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Development of an optimal strategy for monitoring antimicrobial resistance in bacteria from food animals in Switzerland

Objectives As one way to protect humans against antibiotic resistant bacteria, we must prevent the transfer of resistant microorganisms through animal-derived food. An optimal and cost-effective strategy is being developed for continuous monitoring of antibiotic resistant bacteria in food animals to allow early detection of changes in resistance patterns and timely interventions.

Conclusions A simulation model (Monte Carlo simulation model) was developed to assess the effect of various sampling strategies on the estimation of the national prevalence of resistance in bacteria from Swiss livestock (taking imperfect sensitivity and specificity of diagnostic tests into account). The model is based on random sampling from the health population and will be further expanded to support risk-based sampling strategies. Accounting for the costs for sample collection, transportation and laboratory analysis, the number of samples to be taken and the respective step in the production line were optimised, allowing discovery of the optimal and cost-effective sampling strategy for a given bacterial species and an antibiotic to be monitored. For bacterial species with clonal spread among animals within a farm (e.g. *Campylobacter*), increasing the number of herds is the most efficient strategy to obtain a precise estimation for antimicrobial resistance. On the other hand, for bacteria with more varying resistance patterns within one farm (e.g. *E. coli*), it is more efficient to sample fewer herds but to increase the number of samples per herd. This cost-effective and well established resistance monitoring programme for farm animals in Switzerland functions as an early warning system for the emergence of antimicrobial resistance patterns of human health relevance. Practically, sample collection is co-ordinated by the Federal Veterinary Office (FVO), and susceptibility testing is performed at the Centre for Zoonoses, bacterial animal diseases and antibiotic resistance (ZOBA) of the University of Bern. Consequently, in case of an increase of the frequency of resistance, proper measures can be taken in time to maintain the effectiveness of antimicrobial substances and to minimise risks for consumers. Results of the monitoring will be published by the Federal Veterinary Office in the annual Swiss Zoonoses Report.

For *Campylobacter spp.* in poultry, a decreasing trend over the last four years was observed in the frequency of antibiotic resistance from Swiss live poultry. However, the observation of 19% ciprofloxacin resistant isolates in poultry meat is of concern and needs further monitoring. In imported poultry cuts for retail sale, in contrast, *Campylobacter* isolates showed significantly higher resistance levels than *Campylobacter* isolated from Swiss meat. In pigs, meat labelled as animal-friendly was less likely to harbour *Campylobacter* resistant isolates than conventional production.

Genotyping assays revealed that *Campylobacter jejuni* isolates can show both a high degree of genetic diversity in one animal species but also closely related isolates from different animal species (cattle, pets and poultry) and in humans, suggesting importance of animal reservoirs for human infections.

To conclude, a favourable resistance situation in bacteria from Swiss livestock, as compared to livestock in other countries, was confirmed. To provide an early warning system for the emergence of antimicrobial resistance patterns of human health relevance, it may be more cost-effective to perform more intensive sampling of animals from farm types or areas with a high use of antibiotics than of animals with a lower risk for antimicrobial resistance.

Main results and findings

Antimicrobial resistance in bacteria from poultry A valid and cost-effective routine monitoring programme for antibiotic resistance in *Campylobacter spp.* in poultry production was achieved by randomly collecting a modest number of samples from live animals, at slaughter, and from retail. The results can be summarised as follow:

- The prevalence of *Campylobacter spp.* in live animals decreased from 2002 to 2003 but was stable in 2004 (41.6% in 2002, 24.1% in 2003 and 25.6% in 2004).
- The prevalence of *Campylobacter spp.* isolates with resistance to more than one antibiotic decreased significantly from 2002 to 2004 (8.4% in 2002, 7.6% in 2003 and 3.7% in 2004).
- The prevalence of *Campylobacter spp.* in cloacal swabs of live animals was 33% (mean of the years 2002 and 2003), in neck skin samples from slaughter was 11% (mean of the years 2002 and 2003), and in meat samples was 22% (year 2002).

- Significant differences in resistance patterns between *Campylobacter spp.* from Swiss meat (less likely to be resistant) compared to imported products (more likely to be resistant) were found for ciprofloxacin, ampicillin and tetracycline.
- Type of production was also associated with antimicrobial resistance; that is, *Campylobacter spp.* isolates from meat labelled as animal-friendly were less likely to be resistant than isolates from conventional production.

In 2005, the resistance monitoring was expanded to *Enterococcus spp.* and *E. coli*, with following results:

- Among the 559 *E. coli* isolates, resistance to tetracycline, sulfonamides and streptomycin was most frequent, and 43% of the isolates were resistant to more than one of the tested antibiotics.
- Among the 188 *Enterococcus spp.* isolates, resistance to tetracycline and erythromycin was frequent, 3% (5 isolates) were resistant to vancomycin, and 74% of the isolates were resistant to more than one of the tested antibiotics. However, no co-resistance to penicillin, tetracycline and erythromycin was found.

Antimicrobial resistance in bacteria from pigs To assess the prevalence of resistant bacteria in 60 pig breeding farms (period November 2004 to June 2005), the Monte Carlo simulation model was adapted to pigs. Since *E. coli* isolates within the same pooled sample had various resistance patterns, the precision of the estimate, i.e. for tetracycline resistance, was substantially improved by increasing the number of animal tested per farm, or the number of *E. coli* isolates per pig. It was found that resistance patterns of *E. coli* from pigs were similar to *E. coli* from poultry. Furthermore, compared to traditional farms, a decrease in antibiotic resistance in *Campylobacter* isolates from the faeces of Swiss “animal-friendly” pig fattening farms (both in reduction of resistance to fluoroquinolones as well as reduction in the number of isolates with multiple resistances) was observed.

Antimicrobial resistance in bacteria from cattle In 2005, fresh beef was sampled at retail. A low prevalence of *Campylobacter* (among 110 samples, none was positive) and indicator bacteria (among 320 samples: 2 isolates (0.63%) positive for *E. coli*, both streptomycin resistant, and 3 (0.94%) positive for *Enterococcus spp.*, no resistant isolates) was observed.

Genetic diversity in a *Campylobacter jejuni* and *Campylobacter coli* population isolated from Swiss poultry From 100 randomly selected flocks, 5 live birds per flock (at delivery at the abattoir) were selected. The samples were analysed using three independent typing methods: AFLP (whole genome), *flaA*-RFLP (polymorphism in a single gene) and phenotypic determination of resistance (disk diffusion) to 8 antimicrobial agents commonly used in veterinary and human medicine. The results can be summarised as follows:

- The *Campylobacter* population in Swiss poultry is genetically highly diverse, and genetic typing by AFLP or *flaA*-RFLP was complementary.
- No correlation between genotype and resistance pattern was observed, suggesting that antimicrobial resistance is more variable (sketching the high adaptive potential of *Campylobacter* against antimicrobial pressure) than the combined AFLP/*flaA*-RFLP genotype.
- Sulphonamide resistance was frequently found together with streptomycin resistance, and further experiments are required to assess a potential genetic linkage.

Phenon cluster analysis for investigation of relatedness between *Campylobacter* sources To assess the possible epidemiological significance of possible infection sources for human campylobacteriosis, fluorescent AFLP was used to type 243 apparently epidemiologically unrelated *C. jejuni* isolates (77 humans, 46 cattle, 49 pets and 71 poultry). The results showed that:

- Isolates from different sources were frequently clustered together, underlining the high degree of source mixing and the lack of host specificity of *C. jejuni*.
- A high degree of relatedness between animal and human isolates was observed, indicating the importance of animals as infection source of humans.

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