COMPARISON OF ANTIMICROBIAL RESISTANCE PATTERNS BETWEEN *SALMONELLA ENTERICA* SUBSP. *ENTERICA* AND *ESCHERICHIA COLI* IN DAIRY CALVES

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ABSTRACT

Comparisons of Antimicrobial Resistance Patterns Between *Salmonella enterica* subsp. *enterica* and *Escherichia coli* in Dairy Calves

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The primary objective of this study was to compare antimicrobial resistance patterns of *Salmonella enterica* with *Escherichia coli* isolated from the same dairy calves. Fecal samples were collected from February 2004 to September 2005 from 74 calves. Calves with at least one *Salmonella* isolate and one *E.coli* isolate were included in our analysis. The 148 isolates collected from the 74 calves were tested using a broth tube dilution method for determining resistance to several antimicrobial agents (n=14): AMP, APR, CEF, CHL, ENR, FLO, GEN, NEO, OXY, SPE, SCH, SDI, STH, and TRI. Considering resistance to individual drugs, the percent of *E.coli* isolates with resistance was over 50 for 11 drugs and the percent of *Salmonella* isolates resistant was over 50 for 9 drugs. From the antimicrobial resistance patterns generated for *E.coli* (n=42) and *Salmonella* (n= 22), the bacteria were found to have 6 identical patterns in common. From these 6 patterns the most common pattern showed resistance to AMP, CEF, CHL, FLO, NEO, OXY, SCH, SDI, and STH. The association of antimicrobial resistance between *Salmonella* and *E.coli* for the same calves for individual antimicrobial agents proved not statistically significant. This study showed that resistance to drug classes important in human and animal medicine was common in *Salmonella* and *E.coli* from clinically ill calves. Overall, *E.coli* isolates were more resistant than *Salmonella*. While there were shared patterns of resistance (n=6) between *Salmonella* and *E.coli*, *Salmonella* isolates were not significantly more likely to be resistant to individual antibiotics if a calf had a resistant *E.coli*. Results suggest that selection pressure from recent exposure to drugs or interspecific gene transfer was not resulting in a strong association of resistance between the bacteria.

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ABBREVIATIONS

AHI	Animal Health Institute
AMP	Ampicillan
APR	Apramycin
CDC	Center for Disease Control and Prevention
CEF	Ceftiofur
CHL	Chloramphenicol
CIPARS	Canadian Integrated Program for Antimicrobial Resistance Surveillance Europe
CLSI	Clinical Laboratory Standards Institute
DANMAP	Danish Integrated Antimicrobial Resistance Monitoring and Research Program
EARSS	European Antimicrobial Resistance Surveillance System
ENR	Enrofloxacin
FLO	Florfenicol
GEN	Gentamicin
MDR	Multi-drug Resistant
NARMS	National Antimicrobial Resistance Monitory System
NEO	Neomycin
OXY	Oxytetracycline
PFGE	Pulsed-Field Gel Electrophoresis
SCH	Sulfachloropyridazine
SDI	Sulfadimethoxine
SPE	Spectinomycin
STH	Sulphathiazole
SVARM	National Veterinary Institute of Sweden
TRI	Trimethoprim/Sulfamethoxazole

CRITICAL TERMS

Antibiotics

Drugs produced by a microorganism that inhibit growth or destroy microorganisms. Antibiotics are used to treat infectious disease in humans, animals, or plants.

Antimicrobial

"Agent that destroys or inhibits microorganisms; capable of destroying or inhibiting their growth" (CLSI). Antibiotics are a type of antimicrobial.

Antimicrobial Resistance

Ability of a microorganism to multiply under conditions that would inhibit other members of the strain (Anonymous, 2006). Refers to failure of a given antimicrobial treatment.

Breakpoint

"(interpretive criteria) MIC or zone diameter value used to indicate susceptible, intermediate, and resistant as defined by the interpretive criteria used in CLSI documents M2—Performance Standards for Antimicrobial Disk Susceptibility Tests; M7—Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; and M11—Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria" (CLSI).

Multi-Drug Resistant (MDR)

For this study, MDR is defined as resistance to 5 or more antimicrobial agents.

NARMS

"A collaboration among CDC, U.S. Food and Drug Administration (Center for Veterinary Medicine) and U.S. Department of Agriculture (Food Safety and Inspection Service and Agricultural Research Services). State health departments send *Salmonella, Shigella, Campylobacter* and *E. coli* O157:H7 isolates received at their public health laboratories to CDC for susceptibility testing. The number of participating states increased in 2001 to 27, and the population under surveillance increased to 63 percent of the U.S. residents" (CDC, 2002).

Salmonellosis

An illness that resulted from a *Salmonella* infection. Symptoms of clinically ill dairy cattle include diarrhea, bloody stool, fever, dehydration, anorexia/emaciation, rapid breathing, unusual or foul odor stool, sloughing of skin from extremities, and sudden death.

INTRODUCTION

Recently, many strains of *Escherichia coli* have been found to be resistant to multiple, structurally unrelated antimicrobial classes. This high prevalence of multi-drug resistance could be a significant source of resistance for other bacteria that share the same environment, such as *Salmonella*. *Salmonella* is an important pathogen in both animal and human hosts. If new types of resistance begin to emerge, present forms of treatment may become ineffective resulting in more serious disease in humans and animals. The primary objective of this study was to compare antimicrobial resistance patterns of *Salmonella enterica* with *Escherichia coli* from clinically ill dairy calves.

REVIEW OF THE LITERATURE

Bacteria

Bacteria are an integral part of the world and are ubiquitous to every habitat on Earth, adapting readily to shifts in environmental parameters by means of a short generation period, from minutes to hours (American Academy of Microbiology, 2002). These adaptive capabilities, in fact, account for the ease with which microorganisms respond to culture conditions in the laboratory, which are often radically different from the natural habitat of the organism (Roszak and Colwell, 1987). Most of these microorganisms are harmless. Some are symbiotic and actually protect the host from even more harmful bacteria. However, the emergence of bacterial pathogens that are resistant to medically important antimicrobial drugs is recognized as a significant public health concern (Tragesser et al., 2006).

In 1674, Anton van Leeuwenhoek, a Dutch scientist, was credited as being the first microbiologist when he discovered bacteria. In 1859, Louis Pasteur, the father of modern microbiology, picked up where Leeuwenhoek left off (Fleming, 1946). With the beginnings of microbiology, bacterial pathogens became apparent as the cause of some infectious diseases and were found to have the ability to quickly adapt to new antibiotics. By 1928, Scottish bacteriologist Alexander Fleming accidentally discovered the antimicrobial agent produced by *Penicillin notatum*, when it inhibited the growth of the bacteria *Staphylococcus* (Fleming, 1946). Fleming's discovery won him a Nobel Prize in 1945 and marked the first modern antibiotic.

Antibiotics, compounds produced by microorganisms that either kill or inhibit the growth of bacteria (Fleming, 1946), have been critical in the fight against infections for

over fifty years. However, infections that were once cured by the introduction of an antibiotic are now more difficult to combat because of resistance. Antimicrobial resistance develops as a natural consequence of the bacterial population's ability to adapt. Bacteria's continued exposure to antibiotics has resulted in this inevitable resistance to individual and multiple antimicrobial agents in many types of bacteria.

Antimicrobial Resistance

As genetic material can be transferred between bacteria, there is every reason to suspect that any genes carrying resistance to antibiotics could also be transferred. Resistance, as defined by the Institute of Food Technology (IFT), is the "temporary or permanent ability of a microorganism and its progeny to remain viable and/ or multiply under conditions that would destroy or inhibit other members of the strain" (Anonymous, 2006). By 1940, Abraham and Chain submitted a "letter to the editor" in Nature titled "An Enzyme from Bacteria [*E.coli*] Able to Destroy Penicillin" and warned that the misuse of penicillin could lead to the propagation of mutant strains that would be resistant to antibiotics (Abraham and Chain, 1940). By 1952, Lederberg and Lederberg, confirmed that bacteria could transfer resistance to other bacteria through genetic exchange (Lederberg and Lederberg, 1952) and resistance to penicillin had begun appearing in hospitals. Physicians' treatment options, which decades prior were broad, began diminishing drastically. Although there is much debate on the matter, according to the CDC, the main cause for this resistance is due to the over-prescription and/ or misuse of antibiotics. The CDC advises the use of the "precautionary principle"; the use of antibiotics should be reduced to the minimum necessary.

If bacteria come into contact with, but are not killed by, an antibiotic, they may adapt their cell structure or metabolism to make themselves resistant to that antibiotic in the future. Exposures to antimicrobial agents provide bacteria with opportunities to acquire mechanisms of resistance by changing their cellular physiology and structure. Methods of acquiring resistance include genetic mutation, modification of existing genetic material, or acquisition of new genetic material. Once resistance is acquired, bacteria can share and exchange information by either vertical gene transfer to the bacteria's progeny or by horizontal gene transfer to individual bacteria either by transduction, transformation, or conjugation. Transduction occurs when a virus or bacteriophage transfers its DNA between two bacteria; transformation occurs when parts of the DNA are taken up by bacteria from the external environment; and, conjugation requires direct cell-to-cell contact to transfer small pieces of DNA, called plasmids, into another cell. The development of resistance in one bacterial population may spread to other populations over time.

Multiple uses of antimicrobial agents in medicine, production of food animals, and crop protection are some of the reasons for increasing resistance to those agents (American Academy of Microbiology, 2002). Previous studies have shown that waste effluents from hospitals contain one of the highest levels of antibiotic-resistant bacteria (Grabow and Prozesky, 1973). Other sources can also be found in sewage waste from septic tanks, pharmaceutical production plants, receiving waters, crops, or near farms where antimicrobial agents are used extensively to promote growth or treat and prevent disease (American Academy of Microbiology, 2002). These interconnected ecosystems can lead to the emergence of antimicrobial resistance, which could be transferred back

into human and animal disease organisms. If new forms of resistance start to emerge, the decreasing effectiveness of present forms of treatment and inability to treat certain infections is a distinct possiblity.

Economic and Medical Concern

Every year over 17 million people die of infectious diseases worldwide (Twomey, 2000). More than 70 percent of the bacteria that cause hospital-acquired infections are resistant to at least one of the antibiotics most commonly used to treat them and over 60 percent of deaths are caused by bacteria that have become resistant to at least one antibiotic (Twomey, 2000). The cost to the health care system is enormous. It is estimated that resistant bacterial infections increase health care costs by $4 billion per year in the United States alone (American Academy of Microbiology, 2002). Resistant bacteria cause infections that are more difficult to treat, requiring drugs that are often less readily available, more expensive, and more toxic (American Academy of Microbiology, 2002). Examples of clinically important microbes that are rapidly developing resistance to available antimicrobials include bacteria that cause pneumonia, ear infections, meningitis (e.g., *Streptococcus pneumoniae*), skin, bone, lung, and bloodstream infections (e.g., *Staphylococcus aureus*), urinary tract infections (e.g., *Escherichia coli*), foodborne infections (e.g., *Salmonella*), and infections transmitted in health care settings (e.g., enterococci and *Klebsiella* spp.) (Panlilio A.L. et al., 1992; Hofmann J., Cetron M.S., Farley M.M., et al., 1995; Glynn M.K., Bopp C., Dewitt W., et al., 1998; Martone W., 1998; CDC, 1999; Gupta K., Scholes D., Stamm W.E., 1999; Wiener J., Quinn J.P., Bradford P.A., et al., 1999). Nearly all strains of *Staphylococcus aureus* in the United

States have become resistant to penicillin and 17 percent of all enterococci isolates are vancomycin resistant (Twomey, July 2000). Every year, approximately 40,000 cases of *Salmonella* are reported in the United States and are showing high rates of antibiotic resistance. The CDC (1999) also estimates that approximately 11 percent of *S. pneumoniae* are resistant to third-generation cephalosporin antibiotics and are becoming resistant to the newer fluoroquinolones. Many strains are reportedly becoming multi-drug resistant (MDR).

It is not known how much human and agricultural use of antimicrobial drugs contribute to resistance. The most recent study by the Animal Health Institute (AHI) showed that approximately 24.9 million pounds of antibiotics were used in 1999 (of which 88.3 percent was for therapeutic use). By 2004, the total usage had dropped to 21.7 million pounds (of which 95 percent was for therapeutic use) (Animal Health Institute, 2000, 2002, 2005). (See **Table 1**; ***Figure 1***).

Table 1. 2000 - 2002 Animal Health Institute (AHI) Survey[1]

Active Antibacterial Ingredients Sold by AHI Members			
Antibiotic Class	**2000** (lbs)	**2001** (lbs)	**2002** (lbs)
Ionophores/Arsenicals*	9165043	7758492	9050782
Tetracyclines	6693834	7144523	6649567
Cephalosporins, macrolides, lincosamides, polypeoptides, strptogramins, and other minor classes of antibiotics**	4857896	4268658	5056515
Sulfonamides and Penicillins	2363151	2406072	815298
Aminoglycosides	337819	257252	415219
Fluoroquinolones	38082	36204	33602
Total	**23455825**	**21871201**	**22020983**

* Unique drug products developed for animal production and not related to traditional antibiotics

** Grouping necessary to abide by disclosure agreements

[1] Source: (Animal Health Institute, 2002)

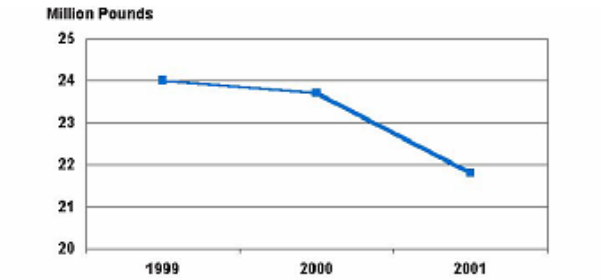

Figure 1. Volume of antibiotic usage in farm and companion animals 1999-2001.[2]

The survey, however, was composed of only AHI members and does not include all generic antibiotic manufacturers. Recent estimates of the amount of antibiotics used in production agriculture ranges from 18.4 million to 30 million pounds compared to the usage in human medicine, which ranges from 4.5 to 32.2 million pounds (Anonymous, 2006).

Although its role in human health is still heavily debated, transfer of antimicrobial-resistant bacteria from food animals to humans has been documented (Sanchez S et al., 2002; Swartz M.N., 2002). Considerable concern exists over antibiotic usage in food animals for drugs classified as critical for human medicine (FDA, Center for Veterinary Medicine). Examples of these antimicrobial agents include fluoroquinolones, such as enrofloxacin, and expanded-spectrum cephalosporins, such as ceftiofur, a third-generation cephalosporin.

Detection of Resistance

Resistance among microorganisms can generally be detected either phenotypically or genotypically. The phenotypic approach is the usual method when testing bacteria for clinical purposes. The Clinical Laboratory Standards Institute (CLSI)

[2] Source: (Animal Health Institute, 2002)

has outlined standard susceptibility testing guidelines. Phenotypic-based antibiotic sensitivity tests most often evaluate resistance using growth inhibition, such as broth or agar disc diffusion assays.

The agar disc diffusion, or Kirby-Bauer, method inoculates an agar plate uniformly with the test organism. A paper disk is impregnated with a fixed concentration of an antibiotic and then placed on the agar surface. Growth of the organism and diffusion of the antibiotic commence simultaneously resulting in a circular "zone of inhibition" in which the amount of antibiotic exceeds inhibitory concentrations. The diameter of the inhibition zone is a function of the amount of drug in the disk and susceptibility of the microorganism. Zone diameter can be then be correlated with susceptibility. Using the zone of inhibition's diameter an organism can be classified as "susceptible", "intermediate", or "resistant" to an antibiotic based on the CLSI criteria. (See ***Figure 2***).

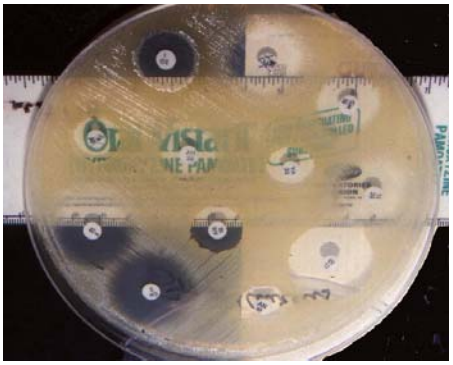

Figure 2. Agar disc diffusion method.[3]

The broth dilution method is the standard method in many laboratories for determining levels of resistance to antibiotics because it can be more easily automated. Serial dilutions of the antibiotic are made in a liquid medium, which are then inoculated with a standardized number of organisms and incubated at 35°C for 16-20 hours. The

[3] Source: (Rollins, 2000)

lowest concentration (highest dilution) of antibiotic that prevents the appearance of turbidity is considered to be the minimal inhibitory concentration (MIC). As with the disk diffusion method, the results can then interpreted as “susceptible”, “intermediate”, or “resistant”.

These “breakpoints” (susceptible, intermediate, or resistant) are determined in part on the basis of achievable drug concentrations at the site of the infection (Anonymous, 2006). The CLSI has established breakpoints for many drugs and organisms. (See ***Table 2***). *Salmonella* breakpoints for most drugs were extrapolated from human data for other *Enterobacteriaceae*. However, because there is no standard breakpoint for identifying resistance versus susceptibility to all clinically important antimicrobial agents, interpretation of the data can be problematic.

Table 2. Antimicrobial agents used in susceptibility testing against *Salmonella* and interpretive criteria of MIC results.[4]

AM Class	Antimicrobial Agent	Interpretive Criteria of MIC (µg/mL)			Source*
		Susceptible	Intermediate	Resistant	
Aminoglycosides	Amikacin	≤16	32	≥64	CLSI
	Gentamicin	≤4	8	≥16	CLSI
	Kanamycin	≤16	32	≥64	CLSI
	Neomycin	≤8		≥16	No CLSI^#
	Streptomycin	≤32		≥64	No CLSI†
Aminocyclitols	Spectinomycin	≤64		≥128	No CLSI#
Beta-Lactams: Penicillins	Amoxicillin/ Clavulanic Acid	≤8/4	16/8	≥32/16	CLSI
	Ampicillin	≤8	16	≥32	CLSI
Beta-Lactams: Cephalosporins (1st Gen.)	Cephalothin	≤8	16	≥32	CLSI
Beta-Lactams: Cephalosporins (2nd Gen.)	Cefoxitin	≤8	16	≥32	CLSI
Beta-Lactams: Cephalosporins (3rd Gen.)	Ceftiofur	≤2	4	≥8	No CLSI†
	Ceftriaxone	≤8	16-32	≥64	CLSI
Phenicols	Chloramphenicol	≤8	16	≥32	CLSI
	Florfenicol	≤8	16	≥32	No CLSI^
Fluoroquinolone	Ciprofloxacin	≤1	2	≥4	CLSI
	Enrofloxacin	≤0.25		≥0.5	No CLSI^
Quinolone	Nalidixic Acid	≤16		≥32	CLSI
Sulfonamide	Sulphizoxazole	≤256		≥512	CLSI
	Sulphachloropyridazine	≤256		≥512	No CLSI‡
	Sulphadimethoxine	≤256		≥512	No CLSI‡
	Sulphathiazole	≤256		≥512	No CLSI‡
	Trimethoprim/ Sulphamethoxazole	≤2/38		≥4/76	CLSI
Tetracycline	Oxytetracycline	≤4	8	≥16	CLSI
	Tetracycline	≤4	8	≥16	CLSI

* CLSI – Clinical Laboratory Standards Institute
^ Breakpoint used in SVARM 2002
# Breakpoint used in DANMAP 2002
† Breakpoint used in NARMS *Salmonella* report
‡ Breakpoint used from sulfizoxazole

Surveillance Programs

Recently many countries established surveillance programs to monitor antimicrobial resistance. Currently in the United States, the National Antimicrobial

[4] Source:(Ray, 2007)

Resistance Monitoring System for Enteric Bacteria (NARMS), established in 1996, is used as the principal organization for monitoring antibiotic resistance in enteric bacteria. NARMS is a collaboration among the CDC, U.S. Food and Drug Administration (Center for Veterinary Medicine) and U.S. Department of Agriculture (Food Safety and Inspection Service and Agricultural Research Services) (CDC, 2002). It monitors the changes among susceptibility patterns for two categories of enteric bacteria: (1) zoonotic bacterial pathogens (*Salmonella* and *Campylobacter*) and (2) usually non-pathogenic bacteria (*E.coli* and *Enterococcus*). The CDC collects isolate samples from state health departments and annual reports of the NARMS surveillance are available at websites from the CDC (human clinical cases) (NARMS, 2003b), FDA (retail meats) (HHS/FDA/CVM, 2003), and USDA (animals and animal products).

Other international surveillance systems include Canada (Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS)), Europe (European Antimicrobial Resistance Surveillance System (EARSS)), Denmark (Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP)), Norway (NORM, for human surveillance, and NORM-VET, for animal and food surveillance), and Sweden (National Veterinary Institute of Sweden (SVARM)).

Salmonella

Salmonella from the family *Enterobacteriaceae* are gram-negative, rod-shaped bacteria. *Salmonella enterica* sub-species *enterica*, which includes serotypes such as Typhimurium, Enteritidis, and Newport, are responsible for most *Salmonella* infections in humans and animals. Both clinical and sub-clinical infections may occur. The source

for the transmission of antimicrobial resistant *Salmonella* is of current interest because of the increase in the prevalence of resistant strains.

In Humans

Clinical signs of salmonellosis in humans include an acute onset of fever, abdominal pain, diarrhea, nausea, sometimes vomiting, and on rare occasions life threatening dehydration (World Health Organization.). It has become one of the most widely distributed food-borne illnesses in the United States. Fluoroquinolones and third-generation cephalosporins (given to children with serious infection) are important antimicrobial agents used for treating salmonellosis. Other drugs given as alternatives to the above treatment include chloramphenicol, ampicillin, amoxicillin, and trimethoprim-sulfamethoxazole. Emerging resistance to these antimicrobials, specifically fluoroquinolones and third-generation cephalosporins, remain of great concern.

In Dairy Cattle

Clinical signs of *Salmonella* in dairy cattle include enteritis, fever, anorexia, depression, reduced milk yield, diarrhea, and, in rare cases, abortion (Ray, 2007). *Salmonella* most commonly causes illness in calves and adult cows and is usually passed from animal to animal by fecal-oral contact (See ***Figure 3***).

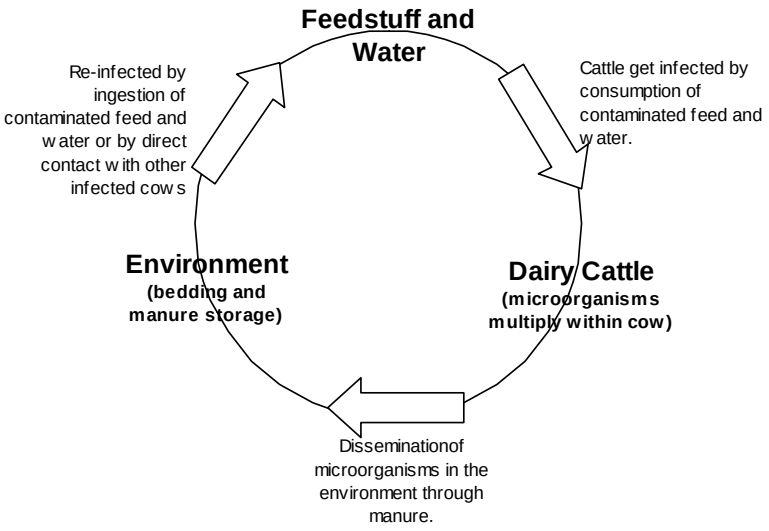

Figure 3. Fecal-oral transmission[5]

Some cattle may harbor *Salmonella* in their gut and exhibit no symptoms. These "carriers" can shed *Salmonella* into the environment in their manure for extended periods of time without giving any indication of an infection. This can result in transmission of the disease to other animals and humans. It is therefore important to investigate antimicrobial resistance in *Salmonella* infected dairy cattle. A previous longitudinal study found that *Salmonella* was found on over 90 percent of a sample of dairy farms in the Midwest and Northeast United States over a one-year period (Fossler et al., 2005).

Escherichia coli

Similar to *Salmonella*, *Escherichia coli* is a gram-negative, rod-shaped bacteria and is considered to be the most common bacterial organism in the world. Some strains are pathogenic and pose an increasing threat to the successful management of calf scours, while most are commensal bacteria that reside in the intestinal tracts of animals and humans without causing illness. In the past few years, many strains of *E.coli* have been

[5] Source: http://babcock.cals.wisc.edu/dwt/en_transmission.pdf

found to be resistant to multiple, structurally unrelated antimicrobial classes, including quinolones, expanded-spectrum cephalosporins, and aminoglycosides (Orden et al., 2001; Donaldson et al., 2006). The high prevalence of these multi-drug resistant *E. coli* in calves could be a significant source of resistance genes for other bacteria that share the same environment, such as *Salmonella* (Donaldson et al., 2006).

METHODS

Recruitment and Herd Selection

The design, herd enrollment, and provided isolates for this investigation were described in detail previously (Cripps et al., 2006; Ray, 2007). As reported earlier, between February 2004 and September 2005, veterinary practices from New York, Vermont, Pennsylvania, Massachusetts, and Connecticut were enrolled to investigate the incidence of clinical salmonellosis in dairy cattle in the northeastern United States. Veterinarians were asked to enroll farms for which they provided routine clinical services and that had at least 30 dairy cattle. Participating veterinarians enrolled client herds by submitting a one-page survey containing contact information, cattle numbers, housing type, salmonellosis herd history, and vaccination practices. Veterinarians were asked to educate their clients about the signs of bovine salmonellosis. Signs included rectal temperature over 103°F, dullness, depression, decreased feed intake, diarrhea containing blood, strands of mucous or intestinal lining, or having a foul odor. Clients were also asked to consider salmonellosis for cattle found dead after a brief period of being off feed in the absence of other specific clinical signs or when an unusual number of deaths occurred in cattle with diarrhea. Most herds were enrolled by September 30, 2004 (90.7%; 754/831). The last herd was enrolled January 26, 2005.

Collection of Samples

Fecal samples were obtained from suspected cases and submitted to the Animal Health Diagnostic Center (AHDC) at Cornell University for *Salmonella* culturing. The cost of shipping and analysis of samples were covered by the study to encourage

sampling of all animals suspected of having salmonellosis. Calves and weaned heifers were also eligible for free laboratory testing of other enteric pathogens, such as *Escherichia coli*, if requested by the client or veterinarian. After the diagnosis of an initial salmonellosis case in a herd was established by bacteriological culture, owners and managers were allowed to make preliminary diagnoses of subsequent suspected cases and submit samples for culture under the supervision of their herd veterinarian.

Processing of Samples

For any cattle that exhibited signs of clinical salmonellosis, 5g of feces was collected from the cows' rectum and put into 30 ml vials containing Cary-Blair transport medium (15ml). Within 24 hours, the samples were taken on ice to the Animal Health Diagnostic Center at Cornell University.

***Salmonella* Isolation**

Standard culturing methods were used to isolate *Salmonella*. A swab was taken of the sample and enriched in 10ml of tetrathionate broth (TTB; Difco, Detroit, MI), which contained 0.2ml of iodine. The broth was incubated at 42°C for 18-24 hours. After incubation, the mixture was streaked onto two agars: Brilliant Green Novobiocin agar (BGN; BBL™, Becton Dickinson and Company, Franklin Lakes, NJ) and Xylose-Lysine-Tergitol-4 agar (XLT-4) and incubated at 37°C for 18-24 hours. H_2S-producing colonies (black) were expected on the XLT-4 plate and lactose-fermenting colonies (red) were expected on the BGN plate. If both black and red colonies appeared in both agars (XLT-4 and BGN, respectively) the colonies were inserted in a Kligler's iron agar (KIA; BBL™) slant. If no colonies appeared, the agars (BGN and XLT-4) were re-incubated for an

additional 18-24 hours. KIA slants were incubated at 37°C for 18-24 hours. If typical *Salmonella* characteristics such as acid butt, alkaline, slant, gas, and H_2S were observed, then the samples were further tested for somatic serogroups, B, C1, C2, D1, and E. After slide agglutination with antisera, Sensititre Automated Microbiology System's A80 panel (TREK Sensititre Microbiology System Division, Westlake, OH) identified positive *Salmonella* colonies. All positive colonies were sent to the National Veterinary Services Laboratory (NVSL), USDA, APHIS, VS, Ames Iowa for complete serotyping.

***Escherichia coli* Isolation**

The Animal Health Diagnostic Center at Cornell University used standard isolation methods for culturing and identifying *E.coli* isolates. Samples were directly plated on Levine EMB agar plates and then incubated at 37°C for 18-24 hours. Characteristic colonies (purple center) were selected and confirmed as *E.coli* using Sensititre Automated Microbiology System's panel (TREK Sensititre Microbiology System Division, Westlake, OH) for identification of gram negative bacteria.

Antimicrobial Susceptibility Testing

A broth microdilution method was used to determine the minimal inhibitory concentration (MIC) of all isolates using a standard panel of antimicrobial agents. Results from 14 of these were used for this study: ampicillin (AMP), apramycin (APR), ceftiofur (CEF), chlortetracycline (CHL), enrofloxacin (ENR), florfenicol (FLO), gentamicin (GEN), neomycin (NEO), oxytetracycline (OXY), spectinomycin (SPE), sulphachloropyridazine (SCH), sulphadimethoxine (SDI), sulphathiazole (STH), and trimethoprim/sulfamethoxazole (TRI). Most isolates were tested for antimicrobial susceptibility within a week of isolation; however a few isolates were recovered for MIC

testing after approximately one year of storage. Isolates stored on TSA slants were recovered for antimicrobial susceptibility testing by a sub-culture within 24 hours on TSA with 5 percent sheep blood (BAP). Lyophilized isolates were recovered for antimicrobial susceptibility testing by reconstitution in water followed by an overnight sub-culture on BAP. The MIC of *Salmonella* and *E.coli* isolates were determined using the Sensititre semi-automated antimicrobial susceptibility testing system (Trek Diagnostic Systems, Cleveland, OH). For each antimicrobial agent, the minimum dilution that inhibited growth of the *Salmonella* isolate was recorded as the MIC. Clindamycin, macrolides, natural penicillins, and tiamulin have poor activity against *Salmonella* so clindamycin, erythromycin, tilmicosin, tylosin, penicillin, and tiamulin were excluded from this analysis (Plumb, 2002). The 8 antimicrobial classes represented by drugs included in the current analysis were aminoglycosides, aminocyclitols, penicillins, cephalosporins, phenicols, fluorquinolones, sulfonamides, and tetracyclines. Quality control was performed every week while the antimicrobial susceptibility testing was conducted using the following four bacteria: *Escherichia coli* ATCC 25022, *Staphylococcus aureus* 29213, *Enterococcus faecalis* 29212, and *Pseudomonas aeruginosa* 27853. Clinical and Laboratory Standards Institute (CLSI) ranges for quality control were used when available (CLSI, 2006a, 2006b). Quality control results were always within expected ranges.

Classifying Antimicrobial Resistance

CLSI interpretive criteria were used to classify *Salmonella* and *E.coli* isolates as resistant or not resistant to individual antimicrobial agents based on MIC panel results

(NCCLS, 2002a, 2002b). CLSI resistant breakpoints were based on human data for *Enterobacteriaceae*. The resistant breakpoints presented in the National Antimicrobial Resistant Monitoring System (NARMS) 2000 Annual Report were used for neomycin, spectinomycin, ceftiofur, florfenicol, enrofloxacin, sulphachloropyridazine, sulphadimethoxine, and sulphathiazole since no interpretive criteria for *Enterobacteriaceae* were available for these antimicrobial agents (U.S. Department of Agriculture, 2000). CLSI resistant breakpoints for sulfizoxazole were used for sulphachloropyridazine, sulphadimethoxine, and sulphathiazole (See ***Table 3***).

Table 3. Antimicrobial agents used to determine the MIC of *Salmonella* and *E.coli* isolates from cattle exhibiting clinical signs of salmonellosis.

AM Class	Antimicrobial Agent	Interpretive Criteria of MIC (µg/mL)			Source*
		Susceptible	Intermediate	Resistant	
Aminoglycosides	Gentamicin (GEN)	≤4	8	≥16	CLSI
	Neomycin (NEO)	≤8		≥16	No CLSI^#
Aminocyclitols	Apramycin (APR)	≤8	16-32	≥32	No CLSI†
	Spectinomycin (SPE)	≥8		≥128	No CLSI#
Beta-Lactams: Penicillins	Ampicillin (AMP)	≤8	16	≥32	CLSI
Beta-Lactams: Cephalosporins (3rd Gen.)	Ceftiofur (CEF)	≤2	4	≥8	No CLSI†
Phenicols	Chloramphenicol (CHL)	≤8	16	≥32	CLSI
	Florfenicol (FLO)	≤8	16	≥32	No CLSI^
Fluoroquinolone	Enrofloxacin (ENR)	≤0.25		≥0.5	No CLSI^
Sulfonamide	Sulphachloropyridazine (SCH)	≤256		≥512	No CLSI‡
	Sulphadimethoxine (SDI)	≤256		≥512	No CLSI‡
	Sulphathiazole (STH)	≤256		≥512	No CLSI‡
	Trimethoprim/ Sulphamethoxazole (TRI)	≤2/38		≥4/76	CLSI
Tetracycline	Oxytetracycline (OXY)	≤4	8	≥16	CLSI

* CLSI – Clinical Laboratory Standards Institute
^ Breakpoint used in SVARM 2002
# Breakpoint used in DANMAP 2002
† Breakpoint used in NARMS *Salmonella* report
‡ Breakpoint used from sulfizoxazole

Samples classified as resistant to 5 or more antimicrobial agents were also classified as multi-drug resistant (MDR).

Calf Samples

Only the calf samples that were tested for both *Salmonella* and *Escherichia coli* were used for this study (n=959). Calves with at least one *Salmonella* isolate and one *E.coli* isolate were included in our analysis (n=74). Resistance patterns (a series of 0's and 1's; with 0=susceptible and 1=resistant) for all 14 antimicrobial agents were generated for each calf to test for resistance similarity among *Salmonella* and *E.coli*. The associations between *Salmonella* resistance and *E.coli* resistance for individual antimicrobial agents and the association of MDR for the two bacterial species were determined using Fisher's Exact tests.

Data Analysis

All results were stored in a Microsoft® Access (2000, Microsoft Corporation, Redmond, WA) database. Data was analyzed using Microsoft® Excel (2000, Microsoft Corporation, Redmond, WA). Statistical analysis was analyzed using Statistix 8 (2006, Analytical Software, Tallahassee, FL) software. Statistical tests with $p<0.05$ were considered significant.

RESULTS

There were 831 dairy herds enrolled in the field study. Samples from 959 calves from 174 herds were used for this part of the investigation. Of the 959 samples, there were 148 isolates from calves with both bacterial types: 74 *Salmonella* and 74 *E.coli* isolates. Only calves with one *Salmonella* isolate and one *E.coli* isolate were included in our analysis. Most herds enrolled in the salmonellosis incidence study were from New York (n=632) and Vermont (n=146), but a few herds were also enrolled from Pennsylvania (n=40), Connecticut (n=8), and Massachusetts (n=5). *Salmonella* was not isolated from calves in Connecticut, Massachusetts, Vermont, and Pennsylvania. Therefore, all isolates included in this study were from herds in New York. Other management characteristics of the herds enrolled in the salmonellosis incidence study and calves with at least one *Salmonella* isolate and one *E.coli* isolate are presented in ***Table 4***.

Table 4. Herd characteristics of herds enrolled in salmonellosis incidence study.

	Herds Enrolled in Salmonellosis Incidence Study	Study Herds
Number of Herds	831	74
State		
New York	632	74
Vermont	146	0
Pennsylvania	40	0
Connecticut	8	0
Massachusetts	5	0
Calf Housing		
Hutch	228	38
Greenhouse	61	21
Curtain Barn	42	6
In Cow Barn	338	3
Other	114	6
No Calves	48	0
Vaccinations		
Endovac-Bovi®[a]	57	5
J-vac® *E.coli*[b]	104	5
J5 *E.coli* Bacterin[c]	190	62
Autogenous *Salmonella* Bacterin	19	2
Commercial *Salmonella* Bacterin	4	0
None of the above	490	6

[a] a vaccine that causes the immune system to make antibodies against Gram Negative bacterial endotoxins, such as *E.coli*, *Salmonella*, *Pasteurella*, and *Moraxella bovis* organisms.
[b] for the vaccination of healthy cattle as an aid in prevention of mastitis due to *E. coli* and the effects of endotoxemia caused by *E. coli* and *Salmonella typhimurium*.
[c] reduces frequency and severity of coliform mastitis

Of the 74 calves in this study, the percent of calves with multi-drug resistant (MDR) *Salmonella* was 68.9 percent (n=51) and with MDR *E.coli* was 90.5 percent (n=67). (See ***Figure 4***). Ninety-four percent (n=48) of the 51 MDR *Salmonella* isolates were also MDR *E.coli* while only 71.6 percent (n=48) of the MDR *E.coli* isolates (n=67) were also MDR *Salmonella*. There was a tendency for *Salmonella* to be more likely to show MDR if the calf had MDR *E.coli*, however, this association was not statistically significant (p=0.19). (See ***Appendix 2***).

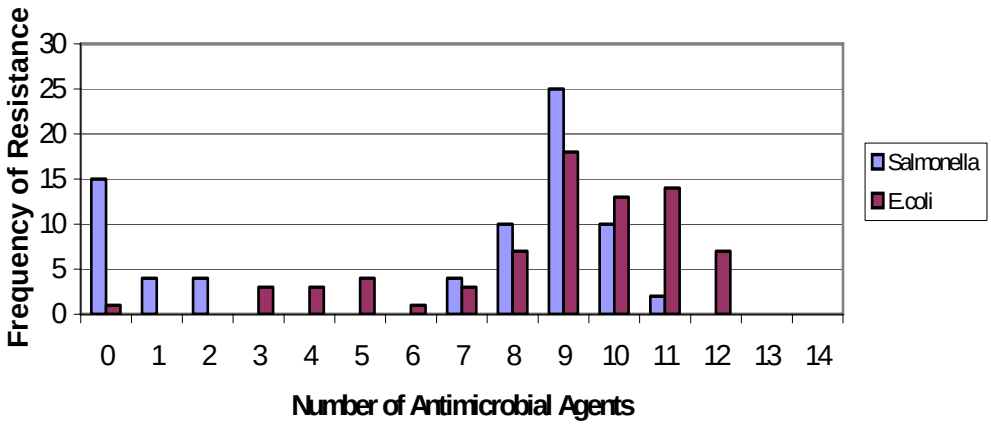

Figure 4. Frequency of resistance to antimicrobial agents among calves

For individual antimicrobial agents, *Salmonella* isolates from this study exhibited a high level of antimicrobial resistance with more than 50 percent of isolates resistant to one or more of the following antimicrobial agents: AMP, CEF, CHL, FLO, NEO, OXY, SCH, SDI, and STH. In contrast, APR and ENR resistance was not found among any *Salmonella* isolates (See ***Table 3*** for breakpoints). *E.coli* isolates also had more than 50 percent of isolates resistant to one or more of the following: AMP, CEF, CHL, FLO, NEO, OXY, SPE, SCH, SDI, STH, TRI. (See ***Table 5***). For each antimicrobial assessed, with the exception of FLO and SCH, the percentage of resistant *E.coli* was higher than the percentage of resistant *Salmonella* sometimes increasing by 2 (e.g. SPE) or even 7 fold (e.g. TRI).

Table 5. Percent of calves with at least one *Salmonella* isolate and one *E.coli* isolate resistant to individual antimicrobial agents

Antimicrobial Agent	Sample Size	% *Salmonella* Resistance	% *E.coli* Resistance
AMP	74	67.6%	91.9%
APR	39	0.0%	2.6%
CEF	72	51.4%	52.8%
CHL	73	65.8%	97.3%
ENR	72	0.0%	1.4%
FLO	71	62.0%	54.9%
GEN	74	2.7%	21.6%
NEO	73	57.5%	90.4%
OXY	73	69.9%	97.3%
SPE	71	28.2%	70.4%
SCH	73	79.5%	74.0%
SDI	71	77.5%	90.1%
STH	71	71.8%	88.7%
TRI	74	8.1%	75.7%

There were 22 unique antimicrobial resistance patterns identified for *Salmonella* isolates and 42 unique antimicrobial resistance patterns identified for *E.coli* isolates. (See ***Table 6***). There were 6 antimicrobial resistance patterns identified among both *Salmonella* and *E.coli* isolates. Pan-susceptibility was the most common individual resistance pattern observed in this study. Of the 6 unique antimicrobial resistance patterns shared by *E.coli* and *Salmonella*, 5 patterns were resistant to 7 or more antimicrobial agents, most commonly including AMP, CEF, CHL, FLO, NEO, OXY, SCH, SDI, STH. All the *Salmonella* and *E.coli* isolates shown in ***Table 7*** were susceptible to APR, ENR, GEN. The most commonly observed resistance pattern for both *Salmonella* (n=8) and *E.coli* (n=3) was sequence number 4 (AMP-CEF-CHL-FLO-NEO-OXY-SCH-SDI-STH) (See ***Table 7***).

Table 6. Antimicrobial resistance patterns for *Salmonella* (n=22) and *E.coli* (n=42) in dairy calves

SALMONELLA	
12345678901234[a]	Count
0_000000001000	4
0_000000001100	4
00000000000000	15
00010101101110	1
1_000100111110	1
1_010001111110	5
1_010101101110	2
1_010101111110	3
1_100101101110	1
1_110000101110	1
1_110001111111	1
1_110100101110	2
1_110101101110	8
1_110101101111	3
10000100111110	1
10010100111110	1
10110100101110	1
10110100111111	2
10110101100110	1
10110101101110	11
10110101111110	4
10110111111110	2
Grand Total	74

E.COLI	
12345678901234[a]	Count
0_010000101000	1
0_010001101000	1
00000000000000	1
00010000110000	1
00010001101111	1
00010101101110	1
1__1__011_0__1	1
1__1__011_1__0	1
1_0_0_1_____1	1
1_0100011011111	1
1_0100011111111	3
1_0100111111111	3
1_1100001011111	1
1_1100011111111	1
1_1101001111111	1
1_1101011011110	3
1_1101011111110	1
1_1101011111111	8
1_1101111111111	3
10000001000111	1
10010000110001	1
10010001100000	2
10010001100110	1
10010001100111	1
10010001101111	3
10010001111111	6
10010101110111	1
10010101111111	2
10010111111111	1
10110011110110	1
10110011110111	1
10110101100110	2
10110101101111	1
10110101110101	1
10110101110110	1
10110101110111	2
10110101111110	1
10110101111111	5
10110110111111	1
10110111111111	3
10111111110111	1
11010011111111	1
Grand Total	74

[a] 1(AMP); 2(APR); 3(CEF); 4(CHL); 5(ENR); 6(FLO); 7(GEN); 8(NEO); 9(OXY); 0(SPE); 1(SCH); 2(SDI); 3(STH); 4(TRI)
0, Not Resistant; 1, Resistant; _, not tested

Table 7. Identical antimicrobial resistance patterns for both *Salmonella* and *E.coli*

Seq #	12345678901234[a]	*Salmonella* Isolates	*E.coli* Isolates
1	00000000000000	15	1
2	00010101101110	1	1
3	1_110001111111	1	1
4	1_110101101110	8	3
5	10110101100110	1	2
6	10110101111110	4	1

[a] 1(AMP); 2(APR); 3(CEF); 4(CHL); 5(ENR); 6(FLO); 7(GEN); 8(NEO); 9(OXY); 0(SPE); 1(SCH); 2(SDI); 3(STH); 4(TRI)
0, Not Resistant; 1, Resistant; _, not tested

There was no obvious association of resistance patterns between *Salmonella* and *E.coli* isolated in the same calf (See ***Appendix 1***).

Fourteen antimicrobial agents were analyzed to observe whether there was an association of resistance of *E.coli* with resistance of *Salmonella*. The majority of the antimicrobial agents (n=8) were not significant at the 0.05 level. There was a significant association of antimicrobial resistance between *Salmonella* and *E.coli* for GEN and SPE, p=0.04 and p=0.05, respectively. (See ***Table 8*** and ***Appendix 2***).

Table 8. Significance of antimicrobial resistance between *Salmonella* and *E.coli* for individual antimicrobial agents

	P-value	Significant
AMP	0.38	No
APR	1.00	No
CEF	0.24	No
CHL	1.00	No
ENR	1.00	No
FLO	0.48	No
GEN	0.04	Yes
NEO	0.07	No
OXY	1.00	No
SPE	0.05	Yes
SCH	1.00	No
SDI	0.11	No
STH	0.30	No
TRI	0.33	No

H_o, *E.coli* resistance has no significant influence on *Salmonella* resistance

Although there was no effect of calf age at the time of testing on the percentage of resistance for either *Salmonella* or *E.coli*, the percentage of MDR *Salmonella* always remained lower than that of MDR *E.coli*. (See ***Table 9***, ***Figure 5***).

Table 9. Number of MDR calves as age increased

Age (days)	Total No. of calves	*Salmonella* Isolates (%)	*E.coli* Isolates (%)
1-4	23	18 (78.3)	20 (87.0)
5-10	29	17 (58.6)	27 (93.1)
11-44	22	16 (72.7)	20 (90.9)
Total:	74		

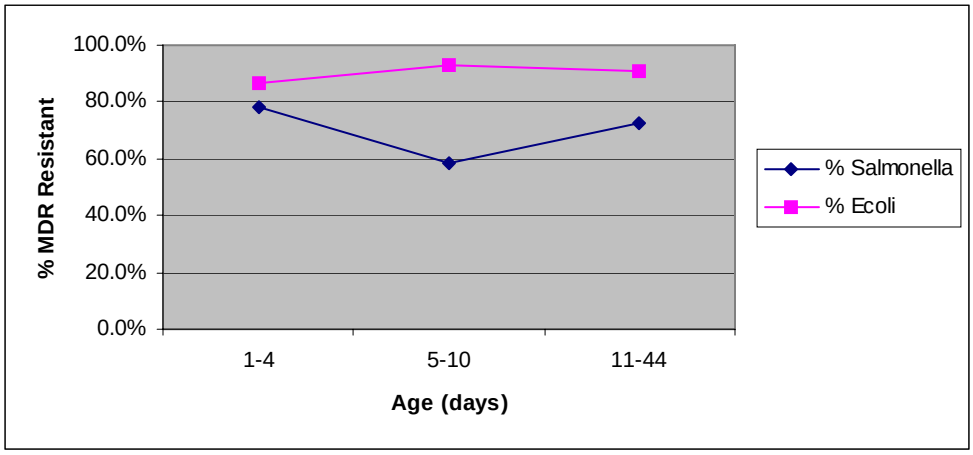

Figure 5. Percentage of calves that were MDR for both *Salmonella* and *E.coli*

DISCUSSION

Samples from this study came from a larger study designed to assess the animal and herd-level incidence of salmonellosis among dairy cattle in the northeastern United States (Cripps et al., 2006; Ray, 2007). This study examined the patterns of antimicrobial resistance for *E.coli* and *Salmonella enterica* in 74 dairy calves.

A high prevalence of antimicrobial resistance among both *Salmonella* and *E.coli* was observed in this study. Isolates tended to be resistant to similar antimicrobial agents, including AMP, CEF, CHL, FLO, NEO, OXY, SCH, SDI, and STH. The occurrence of MDR enteric bacteria could be related to local selection pressures from increased antimicrobial use for either prevention or treatment of diseases, such as salmonellosis. It is also possible that dissemination of highly resistant strains plays a role (Davies, 1999). Factors such as farm size, location, calves born in a building rather than outdoors, and rodent or bird control may have contributed to the occurrence of *Salmonella* within the herds (Warnick et al., 2001). The potential for gene transfer between the two strains of enteric bacteria could also explain the frequency of MDR that was observed in this study.

It was not surprising that *Salmonella* and *E.coli* were mostly susceptible to APR, GEN, and ENR. Apramycin is approved only for use in the swine industry (although not approved, it is still sometimes used to treat calves) (Plumb, 2002) and resistance is rare among gram negative bacteria (Prescott, J.F., et.al., 2000). Although most isolates were susceptible to gentamicin, *in vitro* testing for gentamicin does not relate well to clinical efficacy. It should also be noted that gentamicin use in cattle is discouraged because of prolonged tissue residues. Enrofloxacin, a fluoroquinolone, is not approved for use in

dairy cattle and fluoroquinolone resistance is rare in enteric bacteria isolated from cattle (Plumb, 2002).

Commensal enteric *E.coli* may play an important role of R factor transfer with other bacteria in animals. It is already known that *Salmonella* has mobile genetic elements containing several resistance genes (e.g. integrons or transposons) located on plasmids or integrated into the chromosome. These genetic elements have been found to play an important role in the transmission of resistance to multiple drugs between certain *Salmonella* serotypes (Ray, 2007). It is therefore a concern that *E.coli* may constitute a potential reservoir of resistance genes that could be transferred to pathogenic bacteria, such as *Salmonella* (Donaldson et al., 2006). Dairy calves typically have a relatively high percentage of resistant *E.coli* within 2 weeks after birth (Berge, 2005). In one previous study, resistance decreased after 2 weeks of age (Khachatryan, 2004). In contrast, in our study, resistance did not appear to decrease with age possibly because of isolates were from clinical cases. Among the isolates in our study, most antimicrobial resistant isolates were resistant to five or more antimicrobial agents. *Salmonella's* acquisition of new resistance, specifically from *E.coli,* could result in MDR strains being less responsive to treatment with antimicrobial drugs, resulting in larger outbreaks or more serious disease in people.

Figure 4 suggests a relationship between *Salmonella* and *E.coli*, which tended to show similar trends for resistance to the same antimicrobial agents; however there were few statistically significant associations between *E.coli* and *Salmonella* resistance. In a previous molecular study, for individual antibiotics different genetic properties of R factors were found in *Salmonella* Typhimurium and *E.coli* species isolated from the same

calf. All R factors of *S.* Typhimurium were f_i^-, whereas most of *E.coli* was f_i^+ (Sato and Terakado, 1977). Another study observed drug-resistance patterns of *Salmonella* and *E.coli* strains isolated from the same sample to be the same even though the genetic properties of the R plasmids derived from *E.coli* were different from those of *S.* Typhimuirum (Ishiguro et. al., 1980). A more recent study (Mandal et. al., 2003) concluded that *Salmonella enterica* serovar Typhi's acquired R-plasmid-encoded-resistance from *Escherichia coli*. With the current study's size and cross-sectional design, relatively infrequent transfer of resistant genes occurring over a longer time period can, therefore, not be ruled out.

CONCLUSION / CLINCAL RELEVANCE

Overall, *E.coli* isolates were more resistant than *Salmonella*. While there were some shared patterns of resistance between *Salmonella* and *E.coli*, *Salmonella* isolates were not more likely to be resistant to individual antibiotics if a calf had a resistant *E.coli*. Results suggest that selection pressure from recent exposure to drugs or interspecific gene transfer was not resulting in a strong association of resistance between the bacteria.

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APPENDICES

Appendix 1. Antimicrobial Patterns for *Salmonella* and *E.coli* for Individual Calves (n=74)

Age Group	Accession	SubAcc	Age(in days)	SALMONELLA [a] 12345678901234	E.COLI [a] 12345678901234
1-4 days	70078.04	1	1	00000000000000	10010001100000
	103538.04	1	1	00000000000000	00010001101111
	120321.04	1	1	00000000000000	10110101110111
	15728.05	2	1	10000100111110	10010001100111
	108831.05	1	1	1_110100101110	0_010000101000
	108831.05	3	1	1_110000101110	0_010001101000
	93016.04	3	2	1_110101101110	10010001111111
	121360.04	1	2	10010100111110	10110101110101
	121387.04	3	2	10110101101110	10110101110110
	121370.04	4	2	10110101101110	10010001111111
	23345.05	1	2	1_110101101110	1_110101111110
	52860.05	1	2	1_010001111110	1_110101111111
	53682.05	2	2	1_010001111110	1_110101111111
	82690.05	1	2	1_010001111110	1_110101111111
	90199.05	1	2	1_110100101110	1_110101111111
	88254.05	1	2	0_000000001100	1_010001111111
	130793.05	3	2	1_110101101111	1_110000101111
	130793.05	5	2	1_110101101111	1_110101111111
	85275.04	4	3	10110101111110	10110101111110
	15434.05	1	3	10110100111111	10110110111111
	46245.05	1	3	1_100101101110	1_110101101110
	130793.05	1	3	1_110101101111	1_110101111111
	99293.05	1	4	0_000000001000	1_110100111111
5-10 days	24673.04	1	5	00000000000000	00010000110000
	88465.04	1	5	10110100101110	10110111111111
	68448.05	6	5	0_000000001100	1_110101101110
	135032.04	4	5	10110101101110	10010111111111
	131849.04	1	6	10110100111111	10110101111111
	125225.04	2	7	10110101101110	10110011110110
	25951.05	3	7	1_010101111110	1_010011111111
	25951.05	3	7	1_010101111110	1_010011111111
	102877.05	1	7	1_110001111111	1_110001111111
	137768.04	1	7	00000000000000	10010001111111
	137768.04	2	7	00000000000000	10010000110001
	79914.05	1	7	1_010001111110	1_0_0_1_____1
	13273.05	14	8	10110101101110	10010001100110
	13273.05	15	8	1_110101101110	1__1__011_1__0
	26871.05	5	8	1_010101101110	1_010011111111
	91667.04	1	8	00000000000000	10110101101111
	112232.04	1	9	00000000000000	10110111111111
	121190.04	1	9	10110101101110	10110011110111
	26871.05	4	9	1_000100111110	1_110101111111

	70988.04	1	10	00000000000000	10110111111111
	21529.05	2	10	10110101101110	10110101111111
	26871.05	3	10	1_010101111110	1_010001111111
	32673.05	1	10	0_000000001100	1_010001101111
	32354.05	1	10	1_010101101110	1_010001111111
	55296.05	2	10	0_000000001100	1_110111111111
	133476.04	3	10	1_110101101110	10010001111111
	833.05	1	10	00000000000000	10010001101111
	833.05	2	10	00000000000000	10010001101111
	137192.04	1	10	00000000000000	10010001101111
11-44 days	90676.04	1	11	00010101101110	10010101111111
	10799.05	4	11	10110111111110	11010011111111
	10799.05	5	11	10110111111110	10111111110111
	101139.05	1	11	1_110101101110	1_110111111111
	103917.05	1	11	1_110101101110	1_110101101110
	133476.04	4	11	10110101101110	10010101111111
	135032.04	5	11	10110101100110	10010001111111
	79193.04	2	12	0_000000001000	10110101111111
	111866.05	1	12	1_010001111110	1_110101111111
	126712.04	1	13	10110101101110	10110101100110
	106861.04	2	14	10110101111110	10000001000111
	126712.04	3	14	10110101101110	10110101100110
	76286.04	1	16	00000000000000	10110101111111
	110020.04	1	16	10110101111110	10010101110111
	110783.04	1	17	10110101111110	10110101110111
	126712.04	2	20	10110101101110	00010101101110
	79193.04	1	21	00000000000000	10010001111111
	101139.05	2	21	1_110101101110	1_110111111111
	13273.05	13	27	1_110101101110	1__1__011_0__1
	83084.04	6	40	00000000000000	10110101111111
	83084.04	9	43	0_000000001000	00000000000000
	83084.04	7	44	0_000000001000	10010001100000

[a] 1(AMP); 2(APR); 3(CEF); 4(CHL); 5(ENR); 6(FLO); 7(GEN); 8(NEO); 9(OXY); 0(SPE); 1(SCH); 2(SDI); 3(STH); 4(TRI)
0, Not Resistant; 1, Resistant; _, not tested

Appendix 2. Relationship of resistance for *Salmonella* and *E .coli* isolates

AMP:

		Salmonella 0	Salmonella 1
Ecoli	0	3	3
	1	21	47

74

OXY:

		Salmonella 0	Salmonella 1
Ecoli	0	1	2
	1	22	49

74

APR:

		Salmonella 0	Salmonella 1
Ecoli	0	73	0
	1	1	0

74

SPE:

		Salmonella 0	Salmonella 1
Ecoli	0	21	3
	1	33	17

74

CEF:

		Salmonella 0	Salmonella 1
Ecoli	0	21	15
	1	16	22

74

SCH:

		Salmonella 0	Salmonella 1
Ecoli	0	4	16
	1	12	42

74

CHL:

		Salmonella 0	Salmonella 1
Ecoli	0	1	2
	1	25	46

74

SDI

		Salmonella 0	Salmonella 1
Ecoli	0	5	5
	1	14	50

74

ENR:

		Salmonella 0	Salmonella 1
Ecoli	0	73	0
	1	1	0

74

STH:

		Salmonella 0	Salmonella 1
Ecoli	0	5	6
	1	18	45

74

ERY:

		Salmonella 0	Salmonella 1
Ecoli	0	14	0
	1	0	60

74

TIA:

		Salmonella 0	Salmonella 1
Ecoli	0	0	3
	1	0	71

74

FLO:

		Salmonella 0	Salmonella 1
Ecoli	0	16	19
	1	14	25

74

TIL:

		Salmonella 0	Salmonella 1
Ecoli	0	0	3
	1	0	71

74

GEN:

		Salmonella 0	Salmonella 1
Ecoli	0	58	0
	1	14	2

74

TRI:

		Salmonella 0	Salmonella 1
Ecoli	0	18	0
	1	50	6

74

NEO:		Salmonella	
		0	1
Ecoli	0	6	2
	1	26	40

74

MDR:		Salmonella	
		0	1
Ecoli	0	48	19
	1	3	4

74

0, not resistant; 1, resistant