Quantitative measurement of antimicrobial resistance in *Escherichia coli* populations

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Summary:
A survey to monitor antibiotic resistance of *E. coli* in cattle at various ages from randomly selected farms indicated that resistance may be associated with total bacterial counts, and this resulted in the group questioning the definition of antibiotic resistance. We therefore combined newly acquired technology with a novel quantitative method for describing the resistance of large numbers of bacteria (Humphry, *et al.*, 2002). Using a spiral-plater, our method enumerates the number of bacteria that grow at different concentrations of antibiotic and using non-linear regression fits a cumulative density function of the normal curve to these data. The result is a method that quantifies the total bacterial density, the mean population minimum inhibitory concentration, the phenotypic variation of those bacteria and the proportion of bacteria for each phenotype.

The randomised study mentioned above demonstrated a significantly higher proportion of resistant samples in calves compared to fattening cattle using a traditional disc diffusion method (Table1). However we had no indication of the relative density of bacteria in the samples. Our alternative method would have demonstrated the proportion of resistant *E. coli* in each bacterial sub-population. An associated study found that the proportion of samples from farms undergoing an outbreak of calf diarrhoea was greater than those from unaffected farms (Gunn, *et al.*, 2003) and the additional information provided by our method would have resulted in a better understanding of the reasons for this.

	Proportion resistant samples ± s.e.	
	Calf	**Fattening**
Ampicillin	0.88±0.02	0.49±0.05
Apramycin	0.13±0.03	0.02±0.02
Nalidixic Acid	0.07±0.02	0.02±0.02

Table 1 Proportion of resistant samples for calves and fattening cattle

However these are only two examples where the new method is likely to provide a powerful new tool. The method is currently being applied to test several hypotheses. For example, we collected 4 samples over a nine-week period from a pig holding. Of particular interest was a *post hoc* examination of two samples from different animals taken about 14 days apart (Figure 1). In both samples we could show the existence of two phenotypically different *E. coli* sub-populations. The mean minimum inhibitory concentration for the two sub-populations was the same in both samples. However, in contrast to traditional methods, we observed a difference in the proportion of the total

commensal *E. coli* flora in each phenotypic sub-population. We now have the ability to show very subtle differences and changes in antibiotic resistance and this paves the way for experimental and survey work with much greater statistical power.

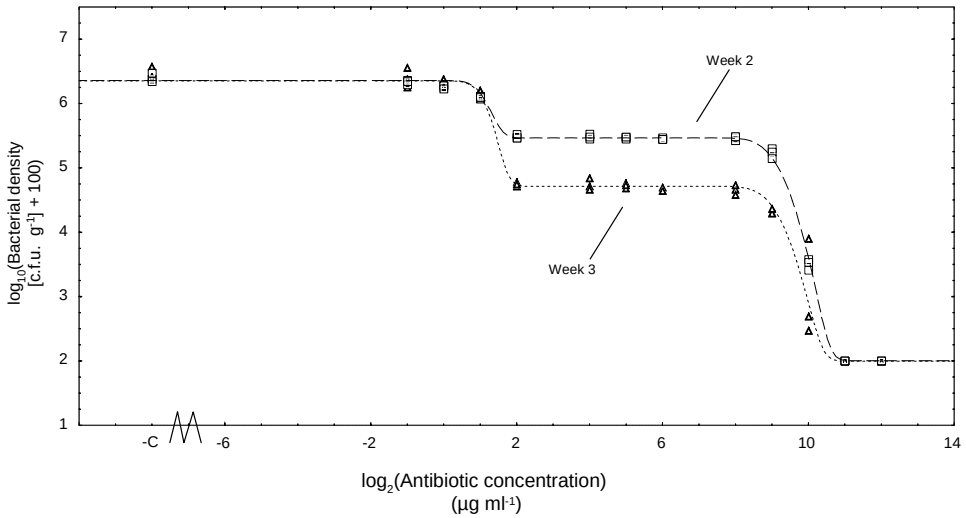

Figure 1. Comparison of the bacterial counts from pig samples from weeks two and three when tested against ampicillin.

References:

Gunn, G. J., Hall, M. & Low, J. C., (2003). Comparison of Antibiotic Resistance for Escherichia coli Populations Isolated from Groups of Diarrhoeic and Control Calves. *The Veterinary Journal* **165**, 172-174.

Humphry, R. W., Blake, D., Fenlon, D., Horgan, G., Low, J. C. & Gunn, G. J., (2002). The quantitative measurement of antimicrobial resistance in Escherichia coli at the meta-population level (meta-population analysis). *Letters in Applied Microbiology* **35,** 326-330.