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Comparison of antimicrobial resistance in *E. coli* isolated from rectal and floor samples in pens with diarrhoeic nursery pigs in Denmark

Weber Nicolai Rosager[a,*], Nielsen Jens Peter[a], Jorsal Sven Erik Lind[d], Haugegaard Svend[c], Denwood Matthew[a], Pedersen Ken Steen[a,b]

[a] University of Copenhagen, Faculty of Health and Medical Sciences, Department of Large Animal Sciences, Grønnegårdsvej 2, 1870, Frederiksberg C, Denmark
[b] Øvet A/S, Køberupvej 33, 4700, Næstved, Denmark
[c] Laboratory for Pig Diseases, Danish Pig Research Centre, Vinkelvej 13, 8620, Kjellerup, Denmark
[d] National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, 1870, Frederiksberg C, Denmark

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ABSTRACT

Introduction: The prudent use of antibiotics in veterinary medicine necessitates the selection of antibiotic compounds with narrow-spectrums targeted against the specific pathogens involved. The same pathotype of enterotoxigenic *E. coli* (ETEC) was recently found both in diarrhoeic pigs and in samples from the pen floor where the pigs were housed. The first objective of this study was to compare resistance profiles from ETEC isolates and Non-ETEC isolates. The second objective was to evaluate the agreement between resistance profiles of ETEC isolated from pen floor samples and from individual rectal samples from pigs.

Across three Danish pig herds, faecal samples were collected from the floors of 31 pens that had a within-pen diarrhoea prevalence of > 25%, and from rectal samples of 93 diarrhoeic nursery pigs from the same pens. A total of 380 *E. coli* isolates were analysed by PCR and classified as ETEC when genes for adhesin factors and enterotoxins were detected. Minimum inhibitory concentrations of 13 antimicrobial agents were determined by the broth micro dilution method. Isolates were classified as resistant based on clinical breakpoints.

Results: Based on logistic regression models, the odds of Non-ETEC isolates (n = 291) being pan-susceptible were significantly higher compared to ETEC isolates (n = 89), (P < 0.001, OR = 20.22, CI95% = 6.35-64.35). The odds of ETEC isolates having multidrug resistance were significantly higher compared to Non-ETEC isolates (p < 0.001, OR: 7.21, CI95%: 2.87-18.10). The odds of an isolate being resistant were significantly higher in ETEC isolates compared to Non-ETEC isolates for ampicillin (p < 0.001), apramycin (p = 0.003), sulphamethoxazole (p < 0.001) and trimethoprim (p < 0.001). No overlap of resistance patterns between the three study herds was observed in the sampled ETEC isolates.

In addition, there was generally good or excellent agreement when comparing resistance profiles from isolates from the same pen (pen floor and pig samples), and perfect agreement (Kappa = 1.000, SE = 0.316) was observed for ampicillin, apramycin, gentamycin, sulphamethoxazole, tetracycline and trimethoprim.

Conclusions: We found that ETEC isolates were more resistant than Non-ETEC isolates. Furthermore, this study indicates that resistance testing of ETEC isolates from pen floor samples can be used as a convenient sampling method for resistance testing and in the selection of clinically relevant antimicrobial agents in the treatment of diarrhoeic pigs. The herd-level variation of resistance in ETEC isolates emphasises the importance of performing antimicrobial susceptibility testing at farm level when selecting antimicrobial agents for the treatment of *E. coli*-related diarrhoea.

1. Introduction

The risk of antimicrobial resistance (AMR) spreading from food-producing animals to humans is a major concern that attracts considerable political attention. The World Health Organisation (WHO) has highlighted antimicrobial resistance as a global threat for human health, and action to combat AMR must be taken to avoid a post-antibiotic era (WHO, 2014). The prudent use of antimicrobials for production animals is therefore a focus point throughout the world (European Commission, 2015; OIE, 2016). Prudent use is defined as the

* Corresponding author.
E-mail addresses: weber@sund.ku.dk (W.N. Rosager), jpni@sund.ku.dk (N.J. Peter), selj@vet.dtu.dk (J.S. Erik Lind), svh@seges.dk (H. Svend), md@sund.ku.dk (D. Matthew), ken@oevet.dk (P.K. Steen).

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choice of antimicrobials based on combined information from clinical experience, the expected susceptibility of the target pathogen, the route of administration, expected activity at the site of infection and the epidemiological history of the production unit, in particular previous antimicrobial resistance profiles (OIE, 2016). By using antimicrobial resistance profiles, veterinarians are able to select antimicrobial compounds with the narrowest spectrum of activity sufficient to target the pathogen (European Commission, 2015).

An important element in achieving prudent use is the development of new and precise diagnostic tools in veterinary pig practice, in order to decide whether antimicrobial treatment is necessary and to achieve the most efficient treatment of diseased animals. Previous published results from our group have shown that faecal pen floor samples can be used to diagnose enteric diseases from groups of pigs (Pedersen et al., 2015; Weber et al., 2017b). Furthermore, in outbreaks of ETEC-induced diarrhoea, the same pathotype of ETEC was demonstrated in rectal faecal samples from diarrhoeic pigs and in faecal samples from the pen floor where the pigs were housed (Weber et al., 2017a). We therefore hypothesise that using ETEC isolated from pen floor samples could be a convenient and relevant method for resistance testing and selection of antimicrobial agents.

The aim of this study was to investigate resistance profiles in ETEC and Non-ETEC isolates and to evaluate whether ETEC isolates from faecal pen floor samples could be used for resistance profiling. This was achieved by comparing resistance profiles in ETEC isolates from pen floor samples to faecal samples obtained per rectum from individual pigs in the same pens. Resistance profiling of pathogenic *E. coli* is highly relevant in veterinary practice when choosing the type of antimicrobial agent for treatment.

The first objective of the study was to compare resistance profiles from ETEC isolates and Non-ETEC isolates.

The second objective was to evaluate the agreement between resistance profiles of ETEC isolated from pen floor samples and from individual rectal samples from pigs.

2. Method

2.1. Design

A cross sectional study was performed in three commercial production herds in 2014. A total of 31 pens were selected and 93 pigs from these pens were sampled 14–28 days after weaning.

2.2. Herd description

A thorough description of the herds included in the study is published in Weber et al. (2017b). The herds were previously selected for a clinical trial investigating batch medication for intestinal diseases in nursery pigs. In brief, the herds were characterised as high-health herds declared free of *Actinobacillus pleuropneumoniae* type 2, 6 and 12, porcine reproductive and respiratory syndrome virus, mange mites and lice (SPF-sus, 2015), but with outbreaks of diarrhoea in nursery pigs requiring antimicrobial treatment (Pedersen et al., 2014). All herds had all-in all-out batch production in sectioned compartments, and the flooring consisted of 1/3 solid floor and 2/3 slatted floor. Feed was home-mixed and formulated with wheat, barley and soybean meal as the main ingredients, and fulfilled the Danish nutrient standards (Tybirk et al., 2015). The nursery pigs were DanAvl crossbreds of Yorkshire/Landrace and Duroc. All herds used 3000 ppm zinc oxide in the feed during the first 14 days after weaning.

2.3. Sampling procedure

The inclusion criteria for individual pens and pigs are described in detail in Weber et al. (2017a). In brief, rectal samples from 15 randomly selected pigs were obtained by digital manipulation. A diarrhoeic pig was identified by scoring the rectal sample using a faecal consistency scale with four categories, where scores of 1 and 2 represented normal faeces and scores of 3 and 4 represented diarrhoea (Pedersen and Toft, 2011). In pens with a diarrhoea prevalence of 25% or above among the sampled pigs, rectal samples from three diarrhoeic pigs and a faecal pen floor sample were collected and stored in sealed plastic containers. The pen floor samples were collected by running a gloved hand across the full length of the slatted floor. The cooled faecal samples were transported for bacteriology to the Laboratory for Pig Diseases in Kjellerup, Denmark in a polystyrene box containing ice packs.

2.4. Laboratory analyses

2.4.1. Bacteriology

In this study, bacterial culture of faecal samples was used to identify presence of *E. coli* colonies. The pig and pen floor samples were aerobically cultured for *E. coli*. Parallel culturing was performed on Drigalski (in-house selective and indicative medium for coliforms) and blood agar plates (Columbia agar (Oxoid) supplemented with 5% calf blood). Plates were incubated for 24 h at 37 °C. To identify the excepted higher diversity of *E. coli* isolates in pen floor samples, a larger number of colonies were sampled from pen floor samples than pig samples (Weber et al., 2017a). After culture, two coliform colonies with haemolytic activity (if present) and two coliform colonies with non-haemolytic activity were isolated from each pig sample. Haemolytic isolates were defined as colonies surrounded by a zone of lysis. Up to five coliform colonies with haemolytic activity and five coliform colonies with non-haemolytic activity were isolated from the pen floor samples. The selected isolates were analysed at the Danish Veterinary Institute using the 5′-nuclease assay (TaqMan PCR) previously described for the detection of virulence factor genes: F4, F5, F6, F18, F41, STa, STb, LT and VT2e (Frydendahl et al., 2001).

2.4.2. Antimicrobial susceptibility testing

Susceptibility testing was performed to determine the phenotypic susceptibilities of the sampled *E. coli* isolates to 13 antimicrobial agents. The antimicrobial concentration ranges and clinical breakpoints of the 13 antimicrobial agents included in the panel are shown in Table 1. The panel comprises clinically relevant antimicrobial agents for the treatment of porcine *E. coli* infections, in agreement with international guidelines (Burch et al., 2008; DANMAP, 2010). Minimum inhibitory concentrations (MIC) were determined by the broth micro dilution method in 96-well microtitre plates using the Sensititre system (Thermo Fisher Scientific, Waltham, Massachusetts, USA), as described in the standards manual of the Clinical and Laboratory Standards Institute (CLSI, 2015). The *E. coli* reference strain ATCC 25922 was used as a control organism. The plates were incubated for 20 h at 37 °C in an aerobic atmosphere. The Sensititre plates were manually read by trained laboratory personnel. The MIC was defined as the lowest concentration producing no visible growth. The clinical breakpoints used to interpret MIC values were a combination of CLSI breakpoints if available, and those routinely used by the Laboratory of Swine diseases, Kjellerup, Denmark and by the Danish Veterinary Institute, Frederiksberg, Denmark (CLSI, 2015; DANMAP, 2016).

2.5. Statistical analysis

The presence of resistance in ETEC and Non-ETEC isolates are presented in summary tables. Statistical analyses were performed in R version 3.1.2 with mixed models implemented using the lme4 package (R-Core-Team, 2014; Bates et al., 2015). The susceptibility to the 13 tested antimicrobials for both ETEC and Non-ETEC isolates were evaluated by determination of MIC50 and MIC90. Furthermore, to estimate the effect of the isolates' ETEC status on the occurrence of resistance, a generalised linear mixed model with logit link and binomial response (logistic regression) was used for each antimicrobial agent, with binary