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Antimicrobial consumption on dairy herds and its association with antimicrobial inhibition zone diameters of non-*aureus* staphylococci and *Staphylococcus aureus* isolated from subclinical mastitis

M. Stevens,*[1] S. Piepers,* K. Supré,† and S. De Vliegher*
*M-team and Mastitis and Milk Quality Research Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium
†Milk Control Centre Flanders, 2500 Lier, Belgium

ABSTRACT

The main objectives of this study were to quantify the consumption of antimicrobials on a convenience sample of dairy herds and to determine the association between herd-level antimicrobial consumption and inhibition zone diameters (IZD) of non-*aureus* staphylococci and *Staphylococcus aureus* isolates from subclinical mastitis cases. Also, the association between the IZD of non-*aureus* staphylococci and *Staph. aureus* isolates within a herd was studied. Antimicrobial consumption data on 56 Flemish dairy farms were obtained between 2013 and 2014 by so-called garbage can audits and expressed as antimicrobial treatment incidence (ATI), with the unit of ATI being the number of defined daily doses animal (DDDA) used per 1,000 cow-days. The average total ATI in adult dairy cattle for all active substances was 18.73 DDDA per 1,000 cow-days and ranged from 6.28 to 42.13 DDDA between herds. The ATI of critically important (for human health) antimicrobials was 6.91 DDDA per 1,000 cow-days; that is, 37% of total antimicrobial consumption. The average ATI for intramammary therapy of (sub)clinical mastitis, intramammary dry-cow therapy, and systemically administered therapy was 5.20, 6.70, and 6.73 DDDA, respectively. The IZD of 239 non-*aureus* staphylococci and 88 *Staph. aureus* isolates originating from milk samples from cows with subclinical mastitis collected on selected dairy herds were determined using Kirby-Bauer disk diffusion and ranged between 6 and 42 mm. Because only a limited number of clinical breakpoints (Clinical and Laboratory Standards Institute) and epidemiological cut-off values (European Committee on Antimicrobial Susceptibility Testing) are available for mastitis-causing bacteria in bovine, IZD were used as a proxy for antimicrobial resistance. Inhibition zone diameters of non-*aureus* staphylococci for cefquinome, a critically important β-lactam antibiotic, were negatively associated with the ATI of critically important β-lactam for systemically administered therapy and positively with the ATI for intramammary therapy of (sub)clinical mastitis of critically important β-lactam antimicrobials. Only for neomycin was a positive association between the IZD of non-*aureus* staphylococci and *Staph. aureus* isolates within the same herd observed.
Key words: antimicrobial consumption, staphylococci, subclinical mastitis, inhibition zone diameters

INTRODUCTION

Acquired antimicrobial resistance is a growing global concern in both human and veterinary medicine because it can result in treatment failure. The 2 main factors involved in the development of antimicrobial resistance in bacteria are the presence of resistance genes and selection pressure caused by the use of antimicrobials (Levy, 1997; Acar and Moulin, 2006; Silbergeld et al., 2008; Chantziaras et al., 2014). In dairy cattle, most antimicrobials are being used for the prevention and control of mastitis (Mitchell et al., 1998; Pol and Ruegg, 2007b; Menéndez González et al., 2010; Stevens et al., 2016). Potential associations between antimicrobial consumption on dairy herds and antimicrobial susceptibility of bovine mastitis pathogens have been studied, but not in Europe and not in much detail (Rajala-Schultz et al., 2004; Pol and Ruegg, 2007a; Saini et al., 2012b).

Over the last 10 yr, non-*aureus* staphylococci, also referred to as CNS, have become the most common cause of subclinical mastitis worldwide (Chaffer et al., 1999; Rajala-Schultz et al., 2004; Piepers et al., 2007; Vanderhaeghen et al., 2014; Sztachańska et al., 2016), particularly in heifers (De Vliegher et al., 2012). They form a heterogeneous group of more than 50 species, of which approximately 10 have been associated with IMI in dairy cattle (Vanderhaeghen et al., 2014) and are abundantly present in bulk milk (De Visscher et al., 2017). Non-*aureus* staphylococci are also present in

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[1]Corresponding author: Marina.Stevens@Ugent.be

other bovine-associated habitats such as air, housing, bedding material, milking equipment, and body parts such as teat apices, hair coat, nares, and the vagina (White et al., 1989; Nagase et al., 2002; Paduch and Kroemker, 2011; Piepers et al., 2011; Piessens et al., 2012; Braem et al., 2013; De Visscher et al., 2014, 2016). Their omnipresence, especially on the body of the cows, implies that non-*aureus* staphylococci are generally more exposed to antimicrobial treatments than other mastitis pathogens, which could explain the higher levels of antimicrobial resistance of non-*aureus* staphylococci compared with the more pathogenic *Staphylococcus aureus* (Owens and Watts, 1988; Taponen and Pyorala, 2009; Schmidt et al., 2015). It has been suggested that non-*aureus* staphylococci might act as a potential reservoir for resistance genes that can be transferred and integrated into the genome of *Staph. aureus* (Holmes and Zadoks, 2011; Otto, 2013; Vitali et al., 2014).

One of the most widely used methods for measuring antimicrobial susceptibility of bacteria is the Kirby-Bauer disk diffusion test, in which radial zones of bacterial growth inhibition are cross-referenced to breakpoints to determine whether an organism is susceptible or resistant to therapy with a particular antimicrobial drug (Traub and Leonhard, 1994). Still, the required clinical breakpoints to correctly interpret the results are often unavailable for a specific combination of pathogen and antimicrobial per host species (CLSI, 2008, 2015; http://www.eucast.org/ast_of_veterinary_pathogens/). This is the case particularly for non-*aureus* staphylococci isolated from bovine milk, partly because they are generally considered minor pathogens and typically not treated with antimicrobials. Using inhibition zone diameters (**IZD**) for non-*aureus* staphylococci and for *Staph. aureus* to determine the presence of (acquired) genetic resistance elements as a proxy for potential antimicrobial resistance, rather than relying on (clinical) breakpoints, avoids these issues.

The main objectives of this study were (1) to quantify the use of antimicrobials on a convenience sample of dairy herds, (2) to determine the association between the herd-level antimicrobial consumption and the IZD of non-*aureus* staphylococci and *Staph. aureus* isolates, respectively, and (3) to determine the association between the IZD of the non-*aureus* staphylococci and *Staph. aureus* isolates originating from the same herds.

MATERIALS AND METHODS

Herds and Cows

Data collected for this study originated from a convenience sample of 56 Flemish dairy herds. Herd inclusion criteria were (1) participation in the DHI program in Flanders with an interval of 4 to 6 wk between 2 test-days, and (2) record keeping of all treatments in adult cattle (date of occurrence, identification number of animal, treatment protocol applied). More details about the included herds are described elsewhere (Stevens et al., 2016).

From March to April 2014, approximately 10 lactating cows per herd with subclinical mastitis (defined as a test-day SCC >150,000 cells/mL and >250,000 cells/mL for heifers and multiparous cows, respectively) were randomly selected for milk sampling for bacteriological culturing.

Antimicrobial Consumption Data

Antimicrobial consumption data were retrieved by so-called garbage can audits, as described in a previous paper reporting on the antimicrobial consumption of the herds between January 2012 and February 2013 (Stevens et al., 2016). For the current paper, empty antimicrobial receptacles collected from March 2013 to February 2014 were used. Product name, volume, and number of receptacles were registered using a datasheet that included herd identification number and start and end date of collection for that particular herd. The quantification of the herd-level antimicrobial consumption was defined by the antimicrobial treatment incidence (**ATI**) as described previously (Stevens et al., 2016) and expressed as the number of defined daily doses animal (**DDDA**) used per 1,000 cows per day.

Classification of Antimicrobials

Route of Administration and Indication. Antimicrobials registered for intramammary use were divided into intramammary injectors registered for dry-cow therapy and injectors registered for the intramammary treatment of (sub)clinical mastitis cases. All systemically injectable antimicrobials used for the therapy of various diseases (including mastitis) are hereafter referred to as "systemically administered therapy." Intrauterine use of antimicrobials was very limited and therefore not taken into account in further analyses, as was done previously (Stevens et al., 2016).

Strata of Antimicrobials. Four strata were generated based on classification as β-lactam and non-β-lactam and their importance in human health: critically important (i.e., third- and fourth-generation cephalosporins and fluoroquinolones) or not critically important, according to the Office International des Épizooties (OIE) classification: stratum 1: critically important β-lactam antimicrobials (i.e., third- and fourth-generation cephalosporins); stratum 2: critically