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Antimicrobial resistance monitoring projects for zoonotic and indicator bacteria of animal origin: Common aspects and differences between EASSA and EFSA

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ABSTRACT

Resistance monitoring programmes are essential to generate data for inclusion in the scientific risk assessment of the potential for transmission of antimicrobial-resistant bacteria or their resistance determinants from food-producing animals to humans. This review compares the technical specifications on monitoring of antimicrobial resistance in zoonotic *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus* as performed by the European Food Safety Authority (EFSA) with veterinary pharmaceutical industry's European Antimicrobial Susceptibility Surveillance in Animals (EASSA) programme. The authors conclude that most of EFSA's recent monitoring recommendations have been covered by EASSA since the start of the latter programme in 1998. The major difference between the two programmes is the classification into 'susceptible' versus 'resistant'. While EFSA categorises all isolates with an MIC value above the epidemiological cut-off value as 'resistant', EASSA differentiates between 'percentage decreased susceptible' and 'percentage clinical resistant' strains by applying both epidemiological cut-off values and clinical breakpoints. Because there is still a need to further improve harmonisation among individual EU Member State activities, Animal Health Industry welcomes EFSA's initiative to further improve the quality of resistance monitoring as it is of utmost importance to apply standardised collection procedures and harmonised susceptibility testing, when monitoring antimicrobial resistance across Europe.

1. Introduction

The potential for transmission of antimicrobial-resistant bacteria or their resistance determinants from food-producing animals to humans has been a public health concern for several decades. Antimicrobial resistance monitoring programmes are therefore essential. In Europe, various initiatives for the monitoring of antimicrobial resistance in food-borne bacteria have been undertaken in the past two decades. In the mid-nineties, Denmark began the first national programme, DANMAP (https://www.danmap.org), and in the subsequent years this was followed by similar surveys in other European countries. Concomitantly, a strong increase of single studies has occurred and various individual programmes have been established by several pharmaceutical companies. In the late nineties, activities of individual companies were replaced or supplemented by a shared initiative of veterinary pharmaceutical industry, i.e., the European Antimicrobial Susceptibility Surveillance in Animals

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(EASSA) programme. Over a decade ago, the European Food Safety Authority (EFSA) established an overarching antimicrobial resistance monitoring programme in food-borne pathogens from food-producing animals and food thereof. With few exceptions, this integrated EU monitoring has now replaced the need to establish individual national programmes. Hence, currently two pan-European monitoring programmes of antimicrobial resistance in enteric bacteria are in place.

Among a number of essential parameters for execution of resistance monitoring surveys, particularly the methodologies applied for antimicrobial susceptibility determination and for analysis of the data can have a major impact on the conclusions drawn. Currently, two different types of criteria are available for analysis of in vitro results: clinical breakpoints and epidemiological cut-off values (ECOFFs). In case a strain collection comprises only clinical target pathogens, clinical breakpoints will generally be applied to estimate the likelihood of therapeutic success. ECOFFs are mainly used for the detection of emerging resistance mechanisms and are determined by a different approach than clinical breakpoints. ECOFFs do not take into account the results of clinical efficacy studies nor the molecule's pharmacokinetic and pharmacodynamic characteristics. Further differences between clinical breakpoints and ECOFFs have been extensively discussed elsewhere (Silley et al., 2006, 2011; CLSI, 2011). Clinical breakpoints and ECOFFs are usually similar or even identical for most bacteria/drug combinations, except for a few classes such as fluoroquinolones. For resistance monitoring in zoonotic and commensal bacteria, both ECOFFs and clinical breakpoints of human-use antibiotics should be applied for the early detection of decreased susceptibility, and to estimate potential clinical treatment failures in humans, respectively.

The objective of this review is to compare the technical specifications on monitoring of antimicrobial resistance in zoonotic *Salmonella, Campylobacter* and indicator *Escherichia coli* and *Enterococcus* as performed by EFSA with the EASSA programme.

2. EFSA

Directive 2003/99/EC requires European Union Member States to monitor and report antimicrobial resistance data of *Salmonella* and *Campylobacter* species on a mandatory basis and on indicator bacteria on a voluntary basis. Since 2004, EFSA has collated and analysed the data received from a number of individual Member States, and so far the results have been issued in five EU Summary Reports (EFSA, 2010; https://www.efsa.europa.eu). More recently, these results have been combined with results on *Salmonella* and *Campylobacter* isolates recovered from humans and published together with the European Centre for Disease Prevention and Control (EFSA/ECDC, 2013; https://www.efsa.europa.eu/efsajournal). At national level, healthy food-producing animals (predominantly cattle, pigs and poultry) and derived meat are sampled either at the farm, slaughterhouse, processing plant or at retail. Subsequent bacterial isolation and antimicrobial susceptibility testing are performed by the individual Member States. EFSA has prepared guidelines for the monitoring of antimicrobial resistance including details on sampling strategy (e.g., target number of isolates per animal population and per slaughterhouse), method of susceptibility testing, and panel of antimicrobials and test ranges to be included (EFSA, 2007, 2008), which has gradually improved the comparability of the data generated among Member States. Each Summary Report has published the number of countries submitting antimicrobial susceptibility data for each bacterium, animal species and source, and which method has been applied (EFSA/ECDC, 2013). Over the years, there has been a positive trend in more and more countries moving towards the reporting of quantitative data generated with antibiotic dilution test methods, and there are only a limited number of countries which perform disc diffusion tests and report qualitative data. The number of countries submitting MIC data for more than 10 isolates for a given animal species or meat category varied from 4 to 16 per bacterial species. Some countries provide EFSA with isolate-based data, i.e., data where the susceptibility results for each antibiotic can be linked back to the individual isolate, but from the majority of the Member States EFSA only receives aggregated data, which does not allow further analysis of multi-resistance and co-resistance. Generally EFSA interprets the results by applying epidemiological cut-off values (ECOFFs) resulting in % wild type and % non-wild type, while for the same Summary Reports ECDC predominantly interprets the results of human isolates using clinical breakpoints. Only in a few instances EFSA applies CLSI or EUCAST clinical breakpoints for Critically Important Antibiotics (CIAs).

The importance of harmonisation in resistance monitoring programmes has been highlighted by Silley et al. (2011) who acknowledged that EFSA achieved several improvements in this regard over the years, but also highlighted various examples of the need for further refinements. To further harmonize the work conducted by the individual Member States, thereby improving the comparability of the data, and to make the data more meaningful at the overall EU level, EFSA recently published two additional scientific reports containing technical specifications on how to improve monitoring and reporting, including additional antibiotics to be tested and their test ranges (EFSA, 2012a,b). These two reports recommend taking into account diverse farming practices, including differences in use of antibiotics, when sampling animals and reporting data, since levels of resistance may be different between different production types (e.g., veal calves vs. dairy vs. beef) or between animals of different ages. EFSA recommends that monitoring of antimicrobial resistance in *E. coli*, *E. faecium* and *E. faecalis* becomes mandatory and that it should be mandatory to report *Salmonella* serovars. During the preparation of this paper, the EU Commission implemented most of these proposals (EC, 2013). To date, not all Member States are reporting annually and Member States are not always testing the complete range of antimicrobials recommended by EFSA in 2007 and 2008. Nevertheless, those panels have now even been extended to include molecules like colistin, tigecycline, and carbapenems (EFSA, 2012b). The EU Commission

has additionally revised the antibiotic panels and their test ranges (EC, 2013). EFSA has reinforced that reporting of quantitative data obtained through standardised dilution methods is preferred and that ECOFF values, set by EUCAST, should be used as interpretive criteria. In addition, EFSA considers it essential that data should be reported at isolate level instead of in an aggregated fashion in order to allow analyses of multi-resistance and co-resistance. A pilot study was initiated to achieve this goal (Aerts and Jaspers, 2012) and for the first time, results on multi-resistance to CIAs were included in the latest Summary Report (EFSA/ECDC, 2013). Enhanced investigation of ESBL/AmpC- and carbapenemase-producing *E. coli* is proposed (EFSA, 2012b) and, overall, it is recognised that further harmonisation of national monitoring protocols would facilitate data interpretation at EU level.

3. EASSA

To protect public health, the CVMP Guideline CVMP/VICH/644/01-FINAL (EMEA, 2004) requires veterinary pharmaceutical companies to include antimicrobial susceptibility data of zoonotic and commensal organisms in their antibiotic registration dossiers. Under the umbrella of the European Animal Health Study Centre (CEESA) in Brussels, research-based animal health pharmaceutical companies jointly conduct several resistance monitoring programmes, one of which is EASSA (de Jong et al., 2013). EASSA examines the antimicrobial susceptibility of zoonotic (*Salmonella* spp. and *Campylobacter* spp.) and commensal (*E. coli* and *Enterococcus* spp.) bacteria in healthy food-producing animals (beef cattle, slaughter pigs and broiler chickens) sampled at abattoir facilities (faecal or caecal samples) throughout Europe. CEESA is organising an EASSA strain collection every 2–3 years; EASSA IV has started recently. Each collection comprises at least two years and the results are disclosed together for each collection period, i.e. results are not presented on a yearly basis. Countries included in the programme are representative of major areas of animal production (in total 10 countries; for overview see de Jong et al., 2013). The programme employs one protocol with uniform methods of sampling and bacterial isolation and identification based on biochemical methods, PCR or MALDI-ToF mass spectrometry. Any requirements regarding sampling procedures (e.g., number of abattoirs; number of samples) and bacterial identification are included in the protocol. One central laboratory is used to determine the minimum inhibitory concentrations (MICs) to a panel of antibiotics commonly used in human medicine. The specific antibiotics defined for each bacterial species and the test ranges have been reported previously (de Jong et al., 2012b). *Salmonella* strains are always serotyped and, if applicable, phage types are determined. Susceptibility testing is performed by agar dilution according to the recommendations of the Clinical and Laboratory Standards Institute (M31/VET01 series), including the use of appropriate quality control strains (CLSI, 2008). EASSA complies with the panels described in EFSA's 2007 and 2008 guidance and additionally investigates cefepime, colistin and tigecycline. Both ECOFFs and clinical breakpoints are applied to interpret the results. This enables categorisation of the data into percentage non-wild type (isolates with MICs above the epidemiological cut-off value), percentage decreased susceptibility (non-wild type isolates with MICs below the clinical breakpoint for resistance) and percentage clinical resistance (isolates with MICs above the resistance breakpoint). Interpretation based on clinical breakpoints is of utmost importance for the clinician. Strains with an MIC value above the ECOFF, but below the clinical resistance breakpoint for antibiotic therapy for humans are likely to be clinically susceptible and to respond to therapy and should therefore not be reported as 'resistant'. These isolates can thus be classified as showing decreased susceptibility rather than clinical resistance. EASSA's results include MIC data for each isolate to allow multi-resistance analysis. Additionally, strains from the EASSA culture collection are used to conduct resistance mechanisms studies where relevant. Several studies so far have been conducted. Most of them refer to the characterisation of ESBL/AmpC-producing *E. coli* and *Salmonella enterica* (Thomas et al., 2012), typing of enterococci (Simjee et al., 2012) or understanding of fluoroquinolone resistance in *Enterobacteriaceae* (Friederichs et al., 2008), frequently being conducted in collaboration with external partners. The generated antimicrobial susceptibility results are primarily used by the member companies for regulatory purposes, and are also publically disclosed through symposia (e.g., de Jong et al., 2012a; Moyaert et al., 2012; Simjee et al., 2013) and peer-reviewed journals (e.g., Bywater et al., 2004; de Jong et al., 2009, 2012b).

4. EFSA vs. EASSA survey

When comparing EFSA's recent recommendations with the EASSA programme, it is worth noting that most of EFSA's recent recommendations have been covered by EASSA since the start of the programme in 1998. Particularly, in the EASSA programme MIC data of individual isolates are recorded, allowing the determination of multi-resistance (Bywater et al., 2004). By contrast, resistance is reported by the Member States to EFSA for groups of bacterial isolates and not for each individual isolate within the groups. Additionally, it needs to be emphasised that, already in 2002, EASSA decided to include colistin in their test panel, an antibiotic with renewed interest for human medicine (Moore and Elborn, 2012), and recently re-classified as critically important by the World Health Organisation (WHO, 2012). These data belong to the very few European monitoring data that have recently been used for the purpose of the European Medicine Agency's review and advice to the European Commission on the use of colistin in veterinary medicine.

The major remaining difference between the two programmes is related to the interpretation of the data. While EASSA applies both ECOFFs and clinical breakpoints, EFSA has reinforced to exclusively use ECOFFs (EFSA, 2012b). By comparison, EASSA reports the data as percentage non-wild type, percentage decreased susceptibility and percentage clinical resistance. EFSA (as well as national surveys) designates all isolates beyond the ECOFF value as "resistant" and although they acknowledged at

one point that this corresponds to what they call "microbiological resistance" or "non-wild type", only the abbreviated term "resistance" is used throughout their reports (EFSA/ECDC, 2013). When interpreting data by using ECOFFs, the term "resistant" is inappropriate; bacteria should be reported as "wild type" if their MIC value falls below the ECOFF, and as "non-wild type" if their MIC value is higher than the ECOFF. Similarly, the use of "microbiological resistance" is confusing as this term includes both decreased susceptible and clinically resistant isolates (Silley et al., 2006; Simjee et al., 2008). Only few exceptions were made in the last Summary Report for some CIAs (EFSA/ECDC, 2013), i.e., a comparison of resistance percentages of animal isolates based on CLSI or EUCAST clinical breakpoints was included. Indeed, infections by food-borne bacteria which are resistant to certain antimicrobials may potentially result in treatment failures in humans and thus susceptibility results would need the interpretation by clinical breakpoints of human-use antibiotics. It needs to be re-emphasised that infections with decreased susceptibility isolates will not necessarily result in clinical treatment failures and these isolates might be considered clinically susceptible (EFSA, 2008). It also should be noted that the exclusive use of ECOFFs for veterinary isolates greatly limits the comparison of the results with those from human studies, where clinical breakpoints are used (Magiorakos et al., 2012; EFSA/ECDC, 2013). It would seem that those Member States which are generating quantitative data could easily interpret their results in terms of ECOFFs and in terms of the clinical breakpoints. To conclude, Animal Health Industry welcomes EFSA's recent recommendations for improvements in surveillance and calls for the increased application of clinical breakpoints, which will enable to differentiate between decreased susceptible and clinically resistant isolates. It is of high importance for both EASSA and EFSA to apply standardised collection procedures and harmonised susceptibility testing, when monitoring antimicrobial resistance across Europe.

Conflict of interest

All authors are employees of veterinary pharmaceutical industry and are actively contributing in the EASSA programme.

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