EMERGING NONTYPHOIDAL *SALMONELLA* ANTIBIOTIC RESISTANCE IN OREGON, 2004-2009: RISK FACTORS AND TRENDS

By

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MASTERS THESIS

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CERTIFICATE OF APPROVAL

This is to certify that the Master's thesis of
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II. ABSTRACT

Background: Non-typhoidal *Salmonella* (NTS) infects an estimated 1.4 million persons in the US every year. Studies have demonstrated the health consequences of infection with antimicrobial resistant NTS, but few population level epidemiologic investigations have examined exposures and trends in resistance. Therefore, our objective was to explore NTS resistance in Oregon.

Methods: The Oregon Health Authority, Public Health Division performs surveillance on 3.6 million persons and ascertains patient exposure history, demographics, and outcomes for all culture-confirmed cases of Salmonellosis. Positive isolates are serotyped and antimicrobial susceptibility testing is performed. We defined resistance as clinically important (CIR) if an isolate exhibited decreased susceptibility to ampicillin, ceftriaxone, ciprofloxacin, gentamicin, or trimethoprim-sulfamethoxazole. We analyzed trends in antimicrobial resistance and modeled risk factors for acquiring a resistant isolate compared to cases with pan-susceptible isolates.

Results: During 2004-2009, 80.4% (n=1813) of all Oregon confirmed Salmonellosis cases had exposure information and susceptibility testing. Among these patients, 16.8% (n=305) had isolates resistant to >=1 CIR antibiotic and 55.3% (n=1,002) were susceptible to all antibiotics screened. The following serotypes had increased resistance compared to serotype Enteriditis: Typhimurium (adjusted odds ratio [aOR] 4.51, <0.01), Heidelberg (aOR 5.01, p<0.01), Typhimurium var Copenhagen (aOR 13.86, p<0.01), and Newport (aOR 7.11, p<0.01). Relative to pan-susceptible isolates, the percentage of isolates that were CIR significantly increased (15.7% in 2004 to 26.7% in 2009, p<0.01). Year (aOR 1.15 per year, p<0.01), travel to East or Southeast Asia (aOR 6.86, p<0.01), and outbreak clusters (aOR 0.55, p<0.01) were significantly associated with resistance to >=1 clinically important antibiotic.

Conclusion: Antimibiotic resistance among NTS increased in Oregon between 2004 and 2009. Clinically important resistance was positively associated with international travel to East and Southeast Asia and negatively associated with outbreak cases.

III. INTRODUCTION

The purpose of this study was to retrospectively investigate temporal trends and risk factors that were important for the emergence of antibiotic resistant non-typhoidal *Salmonella* in Oregon from 2004-2009. Antibiotic resistant NTS was defined as resistance to four antibiotics known to be associated with the ACSSuT DT104 phage type *Salmonella* Typhimurium. Separately, resistance was defined as resistance to clinically important antibiotic agents. The population for this study included all Oregon residents from 2004-2009, specifically laboratory confirmed NTS cases that resided in the Oregon catchment area of the Centers for Disease Control and Prevention's (CDC) Foodborne Diseases Active Surveillance Network (FoodNet). The Oregon FoodNet surveillance population included approximately 3.6 million persons (about 1.2 percent of the U.S. population). The study was carried out by (i) identifying subjects with NTS infections isolated from stool, urine, or a normally sterile site, (ii) merging their information with antibiotic susceptibility data from the Oregon State Public Health Laboratory (OSPHL) and the Oregon State University Veterinary Diagnostic Lab (OSU, VDL), and (iii) extracting information about demographic factors and exposure history from case interviews stored in the Oregon State Public Health Division's "Oregon Public Health Epidemiology User System" (AKA. ORPHEUS).

IV. BACKGROUND

Salmonella enterica Epidemiology

Salmonella enterica is a gram negative facultative anaerobic bacteria with over 2,500 serotypes. *Salmonella* can infect humans causing moderate to severe gastrointestinal illness. Commons reservoirs for this pathogen include animals such as reptiles, poultry, pigs, and livestock (Homberg 1984, Boyle 2007, Tauxe 1986, Hopkins 2010). Humans usually contract the *Salmonella* via the fecal oral route and person to person transmission (Tauxe 1986, Homberg, 1984). *Salmonella* has been shown to be fecally excreted for weeks to months after infection (Murase 2000). Estimates suggest that an excess of 1.4 million NTS infections occur each year in the United States (Voetsch 2004, Mead, 1999), with an estimated 98 million cases globally (Majowicz, 2010). These infections ultimately result in an estimated 168,000 physician visits, 15,000 hospitalizations, and 580 deaths in the US annually (Voetsch 2004, Mead, 1999,). Between 2002 and 2009 Oregon had an average of 349 cases of confirmed Salmonellosis each year. The majority of enteric NTS infections are short-lived and self-limiting, resulting in severe diarrhea, nausea, and discomfort. However, the economic impact of such infections is estimated at $2.7 billion annually in the United States (ERS, 2010) and severe cases can lead to serious invasive bloodstream infections, meningitis, and death (Gordon, 2008).

Globally, NTS has shown to represent a major health burden being the number one cause of bacteremia in tropical Africa, (Berkley 2005, Cheesbrough, 1997) with an estimated 38% of bacteremia caused by NTS in Tanzania, (Mtove,

2010) and a case fatality rate of 38%-47% among people infected with HIV (Gordon 2002). Furthermore, a study using international surveillance data estimated that Salmonellosis contributes to 155,000 deaths annually (95%CI 39,000-303,000), and estimated that 86% of these infections are foodborne (Majowicz, 2010). Multiple international studies have shown that susceptible children and especially people with HIV are at high risk of bacteremia and fatality (Gordon 2002, Gordon 2008, Cheesbrough 1997).

Between 2002 and 2009 there were a total of 2,836 cases of NTS and 107 outbreaks in Oregon. Thirty three of these outbreaks had a causative vehicle identified. The most frequently identified vehicles were chicken and alfalfa sprouts followed by melons, eggs, and nut products. While these outbreaks identified some commonly known risk factors for Salmonellosis, the majority of cases were sporadic cases with no known vehicle and no clear risk factor identified.

Treatment for *Salmonella* infections

Recommendations for treatment of *Salmonella* infections are hydration therapy and electrolyte management (Guerrant, 2001). Most *Salmonella* infections are self limiting and the use of antibiotics is contraindicated. Studies have also demonstrated that antibiotic therapy may lengthen the duration and shedding of *Salmonella* (Murase, 2000). Exceptions include immuno-compromised persons or individuals with decreased immune function, enteric fever, or invasive infection where antibiotic therapy may lessen illness severity and be life saving (Hohman,

2001). When antibiotic thereapy is administered to infected patients, fluoroquinolones (e.g., ciprofloxacin) are most commonly prescribed for adults and extended-spectrum cephalosporins (e.g., ceftriaxone) are prescribed in children (Guerrant, 2001). These first line treatments are also used for a wide variety of other enteric pathogens (Helms, 2004).

Emerging Antibiotic resistance

Key studies from the late 1970's and the 1980's demonstrated an alarming increase in the prevalence of antibiotic resistance among *Salmonella* isolates in the US (Homberg 1984, MacDonald 1987). Some of this increase was attributed to the use of antibiotics agents in animal husbandry (Homberg, 1984, Tauxe 1986), while use of antibiotics in humans was also implicated (MacDonald, 1987).

Growing fears about the emergence of highly resistant strains of *Salmonella,* specifically the emergence of the pandemic *Salmonella* Typhimurium DT 104 strain with ACSSuT resistance, and the emergence of multi-resistant *Salmonella* Newport, prompted the creation of the National Antimicrobial Resistance Monitoring System (NARMS), which monitors antibiotic susceptibility patterns for enteric pathogen isolates from around the US (NARMS 2011). Since its inception in 1996, NARMS has reported the emergence of a multi-drug resistant strain of non-typhoidal *Salmonella* Newport, resistant to 9 or more antibiotics. NARMS also found evidence that use of enrofloxacin in poultry may be driving antibiotic resistance among *Salmonella* Typhimurium isolates. A FoodNet/NARMS retrospective study found significant

associations between hospitalization and invasive infection with antibiotic resistance between 1996 and 2001. (Varma, 2005)

A newly published case series analysis found the global distribution of a *Salmonella enterica* serotype Kentucky strain resistant to multiple antibiotics including ciprofloxacin (Le Hello, 2011). Another case series cross-sectional study among patients in Africa also found a temporal relationship between increases in bacteremia and resistant NTS infection (Gordon 2008). Additionally, a cohort study and a cross sectional study indicated that antibiotic resistant infections appear to have increased pathogenicity resulting in the increased reliance on antibiotics for clearance and increase the risk for treatment failure (Helms, 2004, Winoker 2001).

One reason why resistance in *Salmonella* is particularly troubling is related to recent genomic findings that suggest antibiotic resistant isolates have increased virulence. This finding is unique to Salmonella because many other pathogens exhibit decreased virulence in the presence of resistance genes. This increased fitness appears to come from the *Salmonella* Genomic Island-1 (SGI1) and conjugated plasmids which are often co-integrated with virulence genes and resistance genes encoded together (Fluit, 2004). Therefore, reports of the emergence of new strains of NTS that are increasingly resistant to multiple antibiotics as a result of mobile genetic elements or conjugated plasmids (e.g., bla tem-1, bla cmy-2, qnrD) has the potential to cause pandemic levels of illness. (Poppe 2005, Wichard 2007, Cavaco 2009, Le Hello 2011).

Risk Factors for Acquiring a Resistant Infection

Surveillance data and epidemiological studies have demonstrated an increase in antibiotic resistance as well as increased severity and duration of illness. However, numerous studies have called for more epidemiological evidence that's analyzes risk factors for acquiring resistant infections (Kariuki, 2006, Wichard, 2007). Few studies have examined risk factors for the acquisition of a resistant NTS infection in humans outside of outbreak clusters (other than antibiotic use). Many studies have found resistant strains of NTS in poultry, pigs, and cows, however, there is little information on whether these resistant strains infect humans. One of the landmark resistance studies in the 80's found associations with animal-to-human transmission of resistant strains, but they only looked at clearly identified outbreaks with little to no information on the thousands of sporadic cases that occur each year (Homberg, 1984).

In Oregon, between 2002 and 2009, 33 of the 107 identified NTS outbreaks had a vehicle identified. Interestingly, only 4 (12%) of these outbreaks had isolates which were on resistant to 3 or more antibiotics. Within eight (24%) vehicle identified outbreaks, antibiotic resistance differed by at least 3 antibiotics among isolates that were matched by serotype and pulsed field gel electrophoresis (PFGE). Therefore, even within outbreaks there appears to be heterogeneity in the resistance profile, perhaps indicating that other risk factors (eg. antibiotic use, host factors) or effect modifiers may be important.

Three descriptive studies have implicated international travel as a risk factor for resistance among *Salmonella* Stanley, *Salmonella* Concord and *Salmonella* Typhimurium (Hendricksen 2012, Hendricksen 2009, Threlfall 2000). Antibiotic resistance literature suggests that antibiotic selective pressure may act cumulatively on bacterial populations (O'Brien 2011). Therefore, emergent resistance genes may act to increase resistance globally, drive emergent epidemiological trends and reservoirs, and may disseminate to other emergent pathogens. In this context, there is a need to assess risk factors for acquiring resistant NTS infections. Identification of such risk factors may help to elucidate those characteristics responsible for developing antibiotic resistance as well as identify key points for intervention to prevent and control the emergence of multi-drug resistant isolates in the future.

V. RESEARCH QUESTIONS

Question 1: What were the patterns of antibiotic resistance in non-typhoidal *Salmonella* isolates in Oregon from 2002-2009?

Question 2: Are there risk factors that are significant predictors of antibiotic resistance of non-typhoidal *Salmonella* infection in Oregon? Do this risk factors differ for *Salmonella* serotype Typhimurium?

VI. SPECIFIC AIMS

Aim 1: Describe overall resistance trends and data in Oregon for 2002-2009.

Aim 2: Assess whether resistance was associated with increased disease severity.

Aim 3: Elucidate risk factors associated with acquiring an antibiotic resistance infection (ACSSuT and clinically important) from 2004-2009.

Aim 4: Perform a sub-analysis using multiple logistic regression modeling to determine risk factors associated with clinically important resistance in non-typhoidal *Salmonella* Typhimurium from 2004-2009.

Aim 5: Examine the impacts of risk factors for antibiotic resistance in the context of preventative measures and enhanced surveillance procedures in the State of Oregon and the United States.

VII. TESTABLE HYPOTHESES

Hypothesis 1: The National Antimicrobial Resistance Monitoring System (NARMS) collected isolates from Oregon that were statistically representative of the resistance pattern, serotype diversity, and demographic profile seen in Oregon between 2004 and 2009.

Hypothesis 2: Frequencies of antibiotic resistance among NTS isolates from Oregon during 2004-2009 significantly increased.

Hypothesis 3: Antibiotic resistance was significantly associated with worse clinical outcomes (hospitalization, invasive isolates), compared to cases with pan-susceptible isolates.

Hypothesis 4: There are risk factors for acquiring an antibiotic resistant infection.

Hypothesis 5: International travel is significantly associated with acquiring an antibiotic resistant *Salmonella* Typhimurium infection.

VIII. METHODS

Overview of Oregon's Reportable Infectious Disease Data System

The state of Oregon infectious disease surveillance system is multifaceted and has built in redundancies to maximize disease reporting and to accurately capture as many cases of illness as possible.

Firstly, the state of Oregon requires all physicians to report all lab-confirmed and clinically suspected cases of Salmonellosis to the patient's local health department within one working day of the initial diagnosis. Providers are also required to report the patient's name, home address, phone number, date of birth, sex, diagnosis, and date of symptom onset (patient self reported onset).

Secondly, the state of Oregon requires that all clinical laboratories report any positive tests for non-typhoidal *Salmonella* (usually positive microbiological cultures) to the patient's local health department within one working day. Similarly, the laboratories are required to report the patient's name, date of birth, county of residence, specimen collection date, lab test and result, as well as providing the contact information for the clinician who originally ordered the test and the lab name. Additionally, all positive non-typhoidal *Salmonella* isolates are required to be forwarded to the Oregon State Public Health Laboratory (OSPHL) where they are given a unique sample ID (OSPHL ID), processed for stereotyping, and registered with PulseNet via uploading the bacterium's pulsed field gel electrophoresis (PFGE) pattern.

Emerging Infections Program, FoodNet Active Surveillance

The State of Oregon is part of the CDC Emerging Infections Program and is a statewide Foodborne Diseases Active Surveillance Network (Food-Net) site. FoodNet (http://www.cdc.gov/foodnet) conducts laboratory-based active surveillance of non-typhoidal *Salmonella* among 10 state health departments. As a result, epidemiologists at the Oregon State Department of Health give weekly phone calls to clinical labs and hospitals and audits them to find out the number of non-typhoidal *Salmonella* cases (confirmed and clinically suspected) each week, thereby capturing the majority of confirmed and clinically suspected new cases of Salmonellosis in Oregon. The FoodNet surveillance area in Oregon between 2004 and 2009 represented 3.6-3.8 million persons, or roughly 1.2% of the US population. (US census, 2010, PSU PRC, 2010)

Oregon Salmonellosis Case Follow-up

Upon receiving reports of illness, the patient's county health department is required to attempt to contact the patient and complete the "Salmonellosis Case Report Form" (Appendix F), which includes questions about hospitalization status/clinical outcomes, additional demographic information, and risk factor/exposure information for 7 days prior to illness onset. This additional information includes State lab (OSPHL) ID number, hospitalization status, serotype, and PFGE pattern as well as risk factors/exposures which include travel, pets, livestock, reptiles, raw milk, raw eggs, raw meat, raw cheese, soiled diapers/diaper

changing, people with diarrhea, fecal matter, fecal matter at work, restaurant food, event food, and sprouts. Patients with recurrent infection and/or who have multiple positive isolates are only interviewed once and risk questions relate to the 7 days preceding the first positive isolate. For those patients who submitted multiple 1st isolates on the same day, priority for first isolate is as follows: blood/sterile site>stool>urine>other. This data is then uploaded into the Oregon State Health department infectious disease data-system, and the PFGE patterns and case info is uploaded to PulseNet and FoodNet respectively.

Antibiotic Susceptibility Testing

The State of Oregon began performing antibiotic susceptibility testing on all confirmed non-typhoidal *Salmonella* and *Shigella* isolates in 2003. This testing was done independently of the NARMS testing. Between 2003 and 2005 all of the confirmed isolates were forwarded to the Oregon State University Veterinary Diagnostic Lab (OSU, VDL) for susceptibility testing. From 2003-2005 antibiotic susceptibility was determined using CLSI interpretive criteria for the following 12 antibiotic agents: ampicillin, azithromycin, cefuroxime, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, nitrofurantoin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole.

In 2006 the OSPHL began screening isolates from 2006 onward. For 2006-2009, antibiotic susceptibility was determined using CLSI interpretative criteria for the following 14 antibiotic agents: ampicillin, amoxicillin–clavulanic acid,

ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, levofloxacin, nalidixic acid, nitrofurantoin, norfloxacin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole.

Data Collection and Inclusion Criteria

Secondary analysis of these data was performed to test the research hypotheses and fulfill the aforementioned specific aims of the study. Data from 2004-2009 was abstracted from ORPHEUS and was merged with susceptibility information via the OSPHL ID number. For the multivariate analysis data from 2004-2009 was included and for the *Salmonella* Typhimurium subset, an audit of paper state health reports was performed to increase ascertainment of exposure and demographic history for 2004-2009. Inclusion criteria were as follows:

i. Confirmed cases of non-typhoidal *Salmonella* (blood, sterile site, stool, urine-1st specimen only) between 2004 and 2009. Only one isolate per case was included with priority given to the first positive isolate.
ii. Had susceptibility testing performed on their isolate.
iii. Was interviewed regarding exposure history*

**For multivariate hypothesis testing.*

Finally, there has been speculation about the inclusion of outbreak cases where vehicles were identified because this information can heavily bias or confound study results (i.e. Over sampling of strains and/or exposure information is irrelevant since vehicle was identified). One approach to dealing with this potential

problem is to restrict cases to only the first case (by date of onset and completed record) from outbreaks with an implicated vehicle. The other solution can be to adjust and/or assess interaction between resistance profiles seen among outbreak cases, sporadic cases, and household cases. For this analysis the latter method was used to help to represent the true serotype/resistance diversity as well as being the first study to model resistance for cases based on their epidemiological classification.

Outcome Variables

Outcome variables included pan-susceptible (non-case), tetra-resistance (ACSuT as a proxy for ACSSuT resistance), and clinically important resistance (CIR). Pan-susceptible was defined as susceptibility to all 10 of the following antibiotics; ampicillin, cephalosporins (ceftriaxone, cephalothin and cufuroxime), chloramphenicol, ciprofloxacin, gentamicin, naladixic acid, nitrofurantoin, sulfonamides, SXT, and tetracycline.

Tetra-resistance (ACSuT) was defined as resistance to ampicillin, chloramphenicol, sulfonamides, and tetracycline. Streptomycin is usually considered important for the penta-resistant (AC**S**SuT) profile frequently seen in *Salmonella* Typhimurium phage type DT104 and commonly analyzed in the literature.

Since the OSU, VDL did not perform antibiotic susceptibility testing on streptomycin between 2003-2005 a preliminary analysis of data from the OSPHL

(2002 and 2006-2009) was performed to ascertain what proportion of isolates that met the tetra-resistance definition were also resistant to streptomycin (i.e. penta-resistant). The comparison revealed that there were 125 isolates that met the case definition for tetra-resistance and 123 (98.4%) of these isolates were tetra-resistant as well as penta-resistant. Restricting this analysis to the years 2006-2009 there were 88 isolates that met the case definition for tetra-resistance and 87 (98.8%) of these isolates were resistant to streptomycin. Therefore, assuming this proportion remained constant during 2004-2009 we would only expect 2 cases that were not actually penta-resistant to be misclassified if the tetra-resistant definition is used in place of penta-resistant. Thus, the tetra-resistant definition was a valid proxy for the presence of the ACSSuT resistance pattern.

CIR has been previously defined as resistance to any one of the following antibiotics: ampicillin, ceftriaxone, ciprofloxacin, gentamicin, and/or trimethoprim-sulfamethoxazole (Varma, 2005). However our definition departs from this definition slightly. Since the OSU VDL did not screen for ceftriaxone (2004, 2005) we defined cephalosporin resistance as resistance to both cephalothin (1st generation cephalosporin) and cefuroxime (2nd generation cephalosporin) in lieu of ceftriaxone.

For the simple logistic regression analyses on disease severity, the outcomes of hospitalization and invasive infection (blood, sterile sites vs. stool, urine) were used.

Risk Factor Variables

Predictor variables included demographic information, clinical information, and risk factor information recorded in ORPHEUS. Specifically, this included reported and hypothesized confounding variables (Age, gender, serotype, year, hospitalization,) (Varma, 2005). Table 1 lists all the outcome variables and the predictors that were included in this dataset.

Table 1. Outcome and Risk Factor Categorization

Variable Name	Variable Type	Data format	Variable Measurement
ACSSuT Resistance	Outcome	Categorical	0=Pan-susceptible 1=Resistant
Clinically Significant	Outcome	Categorical	0=Pan-susceptible 1=Resistant
Sex	Demographic (confounder)	Categorical	0=Female 1=Male
Age	Demographic(confounder)	Categorical	0=18-64 years (ref) 1=<1 years 2= 1-4 3=5-17 4=65+
Hispanic	Demographic	Categorical	0=Non-Hispanic 1=Hispanic 2=Unknown
Race	Demographic	Categorical	0=White 1=Non-White 2=Unknown
Hospitalization	Outcome	Categorical	0=no 1=yes
Year	Risk Factor	Discrete (continuous)	2004-2009
Serotype	Risk Factor	Categorical	Top 14 + other for univariate analysis Top 9+other for CIR multivariate analysis, Top 5+ for ACSSuT analysis **S. enteriditis*=ref
Diaper Exposure	Risk Factor	Categorical	0=no 1=yes
Livestock Exposure	Risk Factor	Categorical	0=no 1=yes
Pet Exposure	Risk Factor	Categorical	0=no 1=yes
People with Diarrhea	Risk Factor	Categorical	0=no 1=yes

Raw Cheese Exposure	Risk Factor	Categorical	0=no 1=yes
Raw Egg Exposure	Risk Factor	Categorical	0=no 1=yes
Raw Meat Exposure	Risk Factor	Categorical	0=no 1=yes
Raw Milk Exposure	Risk Factor	Categorical	0=no 1=yes
Reptile Exposure	Risk Factor	Categorical	0=no 1=yes
Sprout Exposure	Risk Factor	Categorical	0=no 1=yes
Occupational Fecal Exposure	Risk Factor	Categorical	0=no 1=yes
Specimen Type	Outcome	Categorical	0=Stool or Urine 1=Blood or Sterile Site
International Travel	Risk Factor	Categorical	0=no 1=yes
Case Type	Risk Factor	Categorical	0=SP(sporadic case)-ref 1=OB (part of cluster/outbreak) 2=HH (probable secondary case)

Analysis

Descriptive analyses were performed on data from 2002-2009. We described frequencies of resistance, characteristics of the patient cohort, and the serotype diversity seen in Oregon over this time period.

Hypothesis 1; Likelihood ratio Chi-square tests were performed to see if there were significant differences in resistance, serotype diversity (top 5 serotypes with all others grouped as "other"), and demographic characteristics (age, race, sex, ethnicity) between isolates submitted to NARMS between 2004 and 2009 compared to all Oregon cases during that time period.

Hypothesis 2; The Cochran-Armitage Test for Trend was used to test the hypothesis that resistance has increased over time.

Hypothesis 3; Simple logistic regression and multiple logistic regression was performed to determine bi-variate associations between hospitalization and

invasive illness (adjusted and unadjusted by serotype and age) and resistance outcomes for data abstracted from 2004-2009.

Hypotheses 4 and 5; Simple logistic regression was performed to determine bi-variate associations between risk factors/demographic variables and resistance outcomes. Predictors which had a level of significance at or below 0.25 as well as previously reported confounding variables were included for multivariate logistic regression modeling. This bi-variate analysis was also performed in a subset analysis for *Salmonella* Typhimurium only.

Multivariate logistic regression models were constructed with those variables below the 0.25 level of significance, known confounders, and those predictors that make the model the most parsimonious. Preliminary stratified chi-square and Breslow-Day tests were performed to assess confounding and to identify potential interactions. For the multivariate modeling covariates for which uni-variable and stratified significance was <0.25 were included into a multi-variable logistic regression model. Variables that were not significant were given further consideration on the basis of their clinical/epidemiological importance or relevance for external validity. The resulting preliminary main effects model was then used to explore the need to include other non-linear or interaction terms. Predictor variables with a 0.05 level of significance were retained in the final multivariate model and log odds ratios were calculated.

Model fit was verified using the Hosmer and Lemeshow method. In order to assess the models discriminative ability, we examined the specificity and sensitivity

of the model and calculated and plotted a receiver operating characteristic (ROC) curve. Finally, we assessed the model for outliers, influential points by graphing the leverages, Cooks deletion, change in Pearson's chi-square, change in deviance, and the change in dfbetas. All analyses were performed in SAS v 9.2 (SAS Institute Inc., Cary NC 2008).

IX. HUMAN SUBJECTS PROTECTION AND IRB APPROVAL

Individual patient level data was not analyzed for this study. All data exported from ORPHEUS and all merged susceptibility data was de-identified and analyzed in aggregate. Therefore identification of individual patients was not possible during the analysis. Furthermore this data was collected as part of public health surveillance and therefore is not considered human subjects research. OHSU IRB exemption was submitted and exemption was granted on September 23, 2011. Statement from OHSU IRB:

" Based upon the submitted information, the IRB has determined that the proposed activity:

Is not human subject research because the proposed activity:

- *Does not meet the definition of human subject per 45 CFR 46.102(f)"*

X. RESULTS

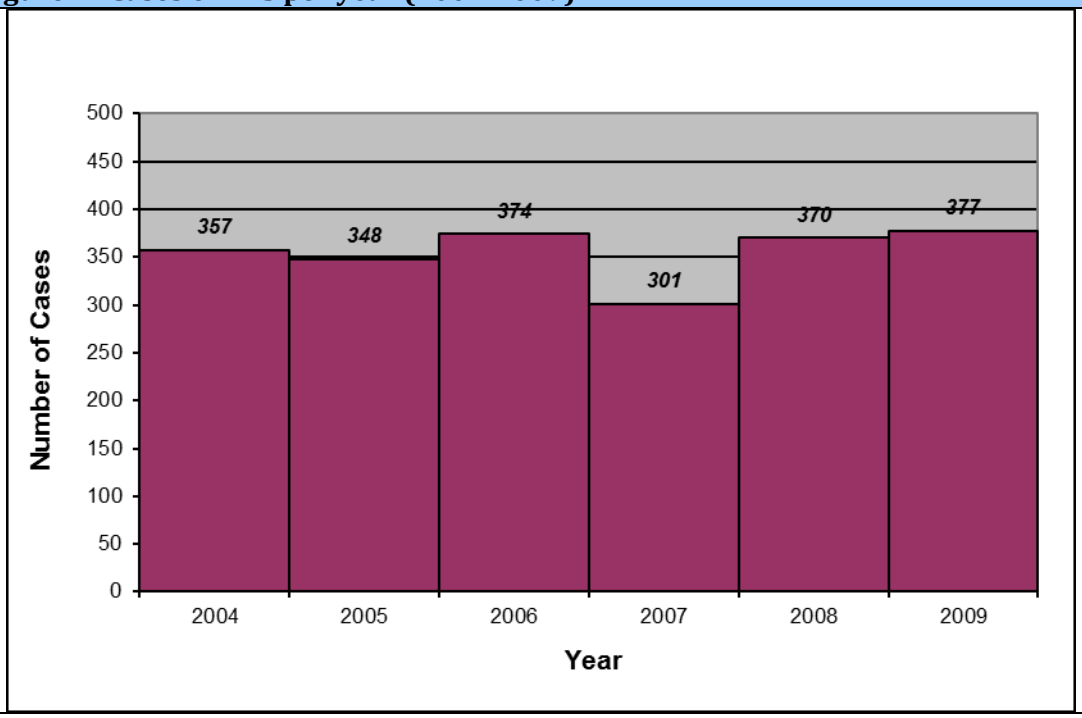
X.1 Oregon Salmonella Cohort Demographics 2004-2009

Between 2004 and 2009, there were 2278 confirmed cases of Salmonellosis among Oregon residents. A total of 23 of these cases were Typhoidal *Salmonella* isolates (*Salmonella* Typhi, Paratyphi A) and were thus excluded from analysis. Additionally, 102 (4.5%) of the NTS cases occurred among Oregon residents in other states or had their isolates submitted to other state laboratories and had no isolate forwarded to the OSPHL and/or didn't have antibiotic susceptibility information. Therefore, 2153 (95.5% of all NTS cases) cases were potentially eligible for enrollment in this study.. Finally, an additional 26 isolates (sputum, wound) were excluded on the basis of the site of isolation. Therefore 2127 (94.3% of all NTS cases) cases of NTS met the broad inclusion criteria for this study and were included in the cohort.

Temporal Trends

From 2004-2009, there were an average of 355 cases of NTS per year in Oregon. The median number of cases per year was 364 (364, 2004-2009). In 2007, only 301 NTS cases were confirmed whereas 2009 had the highest with 377 cases.

Figure 1. Cases of NTS per year (2002-2009)



During this time period 2520 (90%) cases had a defined month of onset. The month of August had the highest number of NTS cases while February had the fewest. In general it was observed that the late spring and summer months had the most cases while the winter months had the fewest.

Epidemiological Classification of Cases

The Oregon Public Health Division differentiates cases according to three epidemiological classifications using serotype, PFGE pattern, and temporal and spatial clustering of cases as the criteria. The most common case classification is "Sporadic" (isolated case with no temporal or spatial matching elsewhere throughout the state or PulseNet). The second case classification is "Outbreak" cases (part of a recognized cluster of at least two cases by serotype , PFGE, and

temporality). The third case classification is "Household" (a temporal and serotype cluster of at least two cases within the same household).

Between 2004 and 2009, there were a total of 109 (5.1%) laboratory confirmed household cases. During this time period 404 (19.0%) cases were linked to an outbreak cluster (166 were part of outbreaks where a causative vehicle was implicated). However, 1614 (75.9%) cases were sporadic infections that were not part of an identified outbreak cluster. (Table 2)

Table 2. Case classification and outbreak investigation success (2004-2009)

Case Type	N	Percent
Household	109	5.1
Outbreak Cluster	404	19.0
Sporadic Case	1614	75.9
Outbreak Demographic	**N**	**Percent**
Vehicle Implicated	166	41.1
Unsolved	238	58.9

Age

The median age was 29 and the IQR was 9-50 years of age. However, age was not normally distributed ($p<0.01$). A categorical variable for age was created called age group. The age categories were chosen to represent clinically meaningful age breakpoints and for literary comparison. 54.2% of cases were between 18 and 64 years of age, 6.5% were less than one year old, 11.6% were between 1 and 4 years of age, 15.8% of cases were between 5 and 17 years of age, and 11.9% of cases were 65 years of age or older at the time of infection.

Race and Ethnicity

From 2004 to 2009, 82.6% of the confirmed cases of NTS were Caucasian, 1.2% were Native American, 3.85% were Asian or Pacific Islander, 1.95% were African American, 1.3% were of other racial makeup, and 9.0% were of unknown racial makeup. Ethnically, 12.6% of cases were of Hispanic descent and 10.5% were of unknown ethnicity. These figures are congruent with Oregon census data.

Since 9.2% of the race data was unknown, 90.8% of the cases with a known racial demographic were white. Therefore, race in Oregon differed from the nationally reported figure of 77.1% in a previous national study. (Varma et al, 2005)

Sex

Between 2004 and 2009, 53.1% of the confirmed cases of NTS were female and 46.9% were male. These proportions are analogous to those figures reported in the Varms et. al. analysis (46.4% Male, 53.6% Female).

Hospitalization

During this time period 412 (19.4%) of confirmed NTS cases had illnesses that required hospitalization, while 20 (0.7%) of cases had unknown hospitalization status. This is slightly less than the national figure published by a NARMS/FoodNet which reported 25% hospitalization. (Varma et al, 2005)

Symptomatic Infection

Among confirmed cases of NTS between 2004 and 2009, 69% had symptomatic infections, 1.8% were asymptomatic, and 29.4% were unknown. Since this variable is one in which the not regularly entered, it was not included in the analysis.

Specimen Type

For the case definition, sputum and wound specimens were not included. Among the 2255 cases, 87.5% of the isolates came from stool samples, 7.5% were urine isolates, 4.6% were blood isolates, and 0.5% came from other sterile sites such as cerebral-spinal fluid (CSF).

This data was collapsed into a binary variable where isolates were classified as either non-invasive (stool and urine) or invasive (blood and sterile site). Therefore, 94.8% of the isolates between 2004 and 2009 were non-invasive whereas 5.2% were invasive.

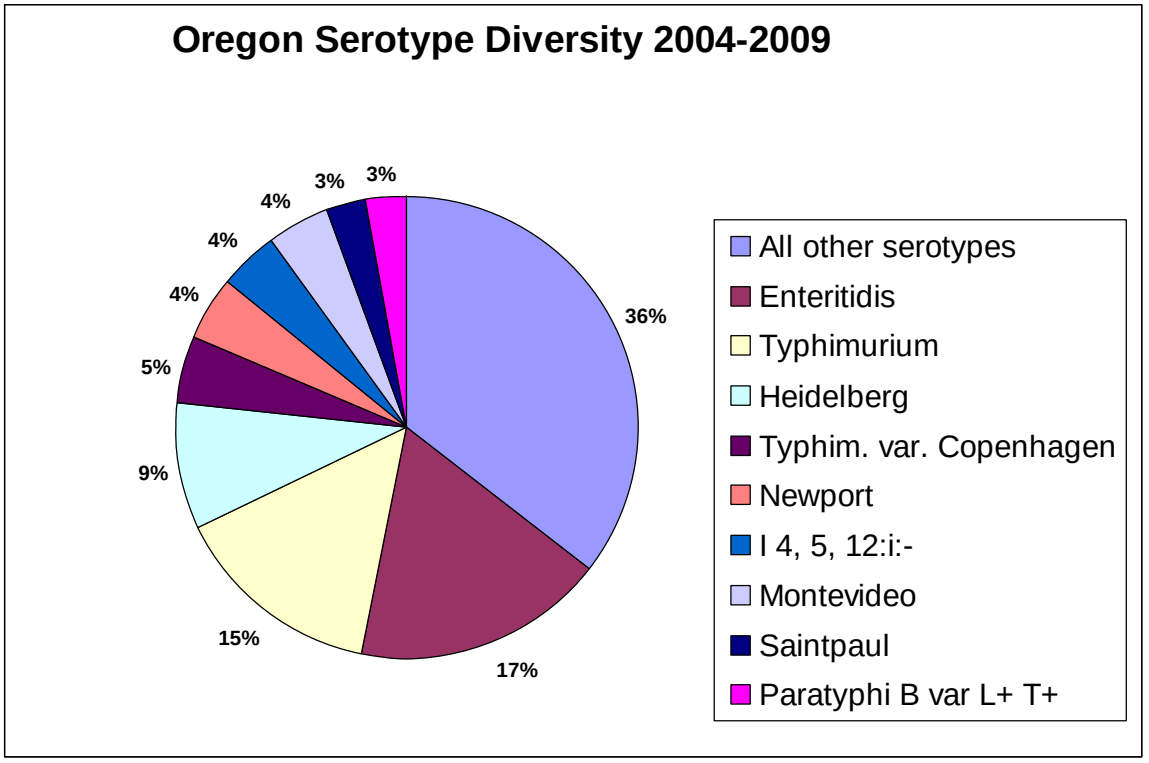
Serotype Diversity

Between 2004 and 2009 there were 197 different serotypes among the 2127 isolates of NTS. The most common serotype was *Salmonella* enterica serotype Enteriditis, followed by Typhimurium, Heidelberg, Typhimurium var Copenhagen, and Newport (Table 8). The top 10 most common serotypes represented 64.0% of the total isolates. During this time period serotypes such as Montevideo, Muenchen, and Staintpaul somewhat decreased whereas there was a dramatic increase in

Typhimurium var. Copenhaagen, and a less pronounced increase in serotype I 4, 5, 1 2:i:-.

Table 3. Most common serotypes from 2004-2009

Serotype	N	Percent
Salmonella Enteritidis	371	17.44
Salmonella Typhimurium	312	14.67
Salmonella Heidelberg	187	8.79
Salmonella Typhimurium var. Copenhaagen	106	4.98
Salmonella Newport	93	4.37
Salmonella I 4, 5, 12:i:-	90	4.23
Salmonella Montevideo	89	4.18
Salmonella Saintpaul	63	2.96
Salmonella Paratyphi B var L(+) tartrate +	58	2.73
Salmonella Muenchen	46	2.16



Antibiotic Susceptibility Frequencies

Between 2004 and 2009, 57.0% of the confirmed NTS isolates were pan-susceptible. This was close to a previously reported national figure of 63% (Varma

et. al.), although a slightly different panel of antibiotics were screened. The resistance profiles differed among the antibiotics that were screened. Tetracycline had the highest proportion of resistance, followed by sulfamethoxazole, nitrofurantoin, and ampicillin. The antibiotic with the smallest percent of resistance was ciprofloxacin followed by trimethoprim-sulfamethoxazole and gentamicin.

Salmonella serovar Typhimurium had the highest frequency of resistance for three antibiotics and was among the most resistant across the other 7 antibiotics tested. Typhimurium had the highest overall frequency of resistance among ampicillin, chloramphenicol, sulfamethoxazole, and tetracycline. The second most resistant serotype was Newport which had the highest frequency of resistance among cephalosporins and trimethoprim-sulfamethoxazole isolates and also was among highest frequency of resistance for chloramphenicol, sulfonamides, and tetracycline. Other serotypes with high frequencies of resistance were Typhimurium var. Copenhaagen, Enteriditis, and Heidelberg (Table 9).

Table 4. Percent of resistance to antibiotics

Antibiotic	Number Resistant	% Resistant
Ampicillin	285	13.40
Ceftrianxone	109	5.12
Chloramphenicol	177	8.32
Ciprofloxacin	13	0.61
Gentamicin	84	3.95
Naladixic Acid	135	6.35
Nitrofurantoin	283	13.31
Sulfamethoxazole	411	19.32
Tetracycline	574	26.99
Trimethoprim-Sulfamethoxazole	60	2.82

The resistance profile observed among the clinically important resistance (CIR) definition revealed that 347 isolates from 2004-2009 were resistant to at least one of these antibiotics.

Using the ACSSuT definition of resistance, there were 158 isolates resistant to ampicillin, chloramphenicol, sulfamethoxazole, and tetracycline between 2004 and 2009. (Table 10) For both of these definitions the serotype with the highest frequency of resistance was *Salmonella* serovar Typhimurium followed by Newport and Typhimurium var. Copenhagen. 100% of the isolates that met the ACSSuT definition also met the definition for CIR since they are all resistant to ampicillin. Therefore 45.5% of CIR isolates met the ACSSuT definition of resistance.

Some serotypes with high proportions of resistant isolates were; Albany (80% CIR, 67% ACSSuT), Concord (86% CIR, 86% ACSSuT), Hadar (75% CIR, 0% ACSSuT), Newport (53% CIR, 50% ACSSuT), and Typhimurium var Copenhaagen (62% CIR, 36% ACSSuT).

Table 5. 2004-2009 CIR and ACSSuT resistance frequencies.

	Resistant N (%)	Pan-susceptible N (%)
CIR	347 (16.3)	1213 (57.0)
ACSSuT	158 (7.4)	1213 (57.0)

**45.55% of CIR cases are also ACSSuT*

Risk Factor Information

Between 2004 and 2009, 85.2% (n=1813) of confirmed cases had exposure history. (Table 11)

Among the 15 risk factors that were collected in the exposure history, the prevalence of exposure ranged from 62.2% (restaurant food) to 0.9% (raw milk). (Table 12) The mean prevalence of exposure among these risk factors was 16.4% and the median prevalence of exposure was 13.4%. Only restaurant food, pets, and travel (domestic and international) had a prevalence of exposure greater that 20%. Only sprouts, occupational fecal exposure (daycare, nursing home), raw cheese, and raw milk had prevalence's of exposure below 10%.

Table 6. Frequency of exposure to risk factors for NTS infection (2004-2009), n=1802

Risks and Exposures	Number Exposed	Percent Exposed
Pet Exposure	666	36.73
Raw Eggs	316	17.43
Diaper Exposure	281	15.50
Reptile Exposure	266	14.67
International Travel	242	13.35
Raw Meat	235	12.96
People with Diarrhea	204	11.25
Livestock Exposure	197	10.87
Sprout Exposure	76	4.19
Occupational Fecal Exposure	52	2.87
Raw Cheese	31	1.71
Raw Milk	16	0.88

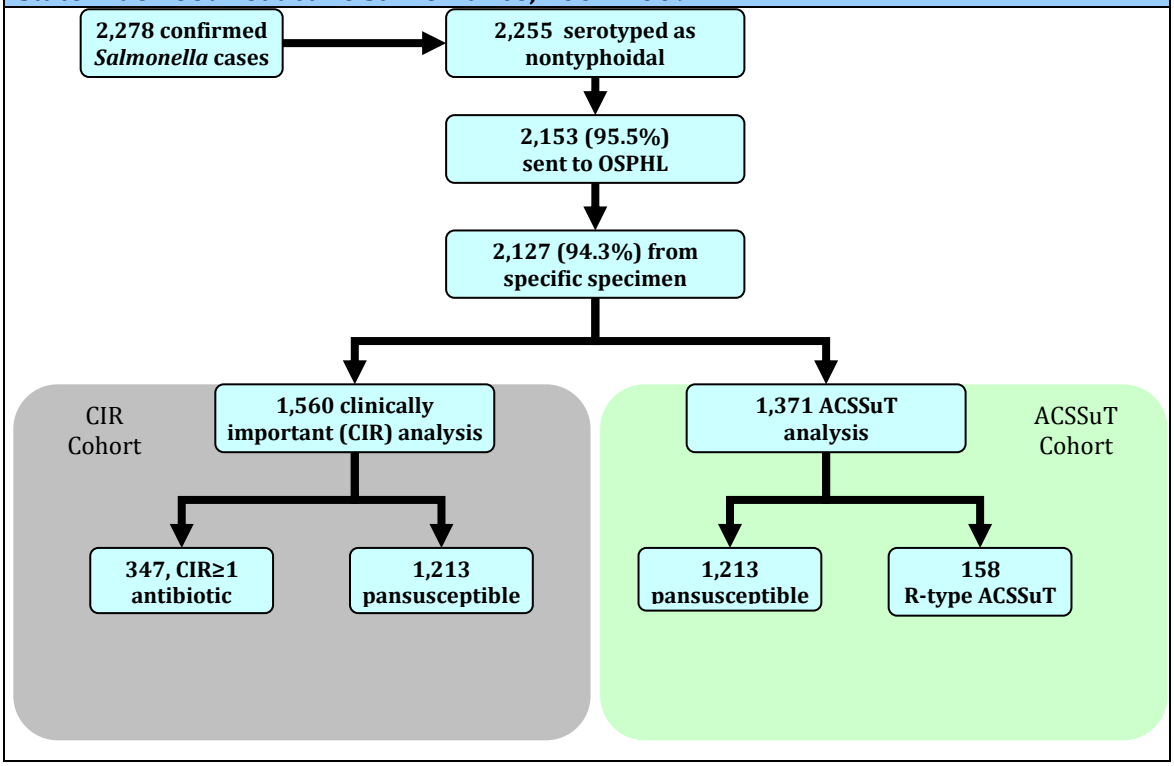
Since international travel was one of the variables of interest this variable was expanded by region of travel for 2004 to 2009. The most frequent destinations were Mexico, South East Asia, Europe, East Asia, and the Caribbean.

Table 7. International travel destinations 2004-2009, n=1802		
Destination	**N**	**Percent**
Mexico	117	48.35
Southeast Asia	27	11.16
Europe	22	9.09
East Asia	16	6.61
Caribbean	16	6.61
Africa	9	3.72
Central America	8	3.31
South America	7	2.89
Canada	5	2.07
Oceania	5	2.07
unknown	4	1.65
Russia	3	1.24
India	2	0.83
Middle East	1	0.41

X.2 Preliminary Analyses

For the years 2004-2009, there were 1560 cases that met the inclusion criteria for the clinically important resistance (CIR) analysis. Among these cases 22.2% met the case definition for CIR and the remaining 77.8% were pan-susceptible. Furthermore, 83.8% of cases in the CIR analysis were interviewed resulting in an N of 1307. (Figure 3) Similarly, there were 1371 cases that met the inclusion criteria for the R-type ACSSuT analysis. However, only 11.5% of these cases met this more stringent resistance definition (Table 8). For the ACSSuT analysis 83.3% of cases were interviewed resulting in an N of 1141.

Figure 2. Oregon patients with culture-confirmed Salmonella infection identified by statewide FoodNet active surveillance, 2004-2009.



In the *Salmonella* enterica serovar Typhimurium cohort, there were 236 and 217 cases that met the inclusion criteria for the CIR and ACSSuT analyses respectively. Within these cohorts respectively 30.9% met the CIR definition and 24.9% met the ACSSuT definition. (Table 8)

Table 8. Number of cases meeting inclusion criteria by analysis and percent meeting definition of resistance (2004-2009)

	Overall		Typhimurium Only	
	CIR	**ACSSuT**	**CIR**	**ACSSuT**
N (total)	1560	1371	236	217
Resistant (%)	347 (22.2)	158 (11.5)	73 (30.9)	54 (24.9)

Resistance in Oregon NARMS isolates 2004-2009 (Hypothesis 1)

Since the Oregon Public Health Division is a participant in the National Antimicrobial Resistance Monitoring System (NARMS), a preliminary analysis was performed to ascertain whether the isolates forwarded on to the CDC between 2004 and 2009, were representative of the resistance profile (CIR, ACSSuT) seen in Oregon during this time period. Between 2004 and 2009, Oregon forwarded 108 isolates to the CDC that met the inclusion criteria for the CIR analysis and 94 that met the inclusion criteria for ACSSuT. The resistance profile (i.e. proportion of CIR, ACSSuT) among isolates sent to the CDC did not significantly differ from the total population of Oregon isolates during this time period (Likelihood Ratio p-value 0.81 for CIR and 0.53 for ACSSuT). (Table 9)

This univariate analysis was expanded to further examine whether the NARMS isolates forwarded to the CDC came from individuals who were representative of the sex, age, race, and ethnicity of the Oregon cases of NTS from 2004-2009. These findings suggest that the Oregon NARMS isolates were sufficiently representative among these demographics, with no p-values approaching the 0.05 level of significance. (Table 9)

Finally, an analysis was performed to see whether the NARMS isolates were representative of the predominant serotype diversity within Oregon over this time period. For this analysis the top 19 most common serotypes (in order of frequency) were included and all other serotypes were grouped as "other." These data indicate that the serotype diversity among the isolates sent to the CDC did not significantly

differ from the overall serotype profile in Oregon over this time period (p-value=0.33). (Table 9)

Therefore, this analysis suggests that the NARMS methodology of forwarding every 20th sample to the CDC, provided a representative sample of the Oregon resistance, serotype diversity, and case demographic profile during that same time period.

Table 9. NARMS analysis of resistance profile, demographic information, and serotype diversity (n=2127), 2004-2009.

Variable	CIR	ACSSuT	Sex	Age	Serotype	Race	Hispanic
Likelihood Ratio Chi-Square p value	0.90	0.62	0.92	0.66	0.33	0.52	0.18

Increasing resistance 2004-2009 (Hypothesis 2)

The Cochran-Armitage test for trend was used to test the hypothesis that antibiotic resistance has significantly increased between 2004 and 2009 compared to pan-susceptible isolates. There were significant increases in resistance to ciprofloxacin, naladixic acid, sulfamethoxazole, SXT and tetracycline. (Table 10) There was also a marginally significant increase in ceftriaxone (p=0.06) and a significant decrease in nitrofurantoin resistance. Upon stratification for outbreak status the results demonstrated that there were significant increases in naladixic acid, sulfamethoxazole, and tetracycline resistance for sporadic cases. There was also a significant decrease in nitrofurantoin resistance for sporadic cases. (Table 10)

In contrast there were significant increases in ceftriaxone, gentamicin, naladixic acid, SXT, and tetracycline resistance with a marginally significant increase in ciprofloxacin resistance (p=0.06) among outbreak cases.

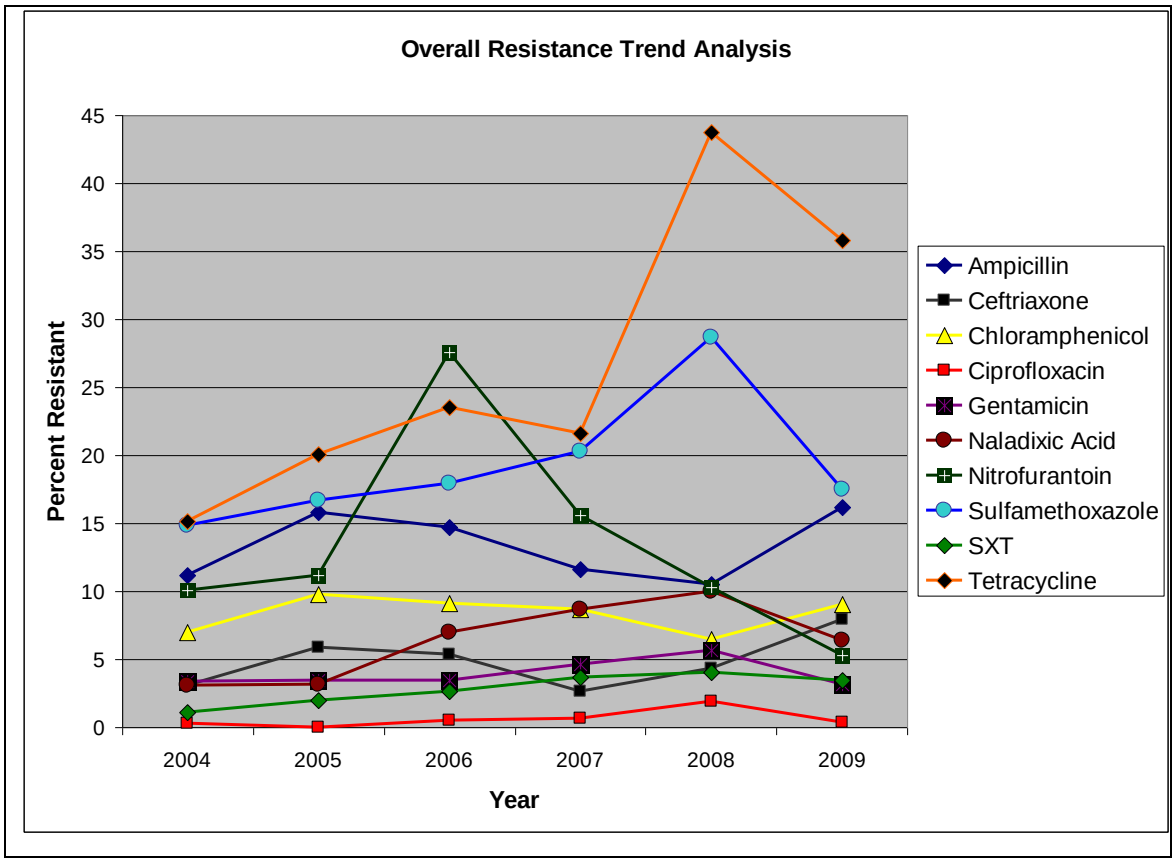
Table 10. Cochran-Armitage Test for Trend of increasing resistance between 2004-2009 by antibiotic and stratification by case status (n=2127)

		Case Status	
Antibiotic	**Overall**	**Sporadic**	**Outbreak**
Ampicillin	0.33	0.49	0.22
Chloramphenicol	0.49	0.42	0.23
Ceftriaxone[†]	0.06	0.41	<0.01*
Ciprofloxacin	0.05*	0.22	0.06
Gentamicin	0.22	0.42	0.03*
Naladixic Acid	<0.01*	<0.01*	0.03*
Nitrofurantoin	(<0.01)	(<0.01)	0.20
Sulfamethoxazole	<0.01*	<0.01*	0.08
SXT	<0.01*	0.03*	0.04*
Tetracycline	<0.01*	<0.01*	<0.01*

** Indicates a significant increase in resistance*

( ) indicate a significant decrease in resistance

[†] *Ceftriaxone resistance was approximated by cephalothin and cefuroxime for 2004-2005*



Upon stratification by serotype, significant increases in ampicillin resistance occurred among the top 5 most common serotypes except the "All Other" group. For chloramphenicol there was only a significant increase in *S.* Typhimurium. Ceftriaxone resistance significantly increased among *S.* Heidelberg and significantly decreased among *S.* Newport. There was no significant increase in gentamicin resistance. However, a significant decrease was found among *S.* Typhimurium isolates. There were significant increases in naladixic acid resistance among *S.* Enteriditis and *S.* Typhimurium Copenhaagen. (Table 11)

Significant increases in sulfamethoxazole resistance were found for *S.* Enteriditis and all other serotypes, while a significant decrease was found in *S.*

Typhimurium var Copenhagen. Significant increases in SXT were found in *S.* Enteriditis, *S.* Typhimurium var Copenhagen, and the All Other serotype group. Finally, tetracycline resistance increased in all serotypes but *S.* Newport between 2004 and 2009.

Table 11. Cochran-Armitage Test for Trend of increasing resistance between 2004-2009 by antibiotic stratified by the 5 most common serotypes (n=2127)

	Stratified by Serotype					
Antibiotic	**Enteriditis**	**Typhimurium**	**Heidelberg**	**Typhimurium var Copenhagen**	**Newport**	**All Other**
Ampicillin	0.01	0.01	<0.01	0.02	0.05	0.43
Chloramphenicol	0.16	0.01	0.37	0.14	0.16	0.24
Ceftriaxone	0.06	0.07	<0.01	0.34	(0.04)	0.22
Ciprofloxacin	0.03	0.31	n/a	n/a	n/a	0.30
Gentamicin	0.28	(0.05)	0.21	0.32	0.18	0.17
Naladixic Acid	<0.01	0.08	0.23	0.01	n/a	0.49
Nitrofurantoin	(0.06)	0.26	0.35	(0.06)	0.15	(0.08)
Sulfamethoxazole	<0.01	0.07	0.14	(<0.01)	0.26	<0.01
SXT	<0.01	0.17	0.41	0.03	0.24	0.01
Tetracycline	<0.01	<0.01	<0.01	0.02	0.5	<0.01

For the primary outcome definitions of resistance (CIR, ACSSuT), the one-sided p-value's were statically significant at the alpha level of 0.05. Therefore, resistance among NTS isolates increased between 2004 and 2009 compared to pan-susceptible isolates. (Table 12) Upon stratification by serotype, significant increases in CIR resistance were noted in *S.* Enteriditis, *S.* Typhimurium, *S.* Heidelberg, and the All Other serotype group. In contrast resistance only increased in *S.* Typhimurium for the ACSSuT analysis. Interestingly, there was a marginally significant decrease in resistance among *S.* Typhimurium var Copenhagen.

Upon stratification by case status we found significant increases in both CIR and ACSSuT for sporadic cases. In contrast there was only a significant increase in resistance to CIR among outbreak cases.

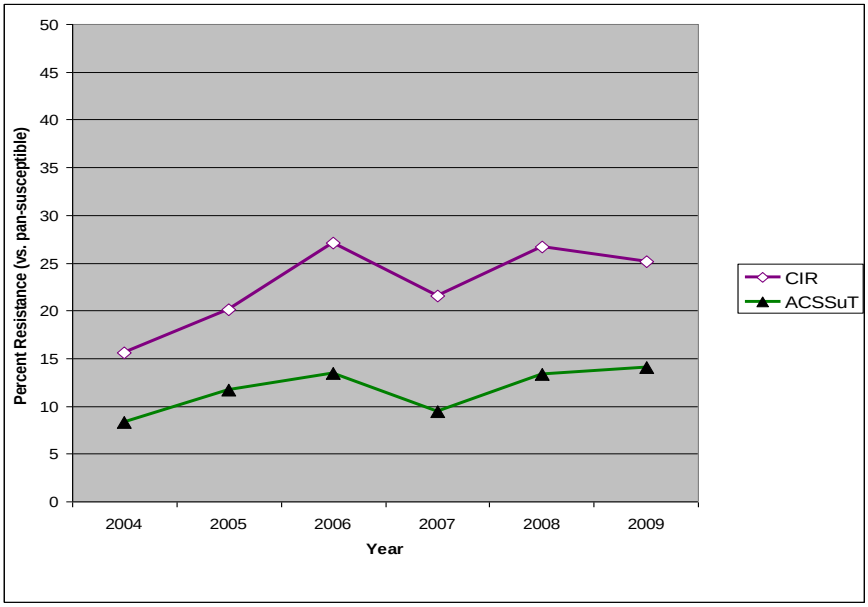
Table 12. Cochran-Armitage Test for Trend of increasing resistance (clinically important [CIR], and R-type ACSSuT) 2004-2009

	CIR[α]	ACSSuT[†]
N	1560	1371
Overall	<0.01*	0.04*
Stratified by Serotype		
S. Enteriditis	0.01*	0.12
S. Typhimurium	0.01*	<0.01*
S. Heidelberg	<0.01*	0.34
S. Typhimurium var Copenhagen	-0.06	-0.10
S. Newport	0.20	0.29
All Other Serotypes	0.05*	0.30
Stratified by Case Status		
Sporadic Cases	0.04*	0.05*
Outbreak Cases	<0.01*	0.48

** Indicates a significant increase in resistance*

[α]CIR resistance included resistance to ampicillin, ceftriaxone, ciprofloxacin, gentamicin, and SXT

[†] ACSSuT resistance was approximated by resistance to ampicillin, chloramphenicol, sulfamethoxazole, and tetracycline



Resistance and Hospitalization (Hypothesis 3)

In order to assess whether the previously reported associations (Varma, 2005) between resistant infections and severe illness (hospitalization, invasive infection) held true in Oregon between 2004 and 2009, Fisher's exact Chi-square tests were performed and odds ratios were calculated.

There was a significant difference between pan-susceptible and resistant isolates with regard to hospitalization ($p<0.01$ for CIR and $p=0.02$ for ACSSuT). For CIR, the odds of being hospitalized with a resistant infection were 50% higher than patients with pan-susceptible isolates. Similarly, cases with ACSSuT isolates were 57% more likely to be hospitalized with a resistant isolate, compared to those cases with pan-susceptible isolates (Table 13). However, this association did not hold true for the *Salmonella* Typhimurium only analysis ($p=0.71$ for CIR and $p=1.00$ for ACSSuT).

Table 13. Chi-square tests and Odds Ratios for hospitalization with a resistant NTS isolate among cases in Oregon 2004-2009

	CIR (n=1560)		**ACSSuT** (n=1371)	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
M-H Chi-Square	1.50 (1.13-1.99)	<0.01*	1.57 (1.07-2.31)	0.02*
Serotype Adjusted	1.60 (1.18-2.17)	<0.01*	1.98 (1.30-3.02)	<0.01*
Age Adjusted	1.65 (1.23-2.21)	<0.01*	1.79 (1.20-2.68)	<0.01*
Typhimurium Only^	1.20 (0.58-2.44)	0.71	1.00 (0.44-2.30)	1

** Indicates significance*

^Sub-analysis data n=236 for CIR and n=217 for ACSSuT

Between 2004 and 2009 there was no significant association between resistance and invasive illness for all isolates ($p=0.19$ for CIR and 0.15 for ACSSuT). This finding also held true for the Typhimurium only analysis ($p=1.00$ for CIR and

0.69 for ACSSuT). (Table 14) However, these findings may be underpowered and also appears to be confounded by serotype and age, so a multivariate analysis adjusting for these variables may elucidate an association.

Table 14. Fisher's exact Chi-square tests for differences between having a resistant isolate that was invasive among Oregon cases with NTS 2004-2009

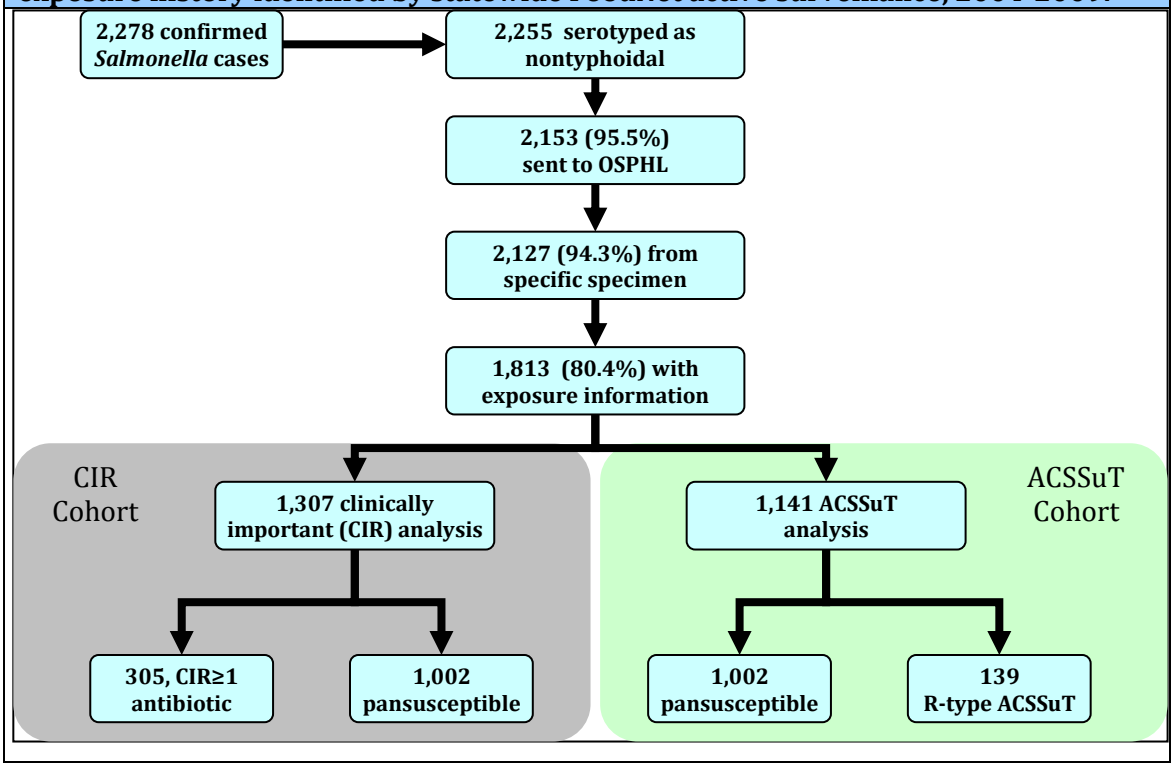
	CIR	ACSSuT
M-H Chi Square	0.19	0.15
Serotype Adjusted	0.15	0.02
Age Adjusted	0.14	0.09
Typhimurium Only	1.00	0.69

X.3 Univariate Analysis

Population for Analysis

The previous analyses utilized a cohort of 2,127 cases. For multivariate modeling 1,813 of those cases (80.4% of all NTS cases) had risk factor and exposure information. Among this subset population, 55.3% of cases were pan-susceptible, 16.8% met the CIR case definition, and 7.7% of cases met the ACSSuT case definition.

Figure 3. Oregon patients with culture-confirmed Salmonella infections with case exposure history identified by statewide FoodNet active surveillance, 2004-2009.



Serotypes

Using *Salmonella* serovar Enteritidis as the referent type (since it was the most common serotype between 2004 and 2009), 13 other serotypes in order of descending frequency, were analyzed for significant differences in resistance. All other serotypes were grouped into the "other" category and were also included. For CIR, serotypes Typhimurium, Heidelberg, Typhimurium var Copenhaagen, Newport, and Paratyphi B var L+ Tartrate significantly differed in from the resistance profile seen in Enteritidis isolates and all had increased odds of resistance. Typhimurium var Copenhagen had an OR of 11.41 followed by Newport (OR 6.67), Heidelberg (OR 3.97), and Typhimurium (OR 3.73). (Table 15)

For the ACSSuT analysis, Typhimurium, Typhimurium var Copenhagen, Newport, Paratyphi B var L+ Tartrate+, and the "Other" serotypes were significantly different from Enteritidis. All of these serotypes had increased odds of resistance relative to Enteritidis with Newport being the highest (OR 25.49), followed by Typhimurium var Copenhaagen (OR 20.95), Typhimurium (OR 14.61), and Paratyphi B var L+ Tartrate+ (OR 12.42). (Table 15) Therefore, for both analyses serotype was a risk factor that was included in the multivariate logistic regression analysis.

Case Type

Outcomes of resistance (CIR, ACSSuT) were analyzed with regard to the type of case they represented, using sporadic cases as the referent. For both CIR and ACSSuT, outbreak cases had significantly reduced odds of resistance (OR 0.52 for CIR and OR 0.39 for ACSSuT) compared to sporadic cases. Household clusters also had protective point estimates (OR 0.71 for CIR and 0.46 for ACSSuT) although the p-values did not reach the 0.05 level of significance likely due to low sample size. (Table 15)

During 2004-2009 there were 88 outbreaks representing 404 total cases. The mean number of cases per outbreak was 4.7 and the median was 3.0. The minimum number of cases that were included in the eligible study population was 1 and the maximum was 24. Cases that were designated as outbreak cases with only one Oregon case, were listed as outbreak cases because they were epidemiologically

linked to national/regional outbreaks via FoodNet and/or PulseNet. Within individual outbreaks, 42.0% of the isolates differed in their resistance profile by at least one antibiotic. When outbreaks with only one isolate were excluded, 53.6% of the isolates differed in their resistance profile by at least one antimicrobial.

In order to assess whether the protective association between case status could be confounded by interview, a univariate analysis was assessed. (Appendix E) The results indicated that interviewed cases were more likely to have a resistant isolate which would actually confound the association toward the null. Therefore, the protective association between resistance (CIR and ACSSuT) and case status was an underestimate, and could not be explained by interview status.

Secondly, to assess whether over-representation/over sampling of outbreak isolates (ie. Many cases with the same isolate) biased the study results, we restricted the data to include only one case from each vehicle identified outbreak in the analysis (From N=206 to N=130 for outbreak level). The resultant odds ratio was still significantly protective (OR 0.60 p=0.01). Performing this same analysis including cases that were not interviewed increased the significance of this association (OR 0.57 p<0.01).

Thirdly, a more restrictive method was used to assess whether over-sampling biased the association between outbreak cases by randomly selecting one outbreak case for each outbreak. This resulted in an average of 8.46 CIR cases (rounded up to 9) and 56.54 pan-susceptible cases (rounded down to 56). The resultant odds ratio was 0.47 (p=0.04). Finally, performing the same analysis with

the inclusion of all cases including those who were not interviewed resulted in an average of 10 CIR cases and 62 pan-susceptible cases. The resultant odds ratio was 0.49 (p=0.04).

These results demonstrate that there was high heterogeneity of resistance among outbreak cases, the association between resistance and outbreak cases was negatively confounded by interview status, and restriction to assess oversampling preserved the association. Therefore, case type was an important predictor/confounder of resistance that was included in the multivariate logistic regression analyses with all interviewed cases included in the analysis and collapsed together with household outbreak clusters due to the similar direction and magnitude of the association.

Age, Sex, Race, and Ethnicity

For the overall CIR analysis, age, sex, race, and Hispanic ethnicity were not significantly associated with resistance. However sex, and Hispanic ethnicity had p-values below 0.25 and were considered as potential confounders for multivariate analysis. For the overall ACSSuT analysis age, sex and Hispanic ethnicity were not significantly associated with resistance. However, non-white race was significant (p-value 0.02, OR 1.96). (Table 15) Only Hispanic ethnicity met the criteria for inclusion in the multivariate analysis as a potential confounder.

Table 15. Demographic analysis of the frequency of Salmonella isolates meeting two resistance definitions, among Oregon cases with NTS infection between 2004 and 2009

	CIR			ACSSuT		
Variable		**OR (95% CI)**	**p value**		**OR (95% CI)**	**p value**
***Salmonella* serotype**						
Enteritidis		Referent			Referent	
Typhimurium	3.73	2.14-6.48	<0.01	14.61	5.14-41.53	<0.01
Heidelberg	3.97	2.17-7.24	<0.01	1.96	0.48-8.05	0.35
Typhimurium var Copenhagen	11.41	5.98-21.75	<0.01	20.95	6.64-66.09	<0.01
Newport	6.67	3.46-12.86	<0.01	25.49	8.39-77.44	<0.01
I 4, 5, 12:i:-	2.03	0.97-4.25	0.06	1.36	0.24-7.61	0.73
Montevideo	0.41	0.12-1.42	0.16	n/a	n/a	n/a
Saintpaul	2.38	0.95-6.00	0.07	n/a	n/a	n/a
Paratyphi B var L+ Tartrate+	2.98	1.32-6.74	<0.01	12.42	3.65-42.23	<0.01
Muenchen	0.21	0.03-1.64	0.14	1.06	0.12-9.82	0.96
Braenderup	0.78	0.17-3.62	0.76	n/a	n/a	n/a
Agona	1.42	0.44-4.56	0.56	3.55	0.61-20.58	0.16
Thompson	0.28	0.04-2.14	0.22	n/a	n/a	n/a
Infantis	1.12	0.31-4.10	0.87	n/a	n/a	n/a
Other	1.21	0.69-2.11	0.51	2.7	0.91-8.00	0.07
Case Type						
Sporadic		Referent			Referent	
Outbreak Cluster	0.52	0.35-0.78	<0.01	0.39	0.20-0.73	<0.01
Household Cluster	0.71	0.38-1.33	0.28	0.46	0.16-1.30	0.14
Age Groups						
18 to 64 years		Referent			Referent	
<1 year	1.11	0.67-1.83	0.69	0.99	0.49-2.00	0.97
1 to 4 years	0.95	0.63-1.43	0.80	0.76	0.42-1.38	0.36
5 to 17 years	1.18	0.83-1.68	0.35	1.04	0.64-1.70	0.87
65+ years	0.83	0.54-1.29	0.40	0.74	0.40-1.37	0.33
Sex						
Female		Referent			Referent	
Male	1.21	0.93-1.56	0.15	1.05	0.74-1.50	0.77
Race						
White		Referent			Referent	
Nonwhite	1.21	0.77-1.91	0.40	1.86	1.09-3.19	0.02
Unknown	0.98	0.57-1.66	0.93	0.70	0.30-1.65	0.41
Hispanic						
Non-Hispanic		Referent			Referent	
Hispanic	1.18	0.78-1.76	0.43	0.79	0.42-1.48	0.46
Unknown	0.72	0.42-1.22	0.22	0.50	0.21-1.1.16	0.11

Typhimurium Only: Case Type

In the Typhimurium only analysis, similar associations were observed with outbreak cases having significantly reduced odds of resistance (OR 0.30 for CIR, and 0.25 for ACSSuT). Again, household had similar magnitude and direction as outbreak cases, yet failed to reach the 0.05 level of significance (p=0.1 for CIR, and p=0.21 for ACSSuT). (Table 16)

Typhimurium Only: Age, Sex, Race, and Ethnicity

In the Typhimurium only analysis 1-5 years of age was significantly protective for both definitions of resistance (OR 0.31 for CIR, 0.27 for ACSSuT). Sex, race, and Hispanic ethnicity did not meet the 0.2 threshold level of significance for inclusion in the model but may merit further consideration as confounders.

Table 16. Demographic analysis of the frequency of *Salmonella* serovar Typhimurium isolates meeting two resistance definitions, among Oregon cases between 2004 and 2009

		CIR			ACSSuT	
Variable	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**
Case Type						
Sporadic		Referent			Referent	
Outbreak Cluster	0.30	0.13-0.68	<0.01	0.25	0.09-0.67	0.01
Household Cluster	0.28	0.06-1.29	0.10	0.38	0.08-1.75	0.21
Age Groups						
18 to 64 years		Referent			Referent	
<1 year	1.13	0.34-3.71	0.84	0.87	0.22-3.53	0.85
1 to <5 years	0.31	0.12-0.81	0.02	0.27	0.09-0.82	0.02
5 to <18 years	0.98	0.50-1.93	0.94	0.94	0.45-1.98	0.87
65+ years	0.60	0.22-1.65	0.32	0.52	0.16-1.66	0.27
Sex						
Female		Referent			Referent	
Male	0.90	0.52-1.57	0.72	0.90	0.48-1.66	0.73
Race						
White		Referent			Referent	
Nonwhite	0.92	0.28-3.04	0.89	1.23	0.37-4.09	0.74
Unknown	1.91	0.56-6.50	0.30	1.02	0.20-5.23	0.98
Hispanic						
Non-Hispanic		Referent			Referent	
Hispanic	0.92	0.40-2.12	0.85	0.66	0.24-1.84	0.42
Unknown	1.41	0.44-4.49	0.56	1.08	0.28-4.26	0.91

Risk Factors/Exposures

For the risk factor/exposure analysis on CIR, only international travel was significantly associated with resistance (OR 1.51). However, diaper changing, raw meat exposure, sprout exposure, and reptile exposure met the 0.25 threshold for inclusion in the multivariate analysis. For ACSSuT resistance, raw meat exposure was the only risk factor that reached significance (OR 1.92). However, pet exposure met the 0.25 threshold for inclusion in the multivariate analysis. (Table 17)

Table 17. Risk factor univariate analysis of the frequency of *Salmonella* isolates meeting two resistance definitions, among Oregon cases with NTS infections between 2004 and 2009

	CIR			ACSSuT		
Variable	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**P value**
Diaper Exposure						
No		Referent			Referent	
Yes	1.26	0.91-1.76	0.17	1.21	0.77-1.92	0.42
Food at Events						
No		Referent			Referent	
Yes	0.89	0.64-1.24	0.49	0.93	0.58-1.47	0.74
International Travel						
No		Referent			Referent	
Yes	1.57	1.07-2.31	0.03	1.33	0.77-2.31	0.32
Livestock Exposure						
No		Referent			Referent	
Yes	1.00	0.68-1.49	0.99	1.12	0.66-1.90	0.69
Occupational Fecal Exposure						
No		Referent			Referent	
Yes	0.63	0.26-1.52	0.28	0.46	0.11-1.93	0.23
People with Diarrhea						
No		Referent			Referent	
Yes	1.04	0.70-1.54	0.86	0.98	0.56-1.70	0.93
Pet Exposure						
No		Referent			Referent	
Yes	1.08	0.83-1.40	0.59	1.30	0.91-1.86	0.16
Raw Cheese Exposure						
No		Referent			Referent	
Yes	1.45	0.59-3.56	0.43	0.90	0.20-3.96	0.89
Raw Egg Exposure						
No		Referent			Referent	
Yes	0.93	0.66-1.31	0.68	0.87	0.54-1.41	0.57
Raw Meat Exposure						
No		Referent			Referent	

Yes	1.28	0.89-1.85	0.19	2.07	1.33-3.22	<0.01
Raw Milk Exposure						
No		Referent			Referent	
Yes	0.47	0.06-3.82	0.44	1.03	0.13-8.45	0.98
Reptile Exposure						
No		Referent			Referent	
Yes	0.76	0.52-1.11	0.15	1.06	0.66-1.71	0.81
Sprout Exposure						
No		Referent			Referent	
Yes	0.63	0.29-1.36	0.22	0.70	0.25-1.97	0.47

A stratified bi-variate analysis of cases by region of travel revealed that cases who travelled to East Asia and Southeast Asia acquired a CIR isolate more frequently than cases without international travel. (Table 18) In the ACSSuT analysis cases that travelled to Southeast Asia and Canada were significantly more likely to have acquired an isolate that was resistant, although the confidence interval for Canada was quite wide due to extremely small sample size.

Table 18. International travel univariate analysis of the frequency of NTS isolates meeting two resistance definitions, among Oregon cases between 2004 and 2009

			CIR			ACSSuT	
Variable	**No. Cases**	**No. CIR (%)**	**OR**	**95% CI**	**No. Cases**	**OR**	**95% CI**
Travel Destination							
None	1172	264 (22.5)	Referent		1031	Referent	
Mexico	55	11 (20.0)	0.86	0.44-1.69	50	1.01	0.42-2.41
South Asia	21	12 (57.1)	**4.59**	**1.91-11.00**	13	**3.28**	**1.00-10.81**
Europe	13	4 (30.8)	1.53	0.47-5.00	10	0.82	0.10-6.53
East Asia	11	6 (54.5)	**4.13**	**1.25-13.63**	5	n/a	n/a
Carribean	10	1 (10.0)	0.38	0.05-3.03	9	n/a	n/a
Latin America	8	2 (25.0)	1.15	0.23-5.71	7	1.23	0.15-10.31
Africa	8	2 (25.0)	1.15	0.23-5.71	8	2.46	0.49-12.33
Oceania	5	1 (20.0)	0.86	0.10-7.73	4	n/a	n/a
Canada	4	2 (50.0)	3.44	0.48-24.53	4	**7.38**	**1.03-52.88**

Typhimurium Only Risk factors/Exposures

For the Typhimurium only analysis, international travel (OR 10.91 for CIR, OR 11.46 for ACSSuT) and Reptile exposure (OR 2.29 for CIR, OR 2.95 for ACSSuT) were significant. (Table 19) Raw milk was marginally significant in ACSSuT and significant in CIR, although the cell counts were too low to calculate point estimates and therefore would likely not be included in the multivariate analysis. For CIR pet exposure was marginally significant (OR 0.57). Raw meat exposure met the 0.2 threshold for inclusion in the multivariate modeling.

Table 19. Risk factor univariate analysis of the frequency of *Salmonella* serovar Typhimurium isolates meeting two resistance definitions, among Oregon cases between 2004 and 2009

		CIR			ACSSuT	
Variable	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**
Diaper Exposure						
No		Referent			Referent	
Yes	0.81	0.36-1.79	0.59	1.02	0.44-2.36	0.96
Food at Events						
No		Referent			Referent	
Yes	0.86	0.42-1.73	0.66	1.04	0.49-2.20	0.93
International Travel						
No		Referent			Referent	
Yes	10.91	2.99-39.76	<0.01	11.46	3.01-43.62	<0.01
Livestock Exposure						
No		Referent			Referent	
Yes	0.83	0.34-1.98	0.67	1.15	0.47-2.81	0.76
Occupational Fecal Exposure						
No		Referent			Referent	
Yes	1.04	0.19-5.85	0.96	1.43	0.25-8.04	0.69
People with Diarrhea						
No		Referent			Referent	
Yes	0.79	0.31-1.99	0.61	0.60	0.19-1.85	0.35
Pet Exposure						
No		Referent			Referent	
Yes	0.57	0.32-1.05	0.07	0.68	0.35-1.31	0.25
Raw Cheese						
No		Referent			Referent	
Yes	1.38	0.23-8.47	0.73	1.89	0.31-11.66	0.50

Raw Eggs						
No		Referent			Referent	
Yes	1.40	0.67-2.94	0.38	0.88	0.35-2.19	0.77
Raw Meat						
No		Referent			Referent	
Yes	1.72	0.85-3.48	0.14	1.80	0.83-3.89	0.14
Raw Milk						
No		Referent			Referent	
Yes	N/A	N/A	0.03*	N/A	N/A	0.07*
Reptile Exposure						
No		Referent			Referent	
Yes	2.29	0.94-5.59	0.07	2.95	1.17-7.44	0.02
Sprout Exposure						
No		Referent			Referent	
Yes	0.92	0.27-3.11	0.90	1.28	0.38-4.34	0.70

X.4 Multivariate Analysis

The case status variable level for household outbreak clusters had too low of cell counts for model building. However, this level had similar magnitude and direction to the outbreak level and did not significantly differ for predicting resistance for either CIR, or ACSSuT (Wald test Chi square p-value 0.66 for CIR, 0.99 for ACSSuT). Therefore for all of the multivariate analyses the outbreak and household levels of this variable were collapsed together and the resultant variable was a binary categorical variable with sporadic cases remaining the referent.

Clinically important resistance (CIR) multiple logistic regression modeling

To expand upon the bi-variate analysis between the binary dependent variable of clinically important resistance (CIR, 0=pan-susceptible, 1=resistant) those independent variables that were associated at the Chi square alpha level of 0.25 were assessed for inclusion in a multivariate logistic regression model. This

was assessed by analyzing these variables individually stratified by another covariate to assess potential confounding and interaction. For this analysis there were 1307 cases that were included in the dataset, 305 of these cases were resistant (CIR).

International travel was broken into three categories based on sample sizes and associations by travel destination. The three levels included Mexico, Asia (east Asia and southeast Asia combined), and all other destinations were grouped into the "all other" category

The serotype variable was collapsed to include the 9 most common serotypes numbered in descending frequency between 2004 and 2009 in Oregon, and all the other remaining serotypes grouped together. The categorical variables diaper changing (0=no, 1=yes), reptile exposure (0=no, 1=yes), case status (0=sporadic case, 1=outbreak cluster case), sex (0=female, 1=male), serotype (0=Enteritidis, 1=Typhimurium, 2=Heidelberg, 3=Typhimurium var Copenhaagen, 4=Newport, 5=I 4, 5, 12:i:-, 6=Montevideo, 7= Saintpaul, 8=Paratyphi B var L+ Tartrate+, 9=All Other), raw meat exposure (0=no, 1=yes), sprout exposure (0=no, 1=yes), and international travel (0=no, 1=Mexico, 2=Asia, 3=All Other) met the inclusion criteria for this model. The following variables suspected of confounding were also included; year (ordinal), age (0=18-64 years, 1=<1 years, 2=1-4 years, 3=5-17 years, 4=65+ years), race (0=white, 1=nonwhite, 2=unknown), and Hispanic ethnicity (0=Non-Hispanic, 1=Hispanic, 2=unknown).

Serotype was the only significant confounder (OR change>=10%) across all predictors and potential interactions were found between serotype and international travel (Breslow-Day p-value<0.10). After adjusting for serotype and case status, diaper changing, reptile exposure, sprout exposure, raw meat exposure, sex, and ethnicity lost all significance and were removed from the model.

The preliminary main effects model included case status, travel to asia, year of infection, and serotype. Due to highly suspected biological relevance and for external generalizability of the final model, age and non-white race were included (fixed) in the model building process. Therefore, the resultant preliminary main effects model included; case status, international travel, serotype, age, year of infection, and non-white race This model was checked for co-linearity via the variance inflation factor (VIF) and no variables were found to be collinear with the highest value of the VIF being 2.48 for the "all other" serotype level. (Appendix A)

Finally, an interaction was hypothesized between serotypes and international travel destinations since previous studies have reported increased resistance associated with serotypes acquired internationally (Hendriksen 2012, Hendriksen 2009, Threlfall 2000). Taking care not to "over-fit" the model by exceeding the m/10 rule of thumb (Harrell, 1984), interaction terms were entered into the model one at a time. No significant interactions (p<0.05) were found.

This final model was checked and confirmed against both forward and backward stepwise selection procedures holding age and race constant in each model. Therefore the final main effects model had 17 degrees of freedom and

included; case status, age, race (non-white vs. white), serotype, year, and travel to asia. (Appendix B)

Using the Hosmer and Lemeshow method to assess goodness of fit , it was shown that this model had excellent fit for this data (p=0.71). (Table 20) When assessing the discriminative ability of the main effects model, an ROC curve was generated and the area under the curve was found to be 0.76, indicating that this model had acceptable discriminative ability.

Table 20. CIR model fit statistics and ROC curve (N=1307)

H-L Goodness of Fit	Pearson	Deviance	ROC
0.71	<0.01	<0.01	0.76

Residual analysis by graphing the leverages, the Pearson's residual, deviance residual, df betas, and the chi-square deletion difference identified no violation of model assumptions. (Appendix C) However two data-points were found to be potential outliers. These data points represented two un-related cases of resistant *Salmonella* serotype Montevideo who were both <5 years of age. Examination of these cases indicated that their demographic information was plausible. Furthermore, the leverages were below 0.06 and the C deletion difference was <0.5, below the 1.0 threshold for removal. Finally, these two data points did not influence the data in a manner that created or magnified a false association. These points influenced the *Salmonella* Montevideo contrast with Enteriditis towards a null association. Removing these points from the data would result in a significantly protective association between serotype Montevideo and resistance compared to

serotype Enteriditis. Therefore these cases were retained in the dataset since they provided a more conservative point estimate.

Serotype associations with CIR

Serotypes Typhimurium, Heidelberg, Typhimurium var Copenhagen, Newport, I 4, 5, 12:i:-, Saintpaul, and Paratyphi B var L+ Tartrate+ were significantly more likely to be resistant compared to pan-susceptible isolates after adjusting for year, age, race, case type, and travel to asia. (Table 21)

Year association with CIR

Adjusting for serotype, age , race, case type, and travel to Asia revealed that clinically important resistance significantly increased each year from 2004 to 2009 compared to cases with pan-susceptible isolates.

Case status association with CIR

Cases that were part of identified outbreak clusters acquired isolates that were resistant to clinically important antibiotics less frequently than sporadic cases after adjusting for year, serotype, age, race, and travel to Asia.

Travel to Asia was associated with CIR

Travel to asia was significantly associated with the acquisition of an isolate that was resistant to >=1 clinically important antibiotic compared to pan-susceptible cases after adjusting for age, race, case type, and serotype.

Table 21. Multivariate analysis of the frequency of Salmonella isolates that were resistant to >=1 clinically important antibiotic among Oregon patients who had isolates submitted to the Oregon State Public Health Laboratory (OSPHL) compared to cases with pan-susceptible isolates (n=1307), 2004-2009.

Variable	OR	95% CI
Serotype		
S. Enteriditis	Referent	
Typhimurium	**4.51**	**2.53-8.05**
Heidleberg	**5.01**	**2.68-9.38**
Typhimurium var Copenhagen	**13.86**	**7.05-27.23**
Newport	**7.11**	**3.60-14.05**
I 4, 5, 12:i:-	**2.41**	**1.12-5.16**
Montevideo	0.47	0.13-1.68
Saintpaul	**2.89**	**1.13-7.43**
Paratyphi B var L+ Tartrate+	**3.87**	**1.65-9.06**
Other	1.01	0.58-1.77
Travel to Asia		
No	Referent	
Yes	**6.86**	**3.12-15.09**
Case Type		
Sporadic	Referent	
Outbreak Cases	**0.55**	**0.38-0.81**
Year		
Per year	**1.15**	**1.06-1.26**

ACSSuT resistance multiple logistic regression modeling

To expand upon the univariate analysis between the binary dependent variable of ACSSuT (0=pan-susceptible, 1=resistant) those independent variables that were associated at the Chi square alpha level of 0.25 were assessed for inclusion in a multivariate logistic regression model. For this analysis there were 1141 cases that were included, 139 of these cases were resistant (ACSSuT).

The serotype variable was collapsed to include the 5 most common serotypes numbered in descending frequency between 2004 and 2009 in Oregon, and all the other remaining serotypes grouped together. The 5-17 age group was collapsed into the 18-64 age group to minimize the number of covariates due to the much smaller outcome sample size of (139 for ACSSuT vs. 305 for CIR). For the ACSSuT multivariate model covariates were coded as follows: serotype (0=Enteritidis, 1=Typhimurium, 2=Heidelberg, 3=Typhimurium var Copenhaagen, 4=Newport, 5=All other), race (0=white, 1=nonwhite, 2=unknown), raw meat exposure (0=no, 1=yes), pet exposure (0=no, 1=yes), and case status (0=sporadic case, 1= outbreak cluster case) met the inclusion criteria for this model (Univariate <=0.25 p-value). International travel (0=no, 1=yes) was also assessed due to this variable's significance in the Typhimurium only univariate analysis. The following potentially confounding variables were also included; year (ordinal), age (0=5-64 years, 1<=1 years, 2=1-4 years, 3=64+ years), sex (0=female, 1=male) , and Hispanic ethnicity (0=Non-Hispanic, 1=Hispanic, 2=unknown).

Serotype was a significant confounder (OR change>=10%) for raw meat exposure, reptile exposure, and case status. Age, race, reptile exposure, and international travel were confounded by case status. Interactions were suggested (Breslow-day test p-value<0.10) between serotype and international travel and also between serotype and raw meat exposure.

The preliminary main effects model included case status, serotype, race (non-white vs. white only), and raw meat exposure. Despite lacking significance as predictor or confounder, age was also included (fixed) in the model, since this variable was highly suspected as a confounder and was important for external generalizability. Therefore, the resultant preliminary main effects model was; case status, serotype, age, race (non-white vs. white only), and raw meat exposure. This model was checked for co-linearity via the variance inflation factor (VIF) and no variables were found to be collinear with the highest value of the VIF being 2.36 for the "All Other" serotype level. (Appendix A)

Finally, interactions were hypothesized between serotype and international travel since previous studies have reported increased resistance associated with isolates of the same serotypes acquired internationally (Hendriksen 2012, Hendriksen 2009, Threlfall 2000). An interaction was also hypothesized between serotype and raw meat because a number of highly resistant isolates from serotypes such as Typhimurium and Newport have been reported as a result of antibiotic use in animal husbandry (LeHello 2011, Varma 2006, Poppe 2005). Taking care not to "over-fit" the model by exceeding the m/10 rule of thumb for model (Harrell, F. E.,

1984) these interaction terms were entered into the model, one at a time and relationships were assessed. Significant interactions ($p<0.05$) were found between *Salmonella* serotype Typhimurium and international travel, and *Salmonella* serotype Newport.

This final model was checked and confirmed against both forward and backward stepwise selection procedures holding age and race constant in each model. Therefore the final main effects model had 14 degrees of freedom and included; case status, serotype, age, race (non-white vs. white only), raw meat exposure, international travel, Typhimurium*international travel, Newport*international travel. (Appendix B)

Using the Hosmer and Lemeshow method to assess goodness of fit, it was shown that this model had adequate fit for this data ($p=0.65$). When assessing the discriminative ability of the main effects model, an ROC curve was generated and the area under the curve was found to be 0.80, indicating that this model had good to excellent discriminative ability. (Table 22)

Table 22. ACSSuT model fit statistics and ROC curve (n=1141)

H-L Goodness of Fit	Pearson	Deviance	ROC
0.65	0.69	0.70	0.80

Residual analysis by graphing the leverages, the Pearson's residual, deviance residual, df betas, and the chi-square deletion difference identified no violation of model assumptions. (Appendix C) However one data-point was identified as a potential outlier. This data point represented a pan-susceptible *Salmonella* serotype

Typhimurium case that had traveled to Mexico during the exposure period. Examination of this case indicated that their demographic information was plausible and their dates of travel correctly overlapped with the necessary exposure window. The leverages of this model were all below 0.1 and the C deletion difference was less than the 1.0 threshold for outliers. Furthermore, the identified potentially outlying point influenced the data towards a null association for international travel among *Salmonella* Typhimurium cases and thus did not influence the data towards a false association (although it is responsible for the wide confidence interval for the Typhimurim and international travel interaction term). Therefore this data point was retained in the model as a valid observation.

Serotype associations with ACSSuT resistance

After adjusting for year, age, race, case type, raw meat exposure, and international travel it was found that serotype Typhimurium var Copenhagen was significantly more likely to be R-type ACSSuT resistant compared to Enteriditis. Upon stratification by international travel, cases that acquired a *Salmonella* serotype Newport or a Typhimurium isolate without international travel were more likely to have a resistant isolate compared to cases with pan-susceptible isolates, after adjusting for age, race, raw meat exposure, and case type. (Table 21)

Case status association with ACSSuT resistance

Cases that were part of identified outbreak clusters acquired isolates that were ACSSuT resistant less frequently than sporadic cases after adjusting for serotype, age, race, raw meat exposure, and international travel.

Raw meat exposure association with ACSSuT resistance

Cases with raw meat exposure acquired an ACSSuT resistant isolate more frequently that cases without raw meat exposure after adjusting for age, race, serotype, and international.

International travel associations with ACSSuT resistance

International travel was positively associated with the acquisition of a Typhimurium isolate that was ACSSuT resistant compared to pan-susceptible after adjusting for age, race, case type, and raw meat exposure. However, international travel was negatively associated with ACSSuT for *Salmonella* serotype Newport after adjusting for age, race, case status, and raw meat exposure.

Non-white race association with ACSSuT resistance

Non-white individuals acquired isolates that were ACSSuT resistant significantly more frequently than white cases after adjusting for serotype, age, case status, raw meat exposure, and international travel.

Table 23. Multivariate analysis of the frequency of Salmonella isolates that were R-type ACSSuT resistant among Oregon patients who had isolates submitted to the Oregon State Public Health Laboratory (OSPHL) compared to cases with pan-susceptible isolates (n=1141), 2004-2009.

Variable	OR	95% CI	
Case type			
Sporadic		Referent	
Outbreak	0.43	0.23	0.79
Serotype			
Enteriditis		Referent	
Typhimurium (no travel)	14.04	4.81	40.94
Heidelberg	2.19	0.52	9.14
Typhimurium var Copenhagen	21.06	6.54	67.77
Newport (no travel)	33.37	10.46	106.50
All Other Serotypes	2.23	0.78	6.43
Race			
White		Referent	
Nonwhite	2.33	1.25	4.34
Age			
5-64 years		Referent	
<1 years	1.45	0.68	3.11
1-4 years	0.60	0.31	1.17
65+ years	0.79	0.40	1.56
Raw Meat Exposure			
No		Referent	
Yes	2.00	1.20	3.34
International Travel			
No		Referent	
Yes	1.03	0.41	2.58
***S.* Typhimurium**			
No travel		Referent	
International travel	12.82	2.45	60.63
***S.* Newport**			
No travel		Referent	
International travel	0.15	0.03	0.77
Interaction			
Enteriditis, no travel		Referent	
Newport (travel)	5.07	0.82	31.37
Typhimurium (travel)	171.10	26.61	1099.60

***Salmonella* serovar Typhimurium subset-CIR multiple logistic regression modeling.**

To expand upon the univariate Typhimurium analysis between the binary dependent variable of clinically important resistance (CIR, 0=pan-susceptible, 1=resistant), those independent variables that were associated at the Chi square alpha level of 0.25 were assessed for inclusion in a multivariate logistic regression model, by analyzing these variables individually stratified by another predictor to assess potential confounding and interaction. For this analysis there were 213 cases that were included in the dataset, 69 of these cases were resistant (CIR).

For this analysis the <1 and the 1-4 years of age groups were collapsed together due to their small sample sizes. Therefore, covariates included age (0=5-64 years, 1= <5 years, 2=65+ years), International travel (0=no, 1=yes), raw meat exposure (0=no, 1=yes), pet exposure (0=no, 1=yes), reptile exposure (0=no, 1=yes), case status (0=sporadic case, 1= outbreak cluster case), and year (ordinal 2004-2009) met the inclusion criteria for this model (Univariate <=0.25 p-value). The following potentially confounding variables were also included; sex (0=female, 1=male) , race (0=white, 1=nonwhite, 2=unknown), and Hispanic ethnicity (0=Non-Hispanic, 1=Hispanic, 2=unknown).

Year was a significant confounder or raw meat exposure, reptile exposure and international travel exposure, and international travel. Case status was a significant positive confounder of reptile exposure. Due to sample size limitations

no potential interactions were assessed, although none were immediately suggested by the stratified analysis.

The preliminary main effects model included case status, year of infection, international travel, and raw meat exposure. The age predictors and nonwhite race did not confound or effect modify any of the relationships between the predictors and CIR, but were included (fixed) in the model due to highly suspected relevance and for external generalizability of the final model. Therefore, the resultant preliminary main effects model was; case status, race (non-white vs. white only), raw meat exposure, age, international travel, and year of infection. This model was checked for co-linearity via the variance inflation factor (VIF) and no variables were found to be collinear with the highest value of the VIF being 1.09 for <5 years of age. (Appendix A)

This final model was checked and confirmed against both forward and backward stepwise selection procedures holding age and race constant in each model. Due to small sample size, no interactions were assessed or hypothesized. Therefore the final main effects model was; case status, race (non-white vs. white only), year, raw meat exposure, age, and international travel. (Appendix B)

Using the Hosmer and Lemeshow method to assess goodness of fit, it was shown that this model had acceptable fit for this data ($p=0.54$). (Table 24) Analysis of the deviance and Pearson's chi-square tests indicated that this model does not exhibit over-dispersion (Pearson 0.92, Deviance 0.71). When assessing the discriminative ability of the main effects model, an ROC curve was generated and

the area under the curve was found to be 0.79, indicating that this model had good discriminative ability.

Table 24. *Salmonella* serovar Typhimurium model fit statistics and ROC curve (n=213)

H-L Goodness of Fit	Pearson	Deviance	ROC
0.54	0.92	0.71	0.79

Residual analysis by graphing the leverages, the Pearson's residual, deviance residual, df betas, and the chi-square deletion difference identified no violation of model assumptions. (Appendix C) However, one data-point was identified as a potential outlier. This data point represented a pan-susceptible *Salmonella* serotype Typhimurium case that had traveled to Mexico during the exposure period. Examination of this case indicated that their demographic information was plausible and their dates of travel correctly overlapped with the necessary exposure window. Therefore this observation was retained in the dataset as a valid observation.

Case status association with Typhimurium CIR

Typhimurium cases that were part of identified outbreak clusters acquired isolates that were ACSSuT resistant less frequently than sporadic cases after adjusting for year, age, race, raw meat exposure, and international travel. (Table 25)

Raw meat exposure association with Typhimurium CIR

Cases with raw meat exposure acquired an isolate that was resistant to >=1 clinically important antibiotic more frequently that cases without raw meat exposure after adjusting for age, race, serotype, and international. (Table 25)

International travel association with Typhimurium CIR

Typhimurium cases with international travel were significantly more likely to have acquired an isolate that was resistant to >=1 clinically important antibiotic compared to cases without international travel after adjusting for year, age, race, case type, and raw meat exposure. This resistance was seen across destinations with the most common destinations being Mexico and Thailand.

Year association with CIR

Adjusting for serotype, age , race, case type, and international travel revealed that clinically important resistance significantly increased each year from 2004 to 2009 compared to cases with pan-susceptible isolates.

Table 25. Multivariate analysis of the frequency of Salmonella Typhimurium isolates that were resistant to >=1 clinically important antibiotic among Oregon patients who had isolates submitted to the Oregon State Public Health Laboratory (OSPHL) compared to cases with pan-susceptible isolates (n=213), 2004-2009.

Variable	OR	95% CI	
Case type			
Sporadic		Referent	
Outbreak	0.19	0.08	0.48
International Travel			
No		Referent	
Yes	15.94	3.87	65.70
Year	1.47	1.21	1.79
Raw Meat Exposure			
No		Referent	
Yes	2.44	1.07	5.55
Age			
5-64 years		Referent	
<5 years	0.54	0.23	1.26
65+ years	0.91	0.30	2.71
Race			
White		Referent	
Nonwhite	0.97	0.24	3.94

XI. DISCUSSION

We confirmed that the NARMS sampling methodology provided a representative sample of cases, serotypes, and resistance profiles among Oregon patients. We also demonstrated significant temporal increases in antibiotic resistance between 2004 and 2009 in Oregon. The previously reported association between resistance and hospitalization was confirmed while the associations between invasive infection as well as severe illness in *Salmonella* serotype Typhimurium were not confirmed (Varma, 2005, Lee 1994). However the direction and magnitude of the point estimate between resistance and invasive illness was similar to previously published results, suggesting that a greater sample size may have pushed this association towards significance (Varma, 2005).

In the multivariate analyses we found significant associations between travel to Asia and the acquisition of an isolate that was resistant to >=1 clinically important antibiotic. This association held true for the *Salmonella* Typhimurium sub-analysis. The most common destinations where resistant isolates were acquired were Thailand, China, and Malaysia/Indonesia. These findings are congruent with European studies which found increasing resistance among isolates of *Salmonella* serotypes Typhimurium and Stanley from patients with recent international travel to Thailand (Hendriksen 2012, 2009).

Travel to Asia was the only risk factor in the primary analysis that approached significance. Therefore, greater epidemiological information is needed about *Salmonella* transmission and resistance internationally. The factors

responsible for the increased acquisition of highly resistant isolates among travelers to Asia are unclear. Thus, a better understanding of antibiotic use among humans and livestock internationally may elucidate areas where restricted use of antibiotics will prevent transmission to humans and control the emergence of increasingly resistant infections. Monitoring of the global food supply for antibiotic resistant isolates of *Salmonella* is necessary. Finally, it is imperative that agricultural producers adhere to the WHO recommendations for restricting the use of antibiotics in food animals (WHO 1997).

The significantly protective interaction between *Salmonella* Newport with international travel cases in the ACSSuT analysis could be explained by the NARMS finding that increasing S. Newport resistance in the US was associated with domestic use of antibiotics in cattle. Since the US is the primary producer of cattle and exporter of beef and dairy products, it is possible that multiple-resistant *Salmonella* Newport isolates are emerging domestically and have not yet disseminated internationally due to limited international availability of hosts (cattle) and decreased prevalence of large scale beef production operations(ERS, 2012).

The finding that resistance among *Salmonella* Newport isolates did not significantly increase between 2004 and 2009 (ceftriaxone resistance significantly decreased p=0.04), may be suggestive that NARMS has helped to control resistance in this serotype. (Table 11, 12) Alternatively, this could be indicative that *Salmonella* Newport resistance has already approached a maximum and is no longer increasing (this is supported by the finding that among the most common serotypes, S.

Newport is one of the most resistant serotypes besides Typhimurium var Copenhagen). (Table 21)

Raw meat exposure was associated with ACSSuT resistance as well as CIR resistance in the Typhimurium sub-analysis. However, this association did not reach significance in the overall CIR analysis (p=0.15). Perhaps, this discrepancy among the models could be explained by the lack of specificity of this exposure. For example, Typhimurium has been repeatedly isolated from poultry and swine. Therefore a higher proportion of cases with raw meat exposure in the Typhimurim sub-analysis may have had raw poultry or swine exposure (among cases with raw meat exposure) making the raw meat exposure variable more specific for either of these raw meat types. Similarly, the ACSSuT model represents the R-type ACSSuT resistance plasmid first associated with *Salmonella* Typhimurium phage type DT-104. Thus, the higher proportion of Typhimurium cases could have pushed this association to significance. This line of reasoning is supported by the finding that raw meat exposure was a negative confounder of the association between international travel and resistance yet was also associated with CIR in the Typhimurium sub-analysis.

A significantly protective association between cases who were part of outbreak clusters was identified; this association was confirmed and preserved after restricting and assessing the data three different ways. Therefore, outbreak cases that were part of identified outbreaks were less likely to have a resistant isolate. This finding is suggestive that resistant isolates had decreased infectivity.

Alternatively, these data could imply that common sources of resistant isolates are less likely to result in widespread contamination, thereby being less frequently associated with outbreaks. While no interaction was found between year and case status, the trend analysis suggests that resistance to clinically important antibiotics significantly increased across more antibiotics for outbreak cases vs. sporadic cases. Thus, we may expect to see more frequent outbreaks with resistant infections leading to greater illness severity and rates of hospitalization in the future.

Increasing antibiotic resistance has widespread implications for human health. We confirm the results from previous studies by Varma et. al and Lee et al, which suggest that antibiotic resistance is associated with increased frequency of hospitalization. This can lead to an increased economic burden as well as more severe infections potentially resulting in treatment failure, sepsis, meningitis, and even death (Hohmann. 2001). Therefore, if resistance continues to increase by 15% per year (as our data suggest), then we would expect to see increased rates of hospitalization in Oregon representing an important economic and public health burden.

XII. STRENGTHS AND LIMITATIONS

The major strength of this study is that there is no other (known) surveillance system in place that captures almost 100% of clinically confirmed cases of Salmonellosis, has antibiotic susceptibility information on over 95% of confirmed cases, and exposure histories on over 80% of cases between 2004 and 2009. This study is further strengthened by having information on a number of known and potential confounding variables that were collected without prior knowledge of the susceptibility profile of the patient's isolate. The use of the same outcome and similar methodology to a previous NARMS/FoodNet study that looked at antibiotic resistance and increased disease severity allowed for increased the external validty of this study as well as the generalizability.(Varma, 2005) Finally, the ability to expand upon previous small European studies which looked at only one international travel as a risk factor for antibiotic resistance improves the study generalizability and plausibility (Hendriksen 2012, Hendriksen 2009, Threlfall 2000).

This study is further strengthened by the inclusion of an epidemiologic variable that allows adjustment for differences between outbreak and sporadic cases. The use of two case-definitions where one definition represented a genetically distinct outcome (microbiologically relevant) and the other represented a clinically meaningful outcome provided greater utility for intervention and future research as well as maintaining strong internal validity and specificity. Finally, the complementary sub-analysis on *Salmonella* Typhimurium improved study internal

validity and relevance by analyzing an important serotype with a known high prevalence of antibiotic resistance, a global distribution, and a high rate of morbidity/mortality.

A limitation of this study is that it may lack information on a number of key confounding variables. For example, the lack of information on previous/recent antibiotic use, a known risk factor, could be a major confounder of this study (MacDonald 1987, Winokur 2001). However, a study in 2001 found that infection with antibiotic resistant NTS is associated with increased severity even when patients did not receive any prior therapy (Winokur, 2001). Furthermore, recent antibiotic use would be expected to non-differentially confound the observed associations resulting in an underestimation rather than explaining any observed association. Another limitation may be that the use of data on only Oregon patients may decrease the generalizability of the results nationally, and provide little useful information where risk factor information is needed most, in areas such as sub-tropical Africa (Gordon 2008, Brent 2006, Schwartz 2010). Furthermore, the lack of data on HIV status, other co-morbidities, and socio-economic status may also confound the study results (Brent 2006).

Lack of timeliness in reporting could have lead to a lag between case onset and risk factor interview resulting in a non-differential recall bias for case exposure history. Providers may have been more likely to see and/or obtain stool samples from patients with co-morbidities and those who were immunologically vulnerable to infection, which may have resulted in a biased sample of confirmed case-patients.

This could have represented a different population from the average Oregon resident (Voetsch, 2004).

This study could have had a clinical surveillance bias because it is estimated that for every laboratory confirmed case of Salmonellosis there are anywhere from 20-38.6 more undiagnosed cases (Mead, 1999, Voetsch 2004). Taking this clinical surveillance bias into account with findings from studies that show increased disease severity with resistant isolates, could lead one to hypothesize that the proportion of resistant *Salmonella* isolates during this time period could actually overestimate the true resistance profile in Oregon. This bias may be important for the descriptive analysis however it would not be expected to affect the logistic regression analysis (except perhaps increasing the power of the study by over-representing the prevalence of the outcome).

Similarly, the finding that cases with resistant isolates were more likely to be interviewed could also result in an over-estimate of the proportion of isolates that were resistant. However, this was found to negatively confound case status. This meant that cases with resistant isolates that were part of outbreaks were more likely to be interviewed, resulting in a differential underestimate of the protective association between outbreak cases and resistance.

Since two laboratories were used for susceptibility testing (OSU VDL 2003-2005, OSPHL 2002, 2006-2009) a systematic bias could have resulted if their methods differed. The fact that both laboratories were licensed and were using the CLSI standardized methodologies should have minimized this bias. The use of

cephalothin (1st gen cephalosporin) and cefuroxime (2nd gen cephalosporin) instead of ceftriaxone (3rd gen cephalosporin) for 2004 and 2005 could have overestimated resistance if these agents had reduced in-vitro efficacy for susceptibility testing. However, the fact that the trend analysis revealed a marginally significant increase in cephalosporin resistance over the study period is suggestive that this potential bias had little to no effect on the data. Additionally, these biases were minimized via adjustment for year in the multivariate analyses.

In-vitro susceptibility testing may not be sufficiently representative of in-vivo susceptibility. Host conditions may trigger the activation of dormant resistance genes not represented in susceptibility testing. Further, biofilm formation in-vivo is an important consideration for antibiotic resistance, because isolates with few resistance genes can survive at maximum tolerated doses of antibiotics, representing a threat for treatment failure.

The lack of specificity for some of the exposures may have masked true associations. For example, the variable for raw meat exposure could have included pork, beef, wild game, or poultry which are known to carry different serotypes that may have differing resistance profiles. Therefore, this could have masked true associations between individual sources or raw meat. Similarly the collapse of sites of international travel into only a few broad groups for the multivariate analysis may have drastically underestimated resistance from particular regions/countries. However, the univariate analysis stratified by region provided some evidence that

the majority of this resistance was acquired from Central (China) and Southeast Asia (Thailand, Indonesia/Malaysia, and potentially Vietnam).

Therefore, this study had high internal validity, specificity, biological plausibility, a low level of biases (non-differential), and temporality. The weaknesses are that this study may have been underpowered for multiple analyses (for *S.* Typhimurium), lacked information on a few important confounding variables, and lacked generalizability. Thus, while there are some limitations with this study design and the surveillance system, the opportunity to analyze a large sample of population level data with an unprecedented level of exposure information outweighed these concerns.

XIII. CONCLUSION

This study presents evidence that antibiotic resistance is increasing and has important public health and economic implications. Our analyses also elucidated international travel and potentially raw meat exposure as risk factors driving antibiotic resistance. Case data was collected prospectively over 6 years from a statewide surveillance system with unprecedented case ascertainment, and these results highlight the need for enhanced domestic surveillance for antibiotic resistance as well as increased international regulation and vigilance with the use of antibiotics in food animals and humans alike. Additionally, we have shown that improved specificity of exposure questions with regard to raw meat exposure as well as the addition of questions regarding recent antibiotic use may be important for future surveillance and research activities both in Oregon and FoodNet alike. Fortunately, the Oregon Health Authority, Public Health Division has since expanded its exposure questions to differentiate between raw beef, poultry, and wild game. However, including questions about recent antibiotic use (~30 days) and types of antibiotics used would help to address a important gap in our knowledge about antibiotic resistance. Finally, we have illuminated the need for future epidemiological research on international travel to areas such as East and Southeast Asia as well as the need for more international studies that examine agricultural practices, disease reservoirs, and risk factors driving the increased resistance in these regions.

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XV. APPENDIX A. CO-LINEARITY ASSESSMENT

Table A.1 Assessment of co-linearity for clinically important resistance (CIR)

Variable	Tolerance	VIF
Typhimurium	0.53	1.87
Heidleberg	0.64	1.56
Typhimurium var Copenhaagen	0.70	1.42
Newport	0.73	1.36
I 4, 5, 12:i:-	0.74	1.35
Montevideo	0.77	1.30
Saintpaul	0.86	1.17
Paratyphi B var L+ Tartrate+	0.82	1.22
Other	0.40	2.48
<1 years of age	0.92	1.08
1-4 years of age	0.90	1.11
5-17 years of age	0.89	1.12
65+ years of age	0.91	1.10
Nonwhite Race	0.98	1.02
Travel to Asia	0.95	1.05
Outbreak Cases	0.98	1.02
Year	0.98	1.02

Table A.2 Assessment of co-linearity for ACSSuT resistance

Variable	Tolerance	VIF
Typhimurium	0.53	1.88
Heidleberg	0.69	1.45
Typhimurium var Copenhaagen	0.78	1.29
Newport	0.74	1.36
Other	0.42	2.36
Outbreak Cases	0.95	1.05
Raw Meat exposure	0.98	1.02
International Travel	0.94	1.06
<1 years of age	0.95	1.06
1-4 years of age	0.93	1.07
65+ years of age	0.94	1.06
Nonwhite Race	0.98	1.02

Table A.3 Assessment of co-linearity for *Salmonella* Typhimurium clinically important resistance (CIR)		
Variable	**Tolerance**	**VIF**
Outbreak Cases	0.97	1.03
International Travel	0.96	1.04
Year	0.96	1.05
Raw Meat Exposure	0.95	1.05
<5 years of age	0.92	1.09
65+ years of age	0.93	1.07
Nonwhite Race	0.98	1.02

XVI. APPENDIX B. MAIN EFFECTS MODELS AND MODEL COEFFICIENTS

B.1 CIR Main Effects Model (N=1307)

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	202.975	17	<.001
Score	204.750	17	<.001
Wald	165.924	17	<.001

Parameter	Estimate	SE	Wald	Pr > ChiSq
Intercept	-288.7	87.5052	10.8834	0.001
Typhimurium	1.507	0.296	26.018	<.001
Heidleberg	1.612	0.320	25.402	<.001
Typhimurium var Copenhagen	2.629	0.345	58.181	<.001
Newport	1.962	0.348	31.855	<.001
I 4, 5, 12:i:-	0.878	0.390	5.073	0.024
Montevideo	-0.747	0.646	1.336	0.248
Saintpaul	1.062	0.482	4.862	0.028
Paratyphi B var L+ Tartrate+	1.352	0.434	9.692	0.002
Other	0.011	0.287	0.001	0.971
<1 years of age	0.294	0.283	1.081	0.299
1-4 years of age	-0.219	0.231	0.896	0.344
5-17 years of age	-0.037	0.200	0.035	0.852
65+ years of age	-0.143	0.243	0.344	0.558
Nonwhite Race	0.171	0.258	0.437	0.509
Travel to Asia	1.925	0.402	22.887	<.001
Outbreak Cases	-0.594	0.195	9.281	0.002
Year	0.143	0.044	10.733	0.001

B.2 ACSSuT Main Effects Model (n=1141)

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	181.002	14	<0.001
Score	219.389	14	<0.001
Wald	145.472	14	<0.001

Parameter	Estimate	SE	Wald	Pr > ChiSq
Intercept	-3.644	0.523	48.610	<0.001
Outbreak Case	-0.842	0.311	7.328	0.007
Typhimurium	2.641	0.546	23.394	<0.001
Heidleberg	0.782	0.730	1.148	0.284
Typhimurium var Copenhagen	3.047	0.596	26.108	<0.001
Newport	3.508	0.592	35.119	<0.001
Other	0.804	0.539	2.223	0.136

Nonwhite Race	0.844	0.319	7.016	0.008
<1 years of age	0.371	0.389	0.910	0.340
1-4 years of age	-0.504	0.337	2.229	0.135
65+ years of age	-0.232	0.346	0.449	0.503
Raw Meat Exposure	0.695	0.261	7.092	0.008
International Travel	0.028	0.469	0.004	0.953
Typhimurium*International Travel	2.473	0.940	6.925	0.009
Newport*International Travel	-1.911	0.950	4.052	0.044

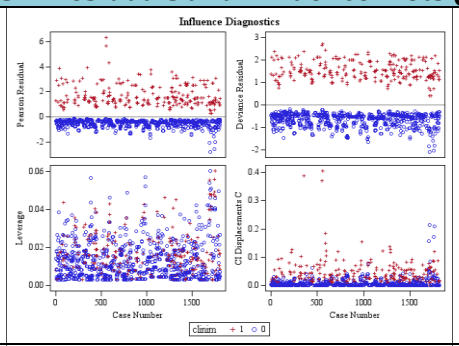
B.3 CIR Typhimurium Only, Main Effects Model (n=213)

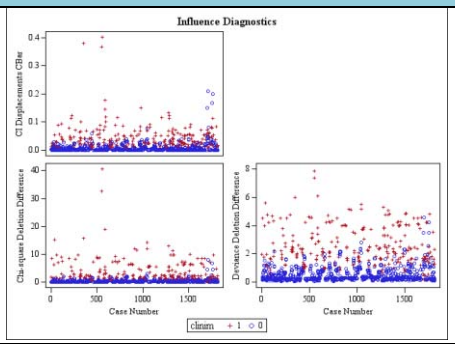
Test	**Chi-Square**	**DF**	**Pr > ChiSq**
Likelihood Ratio	51.311	7	<0.001
Score	46.380	7	<0.001
Wald	34.891	7	<0.001

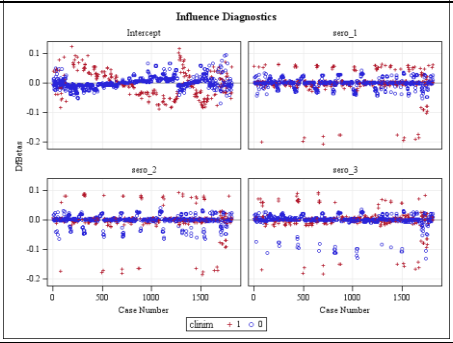
Parameter	**Estimate**	**SE**	**Wald**	**Pr > ChiSq**
Intercept	-773.100	202.300	14.608	<0.001
Outbreak Cases	-1.650	0.462	12.777	<0.001
International Travel	2.769	0.723	14.690	<0.001
Year	0.385	0.101	14.589	<0.001
Raw Meat Exposure	0.891	0.419	4.510	0.034
<5 years of age	-0.617	0.434	2.022	0.155
65+ years of age	-0.096	0.558	0.029	0.864
Nonwhite Race	-0.026	0.713	0.001	0.971

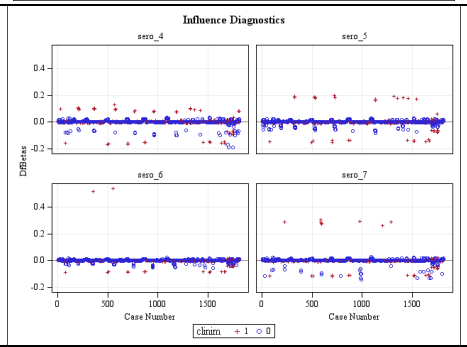
XVII. APPENDIX C. MODEL DIAGNOSTICS

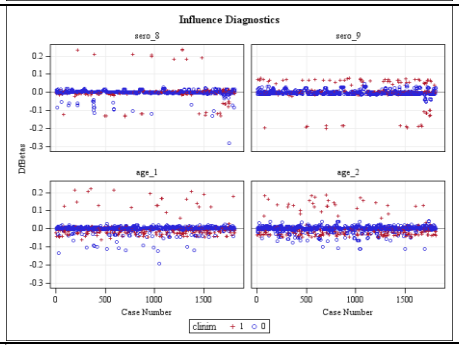
C.1 CIR Residuals and Influence Plots (n=1307)

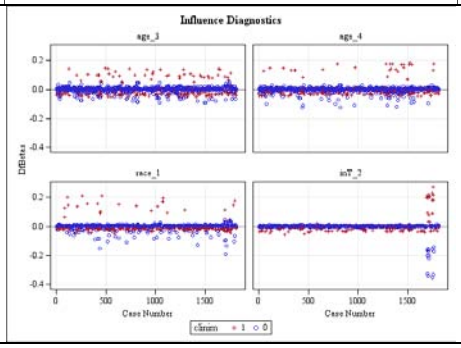


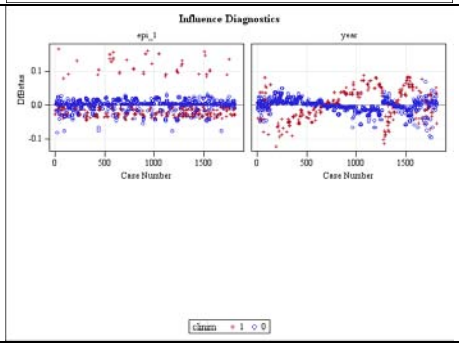




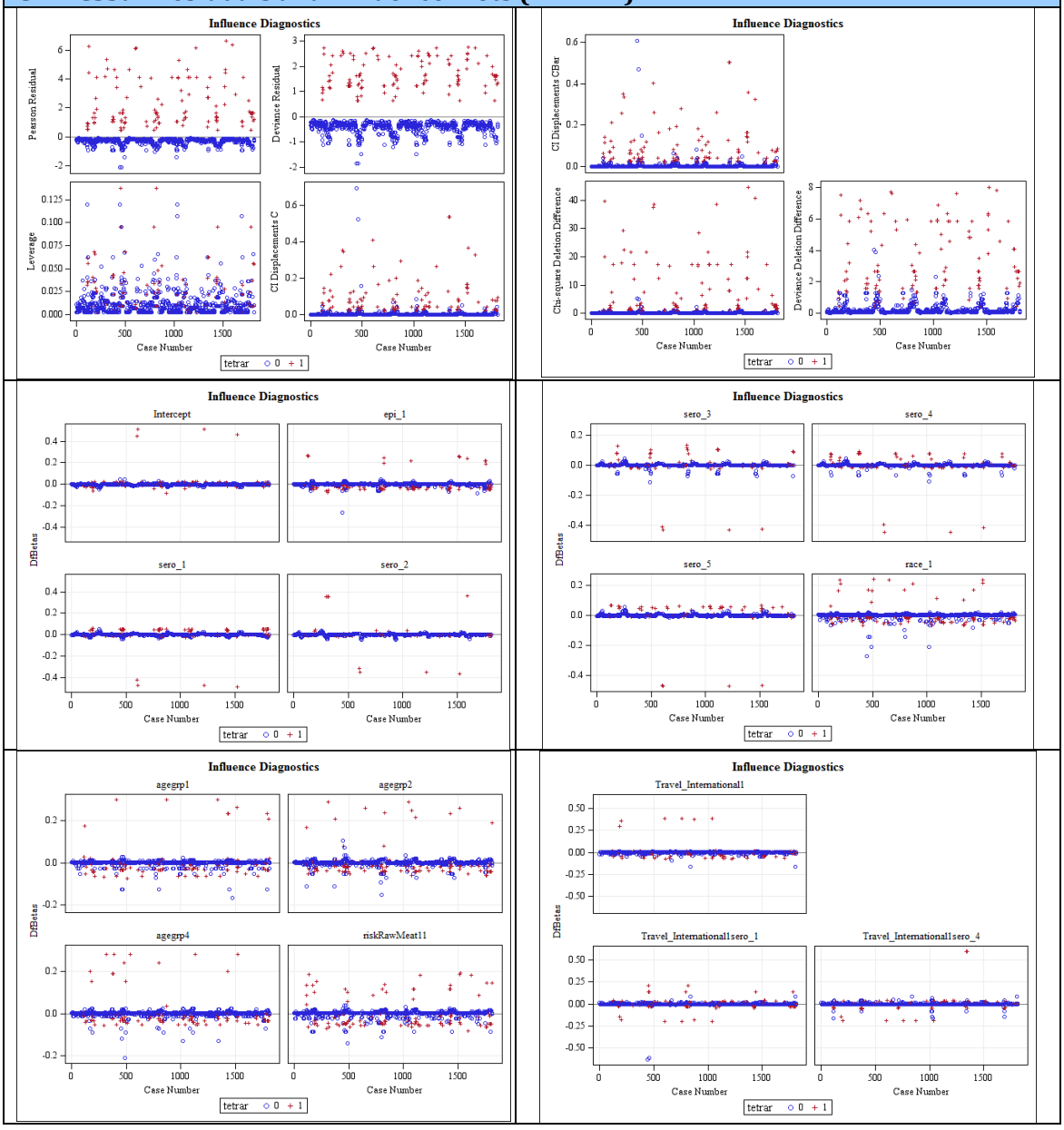




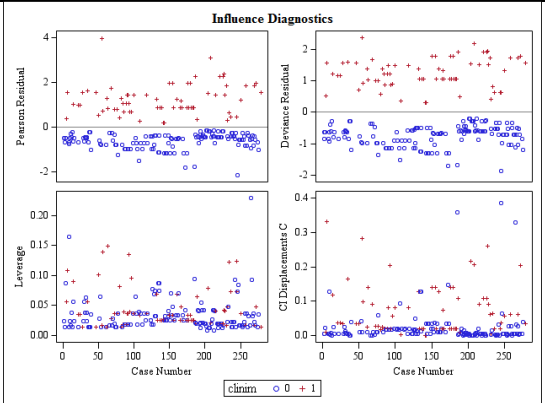


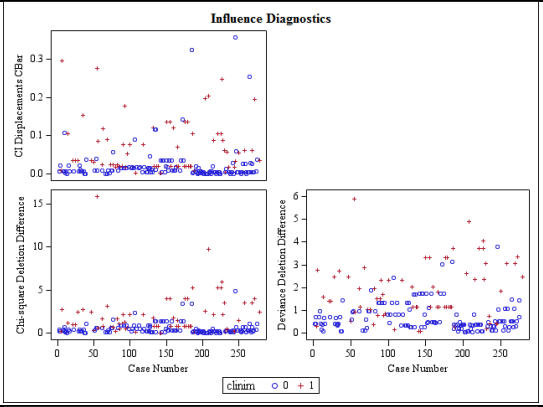


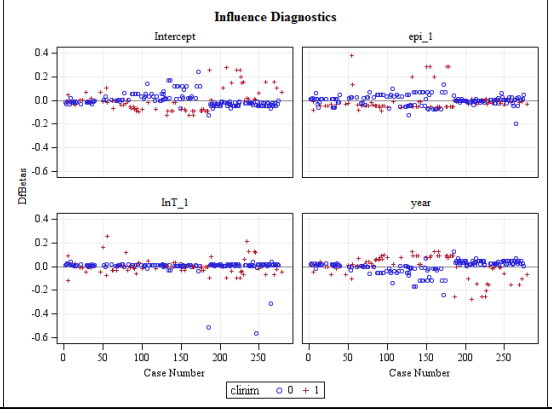
C.2 ACSSuT residuals and Influence Plots (n=1141)

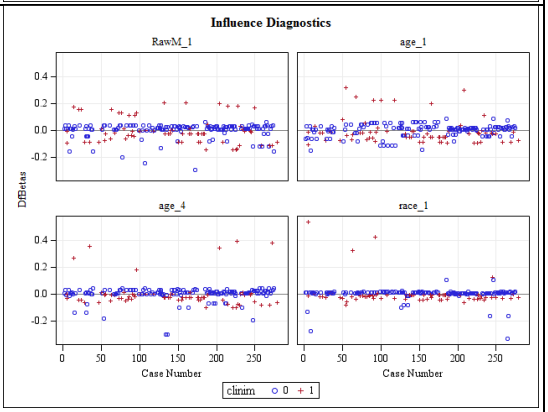


C.3 CIR Typhimurium only residuals and influence plots (n=213)



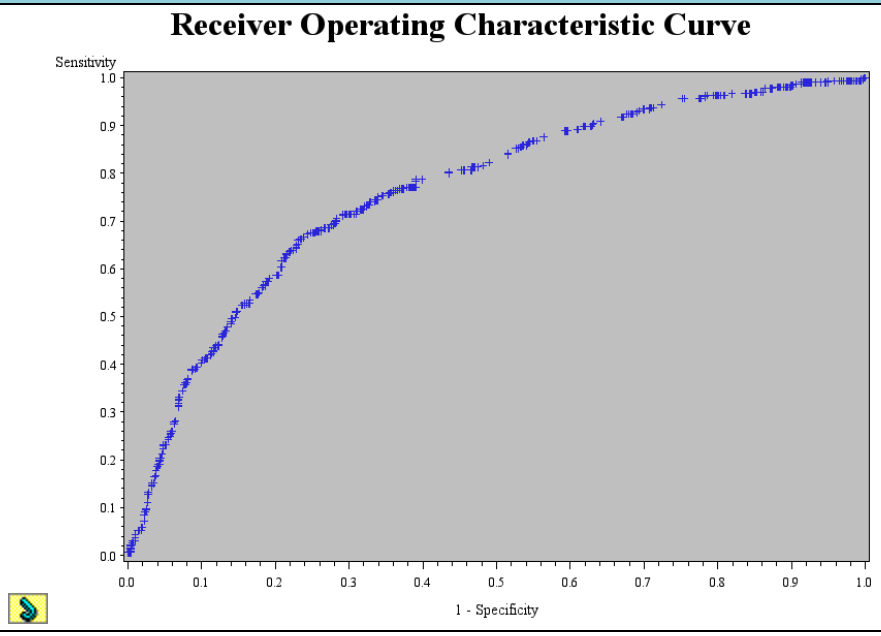




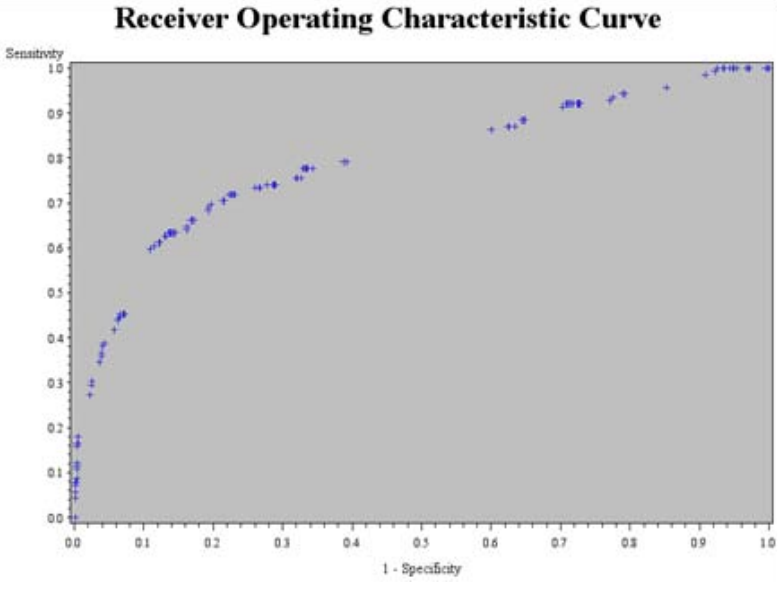


XVIII. APPENDIX D. ROC CURVES

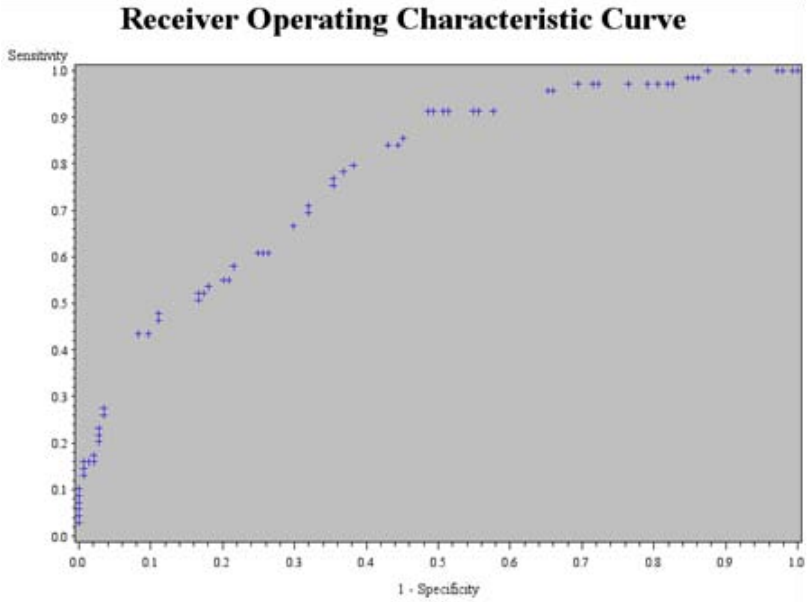
D.1 CIR final main effects model receiver operating characteristic curve (ROC), n=1307



D.2 ACSSuT final main effects model receiver operating characteristic curve (ROC), n=1141



D.3 Typhimurium CIR final main effects model receiver operating characteristic curve (ROC), n=213



XIX. APPENDIX D. POWER AND SAMPLE SIZE

Preliminary power and sample size analysis was performed to estimate the minimum detectable difference within the study population (Table 2). All power calculations were calculated based on an 80% level of power at the 0.05 level of significance. Effect size calculations were conducted with Power Analysis and Sample Size Software (PASS 2008, NCSS, LCC, Kaysville, Utah).

In the primary analysis with tetra-resistance as the outcome, there is at least a 15% prevalence of outcome among at least 750 persons in the population. In the primary analysis with clinically significant resistance as the outcome there is at least a 25% prevalence of outcome among at least 900 persons in the population. Therefore, depending upon the prevalence of the independent variable in the study population, the minimum detectable odds ratio ranges from 1.694 to 2.242 (69% and 124%) for tetra resistance, and from 1.520 to 1.927 (52% and 93%) for clinically significant resistance.

In the secondary analysis on *Salmonella* Typhimurim alone, with tetra resistance as the outcome there is at least a 35% prevalence of resistance among at least 120 people in the population. With clinically significant resistance as the outcome there is at least a 40% prevalence of resistance among at least 140 people in the population. Thus, for the *Salmonella* Typhimurium only analysis, the minimum detectable odds ratio ranges from 2.82 to 5.651 (182% and 465%) for tetra resistance and from 2.659 to 5.161 (166% and 416%) for clinically significant resistance. These estimates are dependent upon the prevalence of the independent variable in the study population.

Table E.1: Power Analysis	**2004-2009 Salmonella isolates**		**2004-2009 Salmonella Typhimurium**	
Prevalence of Independent variable	**ACSSuT Odds Ratio**	**CIR Odds Ratio**	**ACSSuT Odds Ratio**	**CIR Odds Ratio**
10	2.242	1.927	5.651	5.161
20	1.882	1.655	3.625	3.332
30	1.757	1.560	3.085	2.848
40	1.705	1.520	2.877	2.659
50	1.694	1.510	2.820	2.606
60	1.717	1.525	2.882	2.655
70	1.783	1.570	3.093	2.834
80	1.934	1.675	3.617	3.281

XX. APPENDIX F. INTERVIEW ASSESSMENT

A preliminary chi-square analysis was performed to assess a potential information bias on whether people with resistant isolates were more or less likely to be interviewed about their exposure history (although, interviewers had no knowledge of the case's isolate susceptibility profile at time of interview). Between 2004 and 2009 there was a significant difference in the proportion of cases that were interviewed in the CIR analysis (p-value 0.02). A person with a resistant isolate was 52% (95%CI 7%-117%) more likely to be interviewed compared to someone with a pan-susceptible isolate. (Table B.1)

For the ACSSuT analysis there was no significant difference in the proportion of cases that were interviewed with a resistant isolate (p-value 0.08). However, the odds of being interviewed with a resistant isolate were 0.52 times higher (95%CI 0.93 to 2.53). Therefore, this analysis had a similar magnitude and direction to the CIR analysis.

To ascertain whether this association could be confounded by hospitalization status, case status, or serotype (i.e. cases that were hospitalized, from outbreaks, or cases with certain serotypes were more likely to be interviewed), the analysis was re-run adjusting for hospitalization. The results demonstrated that adjusting for hospitalization, did not appreciably confound the magnitude or direction of the association for either analysis. For the CIR outcome, serotype was not a significant confounder but for ACSSuT serotype was a significant positive confounder. Finally, case status was a positive confounder for both CIR and ACSSuT (Adjusted p-value 0.08 and 0.22 respectively). Thus this may be an important point to address in the discussion/limitation section.

F.1. Chi-square tests for differences between having a resistant isolate and being interviewed for Oregon cases of NTS 2004-2009

	CIR			ACSSuT		
Variable	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**
Interviewed	1.52	1.07-2.17	0.02	1.53	0.93-2.53	0.08
Adjusted by Hospitalization	1.51	1.06-2.16	0.02	1.52	0.92-2.52	0.10
Adjusted by Serotype	1.54	1.05-2.24	0.03	1.39	0.82-2.36	0.22
Case status	1.38	0.96-1.98	0.08	1.37	0.82-2.28	0.23

XXI. APPENDIX G. SALMONELLOSIS CASE REPORT FORM

Salmonellosis
(non-typhoidal)

COUNTY

FOR STATE USE ONLY
Orpheus ID
___/___/___ case report ☐ confirmed ☐ presumptive ☐ suspect ☐ no case
___/___/___ interstate

CASE IDENTIFICATION

Name _____ (LAST, first initials) (a.k.a.) Phone(s) _____ home (H), work (W), cell (C), message (M)

Address _____ Street: City Zip

email _____ Language ☐ Spanish ☐ Russian ☐ _____

ALTERNATIVE CONTACT ☐ Parent ☐ Spouse ☐ Friend ☐ _____

Name _____ (LAST, first initials) Phone(s) _____ home (H), work (W), cell (C), message (M)

Address _____ Street: City Zip

SOURCES OF REPORT *(check all that apply)*
☐ ELR ☐ Lab (not ELR) ☐ ICP
☐ Physician ☐ _____
Reporter _____
Date of first report ___/___/___
Primary MD _____
Phone ☐ OK to talk to patient?

DEMOGRAPHICS

SEX ☐ female ☐ male
DOB ___/___/___ (m d y)
if DOB unknown, AGE ____
HISPANIC ☐ Y ☐ N ☐ ?

RACE *(check all that apply)*
☐ white ☐ American Indian
☐ black ☐ Pacific Islander
☐ Asian ☐ refused to answer
☐ unknown ☐ _____

Worksites/school/day care center _____
Occupation/grade

BASIS OF DIAGNOSIS

CLINICAL DATA

Symptomatic ☐ Y ☐ N ☐ ?
first symptoms ___/___/___ (m d y)
first vomit/diarrhea ___/___/___
first V/D time _____ am pm
diarrhea ☐ Y ☐ N ☐ ?
bloody diarrhea ☐ Y ☐ N ☐ ?
Hospitalized ☐ Y ☐ N ☐ ?
Hospital 1 _____
date of admission ___/___/___
date of discharge ___/___/___
Transferred ☐ Y ☐ N ☐ ?
Hospital 2 _____
date of admission ___/___/___
date of discharge ___/___/___
Outcome ☐ survived ☐ died ☐ ?
date of death ___/___/___ (m d y)

PRIVATE LAB DATA

Culture confirmed ☐ Y ☐ N
Specimen collected ___/___/___ (m d y)
☐ stool ☐ urine ☐ blood ☐
Lab _____

PUBLIC HEALTH LAB DATA

Isolate sent to PHL ☐ Y ☐ N ☐ ?
PHL specimenID _____ e.g., G10-1875
Serotype _____
XbaI PFGE _____
BlnI PFGE _____

EPILINKS

At the time of initial report, case appears to be:
☐ sporadic
☐ part of a household cluster
☐ part of a multi-household outbreak OutbreakID _____

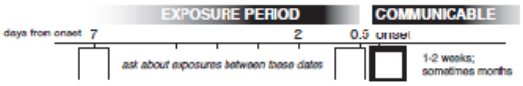
Case appears to be... ☐ primary ☐ secondary (e.g., not first in household)

If a contact of a confirmed or presumptive case, nature of contact:
☐ household ☐ sexual ☐ child care ☐ _____

Specify linked cases; other details as needed.

INFECTION TIMELINE

Enter onset date in heavy box. Count back to figure the probable exposure period.



CASE'S NAME

POSSIBLE SOURCE(S) OF INFECTION DURING EXPOSURE PERIOD

☐ case (or family proxy) could not be interviewed ☐ no risk factors identified ☐ exempt (part of already recognized outbreak, shotgun interview, etc.)

Interviewees ☐ case ☐ parent ☐ physician ☐ other HCP ☐ _____ Interview date(s) _____

Provide ancillary details (names, locations, dates) about possible sources and risk factors checked below.

Y	N	HIGH-RISK FOODS
☐	☐	chicken or turkey consumption*
☐	☐	raw poultry handling by anyone at home*
☐	☐	ground beef* consumption
☐	☐	rare/raw meat
☐	☐	raw or lightly cooked eggs
☐	☐	raw milk**
☐	☐	queso fresco/raw milk cheese**
☐	☐	sprouts (alfalfa, clover, bean, ...)**
☐	☐	venison or other game*
☐	☐	dried meat (salami, jerky, etc.)*
☐	☐	unpasteurized juice/cider**

Y	N	OTHER POTENTIAL SOURCES
☐	☐	food at restaurants
☐	☐	food at other gatherings (potlucks, events)
☐	☐	contact with reptiles or amphibians
☐	☐	contact with baby chicks
☐	☐	other pets, including birds, pocket pets, fish,...
☐	☐	handling pet treats (e.g., dog chews)
☐	☐	livestock, poultry, or farm exposure
☐	☐	animal exhibits (petting zoos, fairs, 4H, etc.)
☐	☐	diaper changing
☐	☐	work exposure to human or animal excreta
☐	☐	exposure to kids in child care settings

Y	N	TRAVEL
☐	☐	outside U.S. to _____
☐	☐	outside Oregon to _____
☐	☐	within Oregon to _____

Provide details about all travel; see Orpheus.

departure ___/___/___

return ___/___/___ (m/d/y)

**Ask about leftovers, including packaging or containers in trash.*

***Collect these leftovers for testing. Contact ACDP epi for details.*

☐ There are no leftovers or packaging that can be tested

TREATMENT Was patient treated with antibiotics or antimotility drugs for this illness? ☐ Y ☐ N ☐ ? *If yes, specify type, dose, and dates given.*

CONTACT MANAGEMENT AND FOLLOW-UP

HOUSEHOLD ROSTER

name	age	sex	occupation	diarrhea Y	diarrhea N	diarrhea ?	onset (m/d/y)	education provided Y	education provided N	education provided ?	comment
				☐	☐	☐	___/___/___	☐	☐	☐	
				☐	☐	☐	___/___/___	☐	☐	☐	
				☐	☐	☐	___/___/___	☐	☐	☐	
				☐	☐	☐	___/___/___	☐	☐	☐	

Does the case know about anyone else with a similar illness? ☐ Y ☐ N *If yes, get contact information, onsets, etc.*

If the case is a food handler, HCW with direct patient contact, or works at or attends daycare, provide details about worksite, job description, dates worked or attended during communicable period (as applicable), supervisor, etc. If the patient attends daycare or nursery school, institute active surveillance.

Contact person/phone number _____

Is the patient in diapers? ☐ Y ☐ N

Are other children or staff ill? ☐ Y ☐ N

SUMMARY OF FOLLOW-UP AND COMMENTS *Provide details as appropriate.*

☐ hygiene education provided ☐ child care restriction ☐ work or school restriction for case ☐ child care inspection ☐ food testing

☐ restaurant evaluation ☐ _____

ADMINISTRATION

Salmonellosis / March 2011

Copy patient's name to the top of this page.

Initial report sent to OPHD or entered into ORPHEUS ___/___/___

Completed by _____ Phone _____ Completed case report ___/___/___