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Discussion

Pan-European resistance monitoring programmes encompassing food-borne bacteria and target pathogens of food-producing and companion animals

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ABSTRACT

Antimicrobial resistance is a concern both for animal and human health. Veterinary programmes monitoring resistance of animal and zoonotic pathogens are therefore essential. Various European countries have implemented national surveillance programmes, particularly for zoonotic and commensal bacteria, and the European Food Safety Authority (EFSA) is compiling the data. However, harmonisation is identified as a weakness and an essential need in order to compare data across countries. Comparisons of resistance monitoring data among national programmes are hampered by differences between programmes, such as sampling and testing methodology, and different epidemiological cut-off values or clinical breakpoints. Moreover, only very few valid data are available regarding target pathogens both of farm and companion animals. The European Animal Health Study Centre (CEESA) attempts to fill these gaps. The resistance monitoring programmes of CEESA have been a collaboration of veterinary pharmaceutical companies for over a decade and include two different projects: the European Antimicrobial Susceptibility Surveillance in Animals (EASSA) programme, which collects food-borne bacteria at slaughter from healthy animals, and the pathogen programmes that collect first-intention target pathogens from acutely diseased animals. The latter comprises three subprogrammes: VetPath; MycoPath; and ComPath. All CEESA projects include uniform sample collection and bacterial identification to species level in various European Union (EU) member states. A central laboratory conducts quantitative susceptibility testing to antimicrobial agents either important in human medicine or commonly used in veterinary medicine. This 'methodology harmonisation' allows easy comparisons among EU member states and makes the CEESA programmes invaluable to address food safety and antibiotic efficacy.

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1. Introduction

Surveillance studies on antimicrobial resistance in bacteria that cause infections in humans and animals (zoonotics and target pathogens) and in indicator bacteria (commensals) are essential when studying changes in the antimicrobial susceptibility patterns of these organisms over time and to identify emerging resistance. Resistance monitoring systems also provide data that assist the authorities in making decisions related to the approval of antimicrobial drugs for animals. In this regard, surveillance is defined as the continuous, randomised collection of data to determine the emergence and prevalence of antimicrobial-resistant bacteria and antimicrobial resistance genes.

Under the umbrella of the European Animal Health Study Centre (Centre Européen d'Etudes pour la Santé Animale; CEESA), the veterinary pharmaceutical industry conducts various projects. CEESA is a Brussels-based, international, non-profit association whose members are global research-based animal health companies. CEESA is a project-driven organisation in which its member companies collaborate with the aim of fulfilling scientific or economic studies on a common basis. Each specific project developed by CEESA is co-owned by the sponsoring companies. Not all member companies participate in all CEESA projects. The participating companies create the specifications of a project and monitor its development in full consensus; they provide on an equal basis the financial resources to achieve the project and share the results.

In the area of antimicrobial resistance monitoring, CEESA member companies have set up several microbial culture collections throughout Europe. These unique collections have enabled CEESA to test the susceptibility of these organisms against numerous antibiotics, depending on the project, commonly used in human medicine or specific to the veterinary field. They have also given their sponsors access to a wide range of well defined zoonotic, commensal and veterinary organisms that can be used by the participating companies for scientific purposes. The number of participating companies per antimicrobial resistance project varies from 6 to 12. As CEESA has no laboratories available in Brussels, external laboratory capacity is identified for the experimental

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work. The data are primarily used by the sponsors to address their individual regulatory requirements. However, in order to fill scientific gaps and to contribute to the resistance debate with high-quality data, CEESA makes them publically available through peer-reviewed scientific conference communications or presentations as well as through more extensive publications in established peer-reviewed journals.

Currently, CEESA is organising culture collections for four antimicrobial resistance monitoring programmes:

- European Antimicrobial Susceptibility Surveillance in Animals (EASSA), which examines the antimicrobial susceptibility of zoonotic and commensal bacteria in healthy food animals;
- VetPath, which examines the antimicrobial susceptibility of major disease-causing bacterial pathogens in food-producing animals;
- MycoPath, which examines the antimicrobial susceptibility of major disease-causing mycoplasma species in food-producing animals; and
- ComPath, which examines the antimicrobial susceptibility of bacterial pathogens in companion animals.

The EASSA and VetPath programmes have been active for more than a decade, whilst ComPath began in 2008. MycoPath is the most recent programme and those isolates, collected from 2010 onwards, have not yet undergone antimicrobial susceptibility testing. For each of the CEESA programmes, isolates are collected in up to 11 countries across the European Union (EU) using uniform collection methodology. So far, more than 34 000 non-duplicate strains have been recovered and investigated. The major characteristics of each programme are described below; first the programme of relevance for public health (EASSA), and next the three programmes collecting samples from diseased food or companion animals.

When monitoring antimicrobial resistance across Europe, it is of utmost importance to apply standardised collection procedures and harmonised susceptibility testing. It has frequently been shown that the outcome of susceptibility testing strongly depends on the criteria for strain collection, the susceptibility testing methodology, the compounds used and the interpretive criteria applied [1]. A thorough analysis has recently been conducted by a Clinical and Laboratory Standards Institute (CLSI) working group chaired by Dr S. Simjee [2], offering guidance on how harmonisation can be achieved in veterinary antimicrobial surveillance programmes with the aim of facilitating comparison of data among surveillance programmes. The analysis emphasises the need to agree on common definitions for resistance, i.e. epidemiological cut-off values and clinical breakpoints. The fact that the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has recently changed the ciprofloxacin epidemiological cut-off value for *Escherichia coli* stresses once again the needs for further harmonisation [1].

2. European Antimicrobial Susceptibility Surveillance in Animals (EASSA)

The potential for transmission of antimicrobial-resistant zoonotic enteric bacteria from food-producing animals to humans via contaminated food has been a public health concern for several decades. Bacteria carrying antimicrobial resistance genes found in the intestinal tract of food animals could potentially contaminate carcasses and food products, which may cause food-borne disease in consumers that may not respond well to antimicrobial treatment. Programmes to monitor antimicrobial resistance in zoonotic bacteria are therefore essential to assist in risk management interventions that are guided by risk assessment. The EASSA programme collects bacteria from healthy food animals and employs a protocol with uniform methods of sampling and bacterial isolation, together with a single central laboratory for minimum inhibitory concentration (MIC) determination to a panel of antimicrobials commonly used in human medicine [3]. The organisms of interest are zoonotic *Salmonella* and *Campylobacter* spp. as well as commensal *E. coli* and *Enterococcus* spp. as the representative Gram-negative and Gram-positive indicator organisms. Faecal or caecal isolates are collected from each of the major food-producing animal species (beef cattle, slaughter pigs and broiler chickens). Both epidemiological cut-off values and clinical breakpoints are applied to interpret the MIC results. So far, three EASSA programmes have been completed (EASSA I, EASSA II and EASSA III); EASSA IV has started recently.

Table 1
Countries included in the European Antimicrobial Susceptibility Surveillance in Animals (EASSA) programme.

Cattle	Pigs	Chickens
Belgium[a]	Denmark	France
France[a]	France	Germany[a]
Germany	Germany	Hungary[a]
Ireland[a]	Hungary[a]	The Netherlands
Italy	The Netherlands	Spain
Poland[a]	Spain	Sweden[a]
UK	Sweden[a]	UK

[a] Not included in all three EASSA programmes.

2.1. Sampling procedures

Countries included in the programme are representative of major areas of cattle, pig and chicken production in the EU, from Scandinavia in the north to Spain and Italy in the south. Four to six countries are selected per animal species (Table 1). The slaughterhouses (per country: 4–9 for cattle, 4–14 for pigs and 4–10 for chickens) are selected based on animal throughput and geographical distribution within the countries. The targeted number of samples is 100–200 per country and per host, with few exceptions. A single animal is randomly selected and sampled as being representative of a whole flock or herd. As the prevalence of *Salmonella enterica* appeared to be particularly low, efforts are made to supplement the collection by purchasing isolates that fulfilled the selection criteria from the national collections. The final number of isolates per country and per animal species as well as the total numbers per host are documented in various papers quoted below.

2.2. Microbiological isolation and identification

One randomly selected isolate for each bacterial species is retained from each sample. Isolates obtained at national microbiology laboratories are sent to the central laboratory, which is the repository for the CEESA culture collection. *Escherichia coli*, *Salmonella*, *Campylobacter* and *Enterococcus* are recovered and identified as described previously [3–5]. *Salmonella* isolates are serotyped according to the Kauffmann–White scheme. If applicable, phage typing is conducted. Identification of *Campylobacter jejuni* and *Campylobacter coli* isolates is based on the ability to hydrolyse sodium hippurate and indoxyl acetate and on susceptibility to cefalothin. Isolates showing unusual MIC patterns (e.g. resistance to nalidixic acid yet full susceptibility to ciprofloxacin) are re-examined by real-time PCR for identification of *C. jejuni* or *C. coli*. Recovery and identification of *Enterococcus faecium* and *Enterococcus faecalis* isolates were conducted by standard phenotypic methods as described previously [4,5] and, in a few countries, by PCR. Furthermore, both for *Campylobacter* spp. and

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