

Food Safety: A look at antimicrobial resistance in dairy and swine

458 Serotype prevalence and anti-microbial resistance of *Salmonella* isolated from dairy cattle in the Southwestern United States. T. S. Edrington*¹, K. M. Bischoff¹, M. L. Looper², T. R. Callaway¹, K. J. Genovese¹, Y. S. Jung¹, R. C. Anderson¹, and D. J. Nisbet¹, ¹USDA-ARS, Food and Feed Safety Research Unit, College Station, TX, ²USDA-ARS, Dale Bumpers Small Farm Research Center, Booneville, AR.

Mature dairy cattle were sampled over a two-year period (2001 to 2002) on six farms in New Mexico and Texas. Fecal samples were collected via rectal palpation, cultured for *Salmonella*, and one isolate from each positive sample serotyped. Three isolates of each serotype, with the exception of *Salmonella* Newport (n = 12), were examined for antimicrobial susceptibility using the broth micro-dilution technique. Twenty-two different serotypes were identified from a total of 393 *Salmonella* isolates. Montevideo was the predominant serotype (27%) followed by Mbandaka (15%), Senftenberg (11.4%), Newport (6.4%), Anatum (4.8%) and Give (4.8%). *Salmonella* Typhimurium and Dublin, two frequently reported serotypes, accounted for only 1% of the observed serotypes in this study. Sixty-four percent of the serotypes were susceptible to all of the antimicrobials, 14% were resistant to a single antibiotic, and 22% were multi-resistant (2 to 11 types of resistance). All isolates tested were susceptible to amikacin, apramycin, imipenem, ceftriaxone, nalidixic acid, and ciprofloxacin. The most frequent types of resistance were to sulfamethoxazole, tetracycline, streptomycin, kanamycin, chloramphenicol, and ampicillin (ranging from 8.9 to 22.4%). Serotypes demonstrating multiple resistance included Dublin and Give (resistant to 3 or more antibiotics), Typhimurium (resistant to 5 antibiotics) and Newport (4 and 2 isolates resistant to 6 and 9 antibiotics, respectively). Class 1 integrons were present in only three isolates, two *Salmonella* Dublin and one *Salmonella* Newport. The most prevalent resistance patterns observed in this study were for antimicrobial agents commonly used in cattle, while all *Salmonella* isolates were susceptible to ceftriaxone and ciprofloxacin, antibiotics used in human medicine.

Key Words: *Salmonella*, Antimicrobial resistance, Dairy cattle

459 Molecular epidemiology of beta-lactam resistant Gram-negative bacteria in dairy cattle. A. A. Sawant* and B. M. Jayarao, Pennsylvania State University, University Park, PA.

Beta-lactam antibiotics are widely used for therapeutic purposes in dairy cattle. A survey on use of beta-lactam antibiotics revealed that ceftiofur (78%), and ampicillin (48.5%) were the most frequently used beta-lactams, however the use of penicillins and cephalosporins were restricted mostly for dry cow therapy. Resistance to ceftiofur and ampicillin was observed in 3 and 60% of dairy farms and 1 and 32% of cows, respectively. It was observed that dairy farms that used ampicillin were 6-fold more likely to have lactating cows shedding ampicillin resistant gram-negative bacteria (AMP-GNB) in feces. A total of 98 of 313 (31.3%) lactating cows on 20 of 33 (60.6%) farms were shedding AMP-GNB. The AMP-GNB accounted for 0.01 to 96.5% of the total GNB in the feces. The diverse AMP-GNB comprised of *E. coli* (n=74), *C. koseri* (n=14), *E. aerogenes* (n=4), *K. oxytoca* (n=3), *M. morganii* (n=1), *P. alcaligenes* (n=1), and *Pseudomonas* spp. (n=6). The 74 *E. coli* isolates showed 12 resistance patterns. Resistance to oxytetracycline (86%), spectinomycin (22%), florfenicol (20%), neomycin (11%), and ceftiofur (8.1%) was observed. A total of 7 isolates of *E. coli* encoded for shiga toxin (*stx*) genes, of which 1, 4 and 2 isolates encoded for *Stx1*, *Stx2* and *Stx1* and 2, respectively. A total of 69 Amp-R *E. coli* isolates belonged to 44 pulsed-field gel electrophoresis subtypes (PFGE). It was observed that PFGE subtypes were unique to each farm, however 2 subtypes were seen in more than one farm. The TEM gene that encodes for resistance to beta-lactams was observed in 94% of *E. coli* isolates. A total of 6 *E. coli* isolates were resistance to one or more of the four extended spectrum beta-lactams (ceftriaxone, cefpodoxime, ceftazidime, cefotaxime), as per NCCLS recommended guidelines for potential extended beta-lactam resistance. It can be inferred that resistance to extended beta-lactams is perhaps an emerging problem in dairy cattle.

Key Words: Beta-lactam resistance, *E. coli*, Dairy cattle

460 Prevalence, distribution, and characterization of oxytetracycline resistant *Escherichia coli* in lactating dairy cattle. A. A. Sawant* and B. M. Jayarao, Pennsylvania State University, University Park, PA.

A study was conducted to gather relevant information on the epidemiology (descriptive and molecular) of oxytetracycline-resistant Gram-negative bacteria (OXY-GNB) in dairy cattle. The findings of our study showed that 97 of 313 (31%) lactating cows on 21 of 33 (63.6%) dairy farms shed OXY-GNB in feces. The OXY-GNB from 23 farms accounted for 0.01 to 100% of the total gram-negative fecal flora. A total of 113 isolates resistant to oxytetracycline from 90 cows on 23 farms were identified as *E. coli*. It was observed that 105 of 113 (92.9%), and 8 (7.1%) isolates encoded for Tet B and Tet A genes, respectively. None of the 113 isolates encoded for shiga-toxin I and II genes. Farms that fed milk replacers containing oxytetracycline were 12-fold more likely to have lactating cattle shed OXY-GNB in their feces as compared to farms that did not use milk replacers. The 113 isolates of *E. coli* belonged to 9 resistance patterns. Resistance to florfenicol (14.2%) was most frequently observed followed by ampicillin (7.1%), spectinomycin (4.4%), and neomycin (2.7%). A total of 99 isolates belonged to 60 pulsed-field gel electrophoresis (PFGE) subtypes. It was inferred that the PFGE subtypes were unique to each farm, however 4 clonal types were seen in more than one farm. Genetic analysis of the Tet B suggested that the gene was not located on a plasmid or an integron. Preliminary results suggest that the Tet B is more likely to be a part of a transposon located on the chromosome. Based on the findings of this study thus far, it can be inferred that prevalence of OXY-GNB is associated with use of milk replacers on dairy farms. Further OXY-GNB from dairy cattle, are likely to be highly diverse and multidrug resistant.

Key Words: Milk replacer, *E. coli*, Dairy cattle

461 The commensal bacterial populations of swine feces and stored swine manure: Reservoirs of antibiotic resistance? T. R. Whitehead*¹, M. A. Cotta¹, G. Whittle², N. Shoemaker², and A. A. Salyers², ¹National Center for Agricultural Utilization Research, Peoria, IL, ²University of Illinois, Urbana, IL.

In order to facilitate improved growth and reduce infection, a number of antimicrobial compounds are commonly added at subtherapeutic levels to the feed of domestic animals in the United States. This practice has come under intense scrutiny of late, as a number of recent reports have suggested that increased microbial resistance to the antibiotics may have an impact on human health. Most investigations have centered on antibiotic resistance in known pathogens and aerobic bacteria. However, the great majority of the bacteria present in the commensal microflora found in the feces and stored manure of domestic animals, including swine, are anaerobic bacteria. These normal residents may serve as natural reservoirs of antibiotic resistance genes. We have initiated an investigation of antibiotic resistant (AR) anaerobic bacteria present in both pig feces and manure storage pits. Samples were collected from a local swine facility where tylosin was used at subtherapeutic levels. AR anaerobic bacteria were enumerated on complex media with and without tetracycline (Tc), erythromycin (Em), or tylosin (Ty) at 10 µg/ml. AR bacteria were found in all samples, and the level of resistance ranged from 4% resistant to 32% resistant. Several Em/Ty resistant strains were also Tc resistant. Results of PCR and DNA sequencing analyses of pure cultures and total DNA from both ecosystems demonstrated the presence of various classes of *erm* and *tet* resistance genes, including the first reported identification of *ermT* in the United States. In addition, new classes of erythromycin (*erm*(35)) and tetracycline (*tet*(36)) resistance genes have been recently identified in the isolates. The *tet*(36) gene has been identified in Gram-positive and Gram-negative isolates, suggesting that the gene is being transferred between microorganisms. The finding of a high number of identified and unidentified AR eubacteria and gene classes in swine feces and manure storage pits suggests that these ecosystems may serve as reservoirs of antibiotic resistance genes. The influence of feeding antibiotics on the levels of AR resistance in these populations has yet to be ascertained.

Key Words: Antibiotic, Resistance, Swine