differences observed in gender or ethnicity.

After adjusting for region and age, CIDTs were more likely to be sent within seven days compared to culture. Effect modification identified by region and age. After adjusting for gender and age, CIDTs were less likely to be hospitalized than culture. Effect modification identified by gender and age groups.

Conclusions: CIDT usage continues to rapidly increase in GA and benefit public health by quicker reporting time and rapid treatment for patients to avoid hospitalization. But CIDT usage also limits GDPH's ability to conduct outbreak investigations. GDPH should continue to monitor if CIDTs usage effects the instance or investigation of foodborne disease outbreaks.

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Acknowledgements

I would like to thank Dr. McNabb for his support and mentorship throughout the thesis process. I am extremely grateful for your insight and expertise as a surveillance expert. Special thanks to Hope Dishman, Elizabeth Hannapel, Lana Jones, Bethany LaClair, Siri Wilson, Nadine Oosmanally, Dr. Melissa Tobin-D'Angelo and my other colleagues at the Georgia Department of Public Health for their support, guidance, and assorted snacks. Time to actually take that camping trip! Finally, thanks to Mom, Dad, Sarah, Emma, Paku, Nana, and my friends for their support and love during this long and rewarding process. Could not have done this without y'all and look forward to the journey ahead.

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Chapter I: Literature Review

1.1 Foodborne Disease

Foodborne disease is a significant, preventable public health problem in the United States. Every year in the United States alone, there are an estimated 9.4 million foodborne illnesses and 900 foodborne outbreaks caused by a variety of pathogens [1]. The Centers for Disease Control and Prevention (CDC) defines a foodborne outbreak as the occurrence of two or more persons with a similar illness resulting from ingestion of a common food. Local, state, and territorial health departments report foodborne disease outbreaks to the CDC through the Foodborne Diseases Active Surveillance Network (FoodNet) [2]. In 2014, FoodNet reported 19,542 infections, 4,445 hospitalizations, and 71 deaths from nine pathogens commonly transmitted through food in 10 participating state health departments. These pathogens *Campylobacter, Listeria, Salmonella, Shigella,* Shiga toxin-producing *Escheria coli* (STEC) serotype 0157, STEC non-0157, *Vibrio, Yersinia,* and the parasites *Cryptosporidium* and *Cyclospora* [3].

Foodborne disease is transmitted by food contaminated by infected persons. Food handlers who exhibit symptoms of diarrhea, vomiting, open skin sores, boils, fever, dark urine, or jaundice and fail to wash their hands after either using the toilet, handling raw meat, cleaning spills, or carrying garbage are the main culprits of disease transmission. Food can also become contaminated during production kept at improper temperatures or prepared by an infected person. Typical foodborne pathogens can also be spread through non-foodborne routes such as person to person contact [4]. Reducing the risk of infection through these modes of transmission is achieved by preventing food contact by persons who have acute diarrheal illness, regularly and thoroughly washing hands, ensuring that food is stored and cooked adequately, and regular sanitization of residential and work environments [5].

The symptoms of foodborne disease also result in significant health and economic costs. Symptoms often include diarrhea, vomiting, cramping, nausea, fever, and head ache that range from a few days to weeks [6]. Treatment includes antibiotics and antidiarrheals. Most cases are treated through supportive care fluid replacement since most foodborne illnesses last for an acute period [7]. Apart from health detriments, the economic costs of foodborne disease amount to between $51 – $77 billion annually in the United States where each case costs $1,626 for medical care, lost wages, and lost quality of life [8].

1.2 Salmonella

Non-typhoidal *Salmonella* spp. ranks second as the most common cause of illness and, leading the causes of hospitalization and deaths related to food in the United States. Ninety-four percent of non-typhoidal *Salmonella* spp. are acquired through food causing around 1 million illnesses, 19,000 hospitalizations, and 400 deaths annually in the United States [9]. *Salmonella enterica* has around 2500 subtypes with the most reported including Typhimurium, Enteritidis, Newport, Heidelburg, and Javiana [10]. The disease typically presents 12 – 72 hours after infection as a febrile illness including diarrhea, abdominal pain, and less commonly vomiting and lasts usually from 4 – 7 days [11, 12].

Salmonellosis is a national notifiable disease reported normally to local, state, and territorial health departments. Patients who present the typical signs of Salmonellosis are confirmed as cases via culture-based testing methods using mainly stool, blood, or urine samples. Patients diagnosed via polymerase chain-based reaction (PCR) testing or enzyme-linked immunoassay testing methods (EIA) are considered suspect cases until confirmed via culture. In Georgia and other FoodNet based sites, speciation of *Salmonella* is carried out at state public health laboratories.

The sources and modes of transmission of enteric nontyphoidal *Salmonella* have been extensively studied and are well known in industrial countries. While *Salmonella typhi* is known to only exist in humans, the reservoirs of nontyphoidal strains can typically be found in the gastrointestinal tracts of many animal species and have even been shown to replicate in the tissues of some plants [10]. Animal products and produce contaminated with animal feces are the most prevalent sources of food-based transmission of *Salmonella* to humans. Apart from spreading through food, transmission also occurs through contact with animals such as chickens, cattle, and reptiles and their environment. Other less common modes of transmission of *Salmonella* occurs through water and human to human contact.

The primary risk factors for nontyphoidal *Salmonella* include having previous gastric surgery, pernicious anemia, and taking medications that reduce the acid barrier in the stomach including antacids, proton pump inhibitors, and H_2 antagonists. Being at the opposite ends of the age spectrum is also a risk factor. Older persons are at risk because of multiple comorbidities and taking multiple medications [11]. On the other end of the spectrum, children under 6 lack a strong immune system necessary to deter infection.

From 1996 – 2011, a total of 608,571 *Salmonella* isolates were reported to the CDC, averaging around 38,000 isolates per year or an annual rate of 13.1 cases per 100,0000 persons. Incidence occurred highest among young children < 5 years (45 cases per 100,000 persons), accounting for 27% of isolates with known demographics. Males, on average, experienced a 10% lower incidence compared to females [10]. Analysis of culture-confirmed infections from the various FoodNet sites around the United States from 2006-2014 shows, however, that overall incidence of *Salmonella* has not changed significantly [3].

The prevention of *Salmonella* spread relies mainly on strong, robust surveillance systems to collect case data, quickly detect outbreaks, and make sure information is disseminated in a quick, prompt manner. Changes in industry standards and regulation have also proved to be effective in reducing the incidence of certain *S. enterica* strains. In 2011, the USDA-FSIS tightened the performance standards for *Salmonella* on poultry carcasses by creating an action plan to decrease contamination in regulated products. These preventive measures not only increased food safety, but also saw a decreased incidence of *Salmonella typhimurium*, a strain associated with contaminated poultry, from 2006 – 2014 [13].

1.3 PulseNet

The primary system responsible for detecting outbreaks of disease caused by bacteria like *Salmonella* is the coordinated effort of CDC and the Association of Public Health Laboratories (APHL) called PulseNet [14]. Since 1996, PulseNet has used "DNA fingerprinting" to detect thousands of local and multi-state outbreaks in the United States. PulseNet USA is made up of 83 federal, regional, state, and local laboratories divided into seven regions with at least one laboratory in every state. In 2016. more than 1 million DNA fingerprints were entered into PulseNet databases. PulseNet monitors the spread of *E. Coli* O157, *Campylobacter, Cronobacter, Listeria monocytogenes, Salmonella, Vibrio cholerae, Vibrio parahaemolyticus,* and *Shigella.* This system annually detects around 1,500 clusters of disease and approximately 30 multistate or national outbreaks are identified [12]. DNA fingerprinting involves using PFGE patterns obtained from cultured isolates to characterize bacterial isolates by a specific organism type and serotype, place, and time.

PulseNet had an enormous impact on improving food safety by initiating food recalls and previously detecting unknown disease clusters. Since the system has been in operation more than

1 billion contaminated food products have been recalled. Including items like beef, eggs, leafy greans, poultry, tree nuts, and other items. This system not only saves lives, but also saves money by quickly alerting companies to recall their products and implement remediation efforts. Improved ability to swiftly recall contaminated food items reduces illnesses from *E. coli* by 2,819 and *Salmonella* by 16,994, leading to $37 million in costs averted each year. Annual costs to public health agencies amount to $7.3 million [15].

1.4 FoodNet

Since 1996, the Foodborne Active Surveillance Network (FoodNet) has conducted active population-based surveillance of laboratory confirmed human infections commonly transmitted through foods [16]. FoodNet is composed of ten sites at the state health departments of Conneticut, Georgia, Maryland, Minnesota, New Mexico, Oregon, Tennessee, California, Colorado, and New York, providing surveillance of 48 million persons. This system allows public health officials to analyze trends in foodborne illness with the goal of reducing them. Apart from monitoring, FoodNet can also estimate the burden of disease in a population, provide numbers of hospitalizations and deaths, demographics, and exposure history.

Beginning in 2014, FoodNet added a case-exposure ascertainment (CEA) initiative to capture exposure information for laboratory-confirmed cases of non-typhoid *Salmonella*, *Campylobacter*, and STEC O-157. This supplement gathers data on a case's food history, environmental exposures, and animal exposures seven days prior to onset of symptoms. These data are crucial to linking specific serotypes of *Salmonella* to various food items such as chicken, beef, or leafy greens during outbreak investigations.

1.5 Testing Methods

Diagnostic testing is not only an important tool to diagnose disease in a patient, but also useful to estimate burden of disease in a population. Currently, two different classes of diagnostic tests are used to detect *Salmonella* in patients: culture-based and culture-independent diagnostic testing. Microbial culture presently is the gold-standard method for diagnosis and is required by the Council of State and Territorial Epidemiologists (CSTE) to confirm a *Salmonella* case [17].While mainly confirming disease, cultures also provide isolates for antimicrobial susceptibility testing, genetic typing by PFGE in systems like PulseNet, and molecular characterization [11]. These typing activities are crucial to detecting outbreaks, understanding which specific serotypes of *Salmonella*, and epidemiologically linking foods and activities with disease. Culture-based methods are also labor- and time-consuming, usually requiring a minimum of 4-6 days awaiting bacterial growth. This delay creates a time lag from when disease is detected to reporting to public health authorities, potentially increasing the risk of continuous transmission of bacterial illness [18]. Culture methods have also been shown to have poor sensitivity for low-level contamination in a sample with a diverse flora background [18].

Since 2011, there has been a steady increase in the clinical use of culture independent diagnostic testing methods (CIDTs) for enteric disease, including *Salmonella*. These tests utilize antigen-based and PCR methods for detecting disease [19]. CIDTs have numerous benefits when compared to culture diagnostic testing. Results from CIDTs can be obtained more rapidly, a feature important to clinical decision making and public health surveillance activities. CIDTs also do not require the same level of technical expertise as require for culture testing [20]. Although costs to switch from culture may be initially expensive, CIDTs wind up cheaper than culture tests over time because of reduced labor costs [21]. CIDTs also have the potential to

improve estimates of disease burden in a population because of their superior sensitivity, ability to detect disease without a practical laboratory test, and capability to detect multiple diseases at once [21].

CIDTs pose a serious risk towards the ability for public health surveillance to detect disease outbreaks. CIDTs testing methods do not produce an isolate or sample that can be further disseminated to other agencies. Isolates are crucial for public laboratories to serotype *Salmonella*, detect outbreaks, and do other typing. The sensitivity to detect clusters of disease and potential outbreaks suffers for systems like PulseNet when isolates are not produced [14]. CIDTs also have an increased tendency to create false-positives due to their increased sensitivity, potentially leading to unnecessary, costly public health interventions [22]. Because of these issues, the CSTE classifies a CSTE positive *Salmonella* case as “suspect”, requiring a culture test to be a confirmed case [17]. While cheaper and quicker, CIDTs lack the detailed information that a culture can provide and that public health officials require to create associations and prevent disease spread.

Chapter II: Manuscript

Introduction

Detection of foodborne disease has reached a cross roads where new technology is replacing older diagnostic methods. For decades, culture testing of specimens has been the gold standard, whereby isolates obtained from patients are submitted from clinical diagnostic laboratories to public health laboratories for serotyping, resistance characterization, and surveillance [23]. The public health laboratory operates at the local, state, and national levels to provide epidemiologists with population-based surveillance data, detect and investigate outbreaks of infectious disease, and to monitor trends in the development of antibiotic resistance and altered pathogenicity [22]. Surveillance, like PulseNet, use pulsed-field gel electrophoresis (PFGE) for molecular subtyping of case isolates obtained from culture to identify clusters of illness [14]. Culture-independent diagnostic tests (CIDT) utilize techniques like polymerase chain reaction (PCR), enzyme immune-linked assays (EIA), and Nucleic Acid-based assays to diagnose patients for a wide range of pathogens quickly and cheaply all at once. The downside of CIDTs, however, is their tendency to create false-positives and rarely be sent to Public Health laboratories for specimen isolate characterization [21].

One of the main causes of foodborne illnesses steadily being diagnosed more often with CIDTs across the United States is non-typhoidal *Salmonella* spp. Every year in the United States, the disease caused by non-typhoidal *Salmonella,* salmonellosis, causes an estimated 1 million foodborne acquired illnesses, more than 19,000 hospitalizations, and 350 deaths [9]. The CDC defines a foodborne outbreak as the occurrence of greater than 2 persons with a similar illness resulting from the ingestion of a similar common food [2]. From 2001 – 2010, 76 outbreaks of *Salmonella enterica* were reported to the CDC [1]. Recent decades of improvements

to surveillance capacity and laboratory techniques has led to the creation of databases like PulseNet that can now use genetic information to link salmonellosis cases and monitor trends of serotypes over time [20]. This information is pivotal to epidemiologists during outbreak investigations and to protect food safety.

The advantages and disadvantages of CIDTs have been well documented as the number of tests administered rises yearly. Since 2013, 50 laboratories in Georgia have started using CIDT to identify enteric pathogens, and, as cost-effective solutions become more apparent, more laboratories are expected to continue CIDT adoption to diagnose enteric infection [20]. As CIDT technology continues to advance, the tendency of testing as a false-positive and have poor or incomplete isolate characterizations leads to unnecessary and costly public health interventions [24]. The Association for Public Health Laboratories (APHL) currently recommends that all positive results from non-culture assays used by clinical laboratories to be confirmed by culture [22].

The Georgia Department of Public Health's State Electronic Notifiable Disease Surveillance System (SendSS) conducts active, laboratory based surveillance of notifiable conditions and disease. A separate network, the Foodborne Disease Active Surveillance Network (FoodNet), uses SendSS to capture information on salmonellosis cases [3]. FoodNet data provide information on reporting dates, demographics like age, race, location, and ethnicity, hospitalization rates, and other risk factors. Comprehensive data gathered on cases make it possible to assess relationships between demographic and clinical factors to consider the implications of increased usage of CIDTs to detect disease.

This study aimed to identify factors associated CIDT methods compared to culture-only methods using data captured by SendSS and FoodNet's CEA initiative. Data gathered from 2014

– 2016 describe the characteristics of culture-independent and culture-only tested non-typhoidal *Salmonella* cases.

Methods

Cases were defined as any person reported in Georgia who tested positive for non-typhoidal Salmonella using only PCR-based methods and reported to the Foodborne Active Surveillance Network (FoodNet) from the period of January 2014 to December 2016. Any case who initially tested positive for *Salmonella* via PCR but was then tested again by culture for confirmation was excluded. Controls were defined as any case who tested positive for non-typhoidal *Salmonella* by culture from January 2014 to December 2016. Data were gathered using a query from the State Electronic Notifiable Diseases System (SendSS) and exported into Microsoft Excel. Region was determined by county of residence for all cases of salmonellosis [**Appendix A**]. Any county of residence not in the Metropolitan Statistical Area (MSA) was considered as the region Georgia Outside Atlanta (GOA) [25]. Timely reporting was calculated as the first day notified to public health subtracted by the disease onset date. Hospitalizations included any person admitted or entered into emergency room care. Any case with missing data on gender, race, county residence, laboratory testing method, reporting dates, or hospitalization status was dropped.

Chi-square tests were used to compare cases from controls to determine any significant differences in any of the demographic or clinical characteristics of non-typhoidal *Salmonella* cases from 2014 – 2016. A p-value of < 0.05 was considered statistically significant. Next, two multivariable logistic regression models were created to calculate odds ratios for assessing the relationships testing method had with timely reporting and hospitalization status.

For the model evaluating the relationship between testing method and timely reporting, an interaction assessment was carried out and identified region and the 18-44, 45-64, and above 65 age groups as significant effect modifiers. After keeping these terms in the model, standard backwards elimination was used to assess potential confounding by gender, region, race, and age groups. Region and the 18-44, 45-64, and above 65 age groups were kept in the final model as confounders. The logistic regression model took the following form:

$$\text{logit } P(Timely\_Reporting = 1) = \beta_0 + \beta_1 CIDT + \beta_2 Region + \beta_3 AgeCat\_Adult + \beta_4 AgeCat\_MidLife + B_5 AgeCat\_Old + \beta_6 CIDT\&Region + \beta_7 CIDT\&AgeCat\_Adult + \beta_8 CIDT\&AgeCat\_MidLife + B_9 CIDT\&AgeCat\_Old$$

Where:

Timely_Reporting: Reported within 7 days = 1, else = 0,
CIDT: Culture-Independent Test = 1, Culture = 0,
Region: MSA = 1, GOA = 0,
AgeCat_Adult: Aged 18-44 years old = 1, else = 0,
AgeCat_Midlife: Aged 45-64 years old = 1, else = 0,
AgeCat_Adult: Aged > 65 years old = 1, else = 0,
CIDT&Region: Interaction term between CIDT and Region
CIDT&AgeCat_Adult: Interaction term between CIDT and 18-44 age group
CIDT&AgeCat_MidLife: Interaction term between CIDT and 45-64 age group
CIDT&AgeCat_Old: Interaction term between CIDT and >65 age group

For the second model evaluating the relationship between testing method and hospitalization status, an interaction assessment was carried out and identified gender and the 18-44, 45-64, and > 65 age groups as significant effect modifiers. After keeping these terms in the model, backwards elimination was used to assess potential confounding by gender, region, race, and age groups. Gender and the 18-44, 45-64, and > 65 age groups were kept in the final model as confounders. The multivariate logistic regression model took the following form:

$$\text{logit } P(Hospitalization=1) = \beta_0 + \beta_1 CIDT + \beta_2 Gender + \beta_3 AgeCat\_Adult + \beta_4 AgeCat\_MidLife + B_5 AgeCat\_Old + \beta_6 CIDT\&Gender + \beta_7 CIDT\&AgeCat\_Adult + \beta_8 CIDT\&AgeCat\_MidLife + B_9 CIDT\&AgeCat\_Old$$

Where:

Timely_Reporting: Reported within 7 days = 1, else = 0,
CIDT: Culture-Independent Test = 1, Culture = 0,
Gender: Male = 1, Female = 0,
AgeCat_Adult: Aged 18-44 years old = 1, else = 0,
AgeCat_Midlife: Aged 45-64 years old = 1, else = 0,
AgeCat_Adult: Aged > 65 years old = 1, else = 0,
CIDT&Gender: Interaction term between CIDT and gender
CIDT&AgeCat_Adult: Interaction term between CIDT and 18-44 age group
CIDT&AgeCat_MidLife: Interaction term between CIDT and 45-64 age group
CIDT&AgeCat_Old: Interaction term between CIDT and >65 age group

Data manipulation, model selection, and analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC, USA). An IRB approval request was submitted to the Emory and Georgia DPH IRB and the study was deemed exempt [**Appendix B**].

Results

From the beginning of 2014 to the end of 2016, 7,345 cases of non-typhoidal *Salmonella* were reported to the GDPH. Over the study period, the annual number of diagnoses made by CIDTs doubled from 186 to 392, while the number of culture-positive tests remained constant [**Figure 1**]. CIDT-positive cases reported an increased number of hospitalizations over the study period while the number of culture cases stayed constant [**Figure 2**]. A similar effect was observed in the number of timely reports [**Figure 3**]. Of the total cases observed, 6,470 had complete demographic, laboratory, and medical information necessary for this study [**Table 1**]. The study population was mainly white, < 5 years of age, residing outside of the metro Atlanta area, and reported to public health within seven days of disease onset [**Table 1**].

The main cohort included 705 diagnoses made by CIDTs and 5,765 diagnoses made by culture or reflex culture methods [**Table 2**]. Cases identified via CIDT were more likely to be < 5 years of age compared to culture (67% vs 34.5% , p-value < 0.0001), Asian (3.8% vs 1.9%, p-value = 0.0014), multi-racial (2.4% vs. 1.2%, p-value = 0.0079), residing in the metro Atlanta (MSA) region (45.5% vs 38.3%, p-value = 0.0002), and reported to public health within seven days of disease onset (65.4% vs 55.1%, p-value < 0.0001). Culture positive cases, however, were more likely to be in the 18-44 (18% vs. 8.5%, p-value < 0.0001), 45-64 (19.5% vs 6.4% , p-value < 0.0001), and greater than 65 years old (14.3% vs 3.7%, p-value < 0.0001) age groups, hospitalized (52.9% vs 23%, p-value < 0.0001), and white (71.3% vs 66.2%, p-value = 0.0073). The proportion of males to females was evenly split for both testing methods.

When assessing the relationship between testing methods and timely reporting, CIDTs were more likely to have been to have sent to public health on time after adjusting for age and region of patient's residence (aOR: 1.63, 95% CI: 1.29-2.05) [**Table 3**]. Among MSA residents

aged 18-44 years old, CIDT results were 1.9 times more likely to be sent on time to public health than culture, after adjusting for age and region. Among GOA residents aged 18-44, there was an even greater effect where CIDT results were 3.4 times more likely to be sent on time compared to culture after adjusting for age and region. Similar increasing effects between the two regions can be seen across all patient age groups, except for cases < five years old [**Table 4**]. Among children aged less than 5, the relationship of testing method with timely reporting was significantly different when comparing region. Among cases diagnosed by CIDT residing in an MSA region, no significant relationship existed between testing method and timely reporting (aOR 0.92 95% CI: 0.69-1.20). Meanwhile, CIDT-positive cases less than five years old residing in a GOA region had a higher likelihood of being reported on time compared to culture (aOR: 1.62 95% CI: 1.28-2.05) [**Table 4**].

Lastly, analyzing the relationship between hospitalizations and clinical testing method revealed that cases diagnosed solely by CIDTs were less likely to be hospitalized than cases diagnosed via culture (aOR: 0.31 95% CI: 0.23-0.42) [**Table 3**]. Gender and age groups were as effect modifiers, and significant differences were found. For male cases < 5 years old, CIDT-diagnosed cases were 0.19 times less likely to be hospitalized compared to positive culture cases after adjusting for age groups and gender (aOR: 0.19 95% CI: 0.14-0.25). Meanwhile, females in the same age category were only 0.31 times less likely to be hospitalized compared to positive cultures cases after adjusting for age groups and gender (aOR: 0.31, 95% CI 0.23-0.42) [**Table 5**]. Significant differences were also observed in the > 65 age group. After adjusting for age and gender, females > 65 years of age had no significant relationship with testing method and hospitalization status, while males diagnosed with CIDT were significantly less likely to be hospitalized than culture (aOR: 0.31 (95% CI 0.14-0.70) [**Table 5**].

Discussion

This study examined data gathered by the GDPH from 2014 – 2016 on non-typhoidal *Salmonella* cases to describe any differences between testing methodologies and demographic information. This study also determined if any significant relationship existed with testing method and hospitalization status or timely reporting to public health status.

Separating non-typhoidal *Salmonella* cases by testing method revealed stark contrasts between the two groups. Children < 5 years of age represented most culture-independent diagnoses. Cases tested with culture, however, had a more evenly spread out range of ages, yet the number of children < 5 nearly doubled any other age group. These data confirmed young children at-risk for salmonellosis and showed that pediatric clinics are using quicker and cheaper methods for diagnosis [11]. Quicker diagnoses may mean quicker treatment, but specimen isolates are less likely to be sent to state public health laboratories for serotyping and antibiotic testing. Favoring reducing diagnosis time also could also mean hampering public health's ability to detect foodborne outbreaks and monitor antibiotic resistance.

Patients tested with culture-independent tests had a higher chance of being sent to public health within seven days of disease onset when compared to patients tested with culture. Numerous challenges factor into the time it takes public health to be notified of a reportable illness, but the difference in time it takes to run a culture versus doing a PCR panel is an important factor to consider in the notification process. Shortening the notification time of disease improves public health's ability to monitor trends and to conduct more timely interviews. Culture-positive patients were more likely to be hospitalized when compared to culture-independent patients. This finding could be explained by the preference of CIDT usage in private

practice whereas hospitals perform more culture within their own laboratory capacity. This effect could also speak to the cost-saving benefits of CIDTs. A quicker diagnosis of salmonellosis allows physicians to rule out more serious disease, to prescribe antibiotic or antidiarrheal medication to rapidly resolve symptoms, and to ensure that patients do not incur hefty medical bills from other diagnostic tests and lengthy overnight stays in the hospital.

This study had limitations. First, some salmonellosis cases lacked data on hospitalization status and certain demographics. In 2016, there were 2,839 cases of salmonellosis reported to the GDPH [26]. Due to the high number of cases, capturing complete clinical and demographic characteristics of every case is a difficult task. Missing data may affect the relationships explored within this study. Second, different types of testing methods fall under the category of culture-intendent diagnostic tests, such as PCR, RT-PCR, or EIA [19]. These tests all have a wide range of characteristics, and the analysis conducted in this study may not be generalizable to all CIDTs used to diagnose *Salmonella*. Third, the unequal distribution of age groups for CIDTs may indicate a bias into our study. Two-thirds of the CIDT-positive cases were < 5 years of age, a potential indication that tests are being administered in different clinical settings. Finally, the disproportionate amount of CIDT versus culture tests utilized currently to diagnose disease may also affect the relationship between the variables analyzed in this analysis. Using CIDTs equally across all age groups and as commonly as culture could better detail the differences between testing methods.

This study also benefited from certain advantages. Grouping together CIDTs based off any PCR positive lab result in the absence of any other culture testing simplified creating the two groups used in this analysis. Also, the linked data of *Salmonella* cases through SendSS on demographics, lab results, clinical characteristics, and exposure information provides a good

opportunity to test the relationships between a wide range of variables. Notifiable disease reporting and a standardized case report form also likely reduce reporting and recall bias. SendSS was also very reliable and simple to pull years off data on specific variables in aa short amount of time with little data cleaning.

Our study was the first analysis of timely reporting, hospitalization status, and other characteristics of Georgia non-typhoidal *Salmonella* cases diagnosed by either culture or culture-independent methods. Though only seeing laboratory testing trends for a relatively short period of time, the results of this study indicate that CIDTs are more likely to quickly report Salmonellosis cases to public health quicker while reducing the likelihood of hospitalizations when compared to culture methods. As the number of CIDTs rise over time, follow-up studies with access to more longitudinal data should reproduce this analysis to validate our findings. Compilation of data from other FoodNet sites may also help to provide more generalizability and precision to this analysis. Expanding our understanding of different testing methods to diagnose foodborne disease will allow us to fully grasp the disadvantages and advantages of the rapid rise of culture-independent diagnostic testing.

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Tables

Table 1. Demographic and Clinical Characteristics of Reported Non-typhoidal *Salmonella* Cases, Georgia State Electronic Notifiable Disease System, 2014 – 2016

	#	%
Total	6,470	100
Gender		
Male	3,200	49.5
Female	3,270	50.5
Age		
< 5	2,458	38
5-17	897	13.9
18-44	1,095	16.9
45-64	1,169	18.1
> 65	851	13.2
Race		
White	4,576	70.7
Black	1,460	22.6
Asian	134	2.1
American Indian	15	0.2
Pacific Islander/Hawaiian	11	0.2
Multi-racial	86	1.3
Other	188	2.9
Region		
MSA	2,526	39
GOA	3,944	61
Hospitalized		
Yes	3,213	49.7
No	3,257	50.3
Reported within seven days		
Yes	3,636	56.2
No	2,834	43.8

Table 2. Demographic and Clinical Characteristics of Reported Non-typhoidal *Salmonella* Cases, Georgia State Electronic Notifiable Disease System, by Test Type, 2014 – 2016

	CIDT (n=705)		Culture (n=5765)		
	#	%	#	%	$\chi 2$ p-value
Gender					
Male	353	50.1	2847	49.4	0.731
Female	352	49.9	2918	50.6	
Age					
< 5	472	67	1,986	34.5	<0.0001[a]
5-17	102	14.5	795	13.8	0.6229
18-44	60	8.5	1,035	18	<0.0001[a]
45-64	45	6.4	1,124	19.5	<0.0001[a]
> 65	26	3.7	825	14.3	<0.0001[a]
Race					
White	468	66.2	4,108	71.3	0.0073[a]
Black	166	23.7	1,294	22.5	0.5094
Asian	26	3.8	108	1.9	0.0014[a]
American Indian	1	0.1	14	0.2	0.5986
Pacific Islander/Hawaiian	2	0.3	9	0.2	0.4377
Multi-racial	17	2.4	69	1.2	0.0079[a]
Other	25	3.6	163	2.8	0.2835
Region					
MSA	321	45.5	2,205	38.3	0.0002[a]
GOA	384	54.5	3,560	61.8	
Hospitalized					
Yes	162	23	3,051	52.9	<0.0001[a]
Reported within seven days					
Yes	461	65.4	3,175	55.1	<0.0001[a]

[a] *Significant result, $p < 0.05$*

Table 3. Adjusted Odds Ratios and 95% Confidence Intervals of Non-typhoidal *Salmonella* Cases Reported within Seven Days and Hospitalized, 2014 – 2016

	CIDT (n=705)		Culture (n=5765)		Adjusted Odds Ratio		
Model	#	%	#	%	aOR	95% CI	χ2 p-value
Hospitalized							
Yes	162	23.0	3051	52.9	0.31[a]	(0.23, 0.42)	<0.0001
Reported within seven days							
Yes	461	65.4	3175	55.1	1.62[b]	(1.29 2.05)	<0.0001

[a] *Multivariate logistic regression, adjusting for 18-44, 45-64, and greater than 65 age groups and gender*
[b] *Multivariate logistic regression, adjusting for 18-44, 45-64, and greater than 65 age groups and region*

Table 4. Adjusted[a] Odds Ratios and 95% Confidence Intervals of Reported Non-typhoidal *Salmonella* Cases reported within Seven Days, 2014 – 2016

Region	MSA aOR (95% CI)		GOA aOR (95% CI)	
Test Type	CIDT	Culture	CIDT	Culture
Age group (years)				
Less than 5	0.92 (0.69-1.20)	ref	1.62 (1.28-2.05)[c]	ref
5-17[b]	-		-	-
18-44	1.90 (1.06-3.38)[c]	ref	3.36 (1.81-6.23)[c]	ref
45-64	1.99 (1.01-3.93)[c]	ref	3.53 (1.72-7.23)[c]	ref
65 and above	4.98 (1.48-16.80)[c]	ref	8.83 (2.55-30.57)[c]	ref

[a] *Multivariate logistic regression, adjusting for age groups listed in table and region of patient address*
[b] *No significant interaction or confounding effects observed in the age category 5-17 years.*
[c] *Significant result in χ2-testing (p-value <0.05).*

Table 5. Adjusted[a] Odds Ratios and 95% Confidence Intervals of Reported Non-typhoidal *Salmonella* Cases Hospitalized, 2014 – 2016, by test type

Gender	Male aOR (95% CI)		Female aOR (95% CI)	
Test Type	CIDT	Culture	CIDT	Culture
Age group (years)				
Less than 5	0.19 (0.14-0.25)[c]	ref	0.31 (0.23-0.42)[c]	ref
5-17[b]	-		-	-
18-44	0.59 (0.34-1.02)	ref	0.98 (0.55-1.72)	ref
45-64	0.60 (0.31-1.14)	ref	0.98 (0.53-1.81)	ref
65 and above	0.31 (0.14-0.70)[c]	ref	0.52 (0.23-1.17)	ref

[a] *Multivariate logistic regression, adjusting for age groups listed in table and gender*
[b] *No significant interaction or confounding effects observed in the age category 5-17 years.*
[c] *Significant result in χ2-testing (p-value <0.05).*

Figures

Figure 1. Number of Reported SendSS Salmonellosis Cases, by Year and Test Type, 2014 – 2016

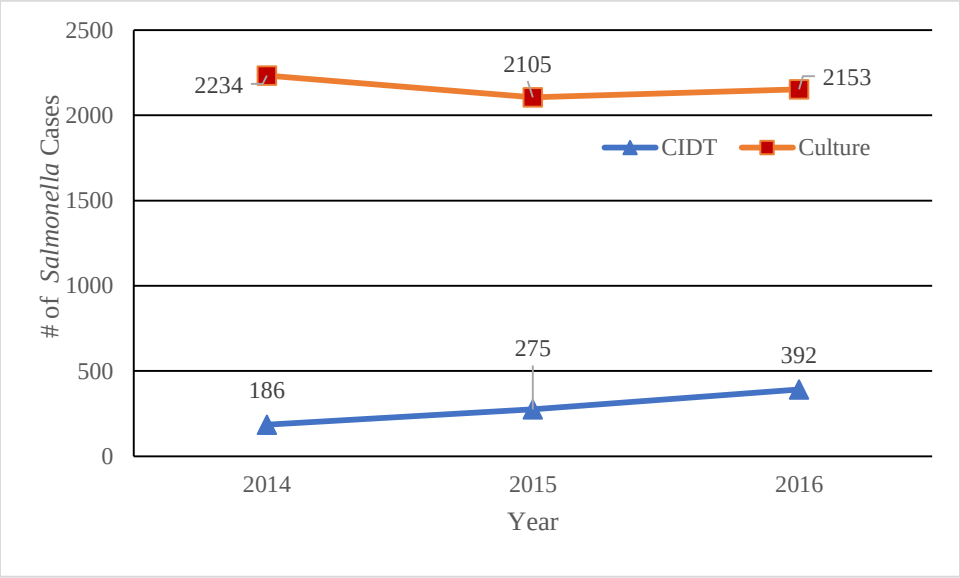


Figure 2. Number of Hospitalized SendSS *Salmonella* Patients, by Year an Test Type, 2014 – 2016

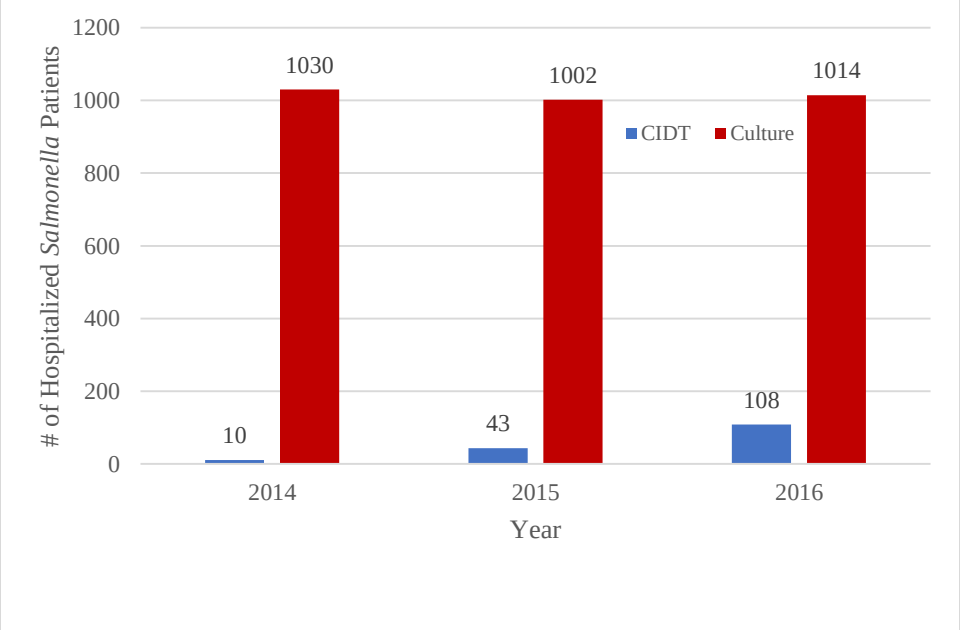
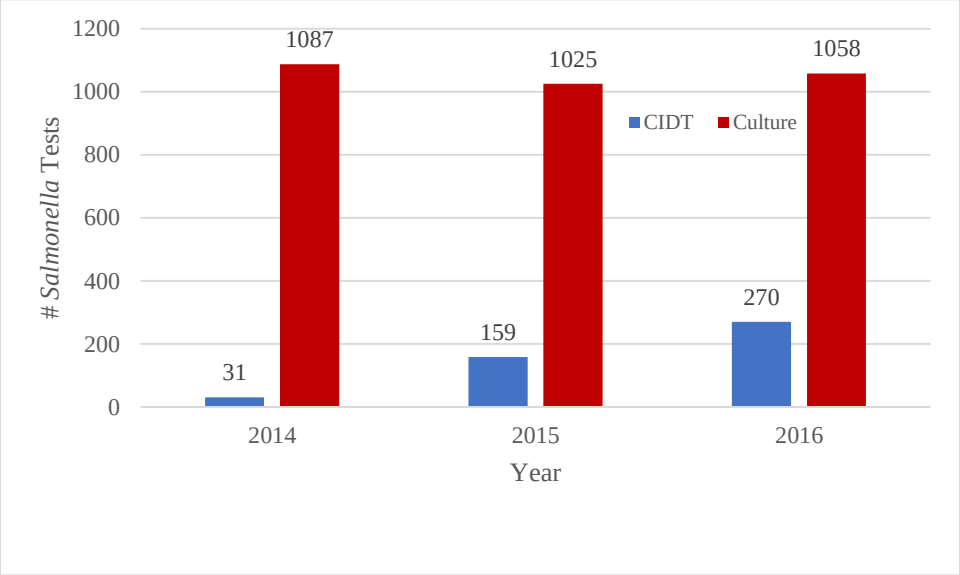


Figure 3. Number of Reported SendSS *Salmonella* Cases within Seven Days, by Year and Test Type, 2014 – 2016



Appendix A

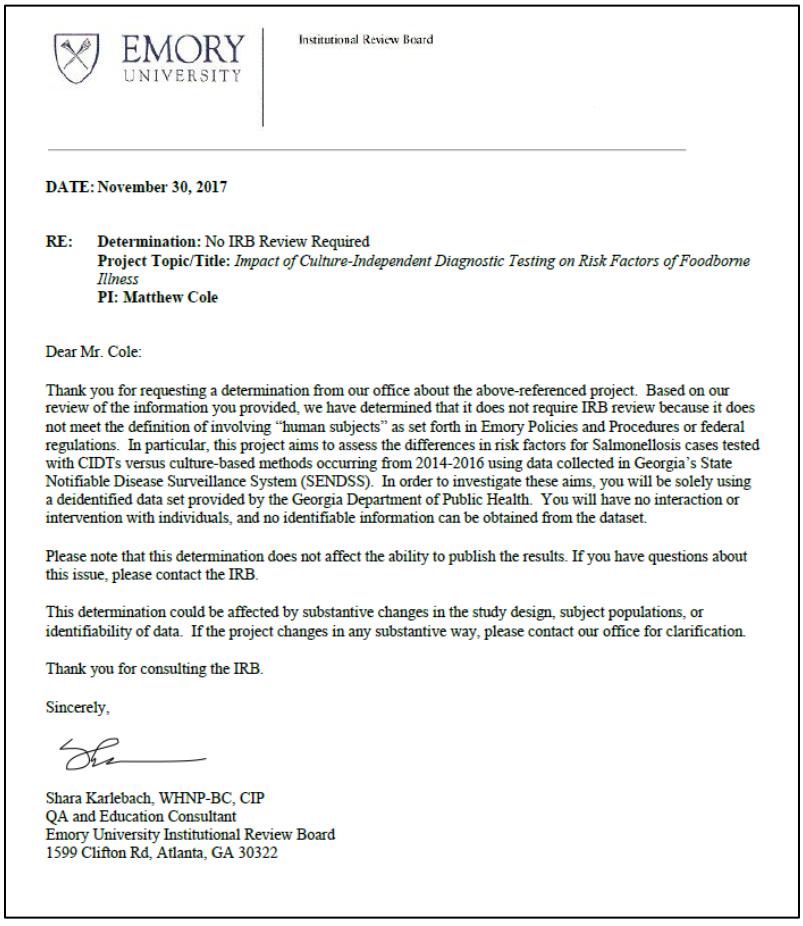
List of Counties in Metropolitan Statistical Area (MSA)

Barrow, Bartow, Butts, Carroll, Cherokee, Clayton, Cobb, Coweta, Dawson, Dekalb, Douglas, Fayette, Forsyth, Fulton, Gwinnett, Haralson, Heard, Henry, Jasper, Lamar, Meriwether, Morgan, Newton, Paulding, Pickens, Pike, Rockdale, Spalding, Walton

Appendix B

IRB Letters

Emory



EMORY UNIVERSITY | Institutional Review Board

DATE: November 30, 2017

RE: **Determination:** No IRB Review Required
Project Topic/Title: *Impact of Culture-Independent Diagnostic Testing on Risk Factors of Foodborne Illness*
PI: Matthew Cole

Dear Mr. Cole:

Thank you for requesting a determination from our office about the above-referenced project. Based on our review of the information you provided, we have determined that it does not require IRB review because it does not meet the definition of involving "human subjects" as set forth in Emory Policies and Procedures or federal regulations. In particular, this project aims to assess the differences in risk factors for Salmonellosis cases tested with CIDTs versus culture-based methods occurring from 2014-2016 using data collected in Georgia's State Notifiable Disease Surveillance System (SENDSS). In order to investigate these aims, you will be solely using a deidentified data set provided by the Georgia Department of Public Health. You will have no interaction or intervention with individuals, and no identifiable information can be obtained from the dataset.

Please note that this determination does not affect the ability to publish the results. If you have questions about this issue, please contact the IRB.

This determination could be affected by substantive changes in the study design, subject populations, or identifiability of data. If the project changes in any substantive way, please contact our office for clarification.

Thank you for consulting the IRB.

Sincerely,

Shara Karlebach, WHNP-BC, CIP
QA and Education Consultant
Emory University Institutional Review Board
1599 Clifton Rd, Atlanta, GA 30322

GDPH

Georgia Department of Public Health

Brenda Fitzgerald, MD, Commissioner | Nathan Deal, Governor

2 Peachtree Street NW, 15th Floor
Atlanta, Georgia 30303-3142
www.health.state.ga.us

April 21, 2017

Matthew Cole
PHCW (Student Intern)
4311 Renaissance Way NE

Project: 170401 - Impact of Culture-Independent Diagnostic Testing on Risk Factors of Foodborne Illness

Project Status: Exempt

Dear Researcher,

The DPH Institutional Review Board has determined that the above-referenced project is **exempt** from the requirement for IRB review and approval.

Reason:
Cat#4 - Research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.

This exemption applies only to the protocol described in your application. Any modification to this protocol may change the status of this project and may require IRB review and approval except where necessary to eliminate apparent immediate hazards to human subjects.

If you have any questions regarding this letter or general procedures, please contact the DPH IRB at irb@dhr.state.ga.us. Please reference the project # in your communication.

Best wishes in your research endeavors,

Brian Kirtland, Ph.D.

Chapter III: Summary, Public Health Implications, Future Directions

Methods used to detect enteric disease in the United States are changing at a rapid pace as technological advances create faster, cheaper, and more efficient diagnostic tests. Since 2011, Culture-independent diagnostic testing (CIDT) methods have become this new generation of tests and continue to increasingly be used to diagnose non-typhoidal *Salmonella* in Georgia. For our study we used FoodNet data from 2014 to 2016 to look for any demographic differences by and to assess the relationships of hospitalizations and timely reporting with testing methodology used to diagnose non-typhoidal *Salmonella* in Georgia.

This study found that the number of positive CIDTs used to identify *Salmonella* nearly doubled from 2014 to 2016, while the number of cultures positive cases remained somewhat constant. Most cases diagnosed were less than five years of age, a known at-risk age group for *Salmonella*. CIDT diagnosed patients were more likely to reported to public health by the required seven days from disease onset than culture-positive cases, especially among CIDT-positive cases residing in areas outside of metro Atlanta. CIDT-positive cases were also less likely to be hospitalized compared to culture cases, especially among males diagnosed with CIDT.

Shortening reporting time is a major benefit to public health. Faster notification mean epidemiologists can potentially interview cases quicker on exposures while trying to reduce as much recall error. Quicker diagnoses by CIDTs also could mean quicker treatment prescribed to patients and reduced likelihood of lengthy hospitalization stays awaiting test results by culture. This effect could also be explained by private practices choice to use cheaper detection methods to cut costs. The continued increase in CIDTs testing positive for *Salmonella* without culture confirmation, however, also shows that more isolates are not being public health laboratories for serotyping, genetic profiling, and antimicrobial susceptibility testing. Public health's ability to conduct epidemiologic activities and monitor disease outbreaks may be negatively affected.

There were limitations in our analysis. Low number of cases in specific age groups may introduce error into our study and affect generalizability. CIDTs encompass a wide range of varying testing methodologies each with their own sensitivities and specificities. Grouping together these tests may also affect the generalizability of our

results. Our study benefited from having access to the State Electronic Notifiable Diseases System (SendSS) that provided linked information on demographic, laboratory, and clinical characteristics of salmonellosis cases.

This study is the first analysis of reporting time and hospitalization status by testing methodology using FoodNet Data. This analysis could be improved by using data on other enteric pathogens that CIDTs have the capability to detect and data from other FoodNet sites. Looking at data over an extended period could also benefit this analysis. In the future, the Georgia Department of Public Health should evaluate if CIDTs have any effect on its ability to detect foodborne disease outbreaks or monitor anti-microbial susceptibility. An improved understanding of CIDTs could underscore the necessity of culture confirmation or specimen characterization.