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Quantitative Estimation of Acquisition of Chlorate Resistance in *Salmonella*; Implications for the Use of Chlorate as a Preharvest Pathogen Reduction Supplement

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Introduction

Chlorate has long been known to be bactericidal against nitrate respiring bacteria¹ but only recently has its use as a preharvest food safety supplement been investigated^{2,3}. Mechanistically, chlorate is reduced intracellularly by respiratory nitrate reductases to cytotoxic chlorite¹. Thus, chlorate exhibits a selective toxicity against nitrate respiring bacteria such as *Escherichia coli* and *Salmonella* while remaining innocuous to most beneficial gut anaerobes that lack respiratory nitrate reductase. This consequence has not escaped the attention of veterinarians and livestock producers who envision using chlorate to treat production diseases such as neonatal scours without depopulating the gut of its functional bacteria. As with any new microbial intervention strategy; however, it is important to know whether or not potential development and propagation of resistance among target populations may render future treatments ineffective. We thus determined the frequency of generating chlorate resistant *Salmonella* in pure and mixed bacteriological culture.

Materials and Methods

The frequency of generating chlorate resistant *Salmonella* in pure culture was determined by plating overnight grown cultures (in tryptic soy broth) of *Salmonella* serovars Typhimurium (NVSL 95-17766) and Choleraesuis (var. kuzendorf 3246pp) directly to Brilliant Green Agar containing 0 or 20 mM added chlorate. The plates were examined after 24 h incubation and the frequency of chlorate resistance was calculated as the ratio of colonies recovered on BGA alone/colonies recovered from chlorate supplemented BGA. The frequency of generating chlorate resistant *Salmonella* in mixed culture was determined by incubating overnight grown cells 6 h in anaerobic suspensions of porcine cecal or fecal contents and then plating to BGA as above.

Results and Discussion

Whether grown in pure or mixed culture, the frequency of recovering *Salmonella* able to grow on Brilliant Green Agar containing added chlorate varied little (Table 1). While these results thus indicate that competition did not increase the rate of spontaneous mutation, they provide no information as

to the competitive fitness of the chlorate resistant *Salmonella* (i.e., in order to determine spontaneity the *Salmonella* had to be grown in TSB and gut contents lacking chlorate). However, earlier work by Callaway *et al.*⁴ had shown that chlorate resistant *E. coli* O157:H7 were not as fit in mixed culture and were not recovered from gut contents of experimentally challenged chlorate treated pigs. In support of their findings, we also were unable to recover chlorate resistant *Salmonella* from gut contents of pigs experimentally challenged with 10⁸ CFU *Salmonella* Typhimurium and allowed ad libitum access to drinking water containing 15 or 30 mM sodium chlorate (n = 15 pigs per treatment).

Table 1. Frequency of recovering chlorate resistant *Salmonella*

	<i>Salmonella</i> serotype	
	Typhimurium	Choleraesuis
Pure culture	1.58 ± 2.0 × 10 ⁶	9.44 ± 8.1 × 10 ⁵
Cecal culture	8.42 ± 5.5 × 10 ⁵	1.48 ± 0.1 × 10 ⁵
Fecal culture	7.11 ± 3.1 × 10 ⁵	1.24 ± 0.2 × 10 ⁵

References

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4. Callaway TR, Anderson RC, Buckley SA, Kubena LF, Nisbet DJ. *Escherichia coli* O157:H7 becomes resistant to sodium chlorate addition in pure culture but not in mixed culture or in vivo. *J. Anim. Sci.* 2000;83 (Suppl. 1):452.