RESEARCH ABSTRACT

PUBLIC HEALTH/WORKER SAFETY

Title: Strategy to preserve efficacies of antimicrobials against important swine pathogens – **NPB #13-116**

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Date Submitted: August 29, 2014

Scientific Abstract: Standardized methods of determining antimicrobial resistance of microbes involves growing the test bacteria in standard medium such as Mueller Hinton broth. Presently, we compared the antimicrobial resistance of multidrug resistant *Salmonella* and select Gram-positive bacteria against a selection of antibiotics after the bacteria had been grown in Mueller Hinton broth as it is commonly done in the laboratory or during growth in Mueller Hinton broth that was modified to contain extracts of fecal contents collected from nonchlorophyll- and chlorophyll-fed pigs. The chlorophyll was added to the pig diets because evidence suggests that certain bacterial degradation products of chlorophyll may accumulate in the gut and inhibit antimicrobial efflux pumps thereby making some multidrug resistant bacteria less resistant. We found that growth of the *Salmonella* strains in Mueller Hinton broth modified to contain fecal extracts from nonchlorophyll-fed pigs had little or only modest effects on their susceptibility to chlortetracycline or norfloxacin but increased resistance of most of the strains to penicillin, carbadox and tylosin. When the Gram-positive bacteria, encompassing strains of *Staphylococcus, Streptococcus* and *Enterococcus* , were grown in Mueller Hinton broth modified to contain fecal extract from nonchlorophyll-fed pigs we found that resistance by most of the strains to chlortetracycline, penicillin, tylosin, ceftazidime and norfloxacin was increased or unaffected, the exceptions being decreased resistance to chlortetracycline by *Staphylococcus hyicus, Streptococcus agalactiae* and *Enterococcus faecium*. Conversely, resistance to carbadox by nearly all of the Gram-positive bacteria grown in the nonchlorophyll-fed extracts, the exceptions being two strains of *Staphylococcus aureus*, was decreased 4 to 16-fold compared to resistance observed in standard Mueller Hinton broth. For the *Salmonella* strains grown in Mueller Hinton broth modified to contain the fecal extracts from chlorophyll-fed pigs, appreciable decreases in resistance were observed only with norfloxacin, with resistance being increased or unchanged from that observed with cultures grown in the standard Mueller Hinton broth for all the other antibiotics tested. Resistance to chlortetracycline, penicillin, carbadox and ceftazidime was lower from that observed during growth in the standard Mueller Hinton broth by nearly all of the Gram-positive strains grown with fecal extracts from the chlorophyll-fed pigs thus supporting earlier work indicating that degradation products of chlorophyll may be more effective against Gram-positive than Gram-negative bacteria.

Results from studies testing individual addition of chlorophyll or its degradation products, pheophorbide-a or pyropheophorbide-a, or with the known efflux pump inhibitor, L-phenylalanyll-arginyl-β-naphthylamide, indicated that these compounds may indeed promote a decrease in antibiotic resistance but like that observed in the studies with added fecal extracts, the activity by these potential effector compounds appears to be highly strain and antibiotic dependent; even affecting increases in resistance in some cases. Results from pig feeding studies indicated that chlorophyll-supplementation had little if any appreciable effect on endogenous populations of penicillin-insensitive, chlortetracycline-insensitive or tylosin-insensitive *E. coli* or enterococci in feces from pigs fed typical commercial grower diets containing penicillin, chlortetracycline and sulfamethazine or containing tylosin. An effect of chlorophyll-supplementation was observed on *E. coli* and enteroccal populations in separate pigs fed a commercial non-medicated diet indicating that chlorophyll itself, or its degradation products, may be inhibitory to these populations by as yet unknown mechanisms. Results indicate that feeding chlorophyll as a source or precursor of efflux pump inhibitors may promote a decrease in antibiotic resistance but the activity of these compounds appears to be highly strain and antibiotic dependent and even affect an increase in resistance in some cases. Based on these results, it is unlikely at the present time that the efflux pump inhibitors tested in the present study can be developed into an inexpensive technology to preserve and enhance the efficacy of currently available antibiotics.