Differences in Antibiotic Resistance in *Escherichia coli*, Isolated from East-European Swine Herds With or Without Prophylactic Use of Antibiotics

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With 7 figures

Summary

The aim of the study was to compare the resistance patterns of *Escherichia coli* isolates from pig herds with or without prophylactic use of anti-microbial substances. The presented pig units received either antibiotics or oregano as preventive feed additives. The trial was performed from April to October 2001, in the large ‘country-corner’, Hungary–Rumania–Serbia. Thirty of 39 evaluated herds suffered *E. coli* O139 K88 ac or ad LT STb caused losses, the remaining were negative for *E. coli* O139. Thirteen of the selected 30 herds produced with oregano feed supplementation (Oregpig® Pecs, Hungary) antibiotic-free pigs. These units had no history of prophylactic antibiotic use since 1995. The remaining 17 herds routinely used prophylactic antibiotic feed supplementation. In each herd, pigs of four different age groups (suckling piglets, weaners, fattening swine and breeding sows), showing the clinical symptoms of wasting, were investigated. *E. coli* O139 K88 ac or ad LT STb were tested for their resistance to antibiotics, available in this region. Oregano-fed herds demonstrated high significantly (*P < 0.001*) lower MICs (µg/ml) for ampicillin, doxycyclin, enrofloxacin, gentamycin, oxytetracycin and sulfamethacin compared to herds with prophylactic use of antibiotics. Resistance to ceftiofur revealed significant (*P < 0.05*) differences between the antibiotic- or oregano-treated units. The present results confirm literature data, that prophylactic use of antibiotics likely plays a role in inducing resistance of *E. coli* and other intestinal bacteria. Thus, imposing greater restrictions on antibiotic use in animal agriculture is likely to not only eliminate the occurrence of resistant isolates.

Introduction

Antibiotics have been used for decades not only to combat disease, but also to improve animal performance (Bilkei and Biro, 1998). Anti-microbial feed additives benefit production by increasing profitability (Bilkei et al., 1995), reducing animal wastes into the environment (Roth and Kirchgessner, 1993) and diminishing pathogen carriage (Kyrakiak et al., 1996). According to World Health Organization (WHO, 1997) and US National Research Council (NRCIM, 1998), the main risk factor for the increase in anti-microbial resistance in pathogenic bacteria is the increased use of antibiotics in both animals and humans. It has been demonstrated (Mathew et al., 1998, 1999, 2001; Bander, 1999) that after introduction of antibiotics in veterinary practice, the resistance in pathogenic bacteria and in faecal flora increases. It has been stated (WHO, 1997) that use of antibiotics in livestock increases not only the resistance in pathogenic bacteria in faecal flora of the animals but also presents a high risk of transfer of this resistance from animals to humans via contaminated food. The large-scale prophylactic use of anti-microbials causes – especially in large units – antibiotic resistance (Cromwell et al., 1996; Mathew et al., 2001). As a practical alternative to prophylactic use of antibiotics against enteric bacterial infections, phytochemical feed additives seem to be of similar value (Bilkei et al., 1995). Oregano feed supplementation positively influences daily feed intake, daily weight gains and feed utilization (Günter and Bossov, 1998; Bilkei and Gertenbach, 2001; Gertenbach and Bilkei, 2001).

The present study was designated to compare the prevalence of antibiotic resistance in *Escherichia coli* on farms where prophylactic antibiotic or oregano feed supplementation were used.

Material and Methods

The trial was performed from April to October 2001, in the large ‘country-corner’ (a territory embracing more than 8000 km²), Hungary–Rumania–Serbia. Thirty-nine large pig production units were chosen for the study.

Experimental design

Thirty of 39 evaluated herds suffered *E. coli* O139 K88 ac or ad LT STb caused losses, the remaining were negative for *E. coli* O139. Only *E. coli* O139 positive herds were subjected for further evaluation. Thirteen of the selected 30 herds produced with oregano feed supplementation (Oregpig® Pecs, Hungary) antibiotic-free pigs. The oregano-using units had no history of prophylactic antibiotic use since 1995. The remaining 17 herds routinely used post-weaning and post-parturient prophylactic antibiotic feed supplementation.

In oregano-using units, 2000 ppm Oregpig® was routinely added to the weaning (for 3 weeks post-weaning) and post-parturient (for 1 week post-partum) diet of the animals. Oregpig® is a dry powder of the leaves and flower of the plant *Origanum vulgare*, enriched with 5% pressed *O. vulgare* ethereal oils consisting of 60–80% carvacrol and 1–2% thymol. Using a diet content of 2000 ppm Oregpig®, the in feed content of carvacrol was 120–160 ppm and that of thymol 2–4 ppm.

In each herd, pigs of four different age groups [suckling piglets (n = 6 per unit), weaners (n = 6 per unit), fattening swine (n = 6 per unit) and breeding sows (n = 3 per unit)], showing the clinical symptoms of wasting, were investigated.
E. coli O139 K88 ac or ad LT STb were tested for their resistance to antibiotics, available in this region.

**Bacteriology**

Rectal swabs were taken. Samples were maintained in Cary–Blair medium (Atlas and Snyder, 1995) on ice until arrival in our laboratory, within 48 h of collection. Samples were cultured and analysed using standard methods for identification of E. coli. Samples were streaked onto McConkey agar (Vet Invest, Zagreb, Croatia) and incubated (37°C) for 24 h. Pink colonies with characteristic E. coli morphology were confirmed by biochemical analysis (Merieux, Syosset, New York, USA). Further analysis of the isolates was performed by multiplex PCR assay for detecting heat-labile toxin (LT), heat-stable toxins (STa, STb) and fimbrial adhesins K88 ac, ad (Bosworth and Casey, 1997; Bosworth et al., 1998). Amplified products were electrophoresed in 2% agarose gel, stained with ethidium bromide and examined under ultraviolet illumination. DNA fragment lengths were verified by a digested lambda-DNA standard run simultaneously. Control for DNA reference strains was included in each reaction.

**Antibiotic testing**

Susceptibility to ampicillin, ceftiofur, doxycyclin, enrofloxacin, gentamycin, oxytetracyclin and sulfamethacin (antibiotics available in this region and of reasonable cost) was determined using standardized minimum inhibitory concentrations broth dilution method (NCCLS, 1997).

**Statistical analysis**

Susceptibility to antibiotics and number of resistant isolates for each herd were compared using a statistical analytic system (SAS, 1990). Two-way analysis of variance, according to Steel and Torrie (1980), was used to compare minimum inhibitory concentrations (MIC, µg/ml) and the percentage of resistant E. coli between herds. The individual herd served as the experimental unit to determine main effects of antibiotic or oregano use.

**Results**

Thirteen oregano and 17 antibiotic-using units revealed the prevalence of E. coli O139 K88 ac or ad LT STb. Only these units were subjected to further investigations. Besides E. coli O139 K88 ac or ad, E. coli serogroups O149 K91 (suckling piglets 41 of 180 samples, weaners 53 of 180 samples, fattening swine 14 of 180 samples and breeding sows 29 of 90 samples) and O147 K89 (suckling piglets 72 of 180 samples, weaners 63 of 180 samples, fattening swine 22 of 180 samples and breeding sows 21 of 90 samples) were registered but not evaluated further. Oregano-fed herds presented lower (P < 0.001) E. coli O139 K88 ac or ad LT STb MICs (µg/ml) (P < 0.001) for ampicillin (Fig. 1), doxycyclin (Fig. 3), enrofloxacin (Fig. 4) gentamycin (Fig. 5), oxytetracyclin (Fig. 6) and sulfamethacin (Fig. 7) compared to prophylactic antibiotic-using herds. Resistance to ceftiofur presented a significant (but only P < 0.05) difference between the antibiotic- or oregano-treated units (Fig. 2). [In Figs 1–7, minimum inhibitory concentration (MIC, µg/ml). Bars with subsequent different letters, differ: P < 0.05; bars with one missing subsequent different letters, differ: P < 0.01; bars with two or more missing subsequent different letters, differ: P < 0.001].

**Discussion**

We were not able to find comparable literature data on bacterial antibiotic resistance in pig units with prophylactic use of phytogenic feed additives. However, Langois et al. (1986) reported on antibiotic resistance in herds having not used prophylactic anti-microbials. It should also be critically mentioned that naturally occurring antibiotics, known to be produced by a variety of organisms (Wiener, 1996; Wim de Koning et al., 1999), might provide a low level of selective pressure for maintenance of resistance elements in field trials.
Resistance is not an all-or-non-phenomenon occurring at only a definite level, but it rather occurs over a wide range of values (Bilkei et al., 1995). In the present trial, while evaluating the data, we concluded that mean MIC (\( \mu g/ml \)) is a suitable indicator of susceptibility to antibiotics. Most of the mean MIC criteria were based on Research Council (NRCIM, 1998) breakpoints and some were slightly relativated by our own experiences in these units. Therefore, the present data may not be indicative of the overall clinical efficacy of all examined substances in other units.

Differences between antibiotic- or oregano-using units in susceptibility of the examined \( E. \) coli strains were dependant on the antibiotic and age of the pig. For example, susceptibility to ampicillin, doxycyclin, enrofloxacin, gentamycin, oxytetracyclin and sulfamethacin differed greatly between antibiotic- or oregano-using herds and between pigs of varying ages, whereas susceptibility to ceftiofur differed little between herds and ages of pigs. Consistent with earlier studies (Bilkei et al., 1995), resistance to oxytetracyclin was greater in isolates from sows, whereas ampicillin, doxycyclin, enrofloxacin, gentamycin showed greater differences in MICs in younger animals. The greater antibiotic resistance observed in \( E. \) coli isolates from young pigs in the present trial is not solely the result of generally more antibiotic use in this stage of the production (Bilkei et al., 1995), as similar trends were noted in pig production units that have not used antibiotics prophylactically for at least 13 years (Langois et al., 1986). It seems to be likely that genetic resistance elements could be widespread among \( E. \) coli even in the absence of antibiotic use (Bilkei et al., 1995). Consistent with literature data (Mathew et al., 1998, 1999, 2001; Bander, 1999), MICs were significantly greater for isolates in antibiotic-using herds (except ceftiofur), indicating that on-farm antibiotic use plays an important role in the susceptibility of resistance of isolates.

Phytogenic feed additives are controversially discussed in the literature (Bilkei et al., 1995; Baumann and Bilkei, 2002). It has been stated that oregano stimulates organic and microbiotic digestion (Wim de Koning et al., 1999). Oregano supports digestion and regulation of gastrointestinal metabolism (Günter and Bossov, 1998) and exerts anti-bacterial properties by hindering dysbiotic processes in the digestive tract of pigs (Kyriakis et al., 1996; Sivropoulou et al., 1996; Tsinas et al., 1998a,b). A study stated that 1000-ppm-dose oregano feed supplementation during the post-weaning period significantly improves weight gain and health of the pigs (Gertenbach and Bilkei, 2001).

The present results confirm literature data (Mathew et al., 2001), that prophylactic use of antibiotics likely plays a role in the susceptibility or resistance of \( E. \) coli and presumably also other enteric bacteria. Exclusion of antibiotics in swine production decreases but does not completely eliminate antibiotic resistance in \( E. \) coli. Thus, imposing greater restrictions on antibiotic use in animal agriculture is likely to reduce but not eliminate the occurrence of resistant isolates.

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