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## Antimicrobial resistance in bacteria isolated from mastitis in dairy cattle in France, 2006–2016

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### ABSTRACT

In dairy cattle, mastitis is the most frequent bacterial disease, and the routine use of antibiotics for treatment and prevention can drive antimicrobial resistance (AMR). The aim of our study was to estimate the levels of AMR of the 3 main bacteria isolated from dairy cattle with mastitis in France (*Streptococcus uberis*, *Escherichia coli*, and coagulase-positive staphylococci) and to investigate their changes over time. Data collected between 2006 and 2016 by the French surveillance network for AMR in pathogenic bacteria of animal origin (called RESAPATH) were analyzed. The proportions of mono- and multidrug resistance were calculated and the trends were investigated using nonlinear analyses applied to time series. Over the whole period, the lowest proportions of resistance in *S. uberis* isolates were observed for oxacillin (2.2%) and gentamicin (2.4%) and most resistance levels were below 20%. The trends in resistance showed some significant variation, mainly for *S. uberis*, but without a common pattern across the various antibiotics examined. For only 2 combinations of bacteria-antibiotic the trend in resistance showed a continuous increase from 2006 to 2016: tetracycline resistance in *S. uberis* isolates and third-generation cephalosporin resistance in *E. coli* isolates. In *E. coli*, the highest proportions of resistance were observed for amoxicillin (28.1%) and tetracycline (23.1%). Resistance to third-generation cephalosporins in *E. coli* from dairy cattle was almost nil in 2006, but reached 2.4% in December 2016. This increase is particularly concerning because these antibiotics constitute one of the latest therapeutic alternatives to fight severe infectious

diseases in humans. Except for penicillin (33.9%), the proportions of resistance in coagulase-positive staphylococci were below 11% during the whole study period. Multidrug resistance (isolates with acquired resistance to at least one antibiotic in 3 or more antibiotic classes) ranged from 2.4% for coagulase-positive staphylococci to 9.9% for *S. uberis*. These findings can serve as guidelines for practitioners in the choice of the most appropriate antibiotic according to the prevailing epidemiological context. Ultimately, our results contribute to risk assessment of AMR and provide a baseline for setting up and evaluating control measures and designing strategies to limit AMR.

**Key words:** antimicrobial resistance, mastitis, time series, dairy cattle, RESAPATH

### INTRODUCTION

Mastitis, the inflammation of the mammary gland, is the most frequent bacterial infection encountered in dairy cattle and is associated with reduced milk production and changes in milk composition. Dairy cattle mastitis is mainly caused by bacteria, and antibiotics are an important component of their treatment and control. In France (Guérin-Faubleé et al., 2003; Botrel et al., 2010), as well as in other European, American, and African countries, *Streptococcus uberis*, *Escherichia coli*, and coagulase-positive staphylococci (CPS) are the most frequent causative pathogens of clinical mastitis (Tenhagen et al., 2006; Bradley et al., 2007; Ericsson Unnerstad et al., 2009; Kateete et al., 2013). For subclinical mastitis, *E. coli* appear to be less prevalent than CPS, CNS, and streptococci (Botrel et al., 2010; Persson et al., 2011). These 4 bacteria are of real concern for public health: they can be a reservoir for antimicrobial resistance (AMR) genes and can play a role in the spread of genes to other pathogenic and

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commensal bacteria in the farm environment (Aarestrup, 2015).

Because the use of antibiotics can lead to resistance (Aarestrup, 2015), AMR occurrence must be carefully monitored to guide prudent prescription (Thomas et al., 2015). Hundreds of publications describe the proportion of AMR in bovine mastitis pathogens in the last decades (Barlow, 2011), highlighting differences in AMR proportions between areas and populations (Erskine et al., 2002; Makovec and Ruegg, 2003; Pitkälä et al., 2004; Osterås et al., 2006; Tenhagen et al., 2006; Botrel et al., 2010; Idriss et al., 2014). But most studies are based on specific selected animal populations and represent point-in-time estimates of AMR in specific geographic regions: they do not provide an overall picture of the situation. In addition, AMR in cow mastitis has never been studied in Europe over a long (at least 4 yr) and continuous period of time to detect emergence and characterize AMR trends. However, the estimation of resistance levels and trends in udder pathogens is crucial to guide the appropriate prescription of antibiotics and thus preserve the therapeutic arsenal ensuring the long-term efficacy of antibiotics.

The aim of our study was to estimate the AMR levels in the 3 most frequent causative agents of mastitis in dairy cattle among those collected by the French national surveillance network for AMR, and to describe their variations from 2006 and 2016.

## MATERIALS AND METHODS

### Source of Data

This retrospective study was performed using data from the French established national surveillance network for AMR in pathogenic bacteria of animal origin, called **RESAPATH**. This network was created in 1982 and monitors AMR in all animal species. It is coordinated by the French Agency for Food, Environmental and Occupational Health and Safety (**ANSES**). **RESAPATH** collects antibiogram results from participating French veterinary laboratories (74 members in 2016 out of 112 in the country). These antibiograms are requested by veterinarians confronted with a clinical case of the pathology for diagnostic and treatment purposes.

The **RESAPATH** laboratories use standard bacteriological cultures and methods for bacterial isolation and identification. Even if each laboratory has its own strategy of bacterial identification, the biggest ones use mass spectrometers (Singhal et al., 2015). Antibiograms are performed using the disk diffusion method, according to recommendations of the Antibiogram Committee of the French Society of Microbiology (**CA-SFM**).

Inhibition zone diameters are communicated to **ANSES** and compiled in a database. In accordance with the breakpoints recommended by the veterinary section of the **CA-SFM** (2017), isolates are then categorized as susceptible, susceptible, or resistant. The **RESAPATH** laboratories have participated in annual quality assurance proficiency tests since 2006, which contributes to the quality control of the data.

From the **RESAPATH** database, we extracted data involving the 3 most frequent bacteria isolated from dairy cattle mastitis from 2006 to 2016, namely *S. uberis*, *E. coli*, and **CPS**. For **CPS** isolates, the information sent by several laboratories did not allow us to strictly distinguish between species so we chose to perform analysis on the category **CPS**. Variables extracted included bacterial species, sampling date, and susceptibility categories (susceptible, susceptible, or resistant) to each tested antibiotic. For the 3 bacteria considered, we selected antibiotics of relevance in veterinary and human medicine (Table 1).

### Data Analysis

From an epidemiological point of view, the event of interest is the nonsusceptibility of the pathogen to the antibiotic tested, indicating that the isolated bacteria is no longer a wild-type strain. Therefore, intermediate isolates were grouped together with resistant isolates in the nonsusceptible bacterial population, referred to as resistant in this study. The indicator of AMR was defined for each bacteria and antibiotic as the proportion of the number of resistant isolates relative to the total number of isolates tested.

In a time series analysis, trends reflect structural dynamics and the detection of trends was used to monitor long-term movement, particularly in AMR (López-Lozano et al., 2000; Mühlemann et al., 2006; Doernberg et al., 2015; Boireau et al., 2018b). A trend is defined as the long-term variation along with seasonality and noise (i.e., the residual components). To compare our results with previous studies, we used generalized additive models (**GAM**; Dominici et al., 2002) to capture trends and seasonality. The time step was chosen according to the number of data available such that there were at least 25 antibiograms in each time step (Barlow, 2011) and to ensure the validity of the model (independent and normally distributed residuals); we used a bimonthly (every 2 mo) time step. Using count data, the bimonthly number of resistant isolates was modeled using a quasi-Poisson regression with an offset equal to the log of the total number of isolates submitted during the same period. The AMR trends were modeled for each bacterial species and ana-

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