# EFFECT OF DIETARY ZINC ON THE HORIZONTAL TRANSFER OF ANTIBIOTIC RESISTANCE GENES IN THE INTESTINE

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Infections with antibiotic-resistant bacteria belong to the emerging threats to human health. We speculated that not only misuse of antibiotics but also nutrition-derived stress factors may directly trigger horizontal transfer of antibiotic resistance genes between gut bacteria. To test this hypothesis, we fed diets containing zinc oxide (ZnO) at either 100 or 1,900 ppm to germ-free mice. The latter ZnO concentration may cause toxic stress to enterobacteria. After adaptation to diet, animals were inoculated with an *Escherichia coli* strain with a non-transmissible sodium azide resistance (experimental day 14) and a *Klebsiella pneumoniae* strain with a plasmid-encoded cefotaxime resistance (experimental day 21). Fecal material was collected each day and plated on agar plates containing either sodium azide, cefoxatime or both antimicrobial agents. Colony forming units ($\log_{10}$ cfu) of sodium azide-resistant *E. coli* (recipient strain), cefoxatime-resistant *K. pneumoniae* (donor strain) and of sodium azide-resistant *E. coli* with acquired cefotaxime resistance (transconjugants) were determined. Conjugation rates were calculated by dividing cfu of the transconjugant by cfu of the recipient strain. The area under the curve (AUC) approach was applied to integrate data obtained for each mouse, strain and diet group over the range of sampling time points.

Both bacterial strains successfully colonized the intestine of previously germ-free mice but fecal bacterial numbers were highly variable between the animals. Median values for the low zinc diet were $\log_{10}$ 9.9 (donor strain), $\log_{10}$ 8.7 (recipient strain) and $\log_{10}$ 6.0 (transconjugant). When the high zinc diet was fed, median values were $\log_{10}$ 10.0 (donor strain), $\log_{10}$ 8.5 (recipient strain) and $\log_{10}$ 5.6 (transconjugant). No effect of diet was observed when the AUC of the donor and recipient values were compared. In contrast transconjugant AUC was significantly higher for the mice fed with 100 ppm of zinc ($P = 0.025$) indicating that high dietary zinc concentrations may rather inhibit horizontal gene transfer. However, this notion was not supported by conjugation rate data and, thus, our study does not support the hypothesis that ZnO at high levels influences horizontal transfer of antibiotic resistance genes in the intestine.