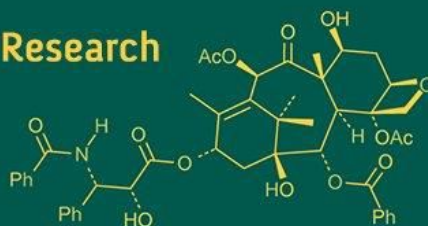


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Rapid field-level antimicrobial susceptibility testing for bovine mastitis under dairy farm conditions in Eluru, Andhra Pradesh

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Abstract

Bovine mastitis remains a major health challenge in dairy herds, resulting in significant economic losses and repeated antimicrobial use. Delayed laboratory diagnosis frequently leads to empirical therapy and inappropriate antibiotic selection. This field-based observational study evaluated a rapid chromogenic antimicrobial susceptibility testing system under dairy farm conditions in Eluru, Andhra Pradesh, India. Seventy milk samples from clinical and subclinical mastitis cases were analyzed, of which 48 samples demonstrated bacterial growth and were subjected to antimicrobial response assessment. Ciprofloxacin showed the highest sensitivity (62.5%), followed by gentamicin (37.5%), enrofloxacin (33.3%) and oxytetracycline (33.3%). Lower sensitivity was observed for amikacin (15.6%), co-trimoxazole (12.5%) and amoxicillin (9.4%). Results were interpretable within 6 hours. The findings suggest that rapid field-level antimicrobial screening may support rational antibiotic selection and improve mastitis management in resource-limited settings.

Keywords: Bovine mastitis, rapid antimicrobial testing, field diagnostics, antimicrobial resistance, dairy cattle

Introduction

Mastitis is one of the most economically important diseases affecting dairy cattle worldwide [1, 5, 7]. Although conventional culture and antibiotic susceptibility testing remain the gold standard [3, 9], they require laboratory facilities and 48-72 hours for result availability. In many field situations, empirical antibiotic therapy is initiated before laboratory confirmation, which may contribute to treatment failure and antimicrobial resistance [4, 8].

The development of rapid antimicrobial susceptibility testing (AST) is an active area of research both internationally and in India to combat antimicrobial resistance (AMR) [4, 7, 8]. Automated phenotypic systems such as Accelerate Pheno and BD Phoenix M50, VITEK® REVEAL™, as well as PCR-based systems such as FilmArray, require substantial infrastructure and trained personnel and are not suitable for in-farm use. National initiatives led by ICAR and regulatory measures under the Drugs and Cosmetics Rules, 1945 emphasize rational antimicrobial use; however, true point-of-care phenotypic AST tools for farm-level deployment remain limited.

The present study aimed to evaluate the field applicability and antimicrobial response patterns observed using a rapid chromogenic testing system.

Materials and Methods

Study Area and Sample Collection

The study was conducted in dairy farms of Eluru, Andhra Pradesh, where bovine mastitis is commonly reported in dairy herds [5, 7]. Milk samples were collected from cows exhibiting clinical signs of mastitis and were tested positive using the California Mastitis Test (CMT). A total of 70 milk samples were included. Approximately 50 µL of freshly collected milk was inoculated per tube as per kit protocol and the Data was analyzed.

Rapid Antimicrobial Susceptibility Testing Kit

Antimicrobial susceptibility testing was performed using a rapid chromogenic-based phenotypic antimicrobial susceptibility testing kit PAS kit (Diagopreutic Pvt. Ltd., Ponda,

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Goa). The kit consists of reconstitutable PAS growth media formulation, chromogenic reagent, antibiotic-containing tubes at two concentrations corresponding to CLSI breakpoint standards (Susceptible and Resistant ranges), and a control tube without antibiotics (Fig.1). All kit components were stored at 2-8 °C and brought to room temperature before performing the assay.

The mastitis antibiotic panel evaluated in the present study included Ciprofloxacin, Ceftiofur, Enrofloxacin, Gentamicin, Oxytetracycline, Amikacin, Co-trimoxazole (trimethoprim/sulfamethoxazole), Amoxicillin, Ceftriaxone,

Cefoperazone-sulbactam, and Levofloxacin.

The PAS growth media was reconstituted using sterile water as per manufacturer instructions. Approximately 50 µL of freshly collected milk sample was inoculated into each antibiotic tubes and the Control containing 450 µL of PAS media. All tubes were incubated at 37 °C for 4-6 hours.

Following incubation, 1 or 2 drops of chromogenic reagent were added to each tube, and colour development was observed after one minute. The control tube served as reference for bacterial growth and colour intensity comparison.

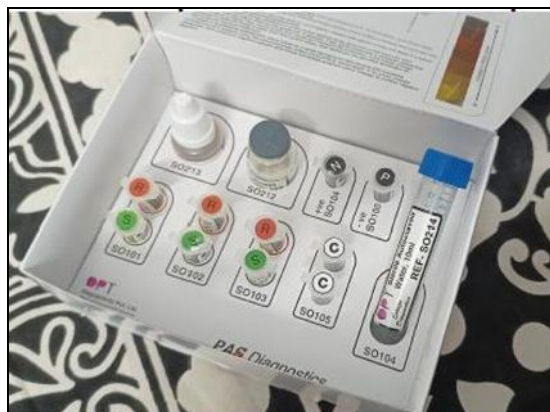


Fig 1: PAS rapid antimicrobial susceptibility testing kit components.

Working Principle and Interpretation

The assay is based on detection of bacterial metabolic activity in the presence of predefined antimicrobial concentrations, consistent with phenotypic antimicrobial susceptibility testing principles described in CLSI guidelines [3, 9]. Turbidity in the control tube confirms bacterial growth. Following addition of chromogenic reagent, colour change

ranging from yellow/orange to pink/red is observed. If the colour intensity in an antibiotic-containing tube is equal to or greater than that of the control, the isolate is interpreted as Susceptible (S). If the colour intensity is lower than that of the control, the isolate is interpreted as Resistant (R) illustrated in Figure 2.



Fig 2: PAS chromogenic susceptibility testing.

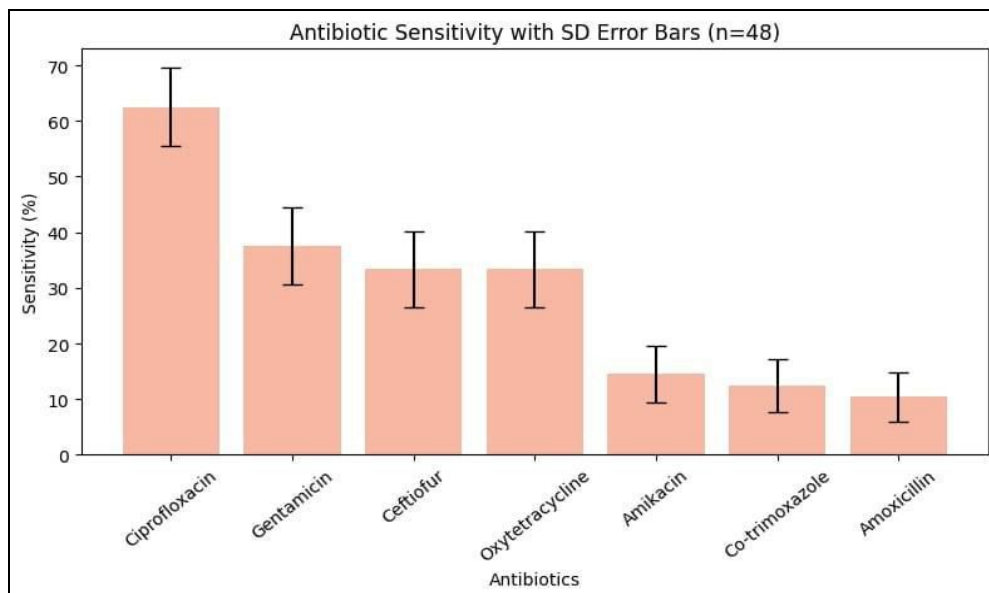
For semi-quantitative assessment, tubes may be photographed against a white background and analyzed using a colour value application (<https://colorpicker-v2.vercel.app/>). The threshold value obtained from the control tube are used for result interpretation. Antibiotic tubes showing values greater than or equal to the threshold are interpreted as susceptible, whereas values below the threshold indicate resistance. The assay detects bacterial metabolic activity based on chromogenic substrate conversion, where metabolic activity produces higher colour intensity corresponding to threshold indicates susceptible isolates (Figure 4b).

Results

Out of 70 milk samples tested, 48 samples demonstrated bacterial growth and were further analyzed for antimicrobial response. The overall growth positivity rate was 68.6%. Considerable variation in antibiotic sensitivity was observed among the tested isolates. Ciprofloxacin showed the highest sensitivity (62.5%), whereas amoxicillin (10.4%) and Co-trimoxazole (12.5%) demonstrated comparatively lower effectiveness. Although overall sensitivity to enrofloxacin was 33.3%, one sample demonstrated an intermediate response suggestive of heteroresistance. The distribution of antibiotic sensitivity percentages is presented in Table 1 and illustrated in Figure 3.

Table 1: Antibiotic sensitivity pattern observed among 48 mastitis-positive milk samples

Antibiotic	Sensitive Isolates (n = 48)	Sensitivity (%)
Ciprofloxacin	30	62.5%
Gentamicin	18	37.5%
Enrofloxacin	16	33.3%
Oxytetracycline	16	33.3%
Amikacin	7	14.6%
Co-trimoxazole	6	12.5%
Amoxicillin	5	10.4%

**Fig 3:** Graphical representation of antibiotic sensitivity percentages among 48 mastitis-positive samples.

Interpretation of result of a sample by PAS kit is shown here and the analysis the same by colour value application has shown similar result (Figure 4 a & b)

**Fig 4a:** Representative chromogenic reactions observed in antibiotic tubes showing susceptible (higher colour intensity) and resistant (lower colour intensity) responses compared to control: Cotrimoxazole was susceptible, Enrofloxacin was intermediate and rest were resistant antibiotics.



Fig 4b: The same sample was analyzed by semi-quantitative assessment with colour value application software (<https://colorpicker-v2.vercel.app/>) and the results are similar.

Discussion

The rapid chromogenic antimicrobial susceptibility system demonstrated practical applicability under field conditions^[4, 7]. The short turnaround time of 3-6 hours allows therapeutic decisions during the same farm visit. Higher sensitivity observed for fluoroquinolones suggests preserved regional efficacy, whereas reduced response to certain beta-lactams may indicate emerging resistance trends reported in mastitis pathogens in Eluru district of Andhra Pradesh, India.

The Kit was validated previously by testing with 47 clinical specimens in the microbiology department of Goa Medical College, Bambolim, Goa, and further revalidated by testing at Veterinary Biological & Research institute, Vijayawada. The data from prior clinical evaluation have reported 91.6% sensitivity, 93.5% specificity, 84.6% positive predictive value, 96.6% negative predictive value, and overall accuracy of 93% compared to standard disc diffusion testing. These values comply with minimal FDA and CLSI performance characteristics (>90% agreement; very major error ≤1.5%; major error <3%).

Conventional antimicrobial susceptibility testing (AST) methods such as disk diffusion and broth-based assays are influenced by several methodological variables including inoculum density, incubation conditions, agar depth, medium composition, and interpretation of inhibition zones, which may affect the reproducibility of results^[3, 6, 9]. Another important microbiological phenomenon relevant to

antimicrobial susceptibility interpretation is heteroresistance, where a bacterial population contains small subpopulations with higher resistance levels than the main susceptible population. These resistant subpopulations may not be detected by conventional susceptibility tests but can expand under antibiotic pressure, potentially leading to treatment failure. Heteroresistance has been increasingly recognized across multiple bacterial pathogens and antibiotic classes and represents an intermediate stage in the evolution of full antimicrobial resistance^[11]. Rapid phenotypic screening methods may therefore assist in identifying antimicrobial response patterns more effectively under field conditions. Such variability has been highlighted in previous studies evaluating the methodological challenges associated with antimicrobial activity testing^[10].

In comparison, the rapid chromogenic PAS kit provides a simplified and standardized workflow with minimal procedural variability, making it more suitable for field-level antimicrobial screening in dairy farm settings, suggesting its potential usefulness as a preliminary field-level screening tool for antimicrobial sensitivity. Due to reduced procedural variability and faster result interpretation, the PAS chromogenic kit provides a more practical alternative to conventional AST under field conditions. Its simplicity and rapid turnaround make it suitable for guiding antimicrobial selection in bovine mastitis management at field level very effectively^[10].

However, there are certain limitations with the PAS kit. The sample must contain a sufficient pathogenic titre to perform the test. The limit of detection of the system is approximately 10³ CFU/mL, therefore samples with very low bacterial load may not yield interpretable results. In typical clinical mastitis infections, the bacterial titre is usually around 10⁶ CFU/mL, which falls within the detectable range for the assay. However, the kit is not suitable for testing healthy milk samples to determine antibiotic selection. If the sample is collected from animals that have already received antimicrobial treatment, certain antibiotics may temporarily reduce the bacterial titre below the detectable threshold. In such cases, the pathogen concentration may fall below the detection limit and the sample cannot be reliably processed for antimicrobial selection. A comparative study involving 30 mastitis-positive samples was also performed using the standard disk diffusion method and the PAS kit. However, the results obtained from the disk diffusion method were inconsistent under field conditions due to the methodological limitations described above; therefore, these data are not presented in detail.

Although the PAS kit has these limitations, they do not significantly affect its overall performance, and the system still provides most reliable and rapid results suitable for field-level antimicrobial decision making.

Conclusion

Rapid chromogenic antimicrobial susceptibility testing offers a practical and time-efficient approach for guiding mastitis therapy in field settings and may contribute to improved antimicrobial stewardship in dairy herds.

Author Contributions

Vineetha Chilukuri and Reddy Navya conducted the study and data collection. Dr. Uday VSP Puligadda supervised the study and reviewed the manuscript.

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