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Changes in the antimicrobial resistance profiles of *Salmonella* isolated from the same Michigan dairy farms in 2000 and 2009

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ABSTRACT

This study compared the antimicrobial resistance profiles of *Salmonella* collected from the same Michigan, USA dairy farms between the years 2000 and 2009. The specific objective was to understand the type and distribution of changes in antimicrobial resistance that occurred within farms over the past 10 years. Multinomial, multilevel models were constructed to estimate the differences in minimum inhibitory concentrations (MICs) between years. The MICs of most antimicrobials were significantly lower in 2009 than in 2000, but were higher for amikacin and gentamicin. Decreases in MICs were in part due to changes in the prevalence of multidrug resistant strains, but were also distributed across the susceptible population of isolates. The type and direction of within-farm changes in MICs were similar for the majority of farms. These results suggest a decrease in antimicrobial resistance and/or a change in the population structure of *Salmonella* that colonize dairy farms in Michigan.

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1. Introduction

Salmonella is a worldwide cause of foodborne illness in people and livestock. Recent data from the Centers for Disease Control show that *Salmonella* is the leading cause of foodborne hospitalizations and death (Scallan, Griffin, Angulo, Tauxe, & Hoekstra, 2011). Persons with suboptimal immune systems, particularly children, are most vulnerable to severe infections (CDC, 2010). Antimicrobial resistance (AMR) in *Salmonella* impairs the ability of physicians and veterinarians to treat serious infections. Patients infected with resistant strains of bacteria have higher hospital costs, a greater likelihood of septicemia, and higher mortality than patients infected with susceptible strains (Maragakis, Perencevich, & Cosgrove, 2008; Varma et al., 2005).

Dairy farms serve as reservoirs of antimicrobial resistant *Salmonella* which can be transmitted to people through food vehicles or direct contact with animals (Fey et al., 2000). Beef and dairy products account for a substantial proportion of traceable *Salmonella* outbreaks (Lynch, Painter, Woodruff, & Braden, 2006). The serotypes and molecular subtypes of AMR *Salmonella* isolated from dairy farms have significant overlaps with those that cause disease in humans (Alcaine et al., 2006; FDA, 2010). Furthermore, resistant *Salmonella* strains may serve as donors of resistance genes to other pathogenic bacteria (Capozzi & Spano, 2009; Mathew, Liamthong, Lin, & Hong, 2009). A study of *Salmonella* shedding on dairy farms conducted from 2000 to 2001 found that 27% of dairy farms harbored one or more AMR *Salmonella* (Ray et al., 2007). Changes in the prevalence of AMR *Salmonella* on dairy farms may have important impacts on human health. Monitoring systems of AMR in the U.S. have shown substantial changes in the types and frequency of resistance in *Salmonella* over the past ten years. The National Antimicrobial Monitoring System (NARMS) has shown increases in the frequency of cephalosporin resistant *Salmonella* in clinically ill cattle (FDA, 2010). Consecutive cross-sectional studies by the National Animal Health Monitoring System (NAHMS) have shown a decrease in the prevalence of AMR *Salmonella*. Approximately 12% of isolates were resistant to at least one antibiotic in 2002, compared to only 1.7% of isolates in 2007 (USDA, 2010). The different populations of *Salmonella* resultant from different sampling methods likely account for the discordant results between NAHMS and NARMS. Nonetheless, both monitoring systems suggest that the population of *Salmonella* on dairy farms and/or the AMR of those organisms has shifted significantly.

Rapid increases in AMR prevalence within farms can occur as a result of the introduction of resistant *Salmonella* strains, as exemplified by the clonal dissemination of *Salmonella* Typhimurium DT104 and MDR AmpC *Salmonella* Newport (Butaye et al., 2006). Changes in AMR may also occur due to the divergence of strain lineages as a result of horizontal gene transfer and genetic recombination (Sangal et al., 2010). Changes in AMR prevalence estimates identified by U.S. AMR monitoring systems could have important impacts on public health. However, it is unknown if these changes were uniformly distributed across farms, or were unevenly distributed, and dependent on farm characteristics. Improved understanding of the within-farm changes

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across years will provide additional insights into the epidemiology and ecology of antimicrobial resistance and *Salmonella* on dairy farms.

The objective of this study was to compare the AMR profiles of *Salmonella* isolates from the same farms at time points ten years apart. The hypothesis tested was that the AMR of *Salmonella* isolates within Michigan dairy farms changed between the years 2000 and 2009.

2. Methods

This study used a retro-prospective study design to identify changes in the AMR profile within Michigan dairy farms. The data for this study consists of two components: retrospective data collected from Michigan dairy farms in the year 2000, and prospective data collected 10 years later from the same Michigan dairy farms. Retrospective data were retrieved from a 2000–2001 multi-center, longitudinal study of *Salmonella* shedding on randomly selected dairy farms in Michigan, New York, Wisconsin, and Minnesota (Fossler et al., 2004).Stored *Salmonella* isolates collected in 2000 were retrieved from the Center for Comparative Epidemiology (CCE) at Michigan State University. Samples from the same farms were collected in August of 2009.

2.1. Farm selection

For data collected in 2000, the number of farms sampled in each state was based on a sample size calculation with the following assumptions: 30% of the farms would be positive for *Salmonella*, a power 0.80, alpha of 0.10, and 2:1 ratio of exposed and unexposed farms for the risk factors of interest. In 2000, 31 dairy farms in Michigan were selected that met the following criteria: less than 100 miles from Michigan State University, milking greater than 30 Holstein cows, raising their own calves for replacements, and shipping milk year-round. For the data collected in 2009, all Michigan dairy farms that participated in 2000 were recruited.

2.2. Sample collection

For the purposes of this manuscript, the word “sample” is used to refer to either animal fecal samples or environmental swabs collected from dairy farms. Comparable sampling plans for collecting fecal and environmental samples were used in both 2000 and 2009. In 2000, farms were sampled every other month, resulting in five sampling events. In 2009, four farms were sampled once, and two farms were sampled twice. Farms were sampled twice if the farm was negative for *Salmonella* on the first round of sampling, and had a greater than 3% shedding prevalence in 2000. Fecal samples were collected from the rectum of dairy cattle using a single use rectal sleeve, and from calves using digital rectal retrieval. In both 2000 and 2009, healthy lactating cows and “target” animals were sampled from each farm. Target animals were defined as dairy animals most likely to be shedding *Salmonella*, including pre-weaned calves, cows identified as sick by the farm management, cows within 14 days of their calving date, and cows scheduled to be culled within 14 days. Target animals were preferentially sampled to increase the number of *Salmonella* isolates recovered and most accurately define the distribution of AMR in *Salmonella* within each farm. The number of samples collected was calculated to provide a 95% probability of recovering at least one *Salmonella* positive sample. Similar sample size calculations for fecal and environmental samples were used in 2000 and 2009, and have been previously described (Fossler et al., 2004). Systematic sampling was used to obtain a representative sample of healthy cows and target animals. Environmental samples were taken using gauze swabs soaked with double-strength skim milk. Samples were taken from cow environments, including the maternity pen, sick pen, cull cow hide, milk filter, and manure storage area. Samples from calf environments included a composite sample from multiple calf pens. All samples were stored in commercial bags,[1] placed in a cooler with ice, and submitted to the microbial epidemiology laboratory the following day.

2.3. Salmonella isolation

Isolation of *Salmonella* was performed in the same laboratory with highly similar protocols in 2000 and 2009. With the exception of a confirmatory step (urea agar used in 2009), and the number of colonies chosen for confirmatory steps (five in 2009, and two in 2000), the protocols for the isolation and confirmation of *Salmonella* from fecal and environmental samples were identical. Samples were enriched by adding tetrathionate broth as to achieve a 1:10 dilution and incubating for 48 h at 37 °C. The enriched sample was streaked onto XLT4 agar and incubated for 24 h at 37 °C. In 2009, up to five suspect colonies from XLT4 agar, (red or yellow with black centers) were inoculated onto TSI and urea agar slants, and incubated for 24 h at 37 °C. In 2000, up to two suspect colonies from XLT4 agar, (red or yellow with black centers) were inoculated onto TSI only, and incubated for 24 h at 37 °C. Colonies with test results typical for *Salmonella* (alkaline/acid/H2S positive and urease negative) were then inoculated onto lysine-iron agar and Simmons citrate agar slants. Colonies that were lysine decarboxylase and hydrogen sulfide positive in lysine-iron agar (purple slant and purple-black butt) as well as positive in Simmons citrate (blue) were considered positive for *Salmonella*. *Salmonella* isolates harvested in 2000 were frozen in tryptic soy broth/glycerol solution at −80 °C and stored in cryovials. In 2009, these were retrieved, and underwent further biochemical confirmation before antimicrobial susceptibility testing. Isolates were stabbed onto a TSA slant, and stored at room temperature prior to antimicrobial susceptibility testing.

2.4. Antimicrobial resistance testing

To enable comparisons of antimicrobial resistance across years, *Salmonella* isolates collected in the 2000 study were tested concurrently with the 2009 isolates using the same commercially prepared microbroth dilution antimicrobial panels.[2] This panel contained a prepared range of concentrations for the following 15 antimicrobials: amikacin, amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole. The tested antimicrobials were those used by NARMS (FDA, 2010), and are considered to be critically important (amikacin, ampicillin, amoxicillin-clavulanic acid, ceftriaxone, ciprofloxacin, gentamicin, nalidixic acid, and streptomycin) or highly important (kanamycin, chloramphenicol, cefoxitin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole,) by the World Health Organization (WHO, 2007). Quality control tests were performed using *E. coli* ATCC 25922 for all panels, and were all within acceptable limits. Colonies identified as *Salmonella* were streaked to Mueller Hinton agar and incubated for 18–24 h at 37 °C. Testing was performed according to the instructions from the manufacturer of the automated microbroth dilution system (Trek Diagnostic Systems, Inc.), and panels were read with an autoreader. Breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) were used to classify isolates as susceptible, intermediate, or resistant (CLSI, 2010). No CLSI interpretive criteria were available for ceftiofur or streptomycin, so breakpoints presented in the NARMS 2007 Annual Report were used (FDA, 2010). Isolates that were classified as intermediate were considered to be sensitive for the purposes of analysis.

[1] WhirlPak®, Nasco, Fort Atkinson, WI.
[2] CMV1AGNF; Trek Diagnostic Systems, Inc.

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