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The intramammary efficacy of first generation cephalosporins against *Staphylococcus aureus* mastitis in mice

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ABSTRACT

Staphylococcus aureus-induced mastitis in cattle causes important financial losses in the dairy industry due to lower yield and bad milk quality. Although *S. aureus* is susceptible to many antimicrobials *in vitro*, treatment often fails to cure the infected udder. Hence, comprehensive evaluation of antimicrobials against *S. aureus* mastitis is desirable to direct treatment strategies. The mouse mastitis model is an elegant tool to evaluate antimicrobials *in vivo* while circumventing the high costs associated with bovine experiments. An evaluation of the antimicrobial efficacy of the intramammary (imam) applied first generation cephalosporins cefalexin, cefalonium, cefapirin and cefazolin, was performed using the *S. aureus* mouse mastitis model. *In vivo* determination of the effective dose $2\log_{10}$ ($ED_{2\log10}$), $ED_{4\log10}$, protective dose 50 (PD_{50}) and PD_{100} in mouse mastitis studies, support that *in vitro* MIC data of the cephalosporins did not fully concur with the *in vivo* clinical outcome. Cefazolin was shown to be the most efficacious first generation cephalosporin to treat *S. aureus* mastitis whereas the MIC data indicate that cefalonium and cefapirin were more active *in vitro*. Changing the excipient for imam application from mineral oil to miglyol 812 further improved the antimicrobial efficacy of cefazolin, confirming that the excipient can influence the *in vivo* efficacy. Additionally, statistical analysis of the variation of *S. aureus*-infected, excipient-treated mice from fourteen studies emphasizes the strength of the mouse mastitis model as a fast, cost-effective and highly reproducible screening tool to assess the efficacy of antimicrobial compounds against intramammary *S. aureus* infection.

1. Introduction

Mastitis provokes detrimental damage to epithelial cells. Hence, it jeopardizes milk production and entails prohibitive costs in the dairy industry (Piepers et al., 2007). *Staphylococcus aureus* is historically one of the most important causes of subclinical mastitis and clinical mastitis that becomes chronic (Piepers et al., 2007; Tenhagen et al., 2009). Despite intensive research and multiple preventive measures (NMC, 2006), the dairy industry has not managed to eradicate *S. aureus* mastitis (Zadoks and Fitzpatrick, 2009). So, antimicrobial therapy remains an essential component of staphylococcal mastitis control programs. The major concern with *S. aureus* infections is that once established they are extremely difficult to eradicate from the mammary gland. Although *S. aureus* is susceptible to numerous antibiotics *in vitro* (Erskine et al., 2002; EUCAST, 2011), this pathogen has

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Table 1
Distribution of the minimal inhibitory concentration 50 (MIC_{50}), MIC_{90}, MIC range and number of samples for cefalexin, cefalonium, cefapirin and cefazolin against *Staphylococcus aureus* species isolated from bovine mastitis cases worldwide. MIC_{50} and MIC_{90} are the epidemiological MICs that indicate that 50% and 90%, respectively, of the *S. aureus* strains have a MIC below this value.

Cephalosporin	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	Number of samples	References
Cefalexin	4.0	4.0	1.0–8.0	119	Guerin-Faublee et al. (2003)
	4.0	128	0.50–256	102	EUCAST (2011)
Cefalonium	0.08	N.R.	0.04–0.08	330	Groothuis and Frik (1982)
Cefapirin	0.50	0.50	≤0.06–1.0	135	Watts et al. (1995)
	0.25	0.50	≤0.06–64	79	Salmon et al. (1998)
	0.125	0.25	≤0.06–0.25	20	Owens et al. (1997)
	0.12	0.25	0.03–0.50	119	Guerin-Faublee et al. (2003)
	≤1	≤1	≤1–4	331	Sato et al. (2004)
Cefazolin	0.39	0.39	0.20–1.56	51	Yoshimura et al. (2002)
	0.25	0.50	0.063–32	105	Brix (2007)
	0.50	0.50	0.12–1.0	119	Guerin-Faublee et al. (2003)
	0.25	1.0	0.125–2.0	30	Nunes et al. (2007)
	0.50	4.0	0.064–32	19,051	EUCAST (2011)

N.R. = not reported.

developed several features to influence the host immune system and to escape from antimicrobial treatment (Barkema et al., 2006).

Over 50% of the single component preparations on the dairy market in Belgium are cephalosporins (BCFI, 2011). Together with penicillins they belong to the group of β-lactam antibiotics, but are less susceptible to inactivation by *S. aureus* β-lactamases. The first generation cephalosporins show good activity against bovine *S. aureus* species *in vitro* (Table 1). Notably, these species show very low resistance rates against first generation cephalosporins (Erskine et al., 2002; Kaspar, 2006; Maran, 2010), an encouraging advantage with regard to public safety and antimicrobial efficacy. The aim of the current study was to assess *in vivo* side-by-side the imam efficacy of the 4 first generation cephalosporins cefalexin, cefalonium, cefapirin and cefazolin in the *S. aureus* mouse mastitis model. In addition, the efficacy of cefazolin was evaluated using two different excipients. Finally, the reproducibility of the mouse mastitis model was statistically analyzed to evaluate its robustness.

2. Materials and methods

2.1. Antibiotics and excipients

Cefalexin (ABCR GmbH & Co. KG, Germany), cefalonium (Sigma–Aldrich, Germany), cefapirin (Fluka Analytical, Switzerland), cefazolin (ABCR GmbH & Co. KG, Germany) and excipients (mineral oil and miglyol 812) were provided as ready to use suspensions by Bayer Animal Health GmbH.

2.2. S. aureus inoculum preparation

S. aureus Newbould 305 (ATCC 29740) was used for infection. This bacterial strain was isolated from a clinical mastitis case in 1958 (Prasad and Newbould, 1968) and is since widely used for experimental intramammary infection of cows (Gudding et al., 1984; Schukken et al., 1999) and mice (Brouillette et al., 2004). Overnight brain heart infusion (BHI; Oxoid Limited, Belgium) cultures of *S. aureus* were diluted in sterile phosphate-buffered saline (PBS; Gibco, Belgium) and quantified by flow cytometry (BD Biosciences, Belgium). Briefly, 1 ml of a 1000-fold PBS-diluted bacterial suspension was added to a TRU count tube (BD Biosciences), which contained a known number of fluorescent beads. The number of bacteria was then calculated using the following equation:

$$\text{Bacteria per ml} = \frac{\text{bacterial counts} \times \text{total beads in tube} \times \text{dilution}}{\text{bead counts}}$$

The actual colony forming units (CFU) of the inoculum was confirmed by overnight culture of a serial logarithmic dilution on tryptic soy agar (TSA; Oxoid Limited) plates.

2.3. Minimal inhibitory concentration (MIC) determination

Determination of the MIC of the 4 first generation cephalosporins for the bovine mastitis isolate *S. aureus* Newbould 305 was performed using the Mueller–Hinton agar dilution assay according to CLSI guidelines (CLSI, 2008). Plates were incubated at 35 °C (±2 °C) for 16–20 h in an aerobic atmosphere.

2.4. Staphylococcal mouse mastitis model

The general procedure for mouse mammary gland infection was adapted from the method used by Brouillette et al. (2004). Briefly, CD-1 lactating mice (Harlan Laboratories Inc., Netherlands) were utilized 12–14 days after birth of the offspring. The mice typically weigh 50 g at that time. The pups were weaned 1–2 h before bacterial inoculation of the mammary glands. A mixture of oxygen and isoflurane (2–3%) was used for inhalational anesthesia of the lactating mice. A syringe with 32-gauge blunt needle (Thiebaud Biomedical Devices, France) was applied to inoculate both L4 (on the left) and R4 (on the right) glands