Discussion: Cross-resistance to different antibacterial agents including quinolones and nalidixic acid, chloramphenicol, trimethoprim and in some cases b-lactam antibiotics is a common phenomenon in Gram-negative bacteria (Gutmann et al., 1995). In this study adaptive resistance in *Salmonella enterica* and *E. coli* O157, O55 and K-12 was readily achieved by passage in sublethal concentrations of antibacterial agents, which conferred cross-resistance to other antibiotics and biocides. Interestingly, adaptive resistance to TLN by *E. coli* O157 appeared to confer a marked increased sensitivity to AMC, AMX and IPM and to a lesser degree to CHL, CS and GEN. Differences between the adaptive- and cross-resistance profiles between K-12, O55 and O157 suggest that strain-specific rather than global mechanisms are underlying the resistance observed, some of which may be facilitated by the additional genes O157 is known to possess over K-12 and potentally O55 (Perna et al., 2001). No obvious correlation could be drawn between *Salmonella* serotype and resistance to a particular class of antibiotics or group of biocides, however, this does not detract from the finding that in particular strain / antibiotic / biocide combination strong evidence of cross-resistance was observed.

Conclusions: These findings support the concern that repeated sub-lethal exposure to biocides not only promotes adaptive resistance but also confers a decreased sensitivity to antibiotics.

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O 67 TETRACYCLINE RESISTANCE GENES IN SALMONELLA FROM GROWING PIGS AND THEIR RELATIONSHIP TO ANTIMICROBRIAL USE AND RESISTANCE TO OTHER ANTIMICROBIALS.

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Summary: The aim of this study was to describe the occurrence of three genes coding for tetracycline resistance in Salmonellae isolated from normal slaughter weight pigs, and to test for relationships between the occurrence of these genes, phenotypic resistance, and the use of antimicrobials in feed and water. Salmonella (1,431) were cultured at slaughter or just before slaughter among slaughter-age pigs, and were isolated using conventional methods. Three tetracycline resistance genes were

tested using denaturing gel electrophoresis (DGGE). Phenotypic tetracycline resistance was observed as the phenotype of 52.0% of isolates. Tetracycline resistance genes, designated A, B and C, were detected in 18.6%, 66.4% and 1.6% of isolates, respectively. When broken down by genetic pattern, resistance/intermediate resistance was found in the following proportion of isolates: A-,B-,C-, 23.0%; A+, B-, C-, 93.1%; A+, B-, C+, 94.7%; and A-, B-, C+, 69.7%. Reported use of antibiotics in feed or water was not significantly correlated with phenotypic resistance or the occurrence of any of the three genes studied. The *tet(C)* gene was positively associated with phenotypic resistance to tetracycline, sulphamethoxazole, streptomycin, ceftiofur, kanamycin and cephalothin. It is concluded that antimicrobial resistance to tetracyclines in growing pigs is common, that the *tet(C)* gene is common, that *tet(C)*is associated with phenotypic resistance to multiple antimicrobials, and that current antimicrobial use is not an important predictor of tetracycline resistance genes.

Keywords: antimicrobials, swine, bacterial genetics

Introduction: Antimicrobial resistance is important for Salmonellae since foodborne illness can have severe health consequences in humans with resistant infections with resistant infections. Understanding the epidemiology of tetracycline resistance in swine isolates can help us understand antimicrobial resistance of other antibiotics that are more crucial in human health. Further, genes coding for resistance to tetracyclines may be coupled to genes coding for resistance to other antibiotics. We designed this study to investigate the occurrence of tetracycline resistance genes, and to examine relationships between these genes and both use of antimicrobials on commercial pig farms and to the expression of resistance to other antimicrobials.

Methods and materials: Herds with a history of delivering at least 30 animals per shipment were solicited from among those supplying slaughter weight pigs to two Midwestern U.S. abattoirs. Among 328 farms solicited, 205 agreed to participate, and 141 were selected based on convenience of scheduling. Farms were collected one to five times over a three year period. After slaughter, 10 g caudal mesenteric lymph node tissue was aseptically collected from 30 randomly selected pigs. In addition, from 30 herds a 10g fecal sample was collected at the farm less than 48 hours prior to shipment to slaughter.

Conventional bacterial culture methods were used to isolate Salmonella from all samples, using a slight modification of a procedure previously described. [Fedorka-Cray et al., 1998] For the first sample from each farm only, five samples were pooled, combining two grams from each pig. Samples were blended and incubated in tetrathionate broth for 48 hrs at 37°C. One ml of this broth was transferred to R-10 broth and incubated 24 hrs at 37°C. XLT-4 plates were streaked for isolation, followed by culture on Brilliant Green agar, and finally suspect colonies were tested for agglutination with polyvalent anti-*Salmonella* sera. From positive pools, retained frozen tissue was cultured individually using two-gram samples in 20 ml of tetrathionate. This same individual procedure, without freezing, was used for subsequent samples collected.

Approximate MIC values were determined for 17 antimicrobials using the Sensititre™ system (TREK Diagnostic Systems, Inc., Cleveland, Ohio, U.S.A.). The antimicrobials were amikacin, amoxicillin/clavulanic acid, ampicillin, apramycin, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulphamethoxazole, tetracycline, and trimethoprim/sulphamethoxazole. Procedures described by the manufacturer were followed. Interpretation of results were as specified by the manufacturer, except that both intermediate and resistant outcomes were considered resistant for purposes of further analysis.

The isolates were tested for the presence of three tetracycline efflux pump genes, termed *tet(A), tet(B) and tet(C)*. PCR based methods were used to identify these genes [Aminov, R.I., et al.]. Farm managers were asked, by a written survey, to describe the use of antimicrobials in the group of pigs

marketed. They described which antimicrobials were used, at what dose, at what age, and whether the drug was used for therapy or for growth promotion. Relationships between gene presence and tetracycline phenotype, and relationships between antimicrobial use and *tet(C)* were assessed by logistic regression, adjusting for the clustering of samples within herd. (Egret, Cytel Software, Inc., Cambridge, Massachusetts, U.S.A). The relationships between *tet(C)* to phenotypes to other antimicrobials were assessed by the Chi-Square statistic.

Results: A total of 1431 Salmonella isolates were tested. Tetracyline resistance was observed in 50.9% of isolates, while 1.4% were intermediate in sensitivity. The *tet(A), tet(B) and tet(C)* genes were detected in 18.6%, 1.6% and 66.4% of isolates, respectively. When broken down by genetic pattern, resistance / intermediate resistance was found in the following proportion of isolates: A-,B-,C-, 23.0%; A+, B-, C-, 93.1%; A+, B-, C+, 94.7%; and A-, B-, C+, 69.7%. The percentage of bacteria with resistant phenotypes, but for which no resistance gene was found was 6.2%. The percentage of isolates with one or more gene detected but with susceptible phenotypes was 29.4%. Because of the small proportion of isolates with *tet(B)* detected, further analysis was not conducted for this gene. The odds of expressing the tetracycline resistance phenotype were higher for isolates with the *tet(A)* (OR = 15.4) and *tet(C)* (OR = 3.8) genes, when compared to isolates where the genes were not detected. There was no statistical evidence of interaction between the genes.

Antimicrobial phenotypic resistance to seven antimicrobials was associated with the detection of *tet(C)*. The antimicrobials and the odds ratio of association for each were sulphamethoxazole (12.7), tetracycline (11.2), streptomycin (6.8), ceftiofur (3.3), kanamycin (2.1), cephalothin (1.9) and chloramphenicol (0.4).

Antimicrobials used in feed or water during the finishing phase by more than 10% of herds were tetracyclines, bacitracin methylene disalicylate and tylosin. No statistical relationship was detected between the use of these antimicrobials and *tet(C)*.

Discussion: The lack of association between antimicrobial use and the most commonly detected tetracycline resistance genes suggests that tetracycline resistance, one acquired, does not rapidly resolve. The historical usage patterns on these farms was not described, so it is not known how long it had been since an antibiotic was used on a farm. However, tetracyclines were reported to be commonly used. It is possible that farms which at the time of the study were not using tetracycline may have used them in the past.

These genes confer the resistance due to the efflux of tetracycline from the cell catalyzed by drug:H+ antiport. [Paulsen IT, et al.] The genes studied here were a sufficient explanation for the majority of the phenotypically tetracycline resistant detected, although a minority of resistant isolates had none of the genes. Conversely, a relatively large proportion of bacteria carried one of the resistance genes, but did not express resistance *in vitro*. The assay used detects genetic fragments from the genes in question, but does not test whether the genes are activated and/or effective. It appears that in some cases these genes are not capable of effectively activating the efflux pumps, or some additional factor effectively renders the pumps ineffective.

We conclude a small number of genes can account for the majority of tetracycline resistance observed on these commercial pig farms, that tetracycline genes remain in the pool of *Salmonella* strains on farms after the withdrawal of the tetracyclines from the diet, and that one gene, *tet(C),* is associated with resistance other antimicrobials, directly or indirectly.

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Reported antimicrobial use and *Salmonella* resistance on 90 Alberta swine farms

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Summary: The study objectives were to describe antimicrobial use (AMU) and *Salmonella* resistance on 90 Alberta swine farms. The vast majority of antimicrobials were used in-feed. In weaners, in-feed use did not vary among farms, suggesting heavy reliance on in-feed antimicrobials. For grow-to-finish production phases, most farms reported heavy reliance on in-feed antimicrobials, but 6 and 14 farms did not report any in-feed AMU in growers and finishers, respectively. The tetracycline-sulphametazine-penicillin combination and carbadox were the most common antimicrobials added to the weaner rations, while tylosin and lincomycin were the most common antimicrobials added to grower and finisher rations. No resistance was observed to nalidixic acid, ciprofloxacin, amikacin and ceftriaxone. A low frequency of resistance (<5%) was observed to gentamicin, apramycin, cephalotin, ceftiofur, amoxicillin/clavulanic acid and trimethoprim-sulphamethoxazole. Most common resistances were detected to tetracycline, streptomycin, sulphamethoxazole, kanamycin and ampicillin. Despite widespread AMU, 40.19% of *Salmonella* isolates were susceptible to 17 antimicrobials.

Keywords: antimicrobial drugs, susceptibility, *Salmonella* serotypes

Background: The emergence of antimicrobial resistance (AMR) is believed to be associated with the use of antimicrobial drugs in human medicine, veterinary medicine and food animal production. The scope and magnitude of the public health impact of antimicrobial use (AMU) in animals remains unclear since there is relatively little information on AMU and the prevalence of resistant bacteria in food animals (McEwen & Fedorka-Cray, 2002). The objectives of this study were to describe AMU and *Salmonella* resistance on 90 Alberta swine farms.
Materials and Methods: Ten swine veterinarians selected 90 Alberta swine farms. AMU data were gathered through a questionnaire, which was completed by the owner or operator of the farm along with the herd veterinarian. Fifteen fecal samples and five environmental samples per farm were collected over a four-month period from the finishing swine and the farm environment. All samples were tested for *Salmonella* using bacteriological culture. *Salmonella* isolates were serotyped by the Health Canada O.I.E Reference Laboratory for Salmonellosis (Guelph, Ontario). Susceptibility testing was performed on all isolates using a Sensititre Custom MIC Panel (Trek Diagnostic Systems Ltd.).