

Comparison of individual and pooled samples for quantification of antimicrobial resistance genes in swine feces by high-throughput qPCR - DTU Orbit (08/11/2017)

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There is a considerable societal interest in the careful monitoring of antimicrobial resistance (AMR) levels in human and animal populations. Sampling and data analysis can be both costly and time consuming. Optimization of sample pooling procedures is therefore important to reduce costs and analysis times. The objective of this study was to estimate how many individual fecal samples are needed to pool to get a representative sample for quantification of AMR-genes in a Danish pig herd. 20 individual fecal samples were collected from one section in a Danish pig herd. One to five rectal fecal samples were taken from each pen with respect to the number of pigs in the pen. A total of 48 pools were made of increasing number of individual samples. The levels of 9 different AMR-genes were quantified using dynamic qPCR arrays on the BioMark HD system (Fluidigm®). DNA was extracted using the Maxwell® 16 Blood DNA Purification Kit (Promega). DNA concentrations were diluted to 40 ng/μl. The efficiency of the primers was determined using standard curves. Obtained results were normalized with 16S ribosomal DNA. There were large variations in the levels of AMR-genes between individual samples. As the number of samples in a pool increased a decrease in sample variation was observed. A steady state in the sample variation was seen when pooling five or more samples. No significant difference was found between pools of five samples and pools of more. There was a significant difference between pools of five or more samples and pools of less ($p < 0.0001$). In order to quantify the level of AMR-genes pools of five individual samples will give a result representative of the pig herd. The findings of this study could be used in planning of observational studies.

General information

State: Published

Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, Section for Epidemiology, University of Copenhagen

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Pages: 27-27

Publication date: 2015

Host publication information

Title of host publication: Proceedings of the Annual Scientific Conference and the Annual General Meeting of the European College of Veterinary Public Health

Place of publication: Belgrade, Serbia

Publisher: European College of Veterinary Public Health

Editor: Blagojevic, B.

Article number: P15

Main Research Area: Technical/natural sciences

Conference: Annual General Meeting of the European College of Veterinary Public Health 2015, Belgrade, Serbia, 07/10/2015 - 07/10/2015

Source: PublicationPreSubmission

Source-ID: 118950868

Publication: Research - peer-review › Conference abstract in proceedings – Annual report year: 2015