

Title: Alternatives to antibiotics? Effects on MRSA prevalence in swine production systems – **NPB #13-021** **revised**

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Date Submitted: 07/09/2015

Scientific Abstract:

Zinc (Zn) is sometimes supplemented at elevated concentrations (2,000-3,000 ppm) in swine diets, ostensibly to prevent enteric infections and promote growth. Studies from Denmark have suggested a genetic linkage and a phenotypic association among Zn resistance, encoded by the *czrC* gene, methicillin-resistance, encoded by the *mecA* gene, and tetracycline resistance (encoded by the *tetM* gene in *S. aureus*). We have previously shown that nasal carriage of *mecA*-positive MRSA exhibits a dose-response to zinc supplementation in pigs. A longitudinal study was performed to evaluate the association of in-feed Zn and chlortetracycline (CTC) supplementation (at two levels) with the prevalence of MRSA in pigs. The study consisted of 240 weaned piglets, housed in 48 pens (5 piglets/pen), randomly assigned to six treatments in a 2×3 complete factorial design. Treatment factors included diets with normal (30 mg/kg) or high (2,500 mg/kg of feed) concentrations of Zn, with or without CTC at low (5 mg/kg) or high (22 mg/kg BW) levels, and the interaction terms for Zn with low or high CTC. Nasal, skin, and tonsillar swabs were collected from all piglets on Days 0, 21, and 42. The samples were inoculated onto MRSA CHROMagar and presumptive MRSA colonies were confirmed by genus (*Staph*) and species (*nuc*) specific PCR. The presumptive MRSA isolates were tested for *mecA* and *czrC* by PCR. Zinc susceptibility was determined by the agar gel dilution method. Statistical analyses were carried out using STATA (v.12.1). Overall treatments and days, the prevalence of *mecA*-positive MRSA was 42.8% (308/720), 37.2% (268/720), and 42.6% (307/720) in nasal, skin, and tonsillar samples, respectively. The prevalence of *czrC*-positive MRSA was 20% (144/720), 21.1% (152/720), and 14.3% (103/720) in nasal, skin, and tonsillar swabs, respectively. The Zn, sampling day (period) and treatment interactions had a significant effect on the prevalence of *mecA* and *czrC*-positive MRSA in all three collection sites ($P < 0.001$). The occurrence of *czrC* gene was strongly associated with *mecA*-positive MRSA isolates ($P < 0.0001$). The median MICs of Zn for zinc resistant and susceptible isolates were 8 and 4 mM, respectively ($P < 0.001$). The prevalence of *mecA*- and *czrC*-positive MRSA was affected by Zn ($P < 0.05$) but not CTC supplementation ($P > 0.05$). The observed association between *czrC* and *mecA* genes points to the importance of elevated Zn supplementation in selection and propagation of antibiotic resistant bacteria such as MRSA in swine production.

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

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