IV. Data Analysis

A. *Salmonella*

1. Recovery of isolates by serotype within commodity

The total number of *Salmonella* isolates tested by year since 1997 is shown in Table 1A.

The top serotypes by commodity for 2010 are shown in Table 2A. Overall, Kentucky, Hadar, Montevideo and Derby ranked as the most prevalent serotype for chickens, turkeys, cattle and swine, respectively. Using 2010 serotypes as the baseline, the relative distributions from 1997-2010 for the top five serotypes per commodity are shown in Figures 1A-4A.

Since 2002, the percentage distribution of Kentucky and Heidelberg from chickens appears divergent (Figure 1A). While an upward trend in Kentucky was observed from 1997 through 2006, a downward trend was observed predominantly for Heidelberg during the same time period. Kentucky declined from 2006 to 2008 before increasing again in 2009 (38.8%) and in 2010 (43.1%). At the same time an increase in Heidelberg was observed from 2006 to 2008 before declining in 2009 (13.4%) and 2010 (4.4%). Since 2002, recovery of Enteritidis has increased to 27.0% of isolates in 2010. Conversely, recovery of Typhimurium and Typhimurium variant 5- has remained at or below 8.7% for all years.

Among isolates recovered from turkeys (Figure 2A) Hadar remained below 18.5% through 2004, increased in 2007 to 43.5%, and has since declined to 19.9% in 2010. The recovery of Saintpaul fluctuated between 0.9% in 1997 and 13.9% in 2010. In general, a steady decline was observed in Heidelberg from 2001 (25.8%) through 2009 (2.5%); however an increase was observed in 2010 (9.3%). Both Senftenberg and antigenic formula III 18:z4,z23:- have remained at or below 9.5% of isolates for all years.

In 2010, the recovery of Montevideo among cattle isolates decreased to 24.7% after a steady incline from 2005 (13.1%) to 2009 (29.5%). With the exception of 2005, Dublin has shown an upward trend from 2005 to 2010 (from 3.6% to 16.6%). Recovery of Anatum, Kentucky and Typhimurium has remained at or below 10.9% since 1998 (Figure 3A).

Recovery of Derby among swine has fluctuated within the years tested from a high of 34.3% in 2002 to a low of 12.3% in 2007. However, a decline has been observed in 2009 (20.0%) and in 2010 (16.2%) (Figure 4A). An increase in Saintpaul was observed in 2010 (9.9%) and represents the highest percent recovered to date. Variations were noted for recovery of Infantis, Johannesburg and Typhimurium variant 5- from 1997-2010, but overall remained at or below 16.2%.

2. MIC distributions

The 2010 MIC distributions by antimicrobial and commodity for all *Salmonella* serotypes combined (macro analysis) are shown in Table 3A. Since it is not unusual for resistance to be driven by only a few serotypes and because the distribution of serotypes between commodities varies greatly, it is important to determine resistance at the serotype and commodity level (micro analysis). However, a macro analysis is often useful to quickly determine any overt change between years prior to conducting a micro analysis of the data.

The overall percent resistance by year (macro analysis), antimicrobial and commodity of all *Salmonella* serotypes combined is shown in Table 4A. Resistance to amikacin has only been observed once in a single isolate from swine in 2007. Similarly, with the exception of one isolate from chicken in 2003, resistance has yet to emerge to ciprofloxacin. However, a breakpoint change for ciprofloxacin for invasive *Salmonella* was recently published in the latest CLSI M100 document (January 2012).[1] Appendix A shows MIC distributions by commodity for all years and percent resistance as calculated with the new lower MIC interpretation breakpoints. A total of only nine additional isolates among all years were classified as ciprofloxacin resistant using the new lower breakpoint (chickens n=3, turkeys n=5, and cattle n=1). Resistance to nalidixic acid remained at or below 0.7% for chickens, turkeys and swine, however, increased from 1.0% to 2.8% among cattle in 2010 and is primarily attributed to an increase in serotype Dublin. Resistance to gentamicin among turkeys increased from 14.9% to 19.9% in 2010 and remains highest among turkeys when compared to other animal sources (not attributed to a particular serotype). In 2010, resistance to the cephems class remained highest among cattle isolates (20.6%, 21.5% and 21.5% for cefoxitin, ceftiofur and ceftriaxone, respectively); additionally, these numbers show an increase from 2009. An increase in resistance to the cephems class was also observed among turkeys from 2009 to 2010; conversely, a decrease in cephem resistance was observed in swine. Ampicillin resistance among turkeys in 2010 (44.4%) has been the highest observed among all commodities and years. Likewise in 2010, resistance to amoxicillin/clavulanic acid increased among turkeys and cattle (15.2% and 21.5%, respectively) and has been the highest observed among all years in each commodity. In 2010, resistance to trimethoprim/sulfamethoxazole was only observed in cattle and swine (4.5% and 1.8%, respectively). Resistance to the other antimicrobials varied by commodity.

A micro analysis of the 2010 data is presented in Tables 5A through 8A which shows total percent resistance and MIC distribution by commodity and serotypes. Data is only presented for those serotypes with at least ten isolates in a particular commodity. Among serotypes from *Salmonella* isolates recovered from chickens (Table 5A), Kentucky (n=243) exhibited varying levels and combinations of resistance to 10 antimicrobials (amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, gentamicin, streptomycin, sulfisoxazole and tetracycline) and showed no resistance to five antimicrobials (amikacin, ciprofloxacin, kanamycin and nalidixic acid trimethoprim/sulfamethoxazole). Conversely, Enteritidis (n=152) was susceptible to amoxicillin/clavulanic acid and to the cephems class while exhibiting $\leq$ 3.3% resistance to all other antimicrobials tested. A contrast in resistance levels among serotypes from *Salmonella* isolates recovered from turkeys is also observed (Table 6A). Hadar (n=30) was susceptible to all antimicrobials

[1] CLSI. 2012. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-second Informational Supplement. CLSI document M100-S22. CLSI, Wayne, PA.

from the cephems class while 35.7% of Heidelberg (n=14) were resistant. Among cattle (Table 7A), Montevideo (n=61) only exhibited resistance to streptomycin (3.3%) and tetracycline (11.5%). Conversely, Dublin (n=41) was resistant to 13 antimicrobials tested and was only susceptible to amikacin and ciprofloxacin.

The frequency and percentage of confirmed *S.* Typhimurium classified as definitive type (DT) 104 and DT104 complex isolates (DT104a, DT104b or U302) is reported separately by food animal source from 1997 through 2010 (Table 9A). In 2010, DT104 isolates were identified among *S*. Typhimurium recovered from cattle (n=5), swine (n=5), turkeys (n=2) and chickens (n=1). In 2010, a total of 13 isolates were confirmed as DT104 or DT104 complex which accounted for 15.1% of all *S*. Typhimurium and *S*. Typhimurium variant 5- isolates tested and for 1.2% of all *Salmonella* (n=1,073) tested in 2010. In previous years, results were presented separately for isolates exhibiting the ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfisoxazole and tetracycline) penta-resistant pattern and the ACSuT quad-resistant pattern; however in 2010, there were no Typhimurium or Typhimurium var. 5- isolates exhibiting the latter pattern.

Specific MDR patterns by commodity are presented in Tables 10A through 13A. Data is presented by CLSI class as well as by phenotype(s) thought to be of clinical importance in humans [at least ACSSuT, ACT/S (ampicillin, chloramphenicol, trimethoprim/sulfamethoxazole), ACSSuTAuCx [ACSSuT, amoxicillin/clavulanic acid and ceftriaxone] or ceftriaxone and nalidixic acid resistance]. Overall, pan-susceptible isolates most often originated (in order of decreasing frequency) from cattle, chickens, swine and turkeys as observed in previous years. Among the clinically important phenotypes reported, resistance was least often observed to ACT/S and to ceftriaxone plus nalidixic acid for all animal sources. Isolates with resistance to phenotypes ACSSuT and ACSSuTAuCx most often originated from cattle (18.6% and 16.2%, respectively in 2010).

B. *Campylobacter*

The number of *Campylobacter* isolates tested from chicken rinsates is shown in Table 1B. *Campylobacter jejuni* were more frequently recovered than *C. coli* for all report years. The distribution of *Campylobacter* species recovered from chicken remained stable from 1998 to 2008. After a decrease in *C. jejuni* from 2008 (73.6%) to 2009 (59.1%), an increase was observed from 2009 to 2010 (59.1% to 67.5%). Conversely, after an increase was observed in *C. coli* from 2008 (31.4%) to 2009 (40.9%), a decrease was observed from 2009 to 2010 (40.9% to 32.5%) (Figure 1B).

MIC distributions by antimicrobial and species are shown in Table 2B. No resistance to florfenicol was observed for either species. In 2010, resistance was higher for *C. coli* than *C. jejuni* for all drugs with the exception of ciprofloxacin and nalidixic acid. For both ciprofloxacin and nalidixic acid, *C*. jejuni resistance was 23.1% while *C*. coli was 22.0%.

Percent resistance by year, antimicrobial, and species are shown in Table 3B. Resistance to gentamicin in 2010 increased among *C. coli* to 5.0% and decreased among *C. jejuni* to 0.5%. Likewise, resistance to clindamicin among *C. coli* increased in 2010 to 4.0% whereas *C. jejuni* isolates continued to be susceptible. In 2010, resistance to the macrolides decreased in *C. coli* to 4.0% while *C. jejuni* isolates remained susceptible. Resistance to the quinolones in *C. coli* remained stable in 2010 following an increase observed in 2009. An increase in *C. jejuni* resistance to the quinolones was observed in 2010 to 23.1% for both ciprofloxacin and nalidixic acid. Tetracycline resistance increased in *C. coli* from 2009 to 2010 and decreased in *C. jejuni;* however, resistance to tetracycline in both species has generally remained stable.

MDR by CLSI class is presented in Tables 4B and 5B. Overall, MDR has been more frequently observed in *C. coli* than *C. jejuni.*

C. *Escherichia coli* (generic)

The number of *E. coli* isolates tested from chicken rinsates is shown in Table 1C. MIC distribution by antimicrobial is shown in Table 2C.

Percent resistance by year is shown in Table 3C. No resistance has been observed to amikacin from 1997 through 2010. Resistance to ciprofloxacin has remained below 0.6% since 1997. With the exception of ampicillin, cefoxitin and ceftriaxone, resistance in 2010 either decreased or remained stable for all other antimicrobials. In 2010, resistance increased to ampicillin (22.2%), cefoxitin (12.5%) and ceftriaxone (12.3%). Resistance in *E. coli* was highest to sulfisoxazole (51.8%), followed by streptomycin (49.1%) and tetracycline (42.9%).

MDR by CLSI class is presented in Table 4C. The percent of isolates that were pan-susceptible decreased in 2010 to 21.5% while resistance to $\geq$ 4 and $\geq$ 5 CLSI classes increased to 15.1% and 8.2%, respectively.

C. *Enterococcus*

The number of *Enterococcus* isolates tested from chicken rinsates is shown in Table 1D. As previously mentioned in Section III- Reporting Methods, the number of isolates tested from 2003-2006 has changed since the last publication of *Enterococcus* data. The top *Enterococcus* species tested are listed in Table 2D. The most frequent species were *E. faecalis* (44.3%), *E. faecium* (30.1%), *E. durans* (7.5%), *E. hirae* (5.9%) and *E. casseliflavus* (4.4%) (Figure 1D).

MIC distribution by antimicrobial and regardless of species is shown in Table 3D. Percent resistance by year is shown in Table 4D. No resistance has been observed to vancomycin or linezolid from 2003 through 2010. In 2010, *Enterococcus* isolates were also susceptible to tigecycline and chloramphenicol while only 0.6% were resistant to daptomycin. No significant variations in resistance to the macrolides occurred from 2009 to 2010, however there was an increase in nitrofurantoin (23.0%) and ciprofloxacin (11.2%) resistance which are the highest percentages observed since *Enterococcus* testing began in 2003.

MIC distributions for the top *Enterococcus* species are shown in Table 5D. Resistance to various antimicrobials was quite distinct between *E. faecalis* and *E. faecium*. Resistance to nitrofurantoin, penicillin and ciprofloxacin was $\geq$ 25.6% for *E. faecium* while resistance to these drugs was < 1.0% for *E. faecalis*. On the contrary, resistance to gentamicin, kanamycin, erythromycin and tylosin was higher among *E. faecalis* ($\geq$ 34.8%) than *E. faecium* ($\leq$ 21.4%).

MDR by CLSI class is presented in Table 6D and 7D for *E. faecalis* and *E. faecium*, respectively. MDR ($\geq$ 3, $\geq$ 4 and $\geq$ 5 CLSI classes) in *E. faecium* was higher than *E. faecalis*.