Comparison of Two Phenotypic Methods for Penicillinase Detection in *Staphylococcus aureus* Isolated from Bovine Mastitis.

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INTRODUCTION

Production of β-lactamase is considered the most frequent mechanism of penicillin resistance among *Staphylococcus aureus* isolated from bovine intramammary infection (IMI) (Haveri et al., 2005). Mastitis diagnostic laboratories usually perform agar dilution or agar diffusion tests to determine S. aureus susceptibility to penicillin. However, recent studies showed that β-lactamase producing S. aureus isolated from bovine IMI had penicillin minimum inhibitory concentrations (MIC) near or below the breakpoint suggested by CLSI (2002); suggesting that this breakpoint could be too high to detect penicillin resistance, mainly in strains close to the detection limit (Haveri et al., 2005; Russi et al., 2008). In such cases, additional testing should be required to correctly identify β-lactamase producing isolates. Detection of the blaZ gene by PCR is considered the reference method, since it correlates well with β lactamase production (Haveri et al., 2005). However, performing this test is usually beyond the capabilities of laboratories involved in routine mastitis diagnosis. The aim of this study was to compare phenotypic methods for penicillinase detection in S. aureus isolated from bovine IMI with MIC values higher and lower than the breakpoint suggested by CLSI.

MATERIALS AND METHODS

Fifty three S. aureus isolates obtained from individual or composite milk samples from clinical and subclinical cases belonging to 32 dairy herds located in the provinces of Buenos Aires, Santa Fe, Córdoba and Entre Ríos were evaluated, including a maximum of four isolates per herd. Isolates were identified to species level according to standard methodology and stored as frozen stocks at -70ºC in tryptic soy broth added with 10% glycerol for 1 month to 3 years before the study was carried out. Agar dilution method was performed according to CLSI (2002) recommendations using S. aureus ATCC 29213 as control. Breakpoint to consider isolates as resistant was ≥0,25 µg/mL (CLSI). Penicillinase was detected by the clover leaf test; performed as described by Bergman et al (1997) using S. aureus Oxford strain (ATCC 9144) as an indicator on Mueller-Hinton agar (Merck& Co., Inc. Whitehouse Station, NJ, USA) and by a chromogenic cephalosporin disk method (DrySlide Nitrocefin, Difco Laboratories, Detroit, USA). This was performed according to the manufacturers directions, following induction of β-lactamase production by streaking each isolate onto one-half Columbia agar base supplemented with 5% defibrinated ovine blood and placing a 1µg oxacillin disk (Laboratorios Britania, Buenos Aires, Argentina) on the streak. Staphylococcus aureus ATCC 29213 and 25923 were included as positive and negative controls for β-lactamase production, respectively. Agreement between tests was analyzed by the Cohen test that evaluates responses of two tests in the absence of a gold standard.

RESULTS AND DISCUSSION

Agreement between CIM and NITROCEFIN		CIM		Total
		Resistant	Susceptible	
NITROCEFIN	β-lactamase producer	21	0	21
	No β-lactamase producer	2	30	32
Total		23	30	53

kappa = 0.873; p<0.001

The two isolates that yield a negative result by nitrocefin disk method had MIC ≥0.25 and ≥0.5 µg/mL.

Agreement between CIM and CLOVER LEAF		CIM		Total
		Resistant	Susceptible	
CLOVER LEAF	β-lactamase producer	23	0	23
	No β-lactamase producer	0	30	30
Total		23	30	53

kappa = 0.966, p<0.001

Determining the accuracy of the CLSI proposed breakpoint to identify penicillin-resistant isolates was beyond the scope of this study. No isolates that could be considered as susceptible by the agar dilution method were found to produce β-lactamase; nevertheless, according to previous findings (Haveri et al., 2005; Russi et al., 2008), it would be advisable to perform additional testing when results of agar dilution or agar diffusion tests are close to the breakpoint. The high percent of agreement and ease to perform in diagnostic mastitis laboratories make both methods eligible to complement agar dilution or agar diffusion testing. However, since color-based methods, as nitrocefin, rely in enzymatic reactions that can be incomplete, a potential for appearance of false-negative results in these cases may exist (Pitkälä et al., 2007).