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Determining an antimicrobial resistance profile in *E. coli* from broiler chickens: what sample size do we need?

Davy Persoons [1,2], Jeroen Dewulf [1], Annemieke Smet [3,5], Patrick Butaye [3,5], Lieve Herman [2], Marc Heyndrickx [2], Boudewijn Catry [4], An Martel [3] and Freddy Haesebrouck [3]

[1] *Department of obstetrics, reproduction and herd health, veterinary epidemiology unit, Faculty of Veterinary medicine, Ghent University, Merelbeke, Belgium*
[2] *Unit Technology and Food, Institute for Agricultural and Fisheries Research, Melle, Belgium*
[3] *Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary medicine, Ghent University, Merelbeke, Belgium*
[4] *Scientific Institute of Public Health, Brussels, Belgium*
[5] *Department of Bacteriology and Immunology, CODA-CERVA-VAR, Brussels, Belgium*

E-mail: davy.persoons@ugent.be

INTRODUCTION

When the prevalence of antimicrobial resistance is to be determined for one specific antimicrobial one can easily use the conventional sample size calculation techniques. However when the aim of the study is to determine the antimicrobial resistance profile (e.g. resistance against 14 different antimicrobials), no ready to use methodology is available to determine the optimal sample size. One could go for the maximum sample size (expected prevalence of 50%). Yet this is often not feasible due to financial constraints. Moreover it is not necessary for most antimicrobials. Besides the number of animals to be sampled one has to determine the number of colonies to be tested within each sample. The aim of this study was to determine the optimal combination of animals and colonies tested and to quantify the increase in uncertainty of the prevalence estimation when the sample size is reduced.

METHODS AND MATERIALS

In five randomly selected broiler flocks (>10.000 animals per flock),100 birds were sampled. In the first flock, the resistance profile of 5 *Escherichia coli* colonies per sample was determined. in the other flocks, one colony per sample was tested. In all samples *E. coli* was identified and antimicrobial resistance testing was done by means of disk diffusion. Subsequently subsets of this large dataset were selected by bootstrapping (1000 iterations per run, @risk). For every selected sample size the width of the 95% confidence interval around the prevalence estimate was monitored.

RESULTS

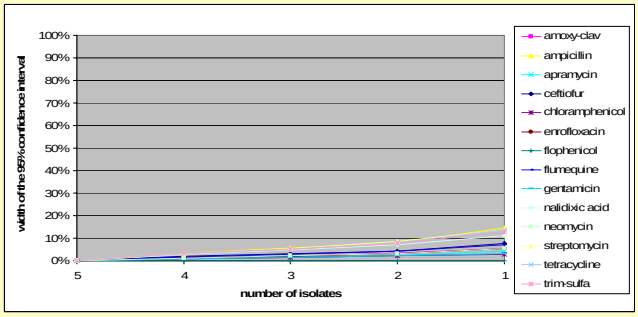

Figure 1: effect of reducing the number of tested colonies per animal sampled on the 95% CI around the prevalence estimate

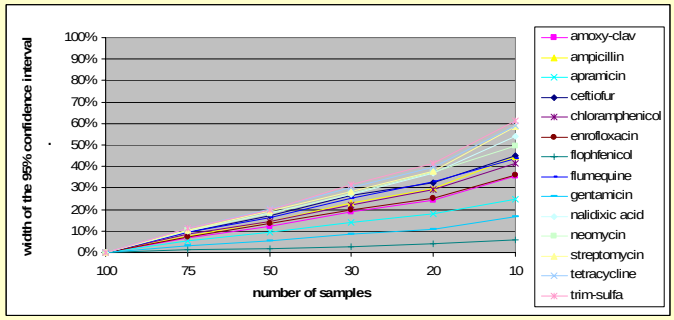

Figure 2: Effect of reducing the number of samples on the on the 95% CI around the prevalence estimate

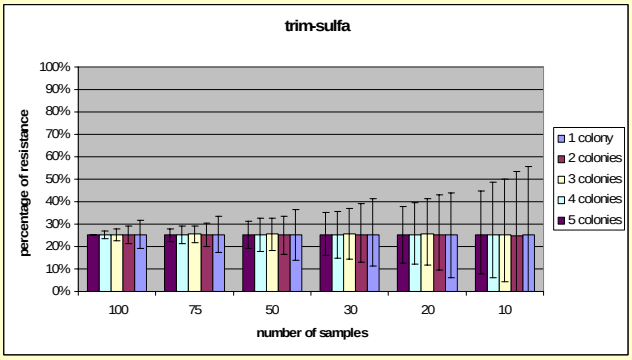

Figure 3: Combined effect of reducing the number of samples and the number of colonies per sample on the 95% CI around the prevalence estimate for trimethoprim-sulfa

DISCUSSION

Results of Figure 1 show that **reducing the number of colonies tested per sample only gives a small increase in uncertainty of the estimated prevalences of antimicrobial resistance**. This is due to the relatively limited variation in resistance profile in *E.Coli* within an animal. Based on these data it was concluded that **it does not seem worth while to examine multiple colonies per sample**. On the contrary, **reducing the number of samples tested has a far greater influence** on repeatability of the prevalence estimate (Figure 2). When less than twenty animals per flock were sampled, the width of the CI increased drastically!

When combining the effect of reducing the number of colonies and the number of samples, it becomes even more clear that **the number of animals sampled has a far greater influence on the repeatability of the outcome than the number of colonies isolated per animal**. These preliminary results are now further processed in order to make some general statements on the minimal sample size for antimicrobial resistance profiling taking into account financial constraints.

ACKNOWLEDGEMENTS

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