

Title: Do probiotics, an antibiotic alternative, mitigate or contribute to emergence and persistence of antimicrobial resistance in gut bacteria? – NPB #15-024

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Scientific Abstract:

Probiotics are live cultures of bacteria or fungi supplemented in swine diets that can beneficially affect the host animal by improving the microbial balance in the gut. *Because probiotics favorably alter bacterial population in the gut, which includes establishment of the 'beneficial bacteria' and exclusion of 'undesirable or harmful bacteria', it is possible that they mitigate emergence and persistence of AMR in gut bacteria. An aspect of feeding probiotics that has not received any attention in the US is whether probiotics, a product generally recognized as safe (GRAS status), could be a source of AMR?* The impact of probiotics on the emergence, spread and persistence of AMR in gut bacteria is an unexplored area. Therefore, we conducted a study to answer two questions: *Do probiotics mitigate AMR in gut bacteria and do probiotics that carry AMR genes contribute to AMR in gut bacteria?*

Initial *in vitro* screening of eight probiotic products containing *E. faecium* revealed the presence of AMR both by phenotypic and genotypic methods. The probiotic *E. faecium* strains were phenotypically resistant to one to three antimicrobials. The pulsed-field gel electrophoresis revealed that *E. faecium* strains from two probiotic products were clonally related. The whole genome sequencing of eight *E. faecium* isolates revealed the presence of macrolide and streptogramin B resistance mediated by *msrC* gene, and tetracycline resistant determinants, *tet(L)* and *tet(M)* genes. For the field study, we selected a probiotic which possessed AMR and one that did not possess AMR to medically-important antibiotics. Study consisted of 60 pens housing 300 weaned piglets with 5 piglets per pen, which were randomly allocated to six treatment groups; control, probiotic A, probiotic B, chlortetracycline (CTC; 22 mg/kg BW), and combination of each probiotic and CTC. Fecal samples were collected from 3 piglets per pen on days 0, 7, 14, 21, 28, 35, and 42 for the isolation of *E. coli*, *Enterococcus* spp., *Campylobacter* spp., and *Salmonella enterica*. Overall prevalence of *Campylobacter* was 21.6% (273/1,260) *C. coli* (49/273) or *C. hyointestinalis* (224/273). The overall prevalence of *S. enterica* was 6.6% (84/1,260). All *E. coli* isolates were susceptible to azithromycin and sulfisoxazole and a majority of the isolates were resistant to tetracycline (93.3%), ampicillin (81.6%), streptomycin (58.5%), ceftriaxone (52.4%), and ceftiofur (36.1%). All the tested *Enterococcus* isolates were susceptible to vancomycin. Majority of the isolates were resistant to tetracycline (95%), erythromycin (86.1%), lincomycin (83.3%), ciprofloxacin (67.2%), nitrofurantoin (53.3%), streptomycin (45.2%) and kanamycin (41.5%). Based on this study, the two probiotic products tested alone or in combination with CTC, had no effects on fecal shedding of *Campylobacter* spp., and *Salmonella enterica* and on antimicrobial resistance in fecal *E. coli*, *Enterococcus* spp., *Campylobacter* spp., and *Salmonella enterica*. However, additional studies are warranted to reevaluate the impact of feeding other probiotic products on the fecal prevalence of food borne pathogens and AMR of gut commensals.

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