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Monitoring of antimicrobial susceptibility of udder pathogens recovered from cases of clinical mastitis in dairy cows across Europe: VetPath results

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ABSTRACT

VetPath is an ongoing pan-European antimicrobial susceptibility monitoring programme collecting pathogens from diseased cattle, pigs and poultry not recently treated with antibiotics. Non-duplicate milk samples were collected from cows with acute clinical mastitis in nine countries and 934 isolates were obtained during 2009–2012 for subsequent antimicrobial susceptibility testing in a central laboratory. CLSI broth microdilution methodology was used, and where available, MICs were interpreted using CLSI approved veterinary-specific (ceftiofur) otherwise human clinical breakpoints.

Among *Escherichia coli* (n = 207) and *Klebsiella* spp., (n = 87), resistance was moderate to tetracycline and high to cephapirin (*E. coli* only) whereas resistance to other β-lactam antibiotics was very low (ceftiofur) to low (amoxicillin/clavulanic acid, cephalexin, cephalonium). The MIC_{90} of enrofloxacin and marbofloxacin was 0.03 and 0.06 μg/mL respectively (*E. coli)* with 0.5% strains displaying higher MICs. *Staphylococcus aureus* (n = 192) and coagulase-negative staphylococci (CNS; n = 165) strains were susceptible to most antibiotics tested except to penicillin (25.0 and 29.1% resistance), respectively. Three *S. aureus* and seven CNS strains were oxacillin-resistant and harboured *mecA*. *Streptococcus uberis* strains (n = 188) were susceptible to the β-lactam antibiotics although 35.6% were penicillin intermediately susceptible, and 20.2% were resistant to erythromycin, 36.7% to tetracycline. For *Streptococcus dysgalactiae* (n = 95) the latter figures were 13.7 and 56.8%, respectively.

For most antibiotics, the percentage resistance among *E. coli*, *S. aureus* and *S. uberis* was comparable to that of the VetPath 2002–2006 survey. This current, expanded VetPath study shows that mastitis pathogens were susceptible to most antibiotics with exceptions of staphylococci tested against penicillin and streptococci against erythromycin or tetracycline. This work highlights the high need to set additional clinical breakpoints for antibiotics frequently used to treat mastitis.

1. Introduction

Intramammary infection (mastitis) is the most costly disease affecting dairy cattle worldwide and the most common reason for the use of antimicrobials in dairy cows. The economic impact of mastitis consists of therapy costs, the cost of discarded milk, increased workload, reduced milk production, and culling and replacement costs. Antimicrobials have been used to treat mastitis for about sixty years, and are important parts of therapy of the disease, although not the solution for health management practices leading to poor udder health. In many dairy herds, mastitis control programmes have been implemented to try to reduce these losses including the antimicrobial therapy for cases of acute clinical mastitis.

Acquired antimicrobial resistance in bacteria or resistance genes is a growing concern in human as well as in veterinary medicine. The resistance issue including monitoring of antimicrobial resistance, therefore, is important from both animal health and human health perspectives and is on many national and international agendas. Recently,

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the General Assembly of the United Nations (UN) has addressed antimicrobial resistance comprehensively and multi-sectorally, in order to curb the spread of antimicrobial resistant infections (United Nations, 2016). The World Health Organization (WHO) considers antimicrobial resistance as an urgent global health threat and has identified various strategies to contain and reduce the threat. The WHO Global Action Plan on Antimicrobial Resistance includes improvements of the quality of resistance monitoring and calls for harmonized surveillance systems (World Health Organization, 2015). The European Community Action Plan has described surveillance systems including monitoring of antimicrobial resistance in animal medicine as one of the key actions (European Commission (EC) (2011)). Similarly, the European Medicine Agency (EMA) has defined various actions for 2016–2020 to ensure the responsible use of antibiotics in animals (European Medicine Agency (2016)).

As any exposure of bacteria to antibiotics for therapy of mastitis may lead to selection of resistance, the antimicrobial susceptibility of udder pathogens should be regularly monitored consistent with stewardship programmes for antimicrobials. Monitoring antibiotic resistance trends over time is important to ensure long-term efficacy of the antibacterial products. Access to recent repository of antimicrobial susceptibility data help guide the veterinarian in selecting the most appropriate antibiotic for treatment of mastitis, particularly given that mastitis therapy is commonly initiated before susceptibility testing of the pathogen. European susceptibility monitoring data of mastitis pathogens, however, is very limited. Although there are a number of national, annual veterinary surveillance programmes for pathogens in place in Europe (e.g., G*ERM*-Vet in Germany, the Swedish Veterinary Antimicrobial resistance Monitoring (SVARM), Résapath in France), these lack harmonisation in relation to sampling schedules, methodology and interpretive criteria. Only a few recent ad hoc studies are available (e.g., Bengtsson et al., 2009; Minst et al., 2012) and their methodologies including test methods and breakpoints also are not harmonized. To help address this problem, monitoring programmes are currently commissioned by the Executive Animal Health Study Centre (CEESA) investigating pathogens from both farm and companion animals (de Jong et al., 2013). CEESA's VetPath programme is dedicated to bacterial pathogens from several types of infections, including dairy mastitis, as well as other types of infections of diseased farm animals (cattle, pigs, poultry) not recently treated with antimicrobials across Europe. The VetPath programme is based on a protocol with harmonized methods of sampling, mastitis case/isolate enrollment and bacterial isolation. As the use of multiple laboratories to conduct susceptibility testing can potentially introduce bias into a surveillance study (Kahlmeter and Brown, 2002; Schwarz et al., 2010), a single central laboratory conducts the determination of minimum inhibitory concentrations (MICs) using a panel of antimicrobials commonly used in veterinary medicine.

The results of the first monitoring period (2002–2006) of three major mastitis pathogens (*Escherichia coli, Staphylococcus aureus, Streptococcus uberis*) were published recently (Thomas et al., 2015). Here we present the findings for isolates of the second monitoring period (2009–2012) recovered pre-treatment from cows with acute mastitis across nine European Union (EU) countries and additionally include data for coagulase-negative staphylococci (CNS), *Streptococcus dysgalactiae* and *Klebsiella* spp. To determine whether resistance has changed over time, the results of the percentage resistance observed were compared to those of the preceding VetPath study.

2. Materials and methods

2.1. Animal criteria and sampling procedures

The design of the survey including the animal populations, clinical history and the sampling procedures were described previously (Thomas et al., 2015). In short, in each of the nine EU countries included in the project (Belgium, Czech Republic, Denmark, France, Germany, Italy, the Netherlands, Spain, the United Kingdom), milk samples were taken from cows with acute clinical signs of mastitis. In each country surveyed, a single national coordinator assumed responsibility for the collection of the samples and their processing according to uniform protocols for pathogen isolation. Records on standard case report forms of all samples indicated that 79.1% of the sampled animals had not been exposed to antibacterial treatment for at least 3 weeks prior to sampling. The remaining 20.9% of the samples were from animals with the treatment status characterized as "unknown". In all cases, only one sample per year was included from each herd sampled, to increase the likelihood of testing epidemiologically unrelated strains. The isolates were identified to genus and species level by standard microbiological and biochemical tests before shipment to the central laboratory (LGC, Fordham, UK). If growth characteristics raised doubts on the identification or unusual susceptibility profiles were observed for the species, Matrix Assisted Laser Desorption Ionization – Time of Flight mass spectrometry (MALDI-ToF MS) on Microflex LT (Bruker Daltonics, Bremen, Germany) was applied to confirm the identity.

2.2. Antimicrobial susceptibility testing

At the central laboratory, MICs for all isolates were determined against 16 antibiotics/antibiotic combinations by broth microdilution in serial two-fold dilutions contained in commercially prepared 96-well microtitre plates (Sensititre; Trek diagnostic Systems Inc., East Grinstead, UK), in accordance with performance standards of the Clinical and Laboratory Standard Institute (Clinical and Laboratory Standards Institute (CLSI), 2013 and preceding version). Quality control strains (*Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *S. aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 49619) were included on each day of testing, and, where applicable, MIC data was only accepted if MICs of the control strains were within the required reference ranges (Clinical and Laboratory Standards Institute (CLSI), 2015). The following antibiotics (test ranges expressed as μg/ml in parentheses), representing five antimicrobial classes, were tested: amoxicillin/clavulanic acid (0.03/0.015–64/32), cefquinome (0.008–16), ceftiofur (0.008–8), cephalexin (0.03–32), cephapirin (0.03–32), cephalonium (0.03–32), cloxacillin (0.03–8), penicillin G (0.004-8), enrofloxacin (0.004–8), marbofloxacin (0.004–8), erythromycin (0.03–64), tylosin (0.03–32), kanamycin (0.12–128), cephalexin/kanamycin (0.12–64), neomycin (0.12–64) and tetracycline (0.06–32). The MICs of penicillin G, cloxacillin, erythromycin and tylosin against Gram-negative bacteria are not reported, due to intrinsic resistance.

2.3. MecA screening

To detect methicillin resistance in staphylococci, oxacillin MICs (range 0.015–16 μg/mL) were additionally assessed only for these species. Subsequently oxacillin-resistant *S. aureus* and CNS strains were examined for the presence of *mecA* gene by PCR according to Bignardi et al. (1996). The oxacillin-resistant, *mecA* positive *S. aureus* strain ATCC 43300 was used for quality control.

2.4. Data analyses

MIC results are expressed as frequency distributions because internationally-endorsed breakpoints are not available for most veterinary antibiotics. The MIC_{50} and MIC_{90} values were determined for each organism-drug combination tested (*E. coli* and *Klebsiella* spp.: 12 antibiotics/antibiotic combinations; *S. aureus,* CNS, *S. uberis* and *S. dysgalactiae*: 16 (or 17: staphylococci) antibiotics/antibiotic combinations). If internationally recognized breakpoints were available, results were also categorized and reported as susceptible, intermediate susceptible and resistant. Such categorizations are currently available only for ceftiofur