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Resistance to selected beta-lactam antibiotics

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ABSTRACT

Susceptibility in vitro and trends in resistance to antimicrobials were determined by a dilution micromethod in a group of *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Mannheimia haemolytica* and *Escherichia coli* isolates from clinical cases of cattle and swine diseases in the Czech Republic from 2007 to 2011. A high susceptibility of pig and cattle respiratory pathogens to antimicrobials was found, with the exception of the moderate prevalence of *M. haemolytica* resistance to ampicillin. In contrast to respiratory pathogens, low susceptibility of *E. coli* of pig and cattle isolates to ampicillin and amoxicillin/clavulanic acid was noted. Regarding resistance trends, an increase in levels of resistance among *E. coli* isolates to ampicillin and amoxicillin/clavulanic acid was identified, but the resistance of respiratory isolates was low, with the exception of *M. haemolytica*.

For the period of 2007–2011, there was a significant and almost continuous increase in sales (compared with population correction unit) of ceftiofur, cefquinome and other beta lactams for pigs. Consumption peaked in 2010. In the case of amoxicillin in combination with clavulanic acid, data showed a significant decrease in sales from 2007 to 2008, followed by a period of fluctuation. In cattle, within the groups of 3rd and 4th generation cephalosporins and for the whole group of other betalactams for the period of 2007–2011, there was a significant and almost continuous increase in sales (compared with population correction unit). Consumption peaked in 2010. In the case of ceftiofur, there was a huge increase noted from 2010. In the case of amoxicillin in combination with betalactamase inhibitor (clavulanic acid) data shows a significant decrease from 2007 to 2008, followed by a period of fluctuation in sales.

1. Introduction

Actinobacillus pleuropneumoniae, *Pasteurella multocida*, and *Escherichia coli* in pigs as well as *P. multocida*, *Mannheimia haemolytica*, and *E. coli* in cattle are bacterial pathogens negatively affecting the economy of animal farming worldwide, and are therefore a common reason for the initiation of treatment or prevention of diseases with antimicrobials. Due to the use of antimicrobials, a certain degree of the risk of selection and dissemination of resistant bacteria, which in recent years represent a serious problem both in veterinary and human medicine, is of concern (Schwarz et al., 2001).

Continuous monitoring of the prevalence of drug resistance in bacterial pathogens to antimicrobials provides essential data on the state and development of susceptibility in pathogens causing bacterial infections.

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These data are necessary for the prediction of the efficacy of antimicrobials in the initial treatment and for the determination of the position of individual substances as drugs of choice and alternative drugs in the therapy of infections. They are therefore a basis for the formulation of targeted antimicrobial treatment and prevention independent of the pharmaceutical industry (AP NAP, 2010).

The increase in exposure of animals to antimicrobials or the inappropriate dosing schedules (e.g. low doses administered for unnecessarily long time periods) can be considered significant risk factors for the increase of bacterial resistance in veterinary medicine (Lees et al., 2008). Therefore, the surveillance systems of antimicrobial resistance, including surveillance of the antimicrobial consumption in livestock have been introduced in many countries with a certain level of details collected and analyzed. These complex systems in Europe include DANMAP in Denmark, SVARM in Sweden, NORM-Vet in Norway, MARAN in the Netherlands and GERM-Vet in Germany. In the Czech Republic, monitoring of antimicrobial resistance among bacterial pathogens of livestock is not currently under the complete central control but it is fragmented into partial initiatives and projects led by universities and research institutes. However, the national sales data for antimicrobials have been collected and analyzed by the Institute for State Control of Veterinary Biologicals and Medicaments, Brno, Czech Republic (ISCVBM) since 2000. Sales data collection and analysis are in conformity with the national legal provisions (Act on Pharmaceuticals in force) and within the framework of the Action Plan of the National Antibiotic Programme (AP NAP). For the period 2010–2013, a new system which is in line with the international project European Medicines Agency (European Medicines Agency, 2011) has been introduced. Professionally competent and cost effective measures at the national, regional and local level based on the relevant data coming from the well-established surveillance of antimicrobial resistance in human and veterinary medicine should be a priority (AP NAP, 2010; Hera et al., 2010).

The aim of this study was to determine the prevalence of clinical resistance and trends in resistance to selected betalactams, including ampicillin, amoxicillin/clavulanic acid, ceftiofur and cefquinome in bovine isolates of *P. multocida*, *M. haemolytica*, and *E. coli* and swine isolates of *A. pleuropneumoniae*, *P. multocida* and *E. coli* that may cause serious infections in animals and to investigate whether there is a correlation among resistance patterns of selected microorganisms to the representatives of certain groups of betalactam antimicrobials and sales patterns of respective antimicrobials. Among the agents selected, both critically important antimicrobials (3rd and 4th generation cephalosporins) and aminopenicillins alone and in combination with betalactamase inhibitors were included to assess both resistance and sales trends and their possible correlations. As no veterinary medicinal product containing cephalosporins of the 1st and 2nd generation is authorized for the treatment of respiratory and enteric diseases of pigs and cattle, and only pathologies of the mammary gland are within the scopes of indications of products with valid authorization for the period of concern, these substances were not included in this article.

2. Materials and methods

2.1. Sampling of isolates

All clinical isolates were obtained from the respiratory and enteric tract of diseased animals in the period 2007–2011. Total number of isolates and numbers of isolates in the respective years are presented in Tables 1–6.

2.2. Determination of resistance to antimicrobials

Resistance to antimicrobials was assessed according to a standardized dilution micromethod of the Clinical and Laboratory Standards Institute (CLSI, 2008) using two commercial kits (Trek Diagnostics Systems Inc., England – including amoxicillin/clavulanic acid 2/1, ceftiofur and cefquinome; Trios, Czech Republic – including ampicillin), with ranges of concentrations of antimicrobials shown in Tables 1–6. Quality control of the kits was performed by using the reference strains of *E. coli* ATCC 25922 and *A. pleuropneumoniae* ATCC 27090. The MICs values were read at the lowest concentration of an antimicrobial agent that inhibited the visible bacterial growth. MIC_{50} and MIC_{90} presented the lowest concentration of antimicrobial substances in mg/L that inhibited the growth of 50% and 90% of isolates as determined by the cumulative conversion (Schwarz et al., 2010). The susceptibility of the isolates was evaluated according to the criteria CLSI and according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in *E. coli*.

2.3. Trends in resistance

Trends in resistance and levels of resistance were assessed on the basis of the percentage of isolates that exceeded the value of the MIC breakpoint for sensitivity in respective years. The level of resistance was assessed as follows: rare (<0.1%), very low (0.1–1%), low (>1–10%), medium (>10–20%), high (>20–50%), very high (>50–70%) and extremely high (>70%).

2.4. Collection of data on the sales of antimicrobials

Data on the sales per package of veterinary medicinal products (VMPs) were collected from all wholesalers and feed mills, licensed in the Czech Republic, that deliver, based on veterinary prescriptions, VMPs directly to final customers (veterinarians, pharmacies or farmers) in the Czech Republic. In the case of medicated premixes (e.g. with amoxicillin), the data reported by manufacturers of medicated feeding stuffs were used for calculation. Sales to wholesalers and manufacturers of medicated feeding-stuffs were used for verification of VMPs' movement in the cross control. VMPs for companion animals were not included in the final counts. For stratification according to the animal species (here pigs and cattle, 3rd and 4th generation cephalosporins and amoxicillin in combination with clavulanic acid) in the cases of products authorized and used for both species, data from Periodic Safety Update Reports (PSURs) and/or inspections were used to estimate the proportion of the final use of each VMP of concern. Within the group of other betalactams, only