

## PORK SAFETY

**Title:** Quantitative evaluation of selective factors impacting the development, amplification, persistence and transfer of macrolide resistance elements within mixed populations of commensal and pathogenic gut bacteria. **NPB #05-198**

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**Abstract**

Continuous flow cultures of feral (culture FC) and domesticated (culture RPCF) pig gut microflora were established in steady state. Cultures, in duplicate, were continuously infused with low (25 µg/ml) or high (100 µg/ml) levels of tylosin and sampled at intervals to assess effects on total culturable anaerobes, *Bacteroides* spp. and *Enterococcus* spp. via plating of serial 10-fold dilutions to anaerobic Brucella blood (BRUC) agar, *Bacteroides* bile esculin (BBE) agar, and M Enterococcus (ME) agar supplemented without or with 100 µg tylosin/ml, the later to assess bacterial sensitivity to tylosin. Concentrations of endogenous tylosin-insensitive anaerobes within the FC and RPCF cultures prior to tylosin administration ranged from 8.8 to 9.2 log<sub>10</sub> CFU/ml and were likely composed with different prominent endogenous tylosin-resistant bacteria, likely a *Clostridium hathawayi* in culture FC and tylosin-resistant *Bacteroides uniformis* and *B. stercoris* in culture RPCF. Consequently, these populations responded differently during the experiments, with tylosin-insensitive concentrations in RPCF cultures becoming enriched more than those in FC cultures during administration of either 25 or 100 µg tylosin/ml to the continuous flow cultures. Moreover, tylosin-insensitive anaerobes persisted at their increased concentrations after cessation of tylosin administration whereas concentrations from FC cultures decreased. Concentrations of *Bacteroides* and endogenous *Enterococcus* spp. decreased to near or below detectable levels (1.0 log<sub>10</sub> CFU/ml) in culture FC following administration of 25 or 100 µg tylosin/ml although tylosin-insensitive populations were present (> 1.0 log<sub>10</sub> CFU/ml) before initiation of tylosin administration. Concentrations of endogenous tylosin-insensitive *Bacteroides* spp. were not enriched in FC cultures during 25 µg tylosin/ml treatment but were enriched to > 5 log<sub>10</sub> CFU/ml in RPCF cultures after 4 days of this treatment. While present at > 2.0 log<sub>10</sub> CFU/ml prior to initiation of tylosin administration, tylosin-insensitive *Bacteroides* spp. were not enriched in one experiment and transiently enriched to > 7.6 CFU/ml in another when 100 µg tylosin/ml was administered to FC and RPCF cultures, with the enriched populations rapidly diminishing upon cessation of tylosin treatment. When administered at 25 µg tylosin/ml, populations of endogenous tylosin-insensitive *Enterococcus* sp. were enriched in RPCF cultures but not in FC cultures. For cultures administered 100 µg tylosin/ml, tylosin-insensitive *Enterococcus* spp. exhibiting two types of colony morphology were recovered at increasing concentrations from two RPCF cultures and from one FC culture, thus

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suggesting the enrichment of the experimentally inoculated tylosin-resistant *Enterococcus faecium* as well as endogenous *Enterococcus*. Further tests of representative isolates confirmed tylosin-resistance in *Enterococcus faecalis* from cultures RPCF and *Enterococcus hirea* from the FC culture and that this resistance was genotypically related to that of *Enterococcus faecium* thus suggesting the occurrence of gene transfer. The results of these studies revealed that tylosin-resistant bacteria, although of different type, were endogenous to gut microbial populations from both a feral and domestically reared pig. These results further illustrate that under the conditions of these tests, administration of tylosin promoted the enrichment of tylosin-insensitive bacterial populations (capable of growing on media supplemented with 100 µg tylosin/ml) within RPCF cultures (originating from a traditionally reared domesticated pig) and FC cultures (originating from a feral pig) and that transfer of resistance between *Enterococcus* spp. likely occurred.