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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.

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

Table 2
Antibiotics tested in the European Antimicrobial Susceptibility Surveillance in Animals (EASSA) programme.

Escherichia coli	*Salmonella* spp.	*Campylobacter* spp.	*Enterococcus* spp.
Ampicillin	Ampicillin	Azithromycin[a]	Ampicillin
Cefepime	Cefepime	Ciprofloxacin	Erythromycin[a]
Cefotaxime	Cefotaxime	Erythromycin	Gentamicin
Chloramphenicol	Chloramphenicol	Gentamicin	Linezolid
Ciprofloxacin	Ciprofloxacin	Nalidixic acid	Quinupristin/dalfopristin
Colistin[a]	Colistin[a]	Tetracycline	Tetracycline[a]
Gentamicin	Gentamicin		Tigecycline[a]
Nalidixic acid[a]	Nalidixic acid		Vancomycin
Tetracycline	Streptomycin		
Tigecycline[a]	Sulfisoxazole[a]		
SXT	Tetracycline		
	Tigecycline[a]		
	SXT		

SXT, trimethoprim/sulfamethoxazole.
[a] Antibiotic not included in all three EASSA programmes.

Enterococcus spp., matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) is employed to confirm the identity.

2.3. Antimicrobial susceptibility testing

All MIC testing is performed at the designated central laboratory. *Escherichia coli*, *Salmonella* and *Enterococcus* are tested by standard agar dilution methods according to the recommendations of CLSI document M31-A3 or a previous version [6]. *Campylobacter* are tested according to CLSI document M45-A2 or a previous version [7]. Reference strains are tested concurrently: for tests with *E. coli*, *Salmonella* and *Enterococcus*, the quality control strains are *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *E. faecalis* ATCC 29212; for tests with *Campylobacter*, *C. jejuni* ATCC 33560 is used. Antibiotics selected for MIC testing are generally in agreement with European Food Safety Authority (EFSA) recommendations, including the antibiotics critically important for human medicine (Table 2). For each of the antimicrobial agents, 12–15 concentrations in a two-fold dilution series are tested. *Escherichia coli* and *Salmonella* isolates exhibiting cefotaxime MICs exceeding 0.25 mg/L and 0.5 mg/L, respectively (epidemiological cut-off values defined by EUCAST; http://www.eucast.org), are tested phenotypically for the presence of extended-spectrum β-lactamases according to CLSI recommendations. Genotypic methods are used to characterise the β-lactamases [8].

The results are presented in a tabulated format and comprise of a number of parameters, including single line listing of the MICs, MIC_{50} and MIC_{90} values (lowest MIC at which at least 50% and 90% of the isolates in a test population are inhibited, respectively) as well as percentage resistance. Clinical resistance for Enterobacteriaceae and enterococci is determined for each drug, organism, animal source and country according to CLSI guidelines as defined by the M100 series, currently M100-S21 [9], and for *Campylobacter* as defined by M45-A2 [7], except for gentamicin and nalidixic acid that follow the M100 series [9]. In the absence of CLSI breakpoints (streptomycin, colistin, tigecycline), the breakpoints adopted by the US National Antimicrobial Resistance Monitoring System (NARMS) (streptomycin) or recommended by EUCAST (colistin, tigecycline) for Enterobacteriaceae are applied. In addition, any detected decreased susceptibility is based on epidemiological cut-off values as defined by the EFSA [10,11]. The number of isolates with MIC values exceeding the wild-type MIC distribution (above the epidemiological cut-off values) but not deemed to be clinically resistant (below the clinical breakpoint) is used to calculate the percentage of decreased susceptibility.

So far, the results show that resistance patterns vary widely depending on bacterial species, antibiotic, host and geographic region. Resistance of enteric organisms varied among countries and was frequently high for older antimicrobials, but clinical resistance to newer compounds essential to treat food-borne disease in humans was generally very low. However, the apparent decreased susceptibility of *E. coli* and *Salmonella* against these newer molecules should be carefully monitored [12,13].

3. European target pathogen resistance monitoring programme of food animals (VetPath)

Acquired antimicrobial resistance in target pathogens is an increasing concern. Monitoring is not only of importance for therapeutic decisions, but additionally provides information on trends in resistance over the years. However, the availability of valid and comparable data across Europe with regard to target pathogens is very limited. The VetPath programme collects major pathogens from the three major food-producing animal species (i.e. cattle, swine and poultry) in various European countries. A fundamental requirement is that isolates exclusively originate from clinically sick animals that have not been exposed to any form of antimicrobial treatment during a given time period prior to sample collection. Similarly, samples from chronically diseased animals are excluded from the VetPath collection programme.

Briefly, the veterinary investigator takes an appropriate sample from the site of infection in the sick animal before antibiotic treatment is initiated. To ensure the selection of pre-treatment strains, the investigator only samples animals that have not been treated for:

- 15 days either individually in the case of cattle, or as a herd or flock in the case of swine and poultry, respectively. For clarity, a herd is defined as the smallest group, e.g. a pen, for which the treatment history is known;
- 21 days for mastitis cases (sample should be taken at least from the 5th day of lactation); and
- for digestive disease, the mother (the cow or sow) should not have been treated either.

3.1. Collection and isolation procedures

The VetPath protocol has strict sample collection guidelines to ensure that similar standards of samples are employed across the sampling countries. The criteria are outlined in Table 3. Samples are sent to a local laboratory for processing and the primary disease-causing bacterial strains are isolated and identified to species level using standard isolation and identification techniques (Table 4).

Table 3
Criteria employed to collect samples from untreated diseased animals in the VetPath programme.

Animal species	Disease	Animal characteristics	Type of sampling
Cattle	Respiratory (BRD)	3 weeks to 12 months	Nasopharyngeal or dead animals (preferably <12 h, max. <24 h)
	Clinical mastitis	Lactating cows	Milk sampling
	Diarrhoea	<3 weeks	Rectal/faecal swab or dead animals (<12 h)
Pig	Respiratory/meningitis	3 weeks to 6 months	Preferably dead animals (preferably <12 h, max. <24 h) (or a swab)
	PPDS (MMA)	Lactating sows	Milk sampling or uterine swab
	Diarrhoea	Neonatal and post weaning	Rectal/faecal swab or dead animals (<12 h)
Poultry	Respiratory/septicaemia	During production period	Sacrificed birds
	Necrotic enteritis	During production period	Cloacal/faecal swab or dead animals (<12 h)

BRD, bovine respiratory disease; PPDS, postpartum dysgalactia syndrome; MMA, mastitis–metritis–agalactia syndrome.

Once the isolates are identified to species level, the national laboratory will transfer the strains to a central laboratory. Upon receipt of the isolates at the central laboratory, all isolates are re-streaked to check for purity and morphology. Mixed cultures are discarded, whilst unfamiliar morphology detected on growth plates result in the isolates being re-identified to species level. Isolates not identified to species level as per Table 4 above are not included in further analysis. For selected strains, MALDI-TOF/MS is used to confirm the identity of the strain. In addition, the central laboratory ensures that the corresponding paperwork related to the animal from which the isolate was recovered is complete. This paperwork should include date of sampling and/or isolation, pre-treatment status confirmation, clinical signs of the animal at presentation, anatomical site from where the sample was taken, age of the animal, and region (area code) plus country of origin. All of the isolates are suspended in a glycerol solution and stored at −80 °C until required for susceptibility testing.

3.2. Antimicrobial susceptibility testing

The collection period for each VetPath programme is usually 24–36 months, depending on the rate of recovery in each country. Once the collection is concluded, the central laboratory performs the MIC testing. MICs are conducted as per CLSI recommendations (M31-A3 and previous versions [6]) against a pre-defined group of antibiotics using Trek 96-well panels. MICs are reported as single line listings and are further summarised in tabular format for each bacterial pathogen and disease state and include MIC range, MIC_{50}, MIC_{90} and, where CLSI interpretive criteria are available, percentage resistance. The VetPath data are used for a multitude of purposes ranging from product registration and regulatory defence, to publications, or to constituting a component of antimicrobial risk assessment and evaluation of implemented risk management policies.

To date, two VetPath programmes have been concluded (VetPath I and VetPath II); the third programme, VetPath III (2009–2012) is expected to be completed 2013. The most extensively studied pathologies are respiratory tract infections and bovine mastitis. Overall results so far show high susceptibility to the compounds tested; exceptions are tetracycline and trimethoprim/sulfamethoxazole [14–18].

Taken together, the VetPath programme is the only ongoing European resistance monitoring programme focusing on a broad range of bacterial pathogens causing important diseases in livestock and poultry. Strict criteria employed during sample collection ensure a harmonised collection process, whereas the use of a single laboratory to conduct antibiotic susceptibility testing minimising the chances of errors and results in uniform data interpretation, finally allowing data comparisons between countries.

4. European target pathogen resistance monitoring programme of *Mycoplasma* (MycoPath)

Mycoplasmas are of considerable veterinary importance around the world, causing infections of the respiratory and urogenital tracts, mammary glands, joints or eyes of various livestock and poultry species [19,20]. Among the various infections by mycoplasmal pathogens, respiratory infections are considered the most prevalent and economically important. *Mycoplasma* spp. (bacterial class Mollicutes) are highly fastidious bacteria, difficult to culture and slowly growing, and working with these bacteria requires extensive experience.

Table 4
Primary pathogens isolated and identified in the VetPath programme.

Cattle		Pigs		Poultry	
Pathology	Pathogen	Pathology	Pathogen	Pathology	Pathogen
Respiratory disease (BRD)	*Pasteurella multocida* *Mannheimia haemolytica* *Histophilus somni*	Respiratory disease (SRD)	*Pasteurella multocida* *Actinobacillus pleuropneumoniae* *Bordetella bronchiseptica* *Haemophilus parasuis*	Respiratory disease	*Escherichia coli* *Pasteurella multocida*
		Respiratory/meningitis	*Streptococcus suis*		
Mastitis	*Staphylococcus aureus* CoNS *Streptococcus uberis* *Streptococcus dysgalactiae* *Escherichia coli* *Klebsiella pneumoniae*	PPDS/MMA	*Escherichia coli* *Staphylococcus* spp. *Streptococcus* spp.		
Digestive disease	*E. coli* *Salmonella* spp.	Digestive disease	*E. coli* *Salmonella* spp. *Clostridium difficile* *Clostridium perfringens*	Digestive disease	*Clostridium difficile* *Clostridium perfringens*

BRD, bovine respiratory disease; SRD, swine respiratory disease; CoNS, coagulase-negative staphylococci; PPDS, postpartum dysgalactia syndrome; MMA, mastitis–metritis–agalactia syndrome.

Table 5
Criteria employed to collect samples from untreated diseased animals in the MycoPath programme.

Animal species	Disease	Animal characteristics	Type of sampling
Cattle	Bovine mycoplasmosis	3 weeks to 12 months	Deep nasopharyngeal swab or lung sample (animal that died preferably <12 h, max. <24 h)
Pig	Enzootic pneumonia	3 weeks to 6 months	Deep nasopharyngeal swab or lung sample preferably (animal that died preferably <12 h, max. <24 h)
Poultry	Avian mycoplasmosis	During production period	Tracheal swabs and swabs from lung and air sack are taken from flocks found to be *Mycoplasma* serologically positive (animal that died preferably <12 h, max. <24 h)

A first programme of *Mycoplasma* collection through CEESA from sick cattle, pigs and poultry has been initiated in 2010. Strains are collected from treatment-naïve animals in six European countries and will be used to determine the susceptibility to commonly used antimicrobial drugs in Europe. The collection can be used for regulatory updates of dossiers and for approvals of new compounds, and to offset the over-representation of isolates taken from treatment failures in existing published resistance data.

4.1. Sampling procedure

Isolates are obtained from cattle, pigs, chickens and turkeys in Belgium, France, Germany, Hungary, Spain and the UK where experts are co-ordinating the collection, isolation and identification of *Mycoplasma* strains. The samples originate from animals with specific respiratory pathology and corresponding clinical signs. Sampling methodology has been standardised per host species and is defined for the involved veterinary practitioners in a specific protocol. The main criteria are summarised in Table 5. All samples are collected before antimicrobial treatment is initiated; the source of the samples, the animals' details and any relevant clinical history are recorded and transmitted together with the sample to the national laboratory in charge of culture, isolation and identification of the *Mycoplasma* strains (biochemical or PCR identification). *Mycoplasma* strains are stored at −70 °C until shipment with the sampling forms to a central laboratory. On receipt, viability and purity testing as well as confirmation of the identity of each strain are performed. The main pathogenic *Mycoplasma* species [19] to be collected are *Mycoplasma bovis* (cattle), *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* (pigs), and *Mycoplasma gallisepticum* and *Mycoplasma synoviae* (poultry).

4.2. Susceptibility testing

Antimicrobial susceptibility determinations will be performed in the central laboratory using a broth microdilution technique, according to a slightly adapted version of the method described by Hannan [21]. The test media will be Eaton's medium for *M. bovis* and Friis medium for other *Mycoplasma* spp. Four *Mycoplasma* strains will be included as quality control organisms: *M. bovis* ATCC 25523; *M. hyopneumoniae* ATCC 25939; *M. gallisepticum* ATCC 19610; and *M. synoviae* ATCC 25204. The major commonly used veterinary antimycoplasma drugs will be tested (Table 6).

5. European target pathogen resistance monitoring programme of companion animals (ComPath)

ComPath is the first pan-European collection of isolates from the main pathologies of diseased dogs and cats. The first programme, named ComPath I, has been run between 2008 and 2010 collecting strains from animals not recently exposed to antibiotics (first-intention strains) and used to determine the susceptibility to antibiotics commonly used in companion animal medicine across Europe. The survey included major aerobic pathogens recovered from three types of bacterial infections in companion animals:

- skin, ear and soft-tissue infections such as pyoderma (dogs), wound infections or abscesses (dogs, cats);
- urinary tract infections (dogs, cats), including prostatitis (dogs); and
- respiratory tract infections (upper tract) (dogs, cats).

The second programme, ComPath II, started recently and additionally includes pathogens from periodontal infections in dogs.

5.1. National procedures

Isolates were obtained from sick dogs and cats in a total of 10 countries representing a major part of the companion animals in the EU: Czech Republic; France; Germany; Hungary; Italy; The Netherlands; Poland; Spain; Sweden; and the UK. The main aerobic pathogen species included are presented in Table 7. In this survey, all species collected (whether major or minor pathogens) have been retained; the most prevalent species are recognised as important canine and feline pathogens, whereas for most of the infrequently recovered strains the pathogenesis has not been clearly established [19,22]. This set-up enables the determination of both the prevalence of pathogens as well as the antimicrobial susceptibility of the organisms.

In each participating country, at least four veterinary clinics were collecting samples from first-intention clinical cases. Sampling has been standardised. Samples were transmitted to local laboratories for isolation and biochemical identification of the strains following standardised procedures documented in a protocol. The outcome of bacteriological identification was registered on sampling forms. Moreover, the time of sampling, the source of the samples, the animals' details and any relevant clinical history were recorded by the veterinary practitioner on the abovementioned forms and were transmitted with the samples to the local laboratory. Strict requirements had to be fulfilled: only one

Table 6
Antimicrobials to be tested on *Mycoplasma* spp. isolated from cattle, pigs and poultry.

	Cattle	Pigs	Poultry
Danofloxacin	x		
Enrofloxacin	x	x	x
Marbofloxacin	x	x	
Florfenicol	x	x	
Tiamulin		x	x
Valnemulin		x	
Spiramycin	x	x	x
Oxytetracycline	x	x	x
Gamithromycin	x		
Tulathromycin	x	x	
Tylosin	x	x	

Table 7
Bacterial species isolated from dog and cat pathologies categorised according to prevalence[a]

Infection	Dogs	Cats
Skin and soft-tissue, ear, abscess	*Staphylococcus pseudintermedius* (47%) *Pseudomonas aeruginosa* (14%) *Streptococcus canis* (11%) *Escherichia coli* (9%) *Staphylococcus aureus* (4%) *Streptococcus dysgalactiae* (3%) *Proteus mirabilis* (3%) CoNS (3%) *Enterococcus faecalis* (2%) *Pasteurella* spp. (1%)	*Pasteurella multocida* (23%) *Staphylococcus intermedius* group (22%) *Staphylococcus aureus* (13%) β-Haemolytic streptococci (11%) CoNS (10%) *Pseudomonas* spp. (8%) *Escherichia coli* (6%)
Urinary tract and prostate	*E. coli* (47%) *S. pseudintermedius* (15%) *P. mirabilis* (11%) *S. canis* (8%) *P. aeruginosa* (4%) *Enterococcus* spp. (3%) *S. aureus* (3%) *Klebsiella pneumoniae* (3%)	*E. coli* (60%) *Enterococcus* spp. (12%) Other *Staphylococcus* spp. (11%) *S. intermedius* group (6%) β-Haemolytic streptococci (4%) *Pseudomonas* spp. (3%)
Respiratory tract	*S. pseudintermedius* (23%) *Bordetella bronchiseptica* (22%) *E. coli* (11%) *Pasteurella multocida* (11%) *S. canis* (10%) *P. aeruginosa* (5%) *S. aureus* (3%)	*P. multocida* (44%) *Bordetella bronchiseptica* (9%) β-Haemolytic streptococci (13%) *Pseudomonas aeruginosa* (6%) *S. aureus* (6%) CoNS (6%) *S. intermedius* group (5%) *E. coli* (5%)

CoNS, coagulase-negative staphylococci.
[a] The frequency of recovery (percentage of total isolates) is indicated for each species and indication in parentheses.

sample per animal was collected and no antimicrobial treatment of the patient during the last 4 weeks prior to sampling was allowed. Chronically diseased animals were excluded. Moreover, pets from the same household or pound, cats from the same breeder, and dogs from the same kennel should not have been sampled; only one animal per group was included in the collection. At the local laboratories, bacteria isolated were stored in deep seed agar at 4 °C for up to 3 months or stored at −70 °C until shipment either in dry ice or at ambient temperature together with the sampling forms to the central laboratory.

5.2. Central laboratory

On receipt at the central laboratory, subculturing was conducted to confirm viability and purity and to confirm the identity of each isolate. Identity checks of the isolates were performed where growth characteristics raised doubts on the identification. PCR or MALDI-TOF/MS was used for confirmation, if required. In addition, specific molecular methods could be applied to study the mechanism of resistance. For instance, oxacillin-resistant staphylococci were screened for the presence of the *mecA* gene according to Bignardi et al. [23]. 16S rRNA sequencing has been used for identification of several *Staphylococcus pseudintermedius* isolates.

5.3. Antimicrobial susceptibility testing

The outcome of the survey was a collection of 2425 isolates (1834 from dogs and 591 from cats). Most isolates were recovered from skin and ear infections ($n = 1408$), followed by urinary/prostate infections ($n = 616$) and the lowest number from respiratory tract infections ($n = 401$).

Table 8
Antimicrobials tested (range in mg/L) on bacteria isolated from diseased dogs and cats.

Antimicrobial	Skin and soft-tissue, ear, abscess	Urinary tract and prostate infection	Respiratory tract infection
Penicillin G	0.004–8		0.004–8
Ampicillin	0.06–32		0.06–32
AMC	0.06/0.03–32/16	0.06/0.03–32/16	0.06/0.03–32/16
Cefalexin	0.032–32	0.032–32	0.032–32
Cefovecin	0.032–32	0.032–32	
Oxacillin[a]	0.25–8	0.25–8	0.25–8
Chloramphenicol	2–16		
SXT		0.008–8	0.008–8
Gentamicin	0.12–32		
Dihydrostreptomycin	0.12–64		
Clindamycin	0.03–8		
Enrofloxacin	0.004–8	0.004–8	0.004–8
Ibafloxacin	0.004–8	0.004–8	0.004–8
Marbofloxacin	0.008–8	0.008–8	0.008–8
Orbifloxacin	0.004–8	0.004–8	
Pradofloxacin	0.002–8	0.002–8	0.002–8
Tetracycline			0.5–16

AMC, amoxicillin/clavulanic acid; SXT, trimethoprim/sulfamethoxazole.
[a] Only tested against *Staphylococcus* spp.

All MIC testing was performed at the central laboratory. The antimicrobial susceptibility of isolates was determined by the standard agar dilution method according to recommendation M31-A3 of the CLSI [6]. Reference strains included in each test run were *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus pneumoniae* ATCC 49619 and *E. faecalis* ATCC 29212. A predefined group of commonly used antibiotics in companion animal medicine was used for MIC testing and is listed in Table 8. MICs were reported as single line listings and were further summarised in tabular format for each bacterial pathogen and disease state and include MIC range, MIC_{50} and MIC_{90} and, where CLSI interpretive criteria were available (M31-A3) [6], percentage of isolates susceptible, intermediate and resistant.

6. Conclusions

The CEESA projects show that it is feasible to realise harmonised surveillance programmes throughout Europe. The merits of the ongoing programmes cannot be entirely estimated yet, but it seems appropriate to conclude that the surveys will contribute to fill gaps in our knowledge and assist regulators in making decisions related to the approval of antimicrobial drugs for animals by science-based antimicrobial risk assessments.

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