Preventive Veterinary Medicine 116 (2014) 129–137

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Prevalence and potential risk factors for the occurrence of cefotaxime resistant *Escherichia coli* in German fattening pig farms—A cross-sectional study

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ARTICLE INFO

Article history:
Received 5 August 2013
Received in revised form 23 June 2014
Accepted 23 June 2014

Keywords:
Antibiotic resistance
ESBL
Epidemiologic study
Pork production
Farm management

ABSTRACT

A cross-sectional study concerning farm prevalence and risk factors for the count of cefotaxime resistant *Escherichia coli (E. coli)* (CREC) positive samples per sampling group on German fattening pig farms was performed in 2011 and 2012. Altogether 48 farms in four agricultural regions in the whole of Germany were investigated. Faecal samples, boot swabs and dust samples from two sampling groups per farm were taken and supplemental data were collected using a questionnaire. On 85% of the farms, at least one sample contained cefotaxime resistant *E. coli* colonies. Positive samples were more frequent in faeces (61%) and boot swabs (54%) than in dust samples (11%). Relevant variables from the questionnaire were analysed in a univariable mixed effect Poisson regression model. Variables that were related to the number (risk) of positive samples per sampling group with a p-value <0.2 were entered in a multivariable model. This model was reduced to statistically significant variables via backward selection. Factors that increased the risk for positive samples involved farm management and hygienic aspects. Farms that had a separate pen for diseased pigs had a 2.8 higher mean count of positive samples (95%-CI [1.71; 4.58], $p = 0.001$) than farms without an extra pen. The mean count was increased on farms with underfloor exhaust ventilation compared to farms with over floor ventilation (2.22 [1.43; 3.46], $p = 0.001$) and more positive samples were observed on farms that controlled flies with toxin compared to farms that did not (1.86 [1.24; 2.78], $p = 0.003$). It can be concluded, that CREC are wide spread on German fattening pig farms. In addition the explorative approach of the present study suggests an influence of management strategies on the occurrence of cefotaxime resistant *E. coli*.

1. Introduction

The number of reports of bacteria producing extended-spectrum β-lactamases (ESBLs) in humans and animals has increased in the last years. ESBLs are plasmid-mediated enzymes that hydrolyse the β-lactam-ring of antimicrobial substances like penicillins and cephalosporines. The

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http://dx.doi.org/10.1016/j.prevetmed.2014.06.014
0167-5877/

genes for ESBL-enzymes are passed to the next generation by cell division (vertical transfer) but also between different bacteria by conjugation or transformation (horizontal transfer) (Shah et al., 2004). ESBLs were identified in human faeces (Goossens and Grabein, 2005; Cantón et al., 2008; Correia et al., 2012), but also in wildlife, zoo and companion animals (Carattoli, 2008; Ewers et al., 2011; Guenther et al., 2011) as well as in food-producing animals (Geser et al., 2012; Hartmann et al., 2012; Hiroi et al., 2012; Friese et al., 2013; Laube et al., 2013). Detailed data on the transmission of ESBL-producing bacteria from animals to humans are not available to date, but high ESBL-*Escherichia coli* (*E. coli*) colonisation rates are reported in humans with contact to animals (Hamscher et al., 2003; Meyer et al., 2012; Dierikx et al., 2013). Another alarming fact is the existence of resistant bacteria in food (Mesa et al., 2006; Guerra et al., 2007; Cohen Stuart et al., 2012; Geser et al., 2012) and the presence of the same ESBL strains in food producing animals, food and humans (Leverstein-van Hall et al., 2011). Also environmental aspects may play an important role in transmission.

A number of studies investigated ESBL *E. coli* in pigs. In Switzerland, 334 faecal samples from pigs were analysed for ESBL-producing bacteria: 15.3% of the swine faecal samples were positive (Geser et al., 2012). In Spain, 36.5% of 131 faecal samples from fattening pigs harboured ESBL-producing *E. coli* (Blanc et al., 2006). In a second study from Spain, 8 out of 10 pig farms investigated contained ESBL-producing enterobacteria (Mesa et al., 2006). A report of ESBL-producing Enterobacteriaceae from Portugal showed a prevalence of 5.7% positive faecal samples taken from 35 healthy pigs (Machado et al., 2008). A lower number of positive animals (3%) was reported in a Japanese study investigating rectal samples of 33 pigs in a slaughterhouse (Hiroi et al., 2012).

In Germany, first examinations showed a high rate of ESBL-producing enterobacteria in healthy fattening pigs as well as in slurry and the farm surroundings. The farm prevalence of 16 investigated fattening pig holdings was 43.8% (Friese et al., 2013).

The aim of the present study was to estimate the prevalence of cefotaxime resistant (ESBL suspicious) *E. coli* on commercial pig farms in Germany and to identify potential risk factors for the number (risk) of positive samples per sampling group on a farm. The study is part of the research network RESET (www.reset-verbund.de).

2. Material and methods

2.1. Study population

For this cross-sectional study four sampling regions in Germany (Fig. 1) were chosen according to the principle management characteristics of livestock breeding (Merle et al., 2012). The northwest region has a high density of livestock kept on large farms with a predominance of pigs and poultry. In the middle of Germany the density of farms is smaller with mainly pig and dairy farms. The eastern region is characterised by few, but huge farms. In contrast, most farms in the south are small dairy and cattle farms. Nearly no livestock is kept in the region Upper Rhine. Therefore, this region was excluded.

From the above-mentioned regions (Fig. 1) representative districts were chosen on the basis of average farm and animal numbers. The contact to the farmers was established via veterinary offices or agricultural associations of the particular districts and via information sessions. The information material provided the inclusion criteria, which were keeping of fattening pigs and commercial animal production. All farmers who volunteered for the study were included.

The sample size was estimated prior to data collection. Based on the approximate two-sided 95%-confidence interval for one proportion, approx. 50 farms are necessary to estimate a prevalence of 10–20% with an error of ±10%.

In total 48 farms with fattening pigs were enrolled in the study. In the northwest, eight farms were included, in the region middle Germany 22 farms, in the eastern region six farms took part and in the south twelve farms participated. On each farm two sampling groups of different ages were investigated. Sampling groups on one farm were defined as animals that were kept in different compartments with separate ventilation and with no contact to each other. If possible, the youngest group and the oldest group on each farm were chosen to test whether age had an effect on the number of positive samples.

2.2. Sampling

The farms were visited between May 2011 and October 2012. In each of the two sampling groups per farm, three mixed faecal samples from the floor of three pens were transferred to a sterile 50 ml centrifuge tube (Schubert Laborfachhandel, Leipzig, Germany). Additionally, one sample was taken with a pair of autoclaved boot swabs (Finnimport GmbH, Hamburg, Germany) from the corridor inside the compartment. The boot swabs were packed in a sterile sample bag (Hele GmbH, Heilsbronn, Germany). To screen the distribution of cefotaxime resistant *E. coli* on the farm, one pooled dust sample per group from the windowsill, pen separation or automatic feeder within each sampled compartment was also transferred to a 50 ml centrifuge tube. The samples were cooled down in a cold box to about 4 °C before being sent to the laboratory in a Süsse Post Box Maxi (Süsse Labortechnik, Gudensberg, Germany) on the day of sampling and analysed within 24 h after sampling.

2.3. Questionnaire

A questionnaire to assess potential risk factors on farm level as well as on sampling group level was developed and pre-tested by different veterinarians and farmers. On the farms a printed version of the questionnaire was filled in by the study epidemiologist. Data was entered in a Microsoft Access 2003 database. The questionnaire consisted of three parts. The general part consisted of questions concerning the management, number of animals, and contact between animals, agricultural and geographical aspects. The second part concentrated on organisation of the stable, feeding attributes and hygiene. The last part contained questions on