PREVALENCE, CHARACTERIZATION AND ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA SPP*. FROM ORGANIC, KOSHER AND CONVENTIONAL CHICKEN IN RETAIL ESTABLISHMENTS

by

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ABSTRACT

Few have reported the prevalence and antimicrobial susceptibility of *Salmonella* in organic and ethnic meats. The objective of this study was to assess the prevalence and antimicrobial susceptibility of *Salmonella spp* from organic (n=120), kosher (n=107), kosher/organic (n=26), and conventional/all natural (n=120) raw retail chicken samples, as well as to characterize these isolates by serotyping. *Salmonella* was isolated from all sample types. Isolates were confirmed by Polymerase Chain Reaction (PCR). Antimicrobial susceptibility was assessed for all confirmed isolates by the agar disk diffusion method and a total of 151 confirmed strains were serotyped. Overall, prevalence of *Salmonella* was higher in organic samples. However, organic and kosher isolates showed higher antimicrobial susceptibility than isolates from the other sample types. The most prevalent serovars were Kentucky, Enteriditis, Heidelberg, and Typhimurium. Findings from this study suggest an influence of poultry production system in the prevalence and antimicrobial susceptibility of *Salmonella* in raw chicken.

RESUMEN

Existen pocos reportes de la prevalencia y susceptibilidad a antibióticos de *Salmonella* en carnes orgánicas y étnicas. El objetivo de este estudio fue evaluar la prevalencia y susceptibilidad a antibióticos de *Salmonella spp* aislada de pollos crudos orgánicos (n=120), kosher (n=107), convencionales/naturales (n=120) y kosher/orgánicos (n=26), además de identificar las especies de *Salmonella* presentes en estas muestras. *Salmonella* pudo ser aislada de todos los tipos de muestras. Todas las cepas aisladas fueron confirmadas por Reacción de Polymerasa en Cadena. A todas las cepas confirmadas se les determinó susceptibilidad a antibióticos utilizando el método de difusión de disco en agar y un total de 151 cepas fueron identificadas a nivel de serotipo. En general, las muestras orgánicas mostraron mayor prevalencia de *Salmonella*. Sin embargo, las cepas aisladas de muestras orgánicas y de muestras kosher mostraron mayor susceptibilidad a antibióticos que cepas aisladas de los otros tipos de muestras. Los serotipos de *Salmonella* mas prevalentes fueron Kentucky, Enteriditis, Heldelberg y Typhimurium. Los resultados de este estudio sugieren que los diferentes sistemas de producción de pollos influyen en la prevalencia y susceptibilidad a antibióticos de *Salmonella spp* en pollos crudos.

DEDICATION

To God, for the blessing of life.

To my husband and my son, for being the motivation to accomplish all my goals and for supporting me and encourage me during this lengthily process.

To my mother and my brother because they are my fans and have always believed that I can do everything and anything.

To my dad. I know you are watching me from heaven, I hope you feel proud of me.

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List of Acronyms and Symbols

^0C	Degrees Celsius
AM	Ampicillin
AmC	Clavulanic Acid/ Amoxicillin
AN	Amikacin
AST	Antimicrobial Susceptibility Test
BGS	Brilliant Green Agar with Sulfadiozine
BPW	Buffered Peptone Water
C	Chloramphenicol
CDC	Center for Disease Control and Prevention
CF	Cephalotin
CIP	Ciprofloxacin
Con/An	Conventional/All Natural
CRO	Ceftrioxone
DdH_2O	Distilled and Deionized Water
ERS	Economic Research Service

FDA	Food and Drug Administration
FoodNet	Foodborne Diseases Active Surveillance Network
FOX	Cefoxitin
FSIS	Food Safety Inspection Service
G	Grams
GM	Gentamicin
GMP	Good Manufacturing Practices
H_2S	Hydrogen Sulfide
HACCP	Hazard Analysis Critical Control Point
HE+N	Hektoen Agar plus Novobiacin
I	Intermediate Susceptibility
K	Kanamycin
Ks	Kosher
MDR	Multi Drug Resistance
mL	Milliliters
Mm	Millimeters
NA	Nalidixic Acid

NARMS	National Antimicrobial Resistance Monitoring System
NCCLS	National Committee for Clinical Laboratory Standards
NOP	National Organic Program
Og	Organic
Og/Ks	Organic/Kosher
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PPIA	Poultry Products Inspection Act
R	Antimicrobial Resistance
RV	Rapport-Vassiliadis Broth
S	Streptomycin
SU	Antimicrobial Susceptibility
SXT	Sulfamethoxazole-Trimethoprim
TAE	Tris Acetated Buffer
Te	Tetracycline
TSB	Tryptic Soy Broth
TT	Tetrathionate Broth Base, Hajna

US	United States
USDA	United States Department of Agriculture
WHO	World Health Organization
WHOCC	World Health Organization Collaborating Center
XNL	Ceftiofur

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1 INTRODUCTION

Salmonella spp. are facultative gram-negative rods belonging to the family *Enterobacteriaceae*. The genus *Salmonella* is composed of two species. *Salmonella enterica,* which is subdivided in six subspecies, and *Salmonella bongori*. Approximately 2,463 *Salmonella* serotypes have been classified between both species (1). *Salmonella* is one of the leading causes of foodborne illnesses, including salmonellosis and Enteric Typhoid Fever, in the United States (US) and worldwide. A report by the Foodborne Diseases Active Surveillance Network (FoodNet) indicated that *Salmonella* had the highest incidence for infections in 2004 with a total of 6,498 cases of salmonellosis reported (2). According to a report from the Center for Disease Control and Prevention (CDC), where data from several surveillance systems were analyzed, *Salmonella* has an incidence of approximately 1.4 million cases each year (3). The pathogen has the highest mortality rate among foodborne pathogens accounting for 31% of all food-related deaths. *Salmonella* is followed by *Listeria* (28%), *Campylobacter* (5%) and *Escherichia coli* O157:H7 (5%) (3).

Salmonellosis is more common in the summer than winter. The lower incidence of the disease during the winter could be explained by seasonal variations in consumer behavior, such as changes in the types of food that are consumed. However, it is likely that the higher incidence of salmonellosis during the summer is also the result of higher temperatures increasing the incidence of infections in animals and favoring the multiplication of the pathogenic microorganism (4, 5). Young children, the elderly, and the immunocompromised are the most likely to develop severe infections. It is estimated that approximately 600

people die each year with acute salmonellosis in the US (6). Since *Salmonella* is an enteric pathogen that colonizes the intestinal tracts of a variety of animals, including chickens, and turkeys, one of the foods that have contributed most to the incidence of salmonellosis is poultry (7).

The term poultry normally includes chicken, turkey, goose, duck, and guinea fowl, although it is sometimes used to encompass species such as pigeon, ostrich, pheasant, and other game birds (8). Over the last 40 years, the poultry meat industry has undergone considerable expansion, and poultry now accounts for some 27% of the world's meat consumption. Chicken and turkey are the principal types of poultry meat, with chicken comprising two-thirds of the total poultry production and an annual output of more than 33,000 million birds world wide (9). Over the years, there has been continuous expansion and diversification of the chicken industry. Today, as consumers increasingly demand more minimally processed foods, organic and ethnic, like kosher, chicken production is a fast-growing segment of agriculture in the US. A major reason for this growth is the belief that organic and natural products are much more healthful than conventional products.

The main difference between organic and conventional meats is based on production. While animals in conventional farming are exposed to chemical substances, such as mineral fertilizers and pesticides, and fed with supplemented feeds, mostly to enhance and accelerate growth rate, organic farming animals are not exposed to these substances (10). Ethnic meats, on the other hand, refer to the content and production requirements indicating that the product meets certain cultural standard. For example, a kosher meat meets the dietary

requirements of Jewish law (11). The process of koshering removes any blood that may remain in meat by several steps of soaking, salting, draining, and rinsing (12).

Protection of the food supply includes considerations of the microbiological quality and safety of commodities available for public consumption. These concerns often address specific pathogenic microorganisms such as *Salmonella*, which according to CDC is a category B biological agent. A category B agent is relatively easy to disseminate and has moderate morbidity rates and low mortality rates; however, it represents a risk to public health (13, 14). Several recent studies have focused on the assessment of the prevalence of pathogens and their antimicrobial susceptibility in retail meats (16, 17, 18, and 20). However, little is known about the microbiological status of organic and kosher products regarding this assessment. In a study conducted by a group of researchers led by Cui (10), retail organic chickens were more frequently contaminated with *Campylobacter* and *Salmonella* than conventional retail chickens. These pathogens when present in organic animal production were found to be more susceptible to certain antimicrobials. On the other hand, a study conducted by Hajmeer and a group of researchers (15) concluded that the salt applied during koshering has a potential for microbial reduction. Therefore, it appears relevant to compare the prevalence and antimicrobial susceptibility of pathogens such as *Salmonella* in organic, kosher and conventional meats in order to evaluate the microbiological safety of the production methods while maintaining product quality.

In this research project the objectives are to: 1) determine the prevalence of *Salmonella spp* in organic, kosher and conventional chickens purchased from retail establishments in Maryland and Northern Virginia, 2) assess the antimicrobial susceptibility of the isolates

from the organic, kosher and conventional retail chickens using the antimicrobial disk-diffusion susceptibility test (AST) with a profile of 15 antimicrobials, and 3) characterize the isolates by serotyping. The main goal of this research is to assess information about the microbiological safety of retail meats, specifically chickens, in the conventional, organic and ethnic (kosher) local markets of the DC metropolitan area. Ultimately, this study will give preliminary information that will contribute to food safety. Such data is necessary to help meat producers to further improve their product quality and safety and also provide the regulatory agencies with the necessary data to formulate base regulatory policies toward such agricultural products.

2 LITERATURE REVIEW

2.1 Foodborne Disease: An Overview

According to the World Health Organization (WHO) a foodborne disease is a disease caused by or thought to be caused by the consumption of food or water (22). Foodborne illnesses are classified in two main categories, foodborne intoxication and foodborne infections. Foodborne intoxications are caused by toxins produced by organisms which have grown to sufficient numbers in the food product. In general, intoxication is manifested rapidly after consumption of the contaminated food and there is no presence of fever (e.g., *Bacillus cereus*). On the other hand, a foodborne infection occurs as a consequence of growth of the pathogen in the human body. Since an incubation period is usually involved, the time from ingestion until symptoms occur is longer than that of intoxications. The two basic categories of foodborne infections are: invasive infections, which are caused by pathogens that invade bodily tissues and organs (e.g., *Salmonella* and *Campylobacter*) and toxicoinfections, which are caused by infective bacteria that are not considered invasive in nature, but are capable of multiplication or colonization of human intestinal tract and produce toxins (e.g., *Escherichia coli* O157:H7) (19).

Foodborne illnesses have been a major cause of human disease for centuries, yet they remain greatly under-reported and their real incidence is often unknown, because most illnesses go undiagnosed. It is only within the past 25 years that there has been a renewed awareness of their public health importance. In the US, each year foodborne illnesses affect 6 to 80 million individuals (23). According to the Economic Research Service (ERS) of the

United States Department of Agriculture (USDA) in 2000, the cost of foodborne diseases caused by *Campylobacter*, *Salmonella*, *Listeria,* and *E.coli* O157:H7 was estimated in 6.9 billion US dollars (21). The loss caused by these diseases included medical costs, costs for premature deaths, costs for chronic conditions, and productivity losses such as value of forgone or lost wages, among others (21). These bacteria and a group of viruses called caliciviruses, are among the organisms that cause the majority of foodborne diseases today. The most common symptoms of foodborne illnesses include but are not limited to diarrhea, vomiting and abdominal cramps (24). Some factors contributing to the incidence of foodborne diseases are: changes in human demographics and behavior, technology and industry; international travel and commerce; microbial adaptation; economic development and land use; the breakdown of public health measures as well as the deficiency in Good Manufacturing Practices (GMP) and the lack of good handling practices (23).

The epidemiology of foodborne diseases is rapidly changing as emerging pathogens and well-recognized pathogens increase in prevalence or become associated with new food vehicles. New pathogens can emerge because of changing ecology or changing food processing technology that connects a potential pathogen with the food chain (25). In the last decade's microorganisms such as *Escherichia coli* O157:H7 and *Vibrio vulnificus* have been described as foodborne pathogens (26). Other known pathogens have been shown to be predominantly foodborne in the last decade. For example, *Listeria monocytogenes,* also an emerging pathogen, was long known as a veterinary pathogen and also as the cause of meningitis and other invasive infections in immunocompromised hosts. However, how these

hosts became infected remained unknown until a series of investigations identified food as the most common source (27).

Almost all foodborne pathogens exhibit several common characteristics: they have an animal reservoir from which they spread to humans; that is, they are foodborne zoonoses, these zoonoses tend to cause little apparent illness in their animal hosts, acting more like commensal organisms (25). They can rapidly spread globally, and they are becoming increasingly resistant to antimicrobial agents, largely because of the widespread use of antimicrobials in the animal reservoir (26). The pathogenic spectrum of these organisms can change substantially over time. Pathogens for which transmission is well understood may ultimately be controlled. Others may emerge through mutation, or may move into a new niche in the food chain. As a result, the frequency of specific infections can change substantially, reflecting a balance between the ecologies that support bacterial populations that contaminate food and the cultural habits and technologies that limit or prevent that contamination from occurring (25).

Along with emerging pathogens, arrays of new food vehicles of transmission have been implicated in recent years. Traditionally, foods most commonly implicated in foodborne diseases were undercooked meat, poultry, seafood, or unpasteurized milk (26); now, additional foods are considered hazardous. For example different serotypes of *Salmonella* have been associated with contaminated unpasteurized orange juice (28), seed sprouts, cantaloupes (29) and chocolate (30). Because of the explosive increase in international food trade, foods produced and processed in one part of the world are now often consumed by people in countries that may be several thousand miles away. While this has greatly

increased the variety, availability and affordability of various foods in many parts of the world, the worldwide distribution of foods has also increased the occurrence of international foodborne disease outbreaks (31).

The prevention of foodborne diseases depends on careful food production, handling of raw products, and preparation of finished foods. Responsibility for food safety has spread from farming procedures to the final cook or consumer, what is often referred to as "from the farm to the table" or "from the farm to the fork". The entire food industry along with the government is involved in developing food safety plans, surveillance systems and new technologies. In the 1990's Hazard Analysis Critical Control Point programs (HACCP) were implemented by the food industry and federal regulatory agencies. Such programs require food industries to identify points in food production where contamination may occur and target resources toward processes that may reduce or eliminate foodborne hazards (23). More recently, Pulse Net, a national molecular subtyping network for foodborne disease surveillance, was created (31). This network facilitates early recognition of foodborne disease clusters that may represent common source outbreaks. This accelerates the outbreak investigation and source identification and provides independent microbiologic confirmation of the epidemiologically implicated source (31). Finally, novel technologies as composting, vaccination, and treatment with ionizing radiation, high pressure, ultrasound and high intensity light are being tested as control and preventive measures of foodborne illnesses (25) in the twenty first century.

2.2 *Salmonella spp.*

2.2.1 Characteristics and Taxonomy

Salmonella spp. are facultative anaerobic Gram negative rods within the family *Enterobacteriaceae*. Members of this genus are motile by peritrichous flagellation except for *Salmonella enterica* subsp. *enterica* serotype Gallinarium, and *Salmonella enterica* subsp. *enterica* serotype Pullorum, which lack flagella (32). Salmonellae grow optimally at 35^0C to 37^0C, and catabolize a variety of carbohydrates into acids and gas. This genus can use citrate as the sole carbon source. These organisms are chemoorganotrophic, oxidase negative and catalase positive and the majority produce hydrogen sulfide (H_2S) (33). There are currently 2,463 serotypes (serovars) of *Salmonella* (1). According to the CDC and the World Health Organization Collaborating Center (WHOCC), the genus *Salmonella* contains two species*: S. enterica* and *S. bongori*. *S. enterica* is divided into six subspecies: *enterica, salamae, arizonae, diarizonae, houtenae* and *indica.* All these subspecies are differentiated biochemically and are divided into different serotypes based on genomic relatedness (1).

2.2.2 Sources of Contamination: Foods

The ubiquity of *Salmonella spp* in the natural environment contributes significantly to the prominence of the bacteria. Principal sources of contamination, as described below, are food commodities such as meat, eggs, shellfish, milk, fruits, and vegetables.

- Organisms of the genus *Salmonella spp*. are found in the intestinal tracts of a variety of animals. Farm animals such as pigs, poultry, cattle, and sheep are carriers of extreme concern to the food industry, because generally they are

asymptomatic carriers. These animals usually pass veterinary slaughterhouse inspection without restrictions. During slaughter however, intestinal material, often containing *Salmonella* bacteria, pollute the surface of carcasses, which in later stages can lead to extensive contamination of meat and meat products (34). A study conducted by Olsen J.E et al., (35) concluded that the slaughter of *Salmonella* positive chickens led to contamination of the processing line, and standard cleaning procedures did not eliminate the contamination in the majority of the cases. Moreover, carcasses can be contaminated due to environmental factors surrounding the animals such as infected manure and contaminated feeds (36), among others. A study conducted by a group of researchers led by Slader J. (37) concluded that contaminated crates could lead to contamination of birds during transport from the farm to the processing plant. In 2002, a Belgian study concluded that feeds given to chickens in broiler houses substantially contribute to spreading of *Salmonella* contamination within a flock (38)

- The presence of *Salmonella* on farms and slaughterhouses leads to contamination of meats in further steps of the food chain such as distribution and retail marketing (20). In 2001 White et al., (18) concluded that *Salmonella* strains were common in retail ground beef from the greater Washington D.C. area, being more prevalent in ground poultry than in any other type of meat. In another study, Zhao et al., (20) found that retail chickens had the highest rate of *Salmonella* contamination compared to turkey, pork and beef. C. Domínguez et al., (39) isolated seven different *Salmonella* serotypes from chicken meats coming from

different retail stores in Spain. The most abundant serotype isolated was *Salmonella* subspecies *enterica* serovar Enteriditis, which agreed with the fact that this serotype was associated with outbreaks in humans in Spain in 1999. USDA Food Safety and Inspection Service (FSIS) reported that commercial chickens processed from 2000 to 2003 showed a *Salmonella* prevalence rate of 9.1 to 12.8% (40)

- Eggs and egg-containing foods are the primary vehicles of *S. enterica* serovar Enteriditis infection, having been implicated in 80% of the known sources of *S. enterica* serovar Enteriditis outbreaks reported to CDC from 1985 to 1999 (41,42). Eggs may be contaminated internally in the ovaries and oviducts of infected hens due to the special facility of *S. enterica* serovar Enteriditis to cause prolonged infection of the avian reproductive tract and its ability to localize in glandular parts of the reproductive tract, such as the magnum, isthmus, and ovarian granulose cells (39, 43). Eggs may be contaminated externally through fecal material (39). Although the aqueous sanitation of shell eggs at 41^0C and pH $\geq$ 10.0 reduces external surface contamination, the practice disrupts the structural integrity of the outer protective cuticle and facilitates penetration of Salmonellae through exposed pores in the egg shell (33). Cross-contamination with *Salmonella* in the egg-packing plants is also a significant contributory factor to the external contamination of shell eggs (44).

- Raw fish and shellfish are often sources of *Salmonella* contamination. Although marine waters generally are considered to be free of *Salmonella* spp, discharges of

urban, agricultural, and rural sewage may represent sources of permanent contamination in this environment (45). Occasional storm-generated discharges may transport the pathogen from their natural reservoirs to the seawater, contaminating the organisms that grow in it and feed through filtration of surrounding water by retention of suspended organic material (45). Other sources of contamination in the fish industry may be poor personal hygiene among non-specialized workers, and repeated manual handling of raw products during the harvesting and packaging operations (33). In a recent study conducted by Brands et al., (46) pathogenic serotypes of *Salmonella* were isolated from oysters harvested in all three U.S. coasts. The oysters sampled were harvested from waters approved for shellfish harvesting, intended for consumers, and sold by shellfish vendors.

- Raw milk is the principal reservoir of *Salmonella spp* in the dairy industry. Contamination arises mainly from inadequately sanitized milking equipment and poor disinfection of teat skin prior to milking (33). Intramammary infections associated with subclinical mastitis also could lead to intermittent shedding of the bacteria in raw milk (33). A multistate outbreak of *Salmonella enterica* subspecies *enterica* serotype Typhimurium in 2003 was linked to the consumption of raw milk from a dairy in Ohio where raw milk was sold by the glass or in milkshakes (47). Although pasteurization, or heat treatment of milk, contributes dramatically to reduce the number of pathogens in milk, outbreaks associated with pasteurized milk continue to occur. These outbreaks can be caused by inadequate

pasteurization or by improper handling of the product after the heat treatment, the latter being the most common cause (48).

- *Salmonella* spp. have been isolated from a wide variety of fresh produce, including alfalfa sprouts, cabbage, cantaloupe, cauliflower, lettuce, spinach, strawberries, tomatoes, and watermelons (49). The factors that contribute to contamination of fresh produce can include spray or canal irrigation of growing fields with contaminated water, fertilization of farmlands with untreated animal wastes, contamination of crops by infected wildlife, rinsing or cooling of harvested products with contaminated water and multiple manual handling during harvesting, processing and packaging (33). In recent years, many outbreaks of *Salmonella* spp have been linked to produce especially, cantaloupes and tomatoes (50, 51).

2.2.3 Antimicrobial resistance

Antimicrobials are naturally occurring, semi-synthetic or synthetic compounds with antimicrobial activity that can be administered orally, parentally, or topically (52). The use of antimicrobials in different fields, such as human and veterinary medicine, agriculture, and as growth promoting agents for food animals has created enormous pressure during the past decades for the selection of antimicrobial resistance among bacterial pathogens, including *Salmonella spp*. (53, 54). According to the infectious-disease report that was released by WHO in 2000, such organisms have become increasingly prevalent worldwide (18). These organisms are mainly transferred to humans through the food chain.

Two conditions are required prior to the development of antimicrobial resistance in an organism. First the organism must come into contact with the antimicrobial. Then, resistance against the agent must develop, along with a mechanism to transfer the resistance to daughter organisms or directly to other bacteria of the same or different species (54). The most common biochemical mechanisms of antimicrobial resistance in bacteria are the following: 1) reduced uptake of the antimicrobial into the cell, 2) active efflux of the drug from the cell, 3) modification of the target site to eliminate or reduce binding of antimicrobial, 4) hydrolysis of the substance, and, 5) sequestration of the antimicrobial by addition of chemical groups (55).

Bacteria have evolved diverse mechanisms to transmit resistance traits. Genetic traits for antimicrobial resistance are coded for in two places in the organism: the chromosomes and mobile genetic elements. Usually a mutation can cause chromosomal genes that normally code for antimicrobial sensitivity to start coding for resistance (54). Mobile genetic elements including plasmids, transposons, and genes cassettes in integrons are efficient routes of acquisition of antimicrobial resistance (53).

There are a large number of genes conferring antimicrobial resistance in *Salmonella* isolates from humans, animals and environmental sources. Many of the resistance genes are located on multi-drug resistant (MDR) plasmids or other integrating elements and thus can be co-transferred and co-selected easily even in the absence of a direct selective pressure. Resistance genes for tetracyclines have been found in plasmids in serovars Enteriditis, Typhimurium, Choleraesuis, Dublin and Typhi (56). Chloramphenicol resistance genes have been found in plasmids or gene cassettes on a plasmid in serovars Typhi, Pullorum,

Enteriditis, Typhimurium, Agona, and Choleraesuis. Other genes are located in the chromosomal DNA of the bacteria. For example gene *aacCA5* located in chromosomal DNA has been associated with the resistance to gentamicin in *S. enterica* serovar Kentucky. In the majority of the cases because more than one gene are involved in conferring the antimicrobial resistance capacity to the bacteria, these genes can be contained in different locations in different serovars. For example, gene *dfrA1* is associated with resistance to trimethoprim in serovar Enteriditis, being located in a gene cassette on plasmid 6/9 in this serovar. Gene *dfrA10* is associated to the same substance but it is located in the chromosomal DNA in serovar Agona (56).

The study of the antimicrobial resistance of *Salmonella spp* is a priority to the food industry mainly because most infections with antimicrobial resistant *Salmonella* are acquired by eating contaminated foods, especially foods of animal origins (18). White et al., (18) isolated 13 different serotypes of *Salmonella spp* from retail ground meats in the Washington D.C. area. They found that 84% of their isolates were resistant to at least one antimicrobial including ceftriaxone, the drug of choice for treating salmonellosis in children. In an Irish study (57) 9 different serovars of *Salmonella* were isolated from local raw retail chickens and imported chicken portions. Among the isolates, the most resistant to antimicrobials was *Salmonella enterica* serovar Typhimurium definitive type DT104, which can cause severe illness. The isolate was resistant to ampicillin, co-amoxiclav, sulfonamide, chloramphenicol, tetracycline, trimethoprim and streptomycin. In both studies all isolates were susceptible to ciprofloxacin. This substance is part of the fluoroquinolones, which are the drugs of choice for treatment of invasive gastrointestinal infections in many parts of the world (58).

However, fluoroquinolone-resistant strains of *Salmonella spp* have been isolated and are becoming a great concern for public health (59). A Portuguese study isolated 10 different serotypes of *Salmonella* from retail chickens where 50% of the isolates were resistant to nalidixic acid (a quinolone) and enrofloxacin (a fluoroquinolone) (60). The first outbreak of fluoroquinolone- resistant *Salmonella* infection in the US was reported in 2001, this outbreak was caused by *S. enterica* serotype *Schwarzengrund* (61). The US Food and Drug Administration (FDA) withdrew on September 12, 2005 its approval for use of fluoroquinolones in treating poultry (62). This action was based on the determination that the use of fluoroquinolones in poultry promotes the development of fluoroquinolone-resistant *Campylobacter* that can be transferred to humans and become a hazard to human health (63).

In the US, The National Antimicrobial Resistance Monitoring System (NARMS) for Enteric Bacteria was established in 1996 as a collaboration between CDC, FDA, and USDA to monitor antimicrobial resistance among foodborne enteric bacteria isolated from humans, foods and animals (64). Its latest published report (2003) states that among retail meats, 27 serotypes of *Salmonella* were found. Among these, serovars Heidelberg, Saint Paul and Typhimurium were the most common. It was also found that these serotypes isolated were more resistant to streptomycin and tetracycline than any other antimicrobial (64).

2.2.4 Salmonellosis

Salmonellosis is the medical term use to describe the illness caused by *Salmonella spp.* The clinical presentation of human salmonellosis may correspond to either the enteric fever syndrome following infection with typhoid or paratyphoid strains or the nontyphoid-

dependent gastroenteritis. In typhoid infections clinical symptoms appear 7 to 28 days following exposure to the pathogens. Watery diarrhea, fever, abdominal pain, headache, nausea, prostration, and a rash of rose spots on the shoulders, thorax, or abdomen may occur. Complications may include intestinal bleeding from ileal ulcers or intestinal perforation. The symptoms of nontyphoid salmonellosis may include nausea, abdominal cramps, diahrrea, fever of short duration, and vomiting that appear 8 to 72 hours after exposure to the bacterium. In both types of infections the sympotomatology and isolation of the organism from blood or stool samples confirms the illness (33).

Most salmonellosis infections are contracted through contaminated food or water, and although it is very rare, direct person to person transmission can occur. In healthy individuals 10^6-10^9 organisms are needed to cause symptomatic illness. Most *Salmonella* cells are killed by the low pH of the stomach, but those that persist, target the colon and small intestine as their portal of entry into the host. The surviving bacteria then direct their internalization by the epithelial cells (both enterocytes and M cells) lining the intestine. *Salmonella* entry into cells is mediated by the ability of this organism to induce its own uptake into epithelial cells, which are not normally phagocytic (65). *Salmonella. enterica* serovar Typhi passes through the intestinal epithelial barrier to gain access to the lymphoid follicles, where the bacteria eventually spread to the bloodstream and more distant organs such as the liver and spleen. Nonthyphoidal *Salmonella* remain primarily at the level of the intestinal epithelium and sub mucosa (except during bacteremia), where they elicit an acute inflammatory response (65).

The treatment of patients with salmonellosis depends on the severity of the illness and the special circumstances of the patient. Antimicrobial therapy is not routinely recommended for the empiric treatment of mild to moderate presumed or proven *Salmonella* infections in healthy individuals (65, 66). Antimicrobial therapy should be initiated for those who are severely ill and for patients with risk factors for extraintestinal spread of infection. Antimicrobials may also be useful when rapid interruption of fecal shedding is needed to control outbreaks and it is always necessary for the treatment of bacteremia, enteric fever and focal infections such as meningitis, septic arthritis, osteomyelitis and pneumonia (66). In case of focal infections further treatments such as surgery can be necessary. Antimicrobials recommended for *Salmonella* infection treatment include fluoroquinolones (e.g.ciprofloxacin) and third-generation cephalosporins (e.g. ceftriaxone) (66). However, as discussed earlier, the emergence of the fluoroquinolones and cephalosporins resistant strains must be carefully considered in choosing antimicrobials for the treatment of salmonellosis.

After appropriate blood and fecal cultures are obtained, usually, 3-7 days of treatment are reasonable for a non-complicated *Salmonella* gastroenteritis. In the case of bacteremia usually a single bactericidial antibiotic drug is prescribed for treatment for 10-14 days. In life threatening infections both kind of drugs, a third generation cephalosporin and a fluoroquinolone are used in patients, until the susceptibilities of antimicrobial agents are known for the culture (66).

Salmonellosis is a very important medical issue to the food industry. According to the latest report of FoodNet a total of 6,498 *Salmonella* infections were reported in 2004, making salmonellosis the foodborne disease with the highest incidence with 14.61 cases per 100,000

population, and more important, having a very high incidence among children under the age of one, with 121.57 cases per 100,000. Of all the *Salmonella* cases reported in 2004, 5% were identified as being outbreak related. Of the outbreak- associated *Salmonella* cases 78% were food-related. Although these numbers do not appear to be significant it is almost certain that thousands of infections went without diagnosis or treatment and consequently were not reported to authorities and surveillance systems.

2.3 Conventional Poultry

2.3.1 Characteristics of the industry

The term poultry normally includes chicken, turkey, duck, goose, and guinea fowl, although it is sometimes used to encompass game birds. Chicken and turkey are the principal types of poultry meat (9). Over the last 40 years, the poultry meat industry has undergone considerable expansion, and poultry now accounts for some 27% of the world's meat consumption. Poultry production is one of the most technologically advanced sectors of agriculture. In many of the larger companies around the world, the operation is partly or completely integrated and a single organization may own breeding farms, hatcheries, feed mills, growing units, and primary and further processing plants (8).

2.3.2 Production

The stages in a typical conventional chicken production chain are as follow: lines of elite stocks are developed, this are family groups of in-bred lines carrying certain desirable genetic traits, for example body conformation and meat yield. Then grandparent stocks are

bred from the best of elite lines to provide parent birds, which produce the chicks that will be reared for meat production. Finally those chicks reared for meat production are called broilers; they are slaughtered at about six week of age or less according to weight requirements. Both breeders and broilers are normally kept on litter (wood shavings or straw) in housing of 20,000 birds where the environmental temperature and ventilation rate are controlled. Elite and grandparent flocks are kept separately but also indoors, with extremely regulated environmental conditions. The flocks are usually fed with a mixture of grains, proteins sources such as meats and bones, minerals and vitamins supplements to prevent any nutrient deficiencies and some sub-therapeutic doses of antimicrobials in order to prevent diseases and promote growth (8). However, USDA regulations do not allow the administration of exogenous steroidal compounds (hormones) in raising poultry (40).

Chicken are then brought to the processing plants in trucks that are especially designated to hold large numbers of birds in crates or modules that can be readily unloaded. The birds are transferred individually to a continuously moving system of shackles, on which they are hung manually by the legs. The birds proceed usually to an electrical stunning machine that either renders them unconscious or actually kills them. This is followed by the bleeding stage, in which the neck is partially severed. After bleeding, the birds are scalded by immersion in hot water in order to loosen the feathers. Feathers are removed from the birds via a series of on-line plucking machines. The next stage is usually to remove the head, which is done by an automatic head puller, and then the feet by means of an automatic feet cutter. Next, the birds are transferred to the evisceration line, where both edible and inedible viscera are removed. During and after the evisceration stages, carcasses are spray washed to

remove any visible spots of blood or feces and then they are chilled. Finally carcasses are weighted, graded and packaged prior to freezing or chill storage, or they may be transferred to a portioning operation (8).

2.3.3 Antimicrobial use

Antimicrobials are used in the poultry industry for therapy, control, prevention, and growth promotion. According to the National Committee for Clinical Laboratory Standards (NCCLS) therapy is the administration of an antimicrobial to an animal or groups of animals, which exhibit frank clinical disease. Control is the administration of an antimicrobial to animals, usually as a herd or a flock, in which morbidity and/or mortality have exceeded baseline norms. Prevention is the administration of an antimicrobial to exposed healthy animals considered to be at risk, but before expected onset of disease and for which no aetiological agent has been yet cultured. Growth promotion is the administration of an antimicrobial, usually as a feed additive, over a period of time, to growing animals that results in improved physiological performance (52). In the US about half of all antimicrobials produced are use for animal husbandry. Only 10% of all these drugs are given to treat infection diseases in animals; the rest are given to promote growth or prevent disease (54), which is likely to cause resistance in the normal and pathogenic intestinal flora and to increase prevalence of the organisms.

The growth promoting effects of antimicrobials in birds were first discovered in the 1940's when chickens fed by-products of tetracycline fermentation were found to grow faster than those that were not fed those by-products. Since then, many antimicrobials have been found to improve average daily weight gain and feed efficiency in livestock in a variety of

applications (52). Whereas the precise mechanisms of growth promoting effects are often unknown, it is known that some growth-promoting effects are mediated through alterations of the normal intestinal microbiota resulting in more efficient digestion of feed and metabolism of nutrients, other effects are mediated through pathogen and disease suppression and immune system release (52). The most common antimicrobials given to poultry as therapy for an infection or, in absence of disease, for sub therapeutic purposes such as growth promotion and enhanced feed efficiency are: penicillins, tetracyclines, cephalosporins, avoparcin, bambermycins, sulphonamides and streptogramins (52, 68).

It is thought that the indiscriminate use of antimicrobials in agriculture, including poultry production is the major contributor to the antimicrobial resistance of pathogens such as *Salmonella spp*. These pathogens acquire their resistance in the food-animal host before onward transmission to humans through the food chain (67). According to Threlfall E.J. (67) the use of antimicrobials in food animals has been a major factor in the development of decreased susceptibility to antimicrobials in zoonotically transmitted Salmonellae. As reported earlier, several antimicrobial-resistant *Salmonella* strains have been isolated from poultry worldwide.

2.4 Organic Poultry

2.4.1 Characteristics of the organic industry

According to the Organic Foods Production Act of 1990 and the National Organic Program (NOP) of the USDA, an organic production is defined as “a production system that

is managed in accordance with the Act and regulations in this part to respond to site-specific conditions by integrating cultural, biological, and mechanical practices that foster cycling of resources, promote ecological balance, and conserve biodiversity" (69). Organic farming systems rely on ecologically based practices such as cultural and biological pest management, and virtually excludes the use of synthetic chemicals in crop production and prohibits the use of antimicrobials and hormones, bioengineering or ionizing radiation in livestock production (40, 69). The term "organic" must not been confused with the term "All Natural" described by the USDA as when no artificial ingredients or pesticides are added at the processing plant (40).

Although organic farming was a small market sector until recently, it became one of the fastest growing segments of US and European agriculture during the 1990's (70). One of the major reasons for the arising interest in organic products lately, is the growing belief that organic and natural products are more nutritious and healthier than conventional products. This perception is mainly due to the principles associated with organic food production, such as the absence of synthetic chemicals and a number of other environmentally sound techniques (70, 71). According to Sundrum A. (72) the basic standards of organic farming provide suitable tools to minimize environmental pollution and nutrient losses at the farm level. This is caused by the emphasis in the use of renewable resources and the conservation of soil and water to enhance environmental quality for future generations (40).

Organic foods are currently sold through three main venues in the US: natural foods stores, conventional grocery stores and direct-to-consumer markets. In 2000, more organic food was purchased in conventional supermarkets than in any other venue (73). The growing

consumer demand for organic products has been manifested in the market in many ways, for example according to USDA estimates, U.S. certified organic cropland doubled between 1992 and 1997, to 1.3 million acres (73). The top selling organic category today is fresh produce, followed by nondairy beverages, breads and grains, packaged foods and dairy products. Organic meat and poultry markets have lagged behind those for crops partly because it was not possible to label these products as organic until February 1999, when USDA approved a provisional label. Food crops and other foods are regulated by the FDA, which allowed food packages to carry an organic label throughout the 1990's (73).

The labeling requirements under the national organic standards apply to raw, fresh, and processed agricultural products that contain organic ingredients and are based on the percentage of organic ingredients in a product. Agricultural products labeled "100% organic" must contain (excluding salt and water) only organically produced ingredients. Products labeled "organic" must consist of at least 95% organically produced ingredients. Products labeled "made with organic ingredients" must contain at least 70% organic ingredients. Products with less than 70% organic ingredients cannot use the term organic anywhere on the principal display panel but may identify the specific ingredients that are organically produced on the ingredients statement on the information panel. The USDA organic seal—the words "USDA Organic" inside a circle—may be used on agricultural products that are "100 % organic" or "organic". Before a product can be labeled "organic" a government-approved certifier inspects the farm where the food is grown to verify that the farmer is following all the rules necessary to meet USDA organic standard. A civil penalty of up to $10,000 per violation can be levied on any person who knowingly sells or labels as

organic a product that is not produced and handled in accordance with these regulations (40, 73).

2.4.2 Production

Organically produced poultry, as defined by the USDA, are retail products made from flocks raised under organic management (73). The chicks have to be raised as organic at least since the second day of life. Some farmers purchase chicks from a certified organic hatchery while others begin raising the chicks organically when they arrive to the farm. The animals are not given growth-promoting antimicrobials. However, they receive preventive care, such as vaccines, and dietary supplements of vitamins and minerals (73). When preventive practices and veterinary biologics are inadequate to prevent sickness, a producer may administer synthetic medications, provided that such medications are allowed under NOP (74). Medical treatment should not be withheld from a sick animal in order to preserve its organic status. The animals consume 100% organically produced grain-based feed, free of animal byproducts and synthetic amino acids and are slaughtered after approximately 11 weeks. Producers must provide living conditions that accommodate the health and natural behavior of the animals. The animals should have access to the outdoors, shade, exercise areas, fresh air, and direct sunlight during the months when it is feasible (73).

The processing of the chickens is also regulated by the USDA, for example, organically produced chickens are processed in certified organic plants (73). The main difference between the processing of conventional and organic poultry are the use of chemicals. The processing of organic chickens should be chemical-free. No unapproved cleaning agents or pest control substances are allowed to be used, according to NOP. In the case where a

processing plant processed both conventional and organic chicken, measures have to be taken in order to maintain the integrity of the organic product. The organic animals should be isolated in a separate pen labeled "organic animals". These animals should be slaughtered first to ensure that equipment is free of remnants from processing conventional animals. The carcasses have to be identified as "organic". Finally, the plant must maintain records, tracked by tag numbers of incoming animals and of all organic slaughter activities. When carcasses are ready to be cut up or further processed, these activities are typically conducted first before other meat is cut up when equipment, knives and other tools are clean, and non-organic meat is not present (74).

2.4.3 Microbiological Assessment

The USDA makes no claims that organically produced food is safer than conventionally produced food, only that organic food differs from conventionally produced food in the way it is grown, handled, and processed (40). However, many consumers perceive organic products as safer than conventional foods (10, 70, 71). According to researchers there is no sufficient scientific evidence to support the claim that organic foods are safer than conventional foods (70, 71). Other researchers, on the other hand, have postulated that organic livestock production is not designed to reduce pathogen loads in food animals and that in fact, the production of organic meats involves potentially higher microbiological safety risks due to raising animals outdoors and the prohibition of antimicrobial use (10, 72). For example, a study conducted by the USDA in 2005 concluded that *Salmonella* was more prevalent in chickens raised outdoors than in chickens from commercial indoor poultry farms (40). One possible explanation is that the outside

environment presents enhanced risk of exposure to wild birds, insects, rodent droppings and other potential carriers of *Salmonella.* In a study conducted by Cui et al. in 2005 the prevalence of *Salmonella* spp and *Campylobacter* spp was assessed in organic and conventional retail chickens (10). They found that organic chickens were more frequently contaminated with the pathogens than the conventional chickens but that the pathogens from organic animal production were more susceptible to certain antimicrobials. It is believed that this susceptibility is the result of the lack of antimicrobial use in the production process of organic chicken (75). However, a Danish study in 2001 found more prevalence of *Campylobacter* in organic chickens than in conventional chickens, the study also found a low level of antimicrobial resistance among both rearing systems failing to relate the resistance pattern and the origin of the isolates (76). It is apparent that more studies are needed to determine the difference in risks of pathogen contamination between conventional and organic poultry as well as the status of organic poultry in the susceptibility or resistance to antimicrobials.

2.5 Kosher meats

"Kosher" is the word that describes the foods that meet the standards of Jewish law. Contrary to popular perception, rabbis or other religious authorities do not bless food to make it Kosher. A series of rules have to be taken in order to produce a kosher food (12).

According to the Jewish law, the only land mammals that can be eaten are those that have cloven hooves and that chew its cud. Any land mammals that do not have either of these qualities are forbidden, for example, the pig, the camel and the hare. Animals that are

permitted are sheep, cattle, goats and deer. Birds of prey or scavengers are forbidden, chicken, geese, ducks and turkeys are permitted. Fin fish such as salmon and tuna are permitted but all shellfish are forbidden. No rodents, reptiles, amphibians, or insects are allowed. Any product derived from these forbidden animals, such as their milk, eggs, fat, or organs, also cannot be eaten (12). The mammals and birds that may be eaten must be slaughtered in accordance with Jewish law. Animals that died from natural causes or that were killed by other animals can not be eaten, in addition, the animal must have no disease or flaws in the organs at the time of slaughter, however, these restrictions do not apply to fish (12).

Ritual slaughter is known as "shechitah", and the person who performs the slaughter is called a "shochet", usually a well-trained man in Jewish law. The method of slaughter is a quick, deep stroke across the throat with a perfectly sharp blade with no nicks or unevenness. This method is painless and causes unconsciousness within two seconds (12). The blood has to be drained from all mammals and birds because according to the Torah (the entire body of Jewish teachings) the consumption of blood is prohibited. The first step of this process occurs during slaughtering, after that, all the remaining blood must be removed, by soaking in water, salting and rinsing. The soaking of the animal is in cold water (8 to 12^0C) for 30 minutes, then the carcasses are salted massively with coarse salt all over their surfaces to allow the blood to drip out, finally the carcasses are rinsed with cold water (77). This final process must be completed within 72 hours after slaughter, and before the meat is chilled, frozen or ground (12). Other rules that are essential for the koshering process are 1) certain parts of animals that are permitted cannot be eaten, for example the sciatic nerve 2) meat

(birds and mammals) cannot be eaten with dairy. 3) utensils have to be kosher 4) the consumption of wines and other grape products made by non-Jews are prohibited. (12).

Products that have been certified as kosher are labeled with a mark called a “hekhsher” that ordinarily identifies the rabbi or organization that certified the product. The process of certification does not involve "blessing" the food, rather, it involves examining the ingredients used to make the food, examining the process by which the food is prepared, and periodically inspecting the processing facilities to make sure that kosher standards are maintained (12).

The USDA also regulates kosher meats, for example poultry is regulated under the Religious Exemption for the Slaughter and Processing of Poultry of the FSIS. The Poultry Products Inspection Act (PPIA) exempts establishments slaughtering or processing poultry or poultry products in accordance with religious dietary laws, from specific provisions of the PPIA. However, FSIS still verifies the establishment’s compliance with the pathogen reduction, sanitation, and HACCP regulations that are applicable (78). The USDA also regulates the labeling of kosher meats; the word kosher may be used only on the labels of meat and poultry products prepared under rabbinical supervision (12).

It is estimated that currently there are about 10 million kosher consumers in the United States, including both Jewish and non-Jewish kosher observers and other consumers attracted by the perceived higher quality of kosher foods (12). Two of the more critical operations that differentiate the production of conventional and kosher birds and that can affect the microbial quality of the end product are the water temperature in the defeathering process and the practice of applying salt in the koshering process. Scald temperatures for the kosher process

cannot exceed ambient water temperature, since higher temperatures may coagulate blood (77,80). According to Clouser et. al the kosher scald temperatures of ~8^0C for the defeathering of birds can cause more cross contamination of *Salmonella* spp than the scald temperature of ~58.6^{o}C for conventional processes. This is explained by skin surface changes occurring during the defeathering process; while conventional defeathering results in a smooth skin surface to which bacteria are loosely attached the kosher defeathering results in a rough skin surface where the bacteria become entrapped or embedded (80). The cross contamination of kosher poultry with *Listeria monocytogenes* is even more likely to persist once it happens. In another study conducted also by Clouser et al. higher levels of *Listeria* from kosher chilled poultry in comparison with conventional chilled poultry where explained by the fact that the pathogen is tolerant to both salt and cold (81).

Salt exerts a drying effect, reducing water activity in foods, and historically have been widely used for food preservation (82). The effect of salt applied during the koshering process on the reduction of microbial growth has been studied. In 1987 a study found that no *Campylobacter jejuni* organisms were isolated after koshering process in a New York processing plant, attributing the results to the sensitivity of *C. jejuni* to salt (83). In 1999 Hajmeer et al. reported reductions in *Salmonella* counts from beef briskets after koshering, concluding that salt application during koshering of meat might reduce or inhibit the microbial growth of pathogens due to associated changes in water activity and ionic strength (15). The use of antimicrobial treatments in kosher meat facilities is limited, mainly due to religious restrictions pertaining to the method of treatment application and acceptability of compounds to be used for meat treatment (84). Zuckerman and Abraham demonstrated that

Nisin and Microgard (MIC^{TM}), a bacteriocin mixture against Gram- positive and Gram-negative bacteria, could be used to improve the microbiological quality of kosher chilled chickens (77). On the other hand, Hajmeer et al. reported that the acidified sodium chloride reduced counts of *E. coli* O157:H7 and *Staphylococcus aureus* when applied to koshered beef briskets (15). There is a lack of scientific data regarding the microbiological safety of kosher meats, therefore, more research on the contamination patterns is needed and its results may lead to safer kosher foods.

3 MATERIALS AND METHODS

3.1 Collection of Samples/ Isolate Recovery

A total of 373 chicken samples, including 12 control samples, (120 organic (Og), 120 conventional/all natural (Con/An), 107 kosher (Ks) and 26 organic/kosher (Og/Ks)) were randomly purchased from different supermarkets and specialty stores in the Baltimore-DC metro area and the Northern Virginia-DC metro area, in 12 different sampling periods between December 2005 and July 2006. On the day of purchase all samples were assigned a number and product information[1] was recorded for each one. A picture of each sample was taken (example shown in Figure 1), and then all samples were stored at 4^{O}C in a walk in cooler (Nor-Lake Scientific, USA) until the next day when they were processed.

Figure 1. Example of Chicken Samples

[1] Product Information: product name, brand, weight, store name, location, sell by date and if frozen or fresh

3.1.1 Recovery and Isolation of *Salmonella spp*.

Each sample was removed from the package using aseptic techniques and transferred to a sterile filter bag (3500mL Lab blender Interscience). One sample was assigned as the control. The control was inoculated with 200 microliters (μL) of 10^{-7}dilution of *Salmonella enterica* serotype Typhimurium strain ISSAGFP. This strain can be easily identified and differentiated in the presence of other strains by fluorescence when excited with UV light (86). This sample was processed in the same way as the other samples.

A total of 150 milliliters (mL) of Buffered Peptone Water (BPW) (Difco/BD, USA) full strength was added to each bag to rinse the samples. After constant shaking for 2 minutes, 25mL of the rinse liquid was transferred to a Whirlpack bag (18oz Nasco, USA) and another 75mL of BPW were added. Each bag was sealed and incubated for 24h at 37^{O}C (Refrigerated Incubator, Model 3710, Forma Scientific, USA). After the incubation period, each Whirlpack bag was homogenized and 1mL of the liquid was transferred to 10mL of Tetrathionate Broth Base, Hajna (TT) broth (Difco/BD, USA) and 0.1mL was transferred to 10mL of Rappaport-Vassiliadis R10 (RV) broth (Difco/BD, USA). Both broths were incubated for 24h at 42^{O}C (Low temperature incubator, Fisher Scientific, USA). A loopfull of each incubated broth was streaked in Hektoen Agar (Difco/BD, USA) plus novobiocin (HE+N) (sodium salt HPLC grade, Sigma Scientific, USA) plates and in Brilliant Green agar w/ Sulfadiazine (BGS) (Acumedia, USA). The plates were incubated for 24h at 37^{o}C. After incubation, typical isolated colonies of *Salmonella* were selected from each plate and re-streaked in HE+N plates to assure purity of the culture. After purity was confirmed, an isolated colony was chosen and transferred to 10mL of Tryptic Soy Broth (TSB) (Difco/BD,

USA); the culture was incubated for 24h at 37°C. After incubation 1.6mL of the culture was mixed with 0.4mL of sterile glycerol (enzyme grade, Fisher, USA) in a cryogenic vial (2.0mL, Nalgene Cryoware ™ Nalge, USA), assigned an isolate number and subsequently stored at -80°C in an ultra-low temperature freezer (Harris, Kendro Laboratories Products, USA).

3.1.2 Confirmation of *Salmonella spp* Isolates

An overnight culture of the isolates was grown in TSB at 37°C, then 10µL of the overnight culture was mixed with 90µL of sterile distilled and deionized water (ddH2O) in a 96 well microplate (Nunc™, Denmark) A PCR master mix, enough for the number of isolates to be tested (x + 1), was prepared:

	for 1 RXN	for X RXNs
Qiagen 10x buffer (Qiagen, USA)	2.5 ul	2.5 (x+1) ul
ddH2O	17.6 ul	
10mM dNTP (Qiagen, USA)	0.2 ul	
Primer mix (0.5nM) (IDT Tech,USA)	2.5 ul	
HotStarTaq (Qiagen, USA)	0.2 ul	
Total	23 ul	23 (x+1) ul

The primers used were:

XN420	AGCCAACCATTGCTAAATTGGCGCA
XN421	GGTAGAATTCCCAGCGGGTACTG
XN422	GTGAAATAATCGCCACGTCGGGCAA
XN423	TCATCGCACCGTCAAAGGAACC

The primer mix working solution was made by mixing each primer in a 1/50 dilution with sterile ddH2O. Two microliters of the diluted overnight culture was mixed with 23µL of the PCR master mix in a 96 well PCR microplate (Biorad, USA). The plate was sealed

and the PCR was run in a thermocycler (Mastercycler, Eppendorf, USA). The PCR program was:

1) One cycle of 10°C for 10 minutes

2) Ten cycles of 95°C for 1 minute then 65°C for 2 minutes

3) Seven cycles of 95°C for 2 minute then 64°C for 2 minutes[2]

4) Fifteen Cycles of 95°C for 0.5 minutes then 57°C for 0.5 minutes and 72°C for 1 minute

5) One cycle of 72°C for 3 min

6) Decrease temperature to 4°C and hold

A 1.5% agarose gel was prepared by mixing 1.5 grams (g) of agarose (1000 Invitrogen, USA) in 100mL of 1X Tris Acetate buffer (TAE) (Sigma, USA) in a microwave for 2 minutes. Twenty microliters of Ethidium bromide (Sigma, USA) was added to the melted mix and the gel was poured and allowed to solidify. After the PCR was done, 7.5µL of loading buffer (24% glycerol in Tris- EDTA with bromophenol blue) (sigma, USA) was added to the PCR reaction. Twenty microliters of the reaction were loaded to the gel; in the first lane 20µL of the standard was also loaded. The gel was run in 1X TAE buffer at 100 volts for 45 minutes. The bands were visualized using a Kodak Gel Logic 200 imaging system. The standard preparation was as followed:

1) Lambda DNA Hind III digest (Biolabs, USA)	100µL
2) Loading buffer	100µL
3) IDTE (IDT Tech, USA)	300µL

[2] This temperature decreases by 1° C each cycle, so it is 58°C in the 7th cycle.

3.2 Antimicrobial Susceptibility Test (AST)

The AST was performed according to the Performance Standards for AST of the Clinical and Laboratory Standards Institute (85). An overnight culture of each isolate was grown in TSB at 37^{o}C. One mL of each culture was diluted in 9mL of 0.01 M Phosphate Buffered Saline (PBS) (Sigma, USA) to visually match the 0.5 McFarland equivalence turbidity standard.

A lawn of the diluted bacteria was spread in Muller Hinton plates (Hardy Diagnostics, USA) with a cotton swab, then the antimicrobial disks (BD, USA) were dispensed into each plate with a disk dispenser (BD, USA) and the plates were incubated at 35^{o}C (Low temperature incubator, Fisher Scientific, USA) for 18h. After incubation the diameter of the halos around each disk were measured (mm) and recorded. The isolates were tested along with control isolates of *Escherichia coli* ATCC 25922 to monitor accuracy and quality control. The antimicrobial disks used are listed in Table I.

Table I. List of Antimicrobial Disks Used

Antimicrobial name	Abbreviation	Doses (µg/disk)
Ceftiofur	XNL	30
Streptomycin	S	10
Kanamycin	K	30
Cephalotin	CF	30
Chloramphenicol	C	30
Sulfamethoxazole-trimethoprim	SXT	1.25/23.75
Ampicillin	AM	10
Tetracycline	Te	30
Ceftriaxone	CRO	30
Gentamicin	GM	10
Nalidixic acid	NA	30
Cefoxitin	FOX	30
Amikacin	AN	30
Ciprofloxacin	CIP	5
Clavulanic acid/ Amoxicillin	AmC	20/10

3.3 Serotyping

A total of one hundred fifty one isolates were sent to the National Veterinary Services Laboratories of the USDA in Ames, Iowa and to the Wisconsin Veterinary Diagnostic Laboratory at University of Wisconsin, Madison to perform the serotyping following standard methods (87).

3.4 Statistical Analysis

A Chi-square fitted logistic model was used to determine statistically significant differences in the prevalence of *Salmonella spp* between chicken samples, this was followed by a multiple comparison based on the same model; this analysis permitted to determine what

types of samples were significantly different from each other. A Poisson regression and Chi-square goodness-of-fit model was used to determine significant differences among the average number of isolates by sample type. Afterwards, a multiple comparison based on the same model was carried out to determine what type of samples presented significant differences in their average number of isolates. Finally, a multinomial logistic model was used to determine differences in the distribution of the number of isolates by sample type (88). All analyses were performed using the statistical software SAS V9.1., Cory, NC (89).

4 RESULTS

4.1 Isolate Recovery

A total of 373 samples were processed in this study (Og= 120, Con/An = 120, K= 107, and Og/Ks = 26). *Salmonella* species were recuperated from only 102 samples (27.3% of all samples) (Og= 54, Con/An = 27, Ks = 17, and Og/Ks = 4). However, a total of 315 *Salmonella spp* isolates were recuperated and confirmed first via phenotypical means and then by PCR (a picture of one of the electrophoresis gels is shown in Figure 2). This was because in some cases more than one isolate was recuperated from each sample. When positive growth was observed in TT (enrichment broth) - HE+N (selective media), TT - BGS plates (selective media) and in RV (enrichment broth)- HE+N and RV- BGS plates of the same sample, each positive plate was considered and recorded as a separate isolate; thus, it was possible to obtain up to 4 different isolates from each sample.

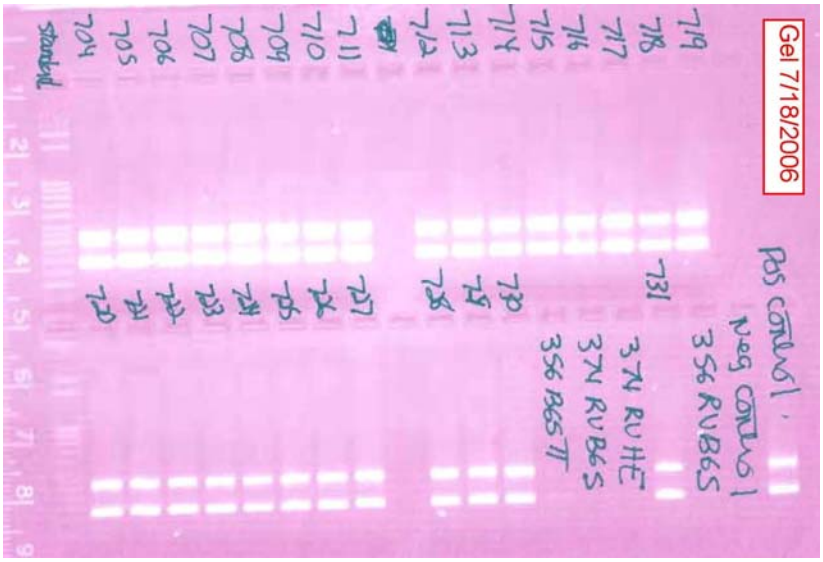

Figure 2 Picture of Electrophoresis (PCR Confirmed *Salmonella spp* Isolates)

Organic samples presented the highest Salmonella *spp* prevalence; 45.4% (54/120) of all organic processed samples presented at least one isolate. This was followed by Con/An samples; 24.8% (27/120) of all Con/An samples were contaminated with *Salmonella spp.* Kosher samples only presented a 15.9% (17/107) prevalence of the bacteria. *Salmonella* was least prevalent in Og/Ks samples; only 15.4% (4/26) of these samples showed presence of *Salmonella spp* isolates. In order to determine whether these differences in prevalence were statistically significant or not, a logistic model was fitted. The response variable in the model was the binary variable defined as presence/absence of *Salmonella spp* (one or more isolates), and the type of chicken was considered as a covariate in the model. After checking the goodness-of-fit of the model, it was possible to determine that statistically significant differences existed among the prevalence of *Salmonella spp* by types of chicken (Chi-square 26.42, Degree of Freedom = 3, P-value <0.0001). Table II shows the 95% confidence intervals for prevalence of *Salmonella spp* by type of chicken, based on the model aforementioned.

Table II. Prevalence of *Salmonella spp* by Type of Chicken

		95% Confidence Interval*		
TYPE	**Prevalence**	**Lower bound**	**Upper bound**	**Sample size**
CONVENTIONAL/ALL NAT.	24.8%	17.6%	33.7%	120
KOSHER	15.9%	10.1%	24.1%	107
ORGANIC/KOSHER	15.4%	6.0%	34.5%	26
ORGANIC	45.4%	36.7%	54.4%	120
			Total	373

*Based on a logistic model

A multiple comparison among the prevalence of *Salmonella spp* by type of chicken was carried out based on the same logistic fitted model. This analysis permitted to determine what types of chicken were significantly different from each other. The results confirmed that the prevalence of *Salmonella spp* in Og chickens was significantly different from the other three types of chicken (Figure 2), such as indicated by the p-values of the comparisons (Table III). For the other types of chicken, there was no statistical evidence of significant differences among them.

Table III. Summary of Significant Differences Among the Prevalence of Contamination by Type of Chickens.
(p-values presented between parenthesis)

	CONVENTIONAL/ ALL NAT.	KOSHER	ORGANIC/ KOSHER	ORGANIC
CONVENTIONAL/ALL NAT.				
KOSHER	No *(0.1074)*			
ORGANIC/KOSHER	No *(0.3118)*	No (0.9497)		
ORGANIC	**Yes *(0.0013)***	**Yes *(0.0001)***	**Yes *(0.0081)***	

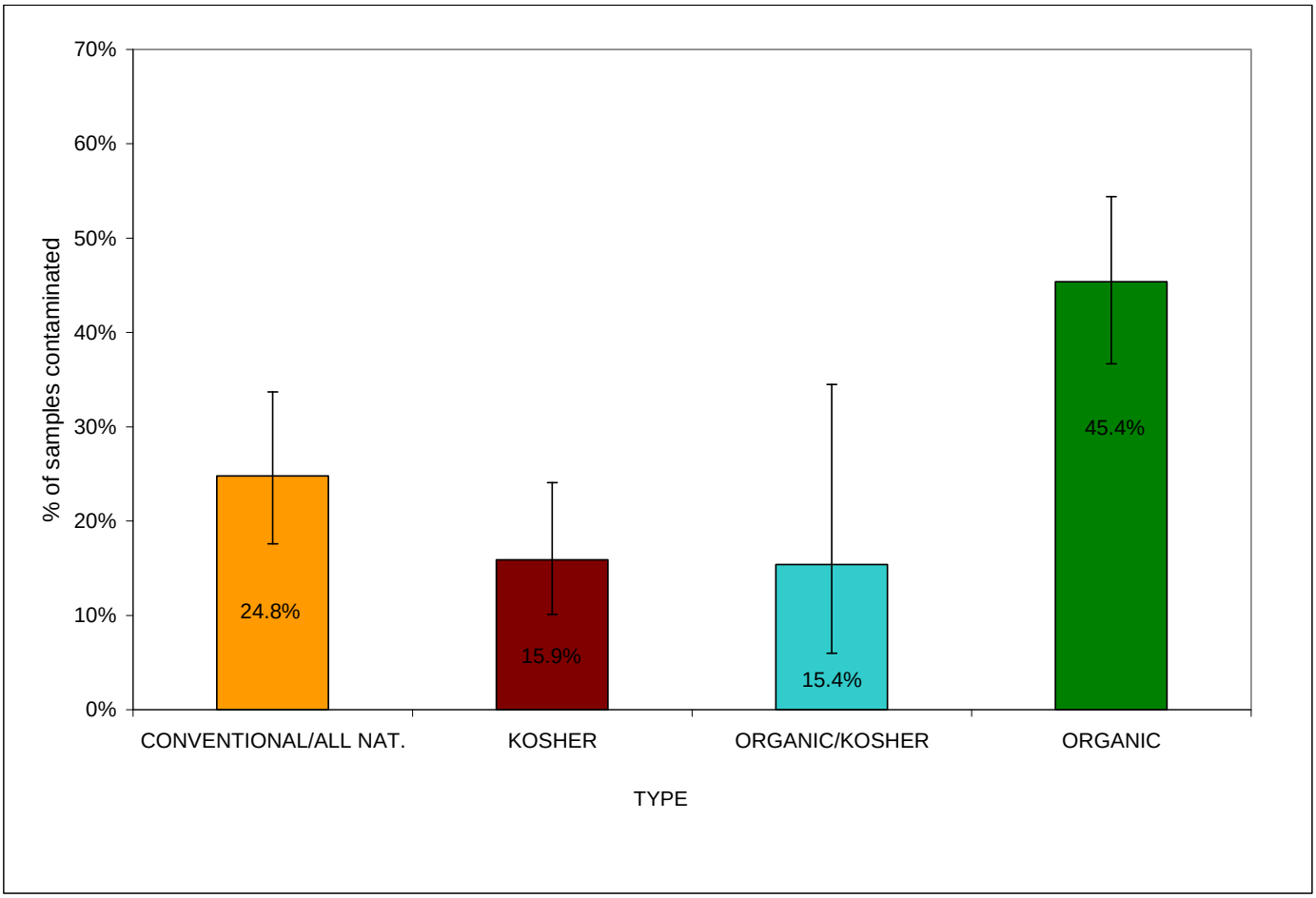

Figure 3. Prevalence of *Salmonella spp* and 95% Confidence Intervals by Type of Chicken

From all isolates recuperated (n=315), 170 isolates were from Og samples, 92 isolates were recuperated from Con/An samples, 43 isolates belonged to Ks samples and only 10 isolates came from Og/Ks samples. Table IV presents the distribution of number of isolates by type of chicken. Likewise, the percentages of this distribution is presented in Figure 3.

Table IV. Distribution of Number of Isolates by Chicken Type

	Number of Isolates				
TYPE	*0**	*1*	*2*	*3*	*4*
CONVENTIONAL/ ALL NAT.	*82*	*2*	*2*	*6*	*17*
KOSHER	*90*	*4*	*5*	*3*	*5*
ORGANIC/KOSHER	*22*	*2*	*0*	*0*	*2*
ORGANIC	*65*	*8*	*6*	*10*	*30*

* without isolates from control samples

A second objective of this research project consisted in determining if the average number of isolates by type of chicken presented significant differences. For this analysis, the response variable considered was the count of isolates by sample among the different types of chicken. In a first approach to this objective, a Poisson regression was used to determine whether there were significant differences among the average number of isolates by chicken type. The goodness-of –fit of the model was adequate and permitted to conclude that the average number of isolates presented significant differences by type of chicken (Chi-square 36.48, Degrees of Freedom = 3, p-value < 0.0001).

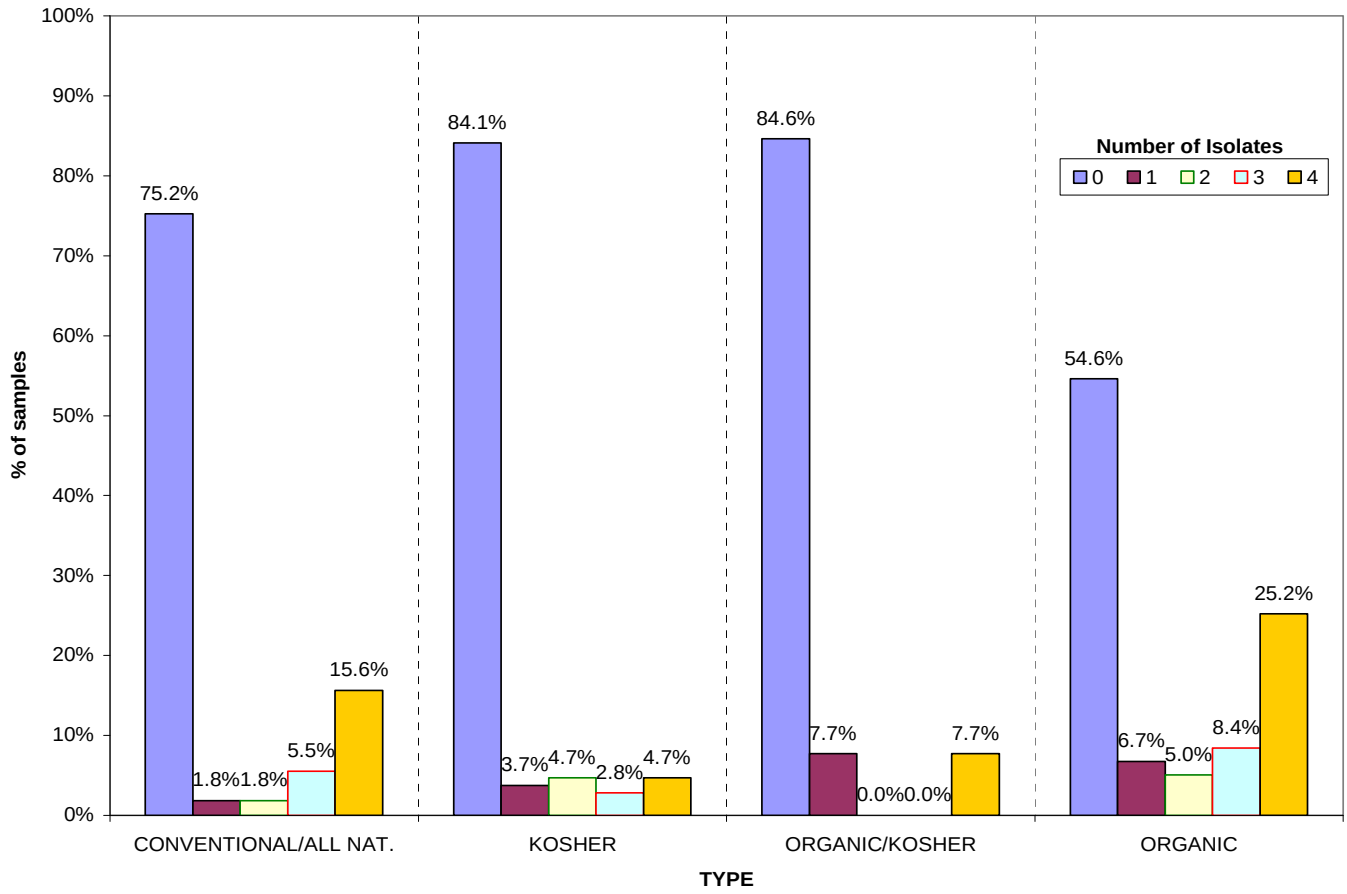

Figure 4. Percentages of the Distribution of Number of Isolates by Chicken Type

The sample mean and standard deviation of the number of isolates is shown in Table V. The 95% confidence intervals for the average number of isolates by type of chicken based on Poisson regression are shown in Table VI.

Table V. Sample Mean and Standard Deviation of the Number of Isolates

	Number of Isolates	
TYPE	Average	Standard Deviation
CONVENTIONAL/ALL NAT.	0.8440	1.5466
KOSHER	0.4019	1.0358
ORGANIC/KOSHER	0.3846	1.0983
ORGANIC	1.4286	1.7446

Table VI. 95% Confidence Intervals of the Average Number of Isolates

TYPE	Lower bound	Upper bound
CONVENTIONAL/ALL NAT.	0.5986	1.1901
KOSHER	0.2719	0.5938
ORGANIC/KOSHER	0.1730	0.8552
ORGANIC	1.0456	1.9519

Following the same analysis scheme, a multiple comparison among the average number of isolates by type of chicken was carried out (Table VII). The average number of isolates between Con/An and Ks samples was significantly different (p-value 0.0062). Likewise, the average number of isolates for Og samples was statistically different from the other types of samples. This last result coincides with the results found for the prevalence of *Salmonella spp.* by type of sample. It is worth to mention that although Og/Ks chickens presented a similar prevalence of *Salmonella spp* to Ks chickens at the sample level, the average number of isolates for Og/Ks was not significantly different from Con/An chickens (unlike Ks isolates) due in part to the small sample size of Og/Ks (n=26).

Table VII. Summary of Significant Differences Among the Average Number of Isolates by Type of Chicken.
(p-values are presented between parenthesis)

	CONVENTIONAL/ ALL NAT.	KOSHER	ORGANIC/ KOSHER	ORGANIC
CONVENTIONAL/ALL NAT.				
KOSHER	**Yes** ***(0.0062)***			
ORGANIC/KOSHER	No *(0.1075)*	No *(0.9321)*		
ORGANIC	**Yes** ***(0.0056)***	**Yes** ***(<0.0001)***	**Yes** ***(0.0060)***	

Finally, as a complement to the previous analysis, it was possible to estimate the probability of getting a number of isolates equal or greater to a certain value based on the type of chicken using a multinomial logistic model. This approach permitted to determine the differences in the distribution of the number of isolates by type of chicken instead of the differences in the distribution of the average number of isolates by type of chicken. For this model, the goodness-to-fit was adequate, therefore, the cumulative probabilities associated with the number of isolates by type of chicken presented statistically significant differences (Chi-square 27.03, Degrees of Freedom = 3, p-value <0.0001). The probabilities estimated by using this model are shown in Figure 4. As an example, the estimated probability of getting two or more isolates in a Con/An sample was 21.4%. this probability was lower than the probability of getting two or more isolates in an Og sample (39.0%). However, the probability of getting two or more isolates in a Con/An sample (21.4%) was greater than the probability of getting two or more isolates in a Ks sample (12.5%) or in a Og/Ks sample

(12.2%). This analysis confirms the previous results about significant differences among the average number of isolates by type of chicken.

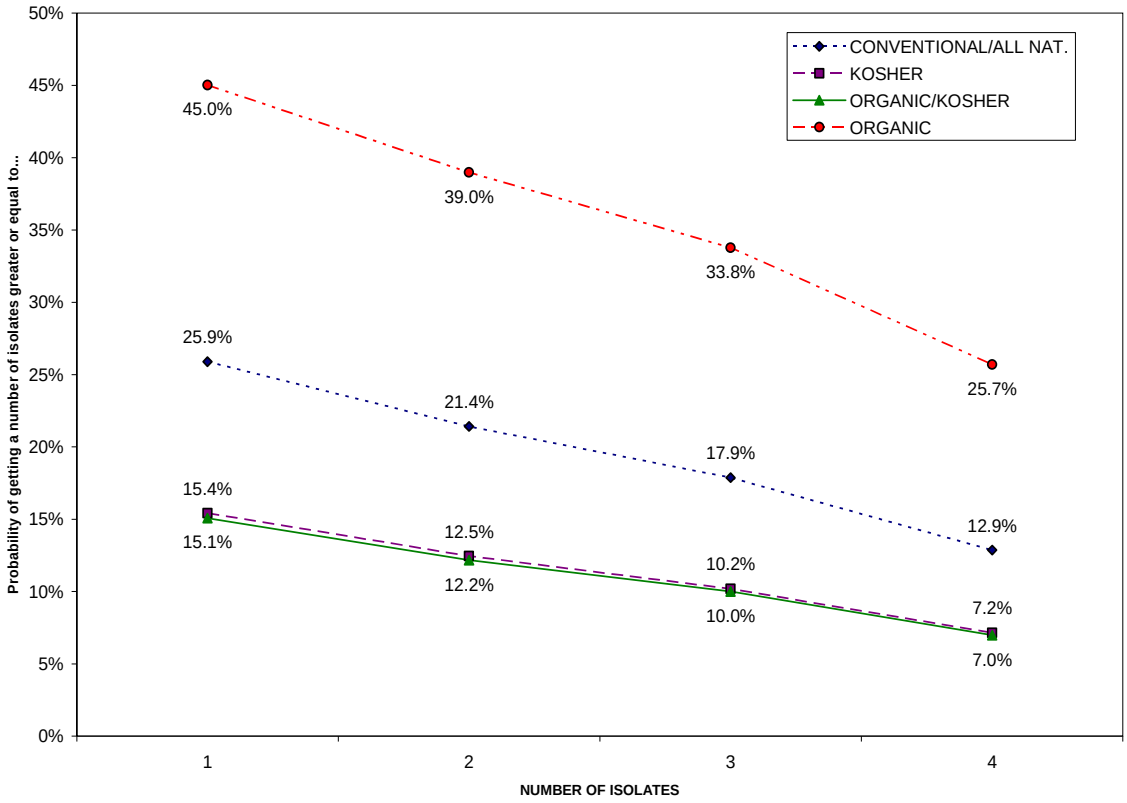

Figure 5. Estimated Probability of Getting a Number of Isolates Equal or Greater to a Certain Value by Chicken Type Based on a Multinomial Logistic Model.

4.2 Antimicrobial Susceptibility Test

Antimicrobial Susceptibility Test was performed for 315 *Salmonella spp* isolates (Og = 170, Ks = 43, Og/Ks = 10, and Con/An = 92). Table XII (page 52) presents a summary of the percentages of antimicrobial susceptibility (SU), intermediate susceptibility (I), and resistance (R) for all isolates by sample type. All percentages were rounded to the nearest whole number for simplicity. Complete antimicrobial profiles for isolates of all sample kinds are presented in Appendixes A, B, C, and D. Detailed results for isolates of each sample kind are presented in the following subsections.

4.2.1 AST for Og Isolates

The complete antimicrobial profiles for all 170 *Salmonella spp* isolates from Og samples is presented in Appendix D. Overall, the highest rates of resistance were observed for S (54%) and Te (51%), while most expressive intermediate susceptibility was observed in S (9%) and CRO (3%). The highest rates of susceptibility were observed for C (100%), AN (100%), CIP (100%), K (98%) and GM (98%).

In general, 54 (32%) of all Og isolates were susceptible to all antimicrobials profiled. One hundred and two (60%) of the isolates were resistant to at least one antimicrobial and multiple resistance[3] was presented in 9 (5%) of the isolates. Among the 9 isolates that showed multiple resistances, 3 were resistant to 7 antimicrobials and 1 was resistant to 8 antimicrobials.

[3] Define as resistance to five or more antimicrobials

Four repeated resistance patterns were identified for the isolates. These patterns are summarized in Table VIII. Pattern B was the most common with 77 isolates followed by patterns A and D (3 isolates). The least common pattern was C with 2 isolates.

Table VIII. Repeated Antimicrobial Resistance Patterns of *Salmonella spp* for Og Samples

Pattern	Phenotype	Number of Isolates for Each Pattern
A	XNL	3
B	S-Te	77
C	XNL-S-CF-AM-FOX-AMC	2
D	XNL-S-CF-AM-Te-FOX-AMC	3

4.2.2 AST for Ks Isolates

The complete antimicrobial profiles for all 43 *Salmonella spp* isolates from Ks samples is presented in Appendix A. Overall, the highest rates of resistance were observed for Te (40%) and S (23%), while most expressive intermediate susceptibility was observed in AmC (12%), SXT (7%) and S (7%). The highest rates of susceptibility were observed for CIP (100%), AN (98%), GM (98%), and K, C, and NA with 95% each.

In general, 23 (53%) of all Ks isolates were susceptible to all antimicrobials profiled. Eighteen (42%) of the isolates were resistant to at least one antimicrobial and multiple resistance was presented in 5 (12%) of the isolates. Among the 5 isolates that showed multiple resistances, 1 was resistant to 7 antimicrobials.

Two repeated resistance patterns were identified for the isolates. These patterns are summarized in Table IX. Pattern A was the most common with 5 isolates followed by patterns B with 2 isolates

Table IX. Repeated Antimicrobial Resistance Patterns of *Salmonella spp* for Ks Samples

Pattern	Phenotype	Number of Isolates for Each Pattern
A	S-Te	5
B	K-AM-Te	2

4.2.3 AST for Og/Ks Isolates

The complete antimicrobial profiles for all 10 *Salmonella spp* isolates from Og/Ks samples is presented in Appendix C. Overall, the highest rates of resistance were observed for Te (80%) and S (50%), while none of the isolates showed intermediate susceptibility to any of the antimicrobials profiled. All isolates (100%) were susceptible to K, C, XNL, AM, CRO, GM, NA, FOX, AN, CIP, and AmC.

In general, none of the isolates were susceptible to all antimicrobials profiled; all of them were resistant to at least one antimicrobial. However, multiple resistance was not shown by any of the isolates.

Only two repeated resistance patterns were identified for the isolates. These patterns are summarized in Table X. Pattern A was the most common with 4 isolates followed by pattern B with 3 isolates.

Table X. Repeated Antimicrobial Resistance Patterns of *Salmonella spp* for Og/Ks Samples

Pattern	Phenotype	Number of Isolates for Each Pattern
A	S-Te	4
B	SXT-Te	3

4.2.4 AST for Con/An Isolates

The complete antimicrobial profiles for all 92 *Salmonella spp* isolates from Con/An samples is presented in Appendix B. Overall, the highest rates of resistance were observed for Te (57%), CF (37%), and XNL and FOX with 34% each, while most expressive intermediate susceptibility was observed for CRO (29%) and AmC (5%). The highest rates of susceptibility were observed for CIP (99 %), NA (99%) and AN (99%).

In general, 25 (27%) of all Con/An isolates were susceptible to all antimicrobials profiled. Sixty six (72%) of the isolates were resistant to at least one antimicrobial and multiple resistance was presented in 31 (34%) of the isolates. Among the 31 isolates that showed multiple resistances, 9 were resistant to 7 antimicrobials.

Ten repeated resistance patterns were identified for the isolates. These patterns are summarized in Table XI. Pattern H was the most common with 15 isolates followed by pattern D with 14 isolates. The least common patterns were pattern B and F with 2 isolates each.

Table XI. Repeated Antimicrobial Resistance Patterns of *Salmonella spp* for Con/An Samples

Pattern	Phenotype	Number of Isolates for Each Pattern
A	Te	6
B	S-CF	2
C	S-GM	3
D	S-Te	14
E	S-Te-GM	4
F	CF-AM-Te-FOX-AmC	2
G	XNL-CF-AM-FOX-AmC	3
H	XNL-CF-AM-Te-FOX-AmC	15
I	XNL-CF-AM-Te-CRO-FOX-AmC	4
J	XNL-S-CF-AM-Te-FOX-AmC	5

Table XII. Summary of Antimicrobial Susceptibility (Su), Intermediate Susceptibility (I) and Resistance (R) of *Salmonella spp* Isolates for All Sample Types.

Antimicrobial Agent	Samples (number of Isolates for each sample)											
	KS (43)			Og (170)			Og/Ks (10)			Con/An (92)		
	SU n(%)	I n(%)	R n(%)	SU n(%)	I n(%)	R n(%)	SU n(%)	I n(%)	R n(%)	SU n(%)	I n(%)	R n(%)
XNL	40 (93)	0 (0)	3 (7)	155 (91)	0 (0)	15 (9)	10 (100)	0 (0)	0 (0)	59 (64)	2 (2)	31 (34)
S	30 (70)	3 (7)	10(23)	62 (36)	16 (9)	92 (54)	5 (50)	0 (0)	5 (50)	61 (66)	1 (1)	30 (33)
K	41 (95)	0 (0)	2 (5)	166 (98)	2 (1)	2 (1)	10 (100)	0 (0)	0 (0)	88 (96)	4 (4)	0 (0)
CF	38 (88)	0 (0)	5 (12)	157 (92)	5 (3)	8 (5)	9 (90)	0 (0)	1 (10)	58 (63)	0 (0)	34 (37)
C	41 (95)	0 (0)	2 (5)	170 (100)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	89 (97)	2 (2)	1 (1)
SXT	40 (93)	3 (7)	0 (0)	157 (92)	3 (2)	10 (6)	7 (70)	0 (0)	3 (30)	90 (98)	2 (2)	0 (0)
AM	35 (81)	1 (2)	7 (16)	158 (93)	3 (2)	9 (5)	10 (100)	0 (0)	0 (0)	57 (62)	2 (2)	33 (36)
TE	25 (58)	1 (2)	17 (40)	82 (48)	1 (0.6)	87 (51)	2 (20)	0 (0)	8 (80)	40 (43)	0 (0)	52 (57)
CRO	39 (90)	2 (5)	2 (5)	154 (90)	6 (3)	10 (6)	10 (100)	0 (0)	0 (0)	59 (64)	27 (29)	6 (7)
GM	42 (98)	1 (2)	0 (0)	167 (98)	3 (2)	0 (0)	10 (100)	0 (0)	0 (0)	84 (91)	0 (0)	8 (9)
NA	41 (95)	1 (2)	1 (2)	164 (96)	5 (3)	1 (0.6)	10 (100)	0 (0)	0 (0)	91 (99)	1 (1)	0 (0)
FOX	40 (93)	0 (0)	3 (7)	160 (94)	0 (0)	10 (6)	10 (100)	0 (0)	0 (0)	59 (64)	2 (2)	31 (34)
AN	42 (98)	1 (2)	0 (0)	170 (100)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	91 (99)	0 (0)	1 (1)
CIP	43 (100)	0 (0)	0 (0)	170 (100)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)	91 (99)	1 (1)	0 (0)
AMC	35 (81)	5 (12)	3 (7)	156 (92)	4 (2)	10(6)	10 (100)	0 (0)	0 (0)	62 (67)	5 (5)	25 (27)

4.3 Serotyping

As shown in Table XIII a total of 7 serotypes were found among the 143 isolates for which serotype identification was possible. Eight other isolates were characterized by an incomplete serotype for a total of 151 isolates to which serotyping was performed. It is worth to mention that all serotypes identified belonged to the specie *Salmonella enterica* sub specie *enterica*.

As shown in Figure 5 the majority of the isolates characterized were described as serotype Kentucky (50%). Enteriditis was the second most prevalent serotype representing 17% of all isolates characterized. Serotypes Heidelberg and Thyphimurium represented 13% and 11% of the isolates respectively, while serotypes Infantis (2%), Montevideo (1%) and Senftenberg (1%) were the least prevalent. Five percent of the isolates were characterized by an incomplete serotype. Seventy-nine characterized isolates were obtained from Og samples. As shown in Table XIII 36 isolates were obtained from Ks samples followed by thirty-one isolates from Con/An samples. Only five characterized isolates were obtained from Og/Ks samples. Figure 6 presents the distribution of *Salmonella* serotypes within the different types of samples. Serotype Kentucky was the only serotype identified in all four-sample types. Kentucky was also the most prevalent serotype identified from isolates obtained from Og samples. Enteriditis was the serotype most prevalent in isolates obtained from Con/An and Og/Ks samples. In isolates obtained form Ks samples the serotype most prevalent was Thyphimurium.

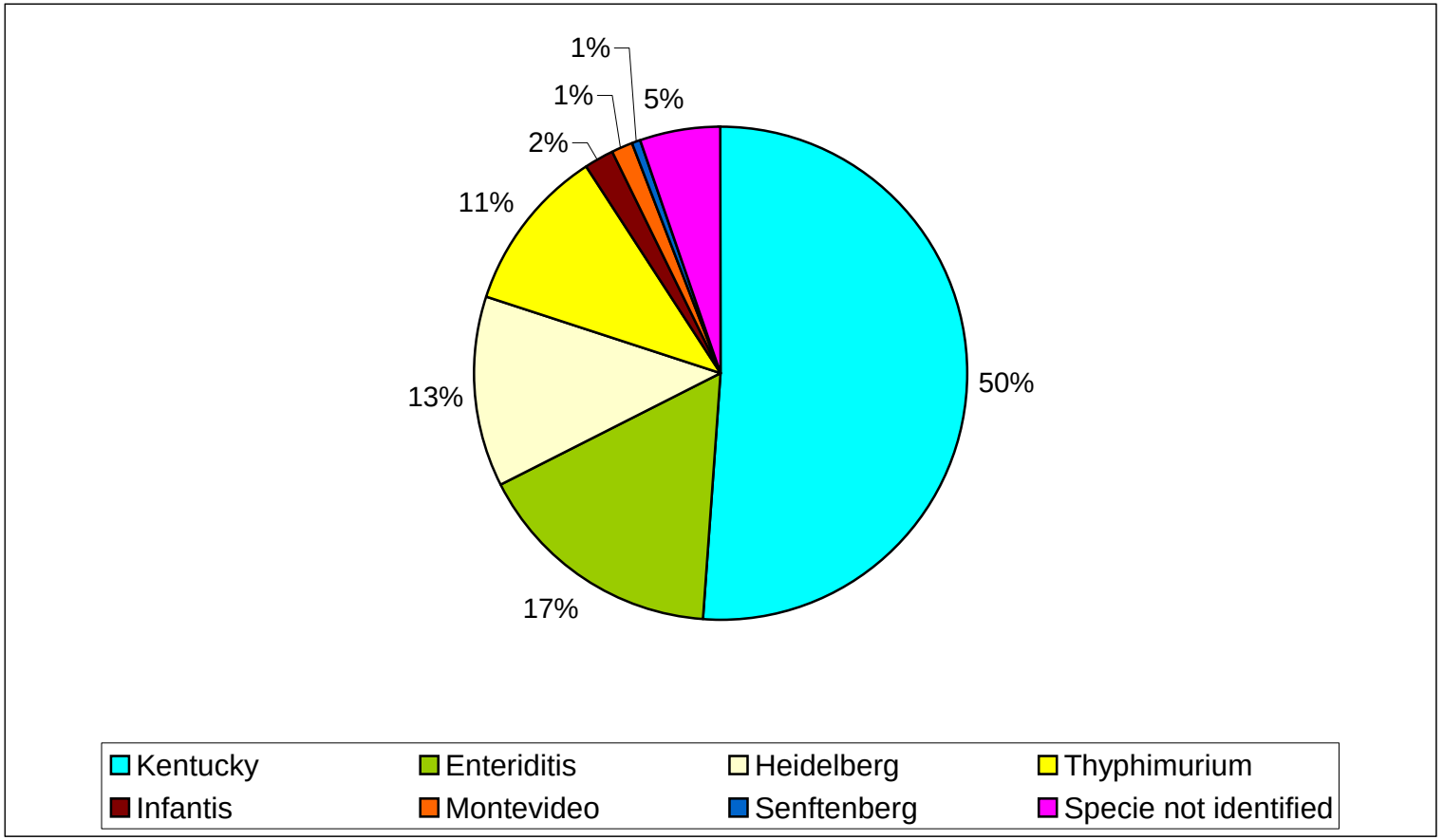

Figure 6. Percentages of *Salmonella* Serotypes Identified from PCR Confirmed Chicken Isolates.

Table XIII. Distribution of *Salmonella spp* Isolates within the Different Types of Samples

Salmonella Serotypes (n)	No. Of Isolates (n=151) Chicken Samples			
	Con/An n(%)*	Og n(%)*	Ks n(%)*	Og/Ks n(%)*
Kentucky (77)	10 (13)	51 (66.2)	12 (15.6)	4 (5.2)
Enteriditis (25)	14 (56)	10 (40)	1 (4)	0 (0)
Heidelberg (19)	0 (0)	9 (47.4)	9 (47.4)	1 (5.2)
Thyphimurium (16)	2 (12.5)	1 (6.3)	13 (81.2)	0 (0)
Infantis (3)	2 (66.6)	1 (33.3)	0 (0)	0 (0)
Montevideo (2)	0 (0)	2 (100)	0 (0)	0 (0)
Senftenberg (1)	0 (0)	0 (0)	1 (100)	0 (0)
Serovar not identified (8)	3 (37.5)	5 (62.5)	0 (0)	0 (0)
Total (n)	31	79	36	5

* Percentages are based on the individual total number of isolates for each serovar.

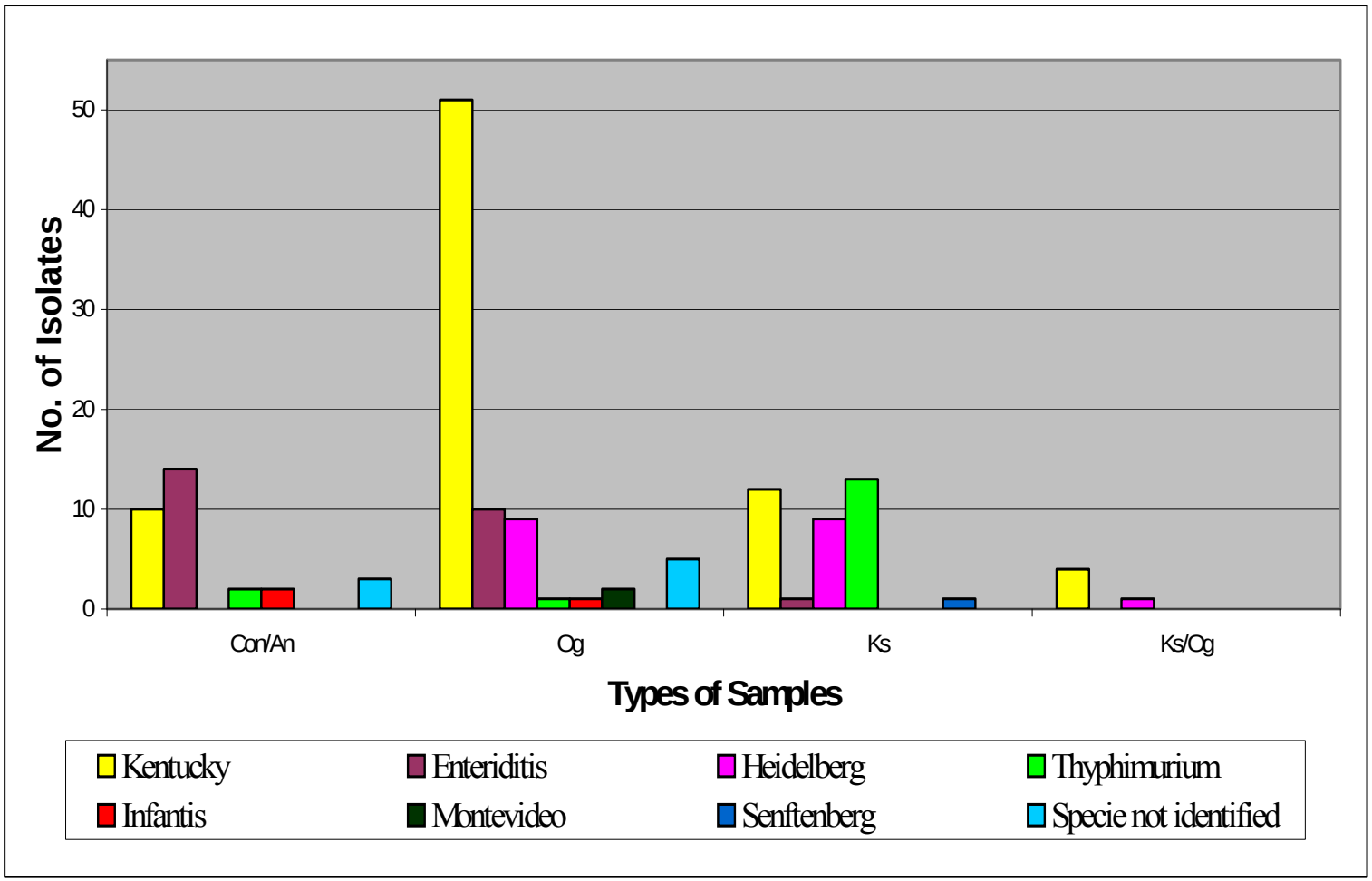

Figure 7. Distribution of *Salmonella* Serotypes within the Different Types of Samples

5 DISCUSSION

5.1 Isolate Recovery

In general, *Salmonella spp* was recovered from 27.3% of all samples processed in this study. This is similar to results from Jorgensen et. al (90) where the overall prevalence of *Salmonella* in raw chickens was 25%. However, other studies have found a higher prevalence of the bacteria in raw chicken products (39, 60). *Salmonella* is commonly found in poultry products in general because although the bacteria is ubiquitous, the primary reservoir is the intestinal tract of animals and the colonization of the bacteria is favored by intensive animal production. Poultry products are frequent vehicles in the transmission of *Salmonella*, dominating other foods of animal origin as potential source of infection (60). Differences in *Salmonella* prevalence among raw chicken studies can be explained through best management practices principles in the production of retail chicken. Good pre-harvest production practices, good sanitary practices in slaughter and dressing, and good sanitary practices of the processing facility must be followed at the processing level in order to reduce the possibility of carcass contamination (91). In addition, good handling practices at the distribution and retail level are necessary in order to avoid cross-contamination of the end product. The level of contamination found in this study indicates a potential breakdown of public health measures at one or various stages of the food processing and distribution chain.

The samples collected in this study were divided in Og, Con/An, Ks, and Og/Ks samples. The highest prevalence of *Salmonella* was showed by Og samples (45.4%) followed by Con/An samples (24.8%), and Ks samples (15.9%). *Salmonella* was least prevalent in Og/Ks samples (15.4%). The only type of sample that showed statistically

significant difference from the other types of samples was Og. This is similar to results from Cui et al. where organic chickens were found to be more frequently contaminated with salmonellae than conventional chickens (10). Bailey and Cosby (40) also found a higher prevalence of *Salmonella* in free-range organic chickens (31%) than in all-natural chickens (25%).

The USDA makes no claims that organically produced food are safer or more nutritious than conventionally produced food, only that organic food differs from conventionally produced food in the way it is grown, handled, and processed. Despite this, there is a perception on the part of consumers that because the conditions for growth for organic foods are more natural, these products will have less *Salmonella* and other pathogenic bacteria (10,40, 70). However, the results of the present study demonstrate the considerable influence that several aspects of the production system may have on food safety.

Animal management in organic farms precludes the use of chemically synthesized allopathic medicines, although vaccines are conditionally permitted in the event of illness (70). Antimicrobials for growth promotion are prohibited and animal health is maintained and promoted mainly through preventive measures (70). The lack of use of antimicrobial and limited vaccination allows the animal to carry a higher microbiological load that can pose a higher risk of microbial contamination than conventional reared animals. On the other hand, organic reared livestock, unlike conventional livestock, have access to the outside, where there is sufficient opportunity for exposure to wild birds, insects, rodent droppings, and other potential carriers of *Salmonella*, thus, promoting a greater chance for contamination. At the processing level, decontamination of food equipment by means such

as irradiation, antimicrobial agents, chemical washes, and other synthetic disinfectants is prohibited in organic production (70). Fewer decontamination alternatives increase the chance of higher risk of microbial contamination in organic production when compared to conventional production.

Another factor that may explain a higher prevalence of *Salmonella spp* in organic poultry is the age of the animal at the time of slaughter. Conventional broilers are slaughtered approximately at 6 weeks of age or less (8), while organic broilers are slaughtered approximately after 11 weeks of age (73). It has been suggested that because an older animal has a well-established colonization of intestinal microflora and a longer exposure to environmental conditions, it may carry a higher chance of pathogen contamination to the processing stage (75). Northcutt et al. suggested an association between *Campylobacter* prevalence in poultry and the age of the birds at the processing plant. They indicated that the prevalence of Campylobacter in poultry was elevated when the age of the birds at the processing plant increased (92).

In the present study, *Salmonella* was least prevalent in Ks (15.9%) and Og/Ks (15.4%) samples. The process of koshering involves a complete blood drain of the animal followed by a soak in cold water for half an hour. After soaking, the carcass is salted with a coarse salt for an hour followed by a triple rinsing in cold water (93). There is a limited amount of scientific data available regarding the microbiological safety of kosher meats. This data is conflicting in its findings. According to Clouser et al. (80) the kosher scald temperature of ~8^0 C for the defeathering of birds can cause more cross contamination of *Salmonella spp* than the scald temperature of ~58.6^0 C for conventional processes. This is explained by skin

surface changes occurring during the defeathering process; while conventional defeathering results in a smooth skin surface to which bacteria is loosely attached, the kosher defeathering results in a rouge skin surface where the bacteria become entrapped or embbedded. However, the results of the present study showed an overall lower prevalence of *Salmonella* in the Ks samples. These results are similar to findings from Hajmeer et. al (15) who reported reduction in *Salmonella* counts from beef briskets after koshering; they concluded that salt application during koshering of meats might reduce or inhibit microbial growth of pathogens due to associated changes in water activity and ionic strength. In a more recent study, Thomas indicated that kosher salt alone did not reduce *Salmonella* contamination in chicken skin. However, when kosher salt was followed by rinsing, *Salmonella* contamination was reduced by 80%. He concluded that the koshering process (kosher salt plus rinsing) is believed to reduce *Salmonella* contamination by preventing attachment of the bacteria or by removing *Salmonella* that are attached to the chicken skin (94). The present study clearly shows that the koshering process reduces the presence of *Salmonella* in raw chickens. The lower prevalence of *Salmonella* in Ks and Og/Ks samples found in this study suggests a possible inhibition of growth or detachment of the bacteria due to the salting and rinsing process involved in the koshering process.

Since in this project, as explained in the results section, it was possible to obtain up to 4 isolates per sample, a comparison between average and raw number of isolates for each type of sample was performed. This analysis revealed that the average number of isolates for Og samples was statistically different from all other types of samples. This result coincided with the results found for prevalence of *Salmonella spp* by type of sample. However, the

average number of isolates for Con/An chickens also showed statistically significant difference from the average number of isolates from Ks samples. This can be explained by a comparison between the analyses performed for prevalence at the sample level and prevalence taking into account the distribution of isolates for each type of sample. When analyzing prevalence in the samples, the parameters used were presence or absence of *Salmonella* in the sample regardless of the amount of isolates obtained from each sample. Thus, no significant difference was found between the amount of Con/An and Ks samples contaminated. On the other hand, when taking into account the amount of isolates obtained for all samples, more isolates were obtained from Con/An samples than from Ks samples. In addition to the explanations aforementioned, one can determine that the reason for obtaining more isolates in Con/An samples involves the salting step of the koshering process. The growth of *Salmonella* is generally inhibited in the presence of 3-4% salt (32). Among its effects on *Salmonella* cells, salt causes plasmolysis, interference with enzymatic activities, and expenditure of cellular energy to maintain homeostasis (33). These effects were likely to contribute to a decrease in growth rate and to presence of injured cells in Ks samples, thus allowing for a lower count of isolates when compared to Con/An isolates counts.

5.2 Antimicrobial Susceptibility Test

Antimicrobial use in animal production systems has been scrutinized as the primary cause of the emergence and dissemination of antimicrobial resistant *Salmonella* (57, 95). In the US, antimicrobial agents are primarily used in humans, animals, and on plants. Human antimicrobial usage in the US has limited impact on resistance among *Salmonella*; on the

other hand, few antimicrobial agents are used on plants. Therefore, a most likely possible cause for the emergence and increasing prevalence of antimicrobial-resistant *Salmonella* is the use of antimicrobial agents in animals, predominately food animals such as poultry (96). In the present study, antimicrobial susceptibility test was performed for 315 *Salmonella spp* isolates from chicken samples (Og = 170, Ks = 43, Og/Ks = 10, and Con/An = 92). Overall, isolates from Og, Ks, and Og/Ks samples were most resistant to S and Te (Og = S (54%), Te (51%); Ks = Te (40%), S (23%); Og/Ks = Te (80%), S (50%)). Although isolates from Con/An samples were also most resistant to Te (57%), they showed intermediate susceptibility to S (33%). Resistance to Te and S of *Salmonella spp* isolated from chicken is not uncommon. Manie et al. (97) reported 97% resistance to Te and 86% resistance to S in Salmonellae isolated from chickens in South Africa. Berrang et al. (98) also found most common resistances to Te (25%) and S (21.5%) in *Salmonella* isolated from retail chicken in Northeast Georgia. Wilson (57) assessed the antimicrobial resistance of *Salmonella* in raw retail chickens, he found highest resistance rates for sulfonamide (52%), streptomycin (26%), and tetracycline (22%). On the other hand, resistance to Te but not to S has also been reported for *Salmonella* isolates from chicken. Cui et al. (10) reported that *Salmonella* isolated from conventional chickens were resistant to Te (69%) but were not significantly resistant to S.

Tetracyclines has been one of the most commonly used antimicrobials for production animals for therapeutic purposes and for subtherapeutic (growth promotion) purposes for a long period of time; members of this class of antimicrobial (chiortetracycline and oxytetracycline) are approved for use in broiler feeds for purposes of growth promotion (75,

98). Thus, resistance to this drug could be expected for Con/An and Ks isolates where subtherapeutic use of antimicrobials is allowed. On the other hand, Og and Og/Ks isolates were also resistant to Te although the use of this antimicrobial is not allowed in organic livestock production. These results concurred with Cui et al. (10) where the highest rate of resistance for organic chickens was to Te (81%). It is possible that Te antimicrobial-resistant *Salmonella* isolates are stable and able to persist in poultry even in the absence of selection pressure. Luangtongkum et al. (75) found a high prevalence of Te resistant *Campylobacter* isolates from organically raised broilers and turkeys; they suggested that is possible that *Campylobacter* strains may have evolutionally become resistant to this class of antimicrobial, leading to the widespread distribution of tetracycline-resistant *Campylobacter* in animal reservoirs regardless of the production system. The present study suggests that animal production system does not seem to influence *Salmonella* resistance to Te in chickens.

Streptomycin was the first aminoglycoside discovered; today it has only limited current usage in clinical medicine. However, it remains important for growth promotion in animals and for bacterial disease control in plants (95, 99). It has been suggested that S resistance in *Salmonella* could be due to an established resistance mechanism genetically linked to other beneficial genes on an integron (95). This may be a possible explanation for the streptomycin resistance in the majority of the isolates in this study. On the other hand, a high resistance rate to streptomycin was expected for Con/An isolates in part because Con/An poultry is exposed to streptomycin for growth promotion and disease prevention in this production system; this was not the case. However as discussed earlier, many studies have found *Salmonella* isolates from conventional chickens with a high resistance to S. The fact

that Con/An isolates were not highly resistant to S excels the importance of considering that many factors can be involved in antimicrobial resistance of bacteria and not all of them are fully understood yet. .

In terms of susceptibility rates, 100% of Og isolates were susceptible to C, AN, and CIP. One hundred percent of all Og/Ks isolates were also susceptible to these antimicrobials. In addition, 100% of Og/Ks isolates were also susceptible to K, XNL, AM, CRO, GM, NA, FOX, and AmC. One hundred percent of Ks isolates were susceptible to CIP and AN. Kosher isolates were also highly susceptible to AN (98%), GM (98%) and NA (95%). The highest antimicrobial susceptibility in Con/An samples was shown for CIP (99%), NA (99%) and AN (99%). Overall, the highest susceptibility for isolates of all sample types was to CIP. Ciprofloxacin is a fluroquinolone, this antimicrobial is normally the drug of choice for treatment of human salmonellosis; treatment failure is rare (57). Fluoroquinolones are neither used in organic or conventional poultry operations (62, 75) thus; a high susceptibility to this antimicrobial was expected. Other studies have found similar susceptibility of *Salmonella* isolates to CIP. Wilson (57) isolated *Salmonella* strains from raw retail chickens, he found that all isolates were susceptible to CIP. In another study, *Salmonella* was isolated from meat, pork, poultry, and shellfish from supermarkets in Vietnam, all isolates in this study were also susceptible to CIP (100).

Isolates from all sample types also showed a high susceptibility rate to AN. Findings by Yashpal et al. showed that 100% of all *Salmonella* isolates obtained from turkey samples were susceptible to AN during a longitudinal study from 1998 to 2002 in Minessota (101). Amikacyn is an aminoglycoside antimicrobial used in treating life threatening infections of

gram- negative bacteria such as *Pseudomonas*; it is also used for veterinary purposes, however, it is more widely used in horses than in poultry (101). One can explain that because the antimicrobial is less used in poultry, this can decrease the probability of resistance for this antimicrobial in poultry in general. It is also known that Amikacin has a high resistance against bacterial inactivation thanks to its L-hydroxyaminobuteryl amide (L-HABA) moiety which inhibit acetylation,, phosphorilation, and adenylation (102).

It is worth to mention that isolates from all sample types were also fairly susceptible to NA (Og=96%, Ks=95%, Og/Ks=100%, Con/An= 99%). Nalidixic Acid is a quinolone (precursor of fluoroquinolones). A recent study by Stevenson et al. correlated *Salmonella* resistance/susceptibility to NA with a decrease/increase in susceptibility to fluoroquinolones such as CIP (103). The present study concurs with Stevenson conclusions by finding high levels of susceptibility for both antimicrobials.

In the present study 32% of all Og isolates, 53% of all Ks isolates, and 27% of all Con/An isolates were susceptible to all antimicrobial tested. None of the Og/Ks isolates were susceptible to all antimicrobials profiled. Sixty percent of all Og isolates were resistant to at least one antimicrobial and 5% of them showed multiple resistances. Forty two percent of all Ks isolates were resistant to at least one antimicrobial; only 12% of them showed multiple resistances to the antimicrobial profile. Conventional/All natural isolates showed the highest resistant to the antimicrobial profile; 72% were resistant to at least one antimicrobial while multiple resistance was shown in 34% of the isolates. Although 100% of Og/Ks isolates showed resistance to at least one antimicrobial, none of them presented multiple resistance to the profile. However, due to the small amount of isolates (n=10) for

Og/Ks samples a correlation between their antimicrobial susceptibility results and the results for all other isolates may be misleading.

In this study, Ks isolates showed the most antimicrobial susceptibility rate in comparison with other isolate types. There is a lack of scientific published data to assess the antimicrobial susceptibility of food pathogens in Kosher foods. A study by Ottaviani et. al found that increasing the salinity of a medium from 1-4% to 5-6% increased the antimicrobial susceptibility in vitro of *Vibrio spp* to penicillin, novobiocin, ampicillin, carbennicillin, nitrofurantoin, sulphamethaxozole, rifompicin, cephalotin, and oxalitic acid (104). It may be possible that the higher presence of salt in Ks sample played a role in *Salmonella* antimicrobial susceptibility patterns isolated from Kosher chickens. However, it is worth to mention that *Vibrio spp* is a moderately halophilic microorganism while *Salmonella spp* is non halophilic; thus, a comparison between both microorganism may be misleading. On the other hand, Coronado et. al found that increasing the salinity of the medium for testing antimicrobial susceptibility of moderately halophilic bacterias in vitro, was dependent on the individual strains and the antimicrobial tested (105). Kosher poultry is raised under the same rules as conventional poultry, therefore, a higher antimicrobial resistance pattern would be expected for Ks isolates if only exposition to antimicrobials during rearing is considered as the cause of overall high prevalence of antimicrobial resistant *Salmonella* isolates. This was not the case in the present study. Baseline data on the antimicrobial susceptibility patterns of foodborne pathogens isolated from Kosher foods are needed to fully understand the rate of antimicrobial resistance of such isolates and the role that salt may have in this matter.

Overall, Og isolates were more susceptible to all antimicrobials profiled than Con/An isolates. This finding concurs with findings by Cui et al. (10) were isolates of *Salmonella spp* and *Campylobacter spp* from organic retail chickens were more susceptible to antimicrobials than isolates from conventional retail chickens. A study conducted by Luangtongkum et. al revealed significant differences in antimicrobial resistant *Campylobacter* isolates between conventional poultry operations and organic poultry operations. In general, they found higher antimicrobial resistance among *Campylobacter* isolates from conventional poultry farms than among isolates from organic poultry farms (75).

Studies comparing antimicrobial susceptibility of food pathogen isolated from organic and conventional production systems have also been done for other types of food animals. Ray et al. studied the antimicrobial susceptibility of *Salmonella* from organic and conventional dairy farms. They found that *Salmonella* isolates from conventional farms were more resistant to tetracycline and sulfamethoxazole than isolates from organic farms (95). Miranda et al. found that the levels of antimicrobial resistant strains of *Escherichia Coli* were higher in conventional pork meat than in organic pork meat (106). It is evident that the antimicrobial susceptibility of food pathogens isolated from food animals varies among different animal production systems. Overall, it seems like isolates from organic production systems have a higher antimicrobial susceptibility than isolates from conventional production systems. This suggests that the practice of antimicrobial usage in conventional production systems influences in the overall prevalence of antimicrobial resistant food pathogens.

The issue of the emergence of multi-antimicrobial resistant bacterial pathogens has become a major public health concern. The use of antimicrobials in any venue, including

disease treatment and growth promotion in livestock, can potentially lead to widespread dissemination of multi-antimicrobial resistant bacteria (75, 96, 107). Consequently, the study of multi-antimicrobial resistance of food pathogens has been a priority topic for research. White et al. found a high prevalence of multi-antimicrobial resistant *Salmonella* (15.6%) in retail ground meats in the greater Washington D.C. area (18). Zhao et al. assessed the antimicrobial susceptibility of *Salmonella enterica* serovar Typhimurium isolated from either animal diagnostic specimens or food animals after slaughtering/processing. They found a high rate (58%) of *Salmonella enterica* serovar Typhimurium from turkeys exhibiting resistance to 10 or more antimicrobials (20). However, the issue of prevalence of multi-antimicrobial *Salmonella* is not unique to foods animals. Multi-antimicrobial resistant *Salmonella* strains have been isolated from numerous other domestic and imported foods including sprouts, spices, frozen seafood, freshwater fish, ice cream, bone meal, herbs, cheese, and lettuce (109). In a study evaluating the antimicrobial susceptibility of 502 *Salmonella* strains isolated from various foods and associated samples between 1999 and 2000, 31.2% (n=77) exhibited multi-antimicrobial resistance (109).

In the present study, 5% of all Og isolates and 34% of all Con/An isolates presented multi-antimicrobial resistance. These findings are similar to those from Cui et al. were *Salmonella* isolates from conventional retail chickens showed higher multi- antimicrobial resistance than isolates from organic retail chickens (10). Another study found that isolates of the food pathogen *Campylobacter spp* from conventionally raised turkey broilers showed significant higher multi-antimicrobial resistance than isolates from organically raised turkey broilers (75). In a recent study, Miranda et al. assessed the antimicrobial resistance in

Escherichia coli strains isolated from organic and conventional pork meat. They found that the presence of multi antimicrobial resistant *E. coli* strains was significantly higher in conventional pork meat (90%) as compared to organic pork meat (41.1%) (106). These findings suggest that the exposition to antimicrobials in the conventional production system may play a role in the prevalence of multi-antimicrobial food pathogens, including *Salmonella*, in livestock production.

Kosher isolates presented higher multi-antimicrobial resistance (12%) than Og isolates (5%). This finding seems to be contradictory to the low overall antimicrobial resistance showed by kosher isolates. However, since kosher chickens are raised in the same way than conventional chickens are, it is possible that although Ks isolates had lower antimicrobial resistance, isolates that did show resistance to the antimicrobial profile were resistance to more antimicrobials due to exposure to a wide variety of these substances during rearing.

The only antimicrobial resistance phenotype pattern that was consistently found in isolates from all sample types was S-Te. This is not surprising because as discussed earlier, in general, isolates in this study were most resistant to these antimicrobials. This finding is not unique; a Danish study characterized streptomycin resistant isolates of *Salmonella enterica* serovar Typhimurium from animal and human origins with respect to co-resistances patterns. The study found nineteen different co-resistance patterns and S-Te was the most commonly observed (110). Various studies have also detected the presence of known genes that encode for resistance to streptomycin and tetracycline respectively in different multi-antimicrobial resistant Enterobacteriaceae such as *Salmonella* (99, 111, 112). One can argue that this phenotype is widely present in *Salmonella* from animal origins due to the wide use

of these antimicrobials in animal husbandry. However, the present study included isolates from organic animals (chickens) were the use of these antimicrobials is forbidden; these isolates also presented the S-Te phenotype. Thus, the use of antimicrobials at the farm level does not seem to be the principal cause of the presence of this particular antimicrobial resistance pattern. It is more likely that genes that encode for resistance to streptomycin and tetracycline are stable and transferred without any selection pressure.

5.3 Serotyping

In the present study, serotyping was performed to 151 *Salmonella* isolates from Og, Ks, Con/An or Og/Ks samples. Complete serovar identification was only possible for 143 isolates. Due to the small number of isolates sent to serotype (less than half of total isolates), a comparison between serovars and antimicrobial susceptibility was not made in order to avoid any misleading conclusions. Overall, 7 serovars of *Salmonella enterica* sub specie *enterica* were identified. The most prevalent serovars were Kentucky (50%), Enteriditis (17%), Heidelberg (13%), and Thyphimurium (11%). These serovars have been identified before as the most prevalent in retail chicken and are typical of those reported in the US broiler industry (98). Although serovar Kentucky is frequently associated with livestock production, it is rarely associated with human illness (113). On the other hand, according to a FoodNet report on the incidence of human infections with pathogens transmitted commonly through foods in US, in 2006 seven *Salmonella* serotypes commonly found in livestock production accounted for 64% of all reported salmonellosis infections in humans (114). Among these serotypes Thyphimurium and Enteriditis accounted for 19% of all infections

each, while Heidelberg accounted for 4% of these infections (114). The presence of these serotypes in retail chickens calls for careful handling and cooking procedures by the consumer in order to avoid contamination/infection.

From all isolates characterized, the majority of Kentucky serovar strains were isolated from Og samples (66.2%). Serovar Enteriditis was most isolated from Con/An samples (56%), while serovar Heidelberg was most commonly found in Og (47.4%) and Ks (47.4%) samples. Serovar Typhimurium was more prevalent in Ks samples (81.2%). Cui et al. (10) also reported a higher prevalence of S. Kentucky in organic chicken samples (59%) compared to Con/An samples (37%). They also found serovar Heidelbeg predominantly in organic samples (33%) compared to Con/An samples (4%). However, Cui et al. did not isolate any strain corresponding to serovar Enteriditis from any sample (10). On the other hand, Atunes et al. reported serovar Enteriditis as the most predominant in their study of incidence of *Salmonella* from poultry products (conventional) (60). There is little data available of the incidence of *Salmonella* serovars in organic poultry and to the best of my knowledge this is the first time *Salmonella* serovars are identified for kosher poultry. It is worth to mention that serovar Typhimurium was predominant in kosher samples. This serotype is of special public health concern because of *Salmonella enterica* serotype Typhimurium Definitive Type 104 virulence and multi-antimicrobial resistance (25,47).

The findings of the present study reveals that retail chicken, regardless of production system, can be contaminated with pathogenic *Salmonella* serovars; thus, raw retail chicken is a potential vehicle for transmitting food-borne diseases, such as salmonellosis. These findings stresses the need to practice better food safety principles from farm to fork.

6 CONCLUSIONS

The purpose of the present study was to compare the prevalence and antimicrobial susceptibility of raw retail chickens from three production systems: conventional, organic, and kosher, as well as to assess what species of *Salmonella* are most commonly found in these types of raw retail chickens.

According to the results obtained, prevalence of *Salmonella spp* in raw retail chickens seems to be influenced by chicken production systems. Organically produced chickens had higher prevalence of *Salmonella* than chickens produced by conventional and kosher approaches. However, regardless of production system it was possible to isolate *Salmonella* from all sample types. This finding is alarming because it indicates potential breakdowns of food safety practices at one or various stages of the food processing and distribution chain. It is imperative that consumers are educated in food safety measures in order to avoid cross-contamination with other food products and ingestion of possibly contaminated poultry.

Antimicrobial susceptibility also seems to be influenced by chicken production system. In the present study, Ks isolates were more susceptible to all antimicrobials profiled than isolates from all other samples. It may be possible that the koshering process has an effect on the antimicrobial susceptibility of *Salmonella* in kosher meats. However, there is a lack of published scientific data of antimicrobial susceptibility of food pathogens isolated from kosher meats. Baseline data on this matter is needed to understand antimicrobial susceptibility trends for food pathogens isolated from kosher meats. On the other hand, Og isolates were most susceptible to antimicrobials profiled than Con/An isolates. This finding

suggests that the lack of use of antimicrobial substances in organic production systems diminishes the overall prevalence of antimicrobial resistant *Salmonella* isolates in organically produced chickens. Nevertheless, resistance to Te and S was found across all sample types, these resistances are believed to be stable and transferred without selection pressure, mainly because their common use in past decades. Multi- antimicrobial resistance was also found for Og, Ks, and Con/An samples. It seems that although the lack of exposure to antimicrobials at the farm level affects the presence of antimicrobial resistant *Salmonella* strains in retail chicken, this is not the only factor involved in the emergence and wide spread of these microorganisms. Other factors such as stability of genes that encode for resistance and the capability of these genes to be transferred without any selection pressure may also influence presence of antimicrobial resistant *Salmonella* in these products.

Salmonella serovars most prevalent in the present study were Kentucky, Enteriditis, Typhimurium, and Heidelberg. All these serovars are common to the U.S. broiler industry. These findings are of concern because servars Enteriditis, Heidelberg, and Typhimurium have been associated with salmonellosis infections in the US. These results indicate that the common consumer perception that organically and ethnic produced chickens are safer is wrong. Pathogenic *Salmonella* serovars can be present in raw retail chickens despite its production system. Is imperative to make consumers aware of this and to stress good handling and cooking procedures in order to avoid possible salmonellosis infections.

7 RECOMMENDATIONS

1. Assess the antimicrobial susceptibility of food pathogens isolated from kosher meats.

2. Study the possible interaction of salt in kosher meats in correlation with antimicrobial susceptibility patterns of isolates from these types of meats.

3. Continue studying the prevalence of food pathogens in kosher meats and identify serovars isolated from these meats.

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9 Appendices

Appendix A Complete Antimicrobial Susceptibility Profile for Kosher Isolates

Kosher Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
1	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
2	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
3	SU	SU	R	SU	SU	I	R	R	SU	SU	SU	SU	SU	SU	I
4	SU	SU	R	SU	SU	I	R	R	SU	SU	SU	SU	SU	SU	I
5	SU	R	SU	SU	R	SU	I	R	SU	SU	SU	SU	SU	SU	I
6	SU	R	SU	SU	R	SU	R	R	SU	SU	SU	SU	SU	SU	R
15	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
17	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
18	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
19	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
20	SU	SU	SU	SU	SU	SU	SU	I	SU	SU	SU	SU	SU	SU	SU
27	R	R	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
65	SU	SU	SU	SU	SU	SU	SU	R	SU	SU	I	SU	SU	SU	SU
68	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
69	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
70	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
71	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
72	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
73	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
74	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
75	R	I	SU	R	SU	SU	R	R	R	SU	SU	R	SU	SU	I

Kosher Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
76	R	I	SU	R	SU	I	R	R	I	I	SU	R	SU	SU	I
77	SU	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
163	SU	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
164	SU	R	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU
165	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
166	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
167	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
239	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
240	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
241	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
254	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
255	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
293	SU	SU	SU	R	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
298	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
299	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
300	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
301	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
302	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
303	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
304	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
305	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
314	SU	R	SU	R	SU	SU	R	R	SU	SU	R	SU	I	SU	R

Cont. Appendix A

Appendix B Complete Antimicrobial Susceptibility Profile for Conventional/All Natural Isolates

Conventional/ All Natural Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
7	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
8	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
9	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
10	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
21	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
22	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
23	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
24	R	R	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
25	R	R	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
26	R	R	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
32	SU	I	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
33	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
34	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
35	R	R	SU	R	I	I	R	SU	R	SU	SU	R	SU	SU	R
45	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
46	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
47	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
48	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
49	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
50	R	SU	SU	R	SU	SU	R	SU	I	SU	SU	R	SU	SU	R
51	R	SU	SU	R	SU	SU	R	SU	I	SU	SU	I	SU	SU	R

Conventional/ All Natural Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
52	R	SU	SU	R	SU	SU	R	SU	I	SU	SU	R	SU	SU	R
53	SU	R	I	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU
54	SU	R	I	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU
55	SU	R	I	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU
56	SU	R	SU	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU
57	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
58	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
59	SU	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	I	R	I	SU
60	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
61	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
62	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
63	SU	SU	SU	SU	SU	SU	I	SU	SU	SU	SU	SU	SU	SU	SU
64	SU	SU	SU	SU	SU	SU	I	R	SU	SU	SU	SU	SU	SU	SU
78	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
79	R	SU	SU	R	I	SU	R	R	R	SU	SU	R	SU	SU	SU
80	R	SU	SU	R	SU	I	R	R	I	SU	SU	R	SU	SU	I
81	SU	SU	SU	SU	SU	SU	R	R	SU	SU	SU	SU	SU	SU	SU
103	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
104	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
105	R	SU	SU	R	R	SU	R	SU	I	SU	SU	R	SU	SU	SU
106	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
107	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU

Cont. Appendix B

Conventional/ All Natural Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
108	R	SU	SU	SU	SU	SU	SU	SU	I	SU	SU	SU	SU	SU	SU
118	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
119	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
120	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
121	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
122	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
128	R	R	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
129	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
130	R	SU	SU	R	SU	SU	R	R	R	SU	SU	R	SU	SU	R
131	R	SU	SU	R	SU	SU	R	R	R	SU	SU	R	SU	SU	R
143	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
144	R	SU	SU	R	SU	SU	R	R	R	SU	SU	R	SU	SU	R
145	R	SU	SU	R	SU	SU	R	R	R	SU	SU	R	SU	SU	R
146	I	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
147	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
148	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
149	I	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
150	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
169	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
170	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
171	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
179	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU

Cont. Appendix B

Conventional/ All Natural Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
180	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
181	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
182	SU	R	SU	R	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
183	SU	R	SU	R	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
184	SU	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
185	SU	R	SU	SU	SU	SU	SU	SU	SU	SU	I	SU	SU	SU	SU
186	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
187	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
188	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
189	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
194	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
195	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
196	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
197	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
219	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
220	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
221	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
222	R	SU	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
235	SU	R	SU	SU	SU	SU	SU	R	SU	R	SU	SU	SU	SU	SU
236	SU	R	SU	SU	SU	SU	SU	R	SU	R	SU	SU	SU	SU	SU
237	SU	R	SU	SU	SU	SU	SU	R	SU	R	SU	SU	SU	SU	SU
238	SU	R	SU	SU	SU	SU	SU	R	SU	R	SU	SU	SU	SU	SU

Cont. Appendix B

Conventional/ All Natural Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
249	SU	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
250	SU	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
251	SU	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
252	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
253	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU

Cont. Appendix B

Appendix C Complete Antimicrobial Susceptibility Profile for Kosher/Organic Isolates

Kosher/Organic Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
190	SU	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
191	SU	SU	SU	SU	SU	R	SU	R	SU	SU	SU	SU	SU	SU	SU
192	SU	SU	SU	SU	SU	R	SU	R	SU	SU	SU	SU	SU	SU	SU
193	SU	SU	SU	SU	SU	R	SU	R	SU	SU	SU	SU	SU	SU	SU
198	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
292	SU	R	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
294	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
295	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
296	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
297	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU

Appendix D Complete Antimicrobial Susceptibility Profile for Organic Isolates

Organic Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
11	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
12	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
13	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
14	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
16	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
28	SU	R	SU	I	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	I
29	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
30	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
31	R	R	SU	I	SU	SU	I	R	R	SU	I	R	SU	SU	I
36	R	R	SU	I	SU	SU	R	R	I	SU	SU	R	SU	SU	R
37	SU	SU	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
38	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
39	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
40	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
41	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
42	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
43	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
44	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
66	SU	SU	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
67	SU	SU	SU	SU	SU	SU	I	SU	SU	SU	SU	SU	SU	SU	SU
82	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU

Organic Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
83	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
84	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
85	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
86	SU	I	R	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
87	R	I	SU	R	SU	I	R	SU	SU	SU	SU	R	SU	SU	I
88	R	I	SU	R	SU	I	R	SU	R	SU	SU	R	SU	SU	SU
89	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	I
90	R	I	R	SU	SU	I	SU	SU	R	SU	SU	SU	SU	SU	SU
91	R	I	SU	SU	SU	SU	SU	SU	I	SU	I	SU	SU	SU	SU
92	R	SU	SU	SU	SU	SU	SU	SU	I	SU	SU	SU	SU	SU	SU
93	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
94	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
95	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
96	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
97	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
98	R	SU	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU
99	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
100	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
101	SU	SU	SU	SU	SU	SU	SU	SU	I	SU	SU	SU	SU	SU	SU
102	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
109	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
110	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU

Cont. Appendix D

Organic Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
111	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
112	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
113	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
114	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
115	SU	R	SU	SU	SU	SU	I	SU	SU	SU	SU	SU	SU	SU	SU
116	R	R	SU	R	SU	SU	R	SU	I	SU	SU	R	SU	SU	R
117	R	R	SU	R	SU	SU	R	SU	I	SU	SU	R	SU	SU	R
123	SU	R	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
124	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
125	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
126	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
127	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
132	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	R
133	R	R	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
134	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
135	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
136	R	R	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
137	R	R	SU	R	SU	SU	R	R	I	SU	SU	R	SU	SU	R
138	SU	R	I	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
139	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
140	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
141	R	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU

Cont. Appendix D

Organic Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
142	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
151	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
152	R	R	SU	R	SU	SU	R	R	R	SU	SU	R	SU	SU	R
153	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
154	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	I	SU	SU	SU	SU
155	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
156	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
157	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
158	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
159	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
160	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
161	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
162	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
168	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	R
172	SU	R	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	R
173	SU	R	SU	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU
174	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
175	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
176	SU	R	SU	SU	SU	SU	SU	R	SU	SU	I	SU	SU	SU	SU
177	SU	I	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
178	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
199	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU

Cont. Appendix D

Organic Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
200	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
201	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
202	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
203	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
204	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
205	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
206	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
207	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
208	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
209	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
210	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
211	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
212	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
213	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
214	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
215	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
216	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
217	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
218	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
223	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
224	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
225	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU

Cont. Appendix D

Organic Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
226	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
227	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
228	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
229	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
230	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
231	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
232	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
233	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
234	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
242	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
243	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
244	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
245	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
246	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
247	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
248	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
256	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
257	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
258	SU	SU	SU	SU	SU	SU	SU	I	SU	SU	I	SU	SU	SU	SU
259	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
260	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
261	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU

Cont. Appendix D

Organic Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
262	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
263	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
264	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
265	SU	I	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
266	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
267	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
268	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
269	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
270	SU	I	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
271	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
272	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
273	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
274	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
275	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
276	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
277	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
278	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
279	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
280	SU	R	I	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
281	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
282	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
283	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU

Cont. Appendix D

Organic Isolate Number	Antimicrobial Profile														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	XNL	S	K	CF	C	SXT	AM	TE	CRO	GM	NA	FOX	AN	CIP	AMC
284	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
285	SU	R	SU	SU	SU	SU	SU	R	SU	SU	R	SU	SU	SU	SU
286	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
287	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
288	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
289	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
290	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
291	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU
306	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
307	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
308	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
309	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
310	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
311	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
312	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
313	SU	R	SU	SU	SU	SU	SU	R	SU	SU	SU	SU	SU	SU	SU
315	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU	SU

Cont. Appendix D