

Assessment of the Impact of the Non-human Use of Antimicrobial Agents on the Selection, Transmission and Distribution of Antimicrobial Resistant Bacteria

Executive Summary

by

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There is mounting evidence showing that the use of antimicrobial agents in animals provides a powerful selective force that promotes the emergence, persistence and dissemination of antimicrobial resistance in pathogenic, zoonotic as well as commensal bacteria. The emergence, persistence and dissemination of antimicrobial resistant bacteria may reduce treatment options for infected hosts, which in turn may lead to an overall increase in disease transmission, morbidity and mortality.

Antimicrobial resistant bacteria not only pose a risk with respect to animal health, but also to human health following the possibility of their transfer to humans as foodborne pathogens via the food chain. However, the relative contribution of the animal use of antimicrobials to the overall problem of antimicrobial resistance in humans has been very difficult to establish. This is due to the fact that other possible indirect sources exist such as the environment or contact with companion animals. In spite of such difficulties, the fact remains that pathogenic bacteria from humans and animals are becoming increasingly resistant to most frontline antimicrobial agents such as aminoglycosides, third generation cephalosporins and fluoroquinolones. This increase has not been met with a corresponding increase in the number of new antimicrobial agents therefore warranting strategies to preserve the efficacy of existing antimicrobial agents.

An approach that has been increasingly used to quantify or qualify the human health risks associated with non-human antimicrobial usage in animals is based on risk assessments. Results from gingerly developed microbial risk assessment models can be used to inform policy-makers and the food production industry on codes of practice relating to food safety. Even though microbial risk assessments use data from surveillance programmes, data gaps do exist regarding analysis along the farm-to-consumption chain. TRAINAU (www.trainau.dk) is a consortium designed to generate data and methods that can be used for conducting microbial risk assessments. It relies on the Danish experience to produce data and develop methods needed to answer questions that are of crucial importance for conducting microbial risk assessments and transmitting the outcomes to other European countries. The current

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project aims at applying statistical and mathematical models to understand the selection, transmission and distribution of antimicrobial resistant bacteria. Attention is focussed on finisher pigs, which are the highest consumers of antimicrobial agents in the animal production industry in Denmark.

In the first part of the project, an in vivo experiment was performed to study the effects of veterinary β -lactam drugs on selection and persistence of ESBL-producing *E. coli* in the intestinal flora of pigs. Twenty pigs were randomly allocated into the three treatment groups and one control group. All pigs were inoculated intragastrically with 10^{10} CFU of a nalidixic acid (NAL)-resistant mutant derived from a CTX-M-1-producing *E. coli* strain of pig origin. Treatment with amoxicillin, ceftiofur or cefquinome according to label instructions was initiated immediately after bacterial inoculation. Rectal faeces were collected before inoculation and on days 4, 8, 15, 22 and 25 after the start of treatment. Total and resistant coliforms were counted on MacConkey agar with and without cefotaxime (CTX). Furthermore, MacConkey Agar with CTX and NAL was used to count the inoculated strain. Significantly higher counts of CTX-resistant coliforms were observed in the three treatment groups compared to the control group up to 22 days after discontinuation of treatment. Ceftiofur and cefquinome exerted a larger selective effect than amoxicillin and the effect persisted beyond the withdrawal times recommended for these cephalosporins. The inoculated strain was only detected in nine animals on day 25. The increase in CTX-resistant coliforms was mainly due to proliferation of indigenous CTX-M-producing strains and possible emergence of strains that acquired CTX-M genes by horizontal transfer. The study provides evidence that cephalosporins used in pig production select for CTX-M-producing *E. coli*. Their usage in animals should be carefully considered in view of the critical importance of cephalosporins and the zoonotic potential of ESBL-producing bacteria.

The next phase was to develop a modelling framework that can be used to assess the impact of antimicrobial usage in pigs on the emergence and transmission of resistant bacteria within a finisher pig farm. The transmission dynamics of drug-sensitive and drug-resistant bacteria among pigs in the herd were characterised by studying the local and global stability properties of steady state solutions of the system. Numerical simulations demonstrating the influence of factors such as initial prevalence of infection, presence of pre-existing antimicrobial resistant mutants, and frequency of treatment on predicted prevalence were performed. Sensitivity analysis revealed that two parameters had a huge influence on the predicted proportion of pigs carrying resistant bacteria: (a) the transmission coefficient between uninfected pigs and those infected with drug-resistant bacteria during treatment ($\beta(2)$) and after treatment stops ($\beta(3)$), and (b) the spontaneous clear-out rate of drug-resistant bacteria during treatment ($\gamma(2)$) and immediately after treatment stops ($\gamma(3)$). Control measures should therefore be geared towards reducing the magnitudes of $\beta(2)$ and $\beta(3)$ or increasing those of $\gamma(2)$ and $\gamma(3)$.

The two previous studies focused on the within and between host transmission dynamics of antimicrobial resistant bacteria. The next two studies aimed at studying trends in the distribution of antimicrobial resistant *E. coli*.

The aim of the third study was thus to analyse and discuss regional, seasonal, and temporal trends in the occurrence of antimicrobial-resistant *E. coli* isolated from pigs at slaughter in Denmark between 1997 and 2005. Data on antimicrobial-resistant *E. coli* were obtained from the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme database. The Cochran-Armitage trend test was used to detect the presence and evaluate the significance of regional, seasonal, and annual trends in the occurrence of antimicrobial-resistant *E. coli* for four drugs. Associations between resistance and explanatory variables region, season, and the year of isolate sampling were analysed using a logistic regression model. The Cochran-Armitage test provided evidence of significant temporal trends for ampicillin-resistant *E. coli* (an increasing trend, $p < 0.0001$) and streptomycin-resistant *E. coli* (a decreasing trend, $p < 0.0001$). The prevalence of ampicillin-resistant *E. coli* increased over time for all seasons ($p < 0.001$) except for winter when no significant variations in prevalence of resistant *E. coli* were captured over time. On the other hand, a significant decreasing trend in prevalence of streptomycin-resistant *E. coli* was observed for the spring, summer, and winter months ($p < 0.001$); however, there were no statistically significant trends for the autumn months ($p > 0.05$). The prevalence of ampicillin-resistant *E. coli* was observed to increase over time for the various regions, whereas prevalence of streptomycin-resistant *E. coli* presented an overall significant decrease over time. The estimated odds ratios from the logistic regression model indicated varying risks of the occurrence of resistance by season and by region. The winter months were associated with an increased risk of the occurrence of resistant *E. coli* as compared to the other seasons of the year. Our study provides evidence of statistically significant regional, seasonal, and temporal variations for ampicillin- and streptomycin-resistant *E. coli* isolated from pigs at slaughter in Denmark between 1997 and 2005.

After studying the trends in occurrence of antimicrobial resistant bacteria, the next step was to determine whether the observed variations in the prevalence of ampicillin-resistant *E. coli* isolated from healthy pigs at slaughter were random or clustered in space and time. Space-time interaction was assessed using the space-time K-function, and detection and location of significant space-time clusters were done using the space-time scan statistic. The space-time K-function analysis provided evidence of space-time interactions in ampicillin-resistant *E. coli* (AREC) isolates in both Funen and Jutland, and Zealand. Significant space-time clusters of resistant *E. coli* isolates were found in the northeastern part of Jutland and Funen and in the southern part of Zealand. Seasonality was found to have a highly significant effect on space-time clustering in Funen and Jutland. The clusters of AREC appeared at the same time as the national consumption of ampicillin in pigs increased, however antimicrobial consumption at the herd level did not appear to have any effects on space-time clustering in this study. The results could serve as a platform to highlight areas where more investigations on the occurrence and spread of AREC in pig herds should be initiated.

Given the information from the intra- and inter-host transmission dynamics, and the spatio-temporal trends in the occurrence of resistant bacteria, it becomes possible to study the between-farm dynamics of antimicrobial resistant bacteria. This will allow an understanding of the national spread of antimicrobial resistant bacteria and the identification of important parameters governing their spread. Between-farm dynamic models can also be used to estimate the national prevalence of antimicrobial resistant bacteria that could serve as input to the release assessment step of risk assessment models for antimicrobial resistant bacteria.

A copy of the dissertation can be obtained from the author by e-mail (enjiabatih@itg.be).