

COMPARISON OF TESTS FOR DETECTION OF β -LACTAMASE *Staphylococcus aureus* IN MASTITIS COWS

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Abstract: This study aims to compare tests for the detection of β -lactamase producing *Staphylococcus aureus*. A total of 116 *Staphylococcus aureus* isolates were collected from clinical mastitis cows and tested for β -lactamase production by nitrocefin test and presence of *blaZ* gene by PCR. Out of 116 *S.aureus*, 12 isolates were positive for nitrocefin test and *blaZ* gene. When compared with PCR results, nitrocefin test showed 100 % sensitivity and 100 % specificity.

Keywords: *Staphylococcus aureus* - β -lactamase production – Nitrocefin test and *blaZ* PCR.

Introduction

Staphylococci causes a wide variety of diseases in animals with the important coagulase negative *Staphylococcus aureus* (CNS), which result in mastitis in cows. In veterinary medicine, penicillin is recommended as the first choice for bacterial infections since most of the bacteria are inherently sensitive to it, with the prevalence of resistance leading to animal disease can be relatively low (Aarestrup and Schwarz, 2006) and is most commonly due to the *blaZ* gene that codes for the production of β -lactamase (Lowy, 2003)

The aim of this study was to compare the performance and evaluate the applicability of nitrocefin and compare the same with the detection of *blaZ* for the determination of β -lactamase production or by determining the presence of β -lactamase gene.

Materials and Methods

A total of 116 *Staphylococcus aureus* isolates were used in this study and the isolates were obtained from clinical mastitis cows.

Production of β -lactamase was tested using nitrocefin (chromogenic cephalosporin) discs (Fluka 49862) as recommended by manufacturer (Sigma Aldrich). The change in colour of disc from yellowish to red was considered as positive (Plate 1).

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To confirm for the presence of *blaZ* gene a single colony from the selective agar was suspended in nuclease free water and lysed by boiling for 10 minutes and the lysate stored at -20°C until use. The lysate were used in a PCR reaction with primers targeting presence of *blaZ* (173 bp) (Plate 2) genes using the following primers: forward 5' ACT TCA ACA CCT GCT GCT TTC 3'(Martineau *et al.*, 2000), and reverse 5' TGA CCA CTT TTA TCA GCA ACC 3'. PCR amplification was performed in a total reaction volume of 25 µl. The reaction mixture contained 12.5 µl of the master mix, 20 pmol of the forward and reverse primer and 15 µl of the test lysate. The amplification profile for the PCR for detection of the different genes were 94°C 5 min; 35 cycles of 95°C for 45 sec, 55°C for 1 min, 72°C for 1 min; final extension of 72°C for 7 min. The PCR products were separated by gel electrophoresis in 1.5 per cent agarose gel and visualized staining with ethidium bromide. The specificity of the amplification was also confirmed by sequencing the amplicons from selected samples and BLAST analysis.

Results and Discussion

The sensitivity and specificity on the nitrocefin compared to *blaZ* PCR results are presented in Table 1. Out of 116 that tested positive for *S.aureus*, 12 isolates were positive for nitrocefin test (Plate 1) and 12 were positive for β-lactamase production by *blaZ* PCR target gene (Plate 2).

The detection of antimicrobial resistance genes with PCR is an interesting possibility for complementing or replacing conventional antibiotic resistance testing. In this study, compared against the reference method, the PCR assay will also be a reliable test. Support for the potential of β-lactamase production by all strain positive for *blaZ* based on PCR was provided by the fact that atleast one method based on β-lactamase identification always detected these strains as positive (Pitkala *et al.*, 2007)

Loeffler and Lloyd, (2010) opined that detection of *blaZ* gene by PCR was gold standard test for confirmation of β-lactamase resistance in MRSA. The results of the present study concurred with the above said authors.

The present observation was in accordance with Pitkala *et al.*, (2007) who reported that testing of β-lactamase production of staphylococci by a nitrocefin test.

We conclude that the PCR targeting the *blaZ* gene and nitrocefin disc can be recommended for routine clinical use in veterinary laboratories.

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Table 1: Sensitivity and specificity on the Nitrocefin compared to *blaZ* PCR

MRSA	Nitrocefin disc	PCR <i>blaZ</i>
True positive (n)	12	12
True Negative (n)	114	114
Sensitivity (%)	100	100
Specificity (%)	100	100



Plate 1

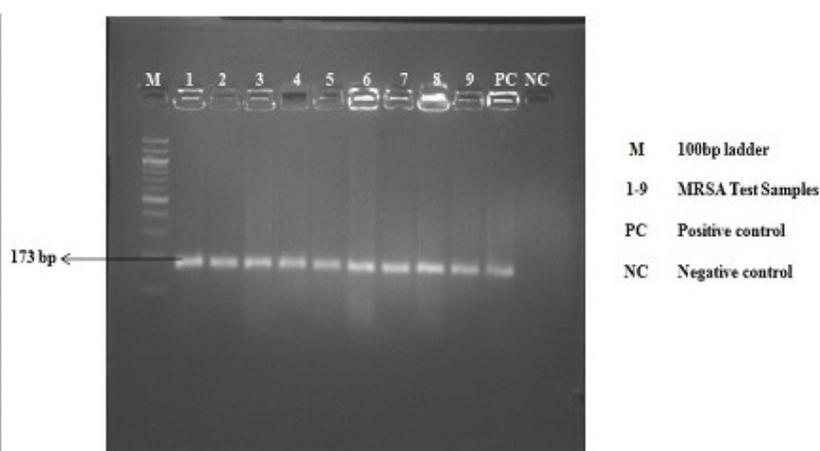


Plate 2

Plate 1: Nitrocefin test-negative and positive

Plate 2: Amplification of *blaZ* gene of Methicillin resistant *S.aureus*