



Management of Antibiotic-Resistant Pathogens in Clinical and Chronic Mastitis using Pashu Ayurveda (Ethnoveterinary) Formulations: A Field-Based Interventional Study

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Abstract: Background: Bovine mastitis remains one of the most prevalent diseases affecting dairy animals worldwide and represents a major cause of economic loss and antimicrobial resistance (AMR). Indiscriminate and prolonged antibiotic usage in mastitis management has resulted in therapeutic failures, drug residues in milk, and the emergence of multidrug-resistant pathogens. Objective: The present study aimed to identify antibiotic-resistant mastitis pathogens and to evaluate the clinical and microbiological efficacy of a standardized Pashu Ayurveda (ethnoveterinary) formulation in the management of clinical and chronic mastitis. Methods: A field-based interventional study was conducted on 100 dairy animals diagnosed with clinical or chronic mastitis between January 2020 and December 2023. Milk samples were subjected to bacteriological culture, antibiotic sensitivity testing, somatic cell count (SCC) analysis, and pH estimation. A standardized ethnoveterinary formulation was administered topically for 10 consecutive days, along with internal herbal supplementation. Clinical and laboratory parameters were recorded before and after intervention. Statistical analysis was performed using paired proportion tests. Results: Significant improvement was observed in clinical parameters, including abnormal milk colour, udder swelling, flakes in milk, pain during milking, and udder fibrosis ($P \leq 0.01$). Normal milk pH increased from 31% to 48% ($P = 0.027$). High and very high SCC categories showed a marked reduction ($P \leq 0.001$). Antibiotic resistance declined from 100% at baseline to 2% post-treatment, with 92% of isolates regaining antibiotic sensitivity. The overall recovery rate was 79.38%. Conclusion: The Pashu Ayurveda (ethnoveterinary) formulation demonstrated significant clinical and microbiological efficacy in managing antibiotic-resistant mastitis, improving milk quality, reducing inflammatory burden, and restoring antibiotic responsiveness. This approach offers a sustainable, residue-free alternative aligned with antimicrobial stewardship and the One Health framework.

Keywords: Clinical mastitis, antimicrobial resistance, Pashu Ayurveda, somatic cell count, One Health.

INTRODUCTION

Mastitis is the most prevalent production disease affecting dairy cattle and buffaloes, with reported prevalence ranging from 29-78% in cows and 27-70% in buffaloes. In India, mastitis alone contributes to an estimated annual economic loss of ₹7,165.5 crores due to reduced milk yield, deterioration of milk quality, treatment costs, and premature culling. Antibiotics remain the cornerstone of mastitis management; however, irrational and repetitive usage has resulted in widespread antimicrobial resistance, particularly among pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas* spp. This trend

poses a serious threat to animal health, public health, and food safety due to antibiotic residues in milk and the transmission of resistant organisms across the food chain.

Pashu Ayurveda (ethnoveterinary medicine), derived from traditional Indian knowledge systems, has been practiced for centuries in livestock healthcare. Increasing scientific evidence indicates that several ethnoveterinary formulations possess antimicrobial, anti-inflammatory, immunomodulatory, and tissue-healing properties. Integration of such approaches into mastitis management may reduce antibiotic dependence and contribute to global AMR mitigation strategies under the One Health paradigm.

The present field-based study evaluates the effectiveness of a standardized Pashu Ayurveda formulation in managing antibiotic-resistant clinical and chronic mastitis under real-world farm conditions.

MATERIALS AND METHODS

Study Design and Duration

A cross-sectional diagnostic study followed by a prospective interventional study was conducted from January 2020 to December 2023.

Study Area and Animals

The study was carried out in dairy farms associated with Gokul Dairy, India. A total of 100 cross-bred dairy animals diagnosed with clinical or chronic mastitis were included.

Inclusion and Exclusion Criteria

Inclusion Criteria:

- Cross-bred dairy animals
- Second calving onwards
- Clinical or chronic mastitis

Exclusion Criteria:

- Indigenous breeds
- Animals below 2 years of age
- Animals beyond sixth calving
- Subclinical mastitis cases

Milk Sample Collection and Bacteriological Analysis

Milk samples were collected aseptically from affected quarters and cultured on nutrient agar and MacConkey agar. Isolates were identified based on colony morphology, Gram staining, and biochemical tests.

Antibiotic Sensitivity Testing

Antibiotic sensitivity was assessed using the Kirby-Bauer disk diffusion method against commonly used veterinary antibiotics.

Pashu Ayurveda (Ethnoveterinary) Intervention

A thick paste was prepared using Aloe vera gel (250 g), Curcuma longa rhizome powder (50 g), and calcium hydroxide (15 g). From this prepared paste, 30 g (approximately one-tenth) was taken and diluted with 200 ml of water to obtain a thin, spreadable consistency. The udder was first washed with clean water, and complete milking of all quarters was performed. The thin paste was then applied uniformly over all quarters of the udder. This procedure was repeated every 3 hours (5-6 times daily). The final application of the day is with 200 ml gingelly (sesame) oil or mustard oil instead of 200 ml of water to make a thin paste, while applying see to it that the moisture on the udder is removed.

Internal Administration:

A powdered herbal mixture consisting of Tinospora cordifolia stem (50 g), Withania somnifera root (50 g), Moringa oleifera leaves (50 g), and Asparagus racemosus root (50 g) was administered orally once daily, mixed with the regular feed throughout the treatment period.

Parameters Assessed

Subjective Parameters:

- Milk colour
- Udder swelling
- Flakes in milk
- Pain on milking
- Blood in milk
- Udder fibrosis

Objective Parameters:

- Milk pH
- Somatic cell count (SCC)

Assessments were recorded on Day 0 and Day 10.

Statistical Analysis

Data were analysed using SPSS v22.0 and R v3.2.2. Paired proportion tests were applied, and $P \leq 0.05$ was considered statistically significant.

RESULTS

Clinical Improvement

Clinical Parameters

Table 1: Distribution of Clinical Mastitis Cases Based on Subjective Parameters

Parameter	Status	Before n (%)	After n (%)	P value
Milk colour	Abnormal	46	5	≤0.001
Udder swelling	Present	53	6	≤0.001
Flakes in milk	Present	74	20	≤0.001
Pain on milking	Present	53	16	0.002
Udder fibrosis	Severe	42	11	0.028

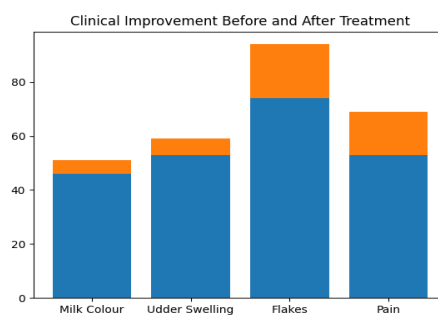


Figure 1: Improvement in major clinical signs before and after ethnoveterinary treatment.

A significant reduction was observed in abnormal milk colour (46% to 5%), udder swelling (53% to 6%), flakes in milk (74% to 20%), pain on milking (53% to 16%), and severe udder fibrosis (42% to 11%) following treatment ($P \leq 0.01$).

Milk pH

Table 2: Changes in milk pH before and after treatment

pH category	Before	After	P value
Clinical pH	28	7	0.027
Normal pH	31	48	

Normal milk pH increased from 31% before treatment to 48% after treatment, while clinical pH reduced from 28% to 7% ($P = 0.027$).

Somatic Cell Count

High SCC cases reduced from 41% to 14%, and very high SCC from 26% to 13% ($P \leq 0.001$).

A highly significant reduction in high and very high SCC categories with a concomitant shift toward lower and moderate SCC ranges was observed following ethnoveterinary treatment, indicating substantial reduction in mammary inflammation.

Table 3: SCC distribution before and after treatment

SCC level	Before	After	P value
Low (≤ 3 lakh)	24 (24%)	36 (36%)	+12.0
Normal (3-5 lakh)	4 (4%)	4 (4%)	0.0
Moderately High (5-10 lakh)	5 (5%)	27 (27%)	+22.0
High (10-50 lakh)	41 (41%)	14 (14%)	-27.0
Very High (≥ 50 lakh)	26 (26%)	13 (13%)	-13.0

Antibiotic Sensitivity Pattern

Table 4: Antibiotic sensitivity pattern

Status	Before	After	P value
Resistant	100	2	≤ 0.001
Sensitive	0	92	

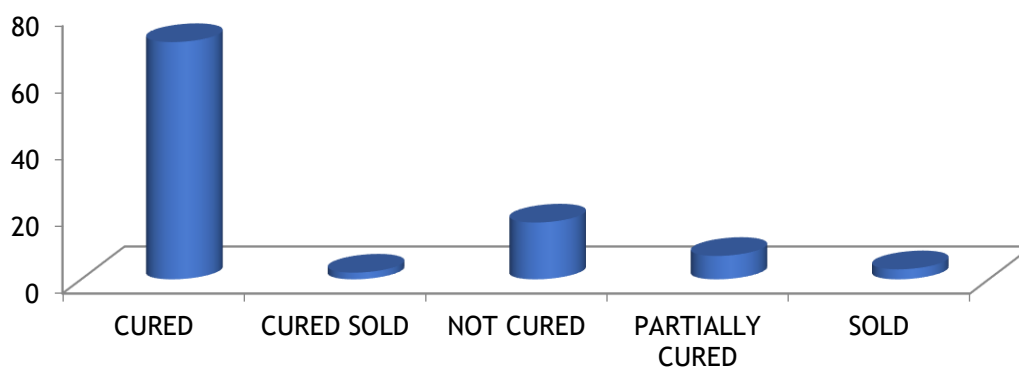
All isolates showed resistance to one or more antibiotics before intervention. Post-treatment, resistant isolates reduced to 2%, while 92% became antibiotic sensitive ($P \leq 0.001$).

Treatment Outcome

Cases Recovery Status:

Table 28: Cases recovery status

Case Status	No. of Patients	%
Cured	71	71.0
Cured but sold after	2	2.0
Cured after 1 month	4	4.0
Total cured	77	77.0
Not cured	13	13.0
Partially cured	7	7.0
Total	97	97.0
Percentage of success	79.38 %	
Sold not completed the course of the study- Drop Out	3	3.0
Total	100	100.0



Out of 100 animals, 77 were completely cured, 2 partially recovered, 18 did not recover, and 3 dropped out. The overall success rate was 79.38%.

DISCUSSION

The findings of the present study demonstrate that the Pashu Ayurveda (ethnoveterinary) intervention was effective in managing clinical and chronic mastitis caused by antibiotic-resistant pathogens. The significant improvement observed in clinical signs, milk pH, and SCC reflects a substantial reduction in mammary inflammation and restoration of udder health.

The marked reduction in antibiotic resistance following treatment suggests a decrease in pathogen burden and improved host-pathogen balance, rather than direct antimicrobial substitution. This observation aligns with antimicrobial stewardship principles, where restoration of host immunity and tissue integrity plays a critical role in reducing recurrence and improving therapeutic responsiveness.

These results are consistent with earlier field-based and experimental studies validating the role of ethnoveterinary formulations in mastitis management. Adoption of Pashu Ayurveda-based approaches offers a cost-effective, residue-free, and farmer-friendly alternative, supporting sustainable dairy production and One Health-oriented AMR mitigation strategies.

Ayurvedic Justification of the Ethnoveterinary Formulation

In Ayurveda, inflammatory and infective conditions of the mammary gland are described under Stana Vikara, Shopha, and Dushta Vrana, predominantly involving Pitta-Kapha Dosha vitiation with association of Ama, Rakta Dushti, and Srotorodha. Chronic and non-healing mastitis conditions, especially those showing fibrosis and recurrence, closely resemble Dushta Vrana, as described by Sushruta, where impaired tissue response and persistent inflammation lead to delayed healing and resistance to treatment (Sushruta Samhita, Sutrasthana 22).

Classical Ayurvedic management of such conditions emphasizes Vrana Shodhana (cleansing), Vrana Ropana (healing), Shothahara (anti-inflammatory measures), and Rasayana (immunomodulation) rather than sole pathogen-directed therapy. This holistic approach is particularly relevant in antibiotic-resistant mastitis, where enhancement of host defense and tissue metabolism is crucial.

Ayurvedic Rationale for External Formulation (with Textual References)

Aloe vera (Kumari)

Kumari is described in Bhavaprakasha Nighantu (Guduchyadi Varga) as Pitta-Kapha Shamaka, Sheetala, and Vrana Ropana. Charaka describes Kumari as useful in Raktapitta, Daha, and inflammatory conditions, supporting its role in reducing udder inflammation, edema, and burning sensation (Charaka Samhita, Chikitsa Sthana 30). Its application promotes Rakta Prasadana and epithelial regeneration, making it suitable for both acute and chronic mastitis.

Curcuma longa (Haridra)

Haridra is extensively described by Charaka as Krimighna, Kandughna, Vishaghna, and Shothahara (Charaka Samhita, Sutrasthana 25). Sushruta recommends Haridra in Vrana Shodhana and Dushta Vrana, where infection and suppuration are present (Sushruta Samhita, Chikitsa Sthana 1). Its Ama-Pachana and Rakta Shodhana properties are particularly beneficial in chronic and resistant mastitis.

Calcium hydroxide (Kshara-based action)

Kshara is described by Sushruta as possessing Lekhana, Bhedana, and Shodhana properties, indicated in Dushta Vrana, Granthi, and chronic inflammatory swellings (Sushruta Samhita, Sutrasthana 11). The inclusion of calcium hydroxide provides a localized Kshara-like effect, facilitating removal of inflammatory exudates, reducing microbial load, and relieving Srotorodha.

Gingelly oil / Mustard oil (Taila)

Sushruta identifies Taila as the best medium for Vrana Ropana, owing to its Sukshma, Vyavayi, and Yogavahi properties (Sushruta Samhita, Chikitsa Sthana 1). Gingelly oil is described as Vata-Kapha Shamaka and is indicated for reducing stiffness, pain, and fibrosis in chronic inflammatory conditions.

Ayurvedic Rationale for Internal Herbal Administration***Tinospora cordifolia (Guduchi)***

Guduchi is classified as a prime Rasayana drug by Charaka and is indicated in Jwara, Tridoshaja Vikara, and chronic inflammatory diseases (Charaka Samhita, Chikitsa Sthana 1). Bhavaprakasha describes Guduchi as Ama-Pachaka and Rakta Prasadaka, enhancing Ojas and immunity, which is essential in recurrent and antibiotic-resistant mastitis.

Withania somnifera (Ashwagandha)

Ashwagandha is described in Bhavaprakasha Nighantu (Guduchyadi Varga) as Balya, Rasayana, and Vata-Pitta Shamaka. Charaka recommends it for Dhatu Kshaya and debility (Charaka Samhita, Chikitsa Sthana 1), supporting tissue regeneration and recovery in chronic mastitis with fibrosis.

Moringa oleifera (Shigru)

Shigru is described as Ushna, Tikshna, and Kapha-Vata Shamaka in Bhavaprakasha Nighantu, and is indicated in Shopha, Granthi, and obstructive inflammatory conditions. Its Deepana-Pachana action helps in resolving induration and improving lymphatic drainage of the mammary gland.

Asparagus racemosus (Shatavari)

Shatavari is described by Charaka as Pitta Shamaka, Rasayana, and Stanya Dhatu Prasadaka (Charaka Samhita, Sutrasthana 27). It nourishes mammary tissue, supports regenerative healing, and balances inflammatory responses, particularly in chronic mastitis.

Ayurvedic Perspective on Antibiotic Resistance

Classical Ayurveda emphasizes correction of host susceptibility (Rogi Bala) over direct eradication of pathogens. By improving Agni, clearing Ama, and strengthening Ojas, the ethnoveterinary formulation restores tissue resistance, thereby reducing recurrence and improving treatment responsiveness. This explains the observed reduction in somatic cell count, improvement in clinical signs, and restoration of antibiotic sensitivity.

CONCLUSION

The formulation was systematically developed based on classical Ayurvedic principles of Dosh pacification, Vrana Shodhana-Ropana, and Rasayana therapy as described in the *Charaka Samhita*, *Sushruta Samhita*, and *Bhavaprakasha Nighantu*. The Pashu Ayurveda intervention demonstrated significant efficacy in the management of chronic and antibiotic-resistant mastitis by improving milk quality, reducing somatic cell count, and restoring antibiotic sensitivity. Integration of such ethnoveterinary approaches into mastitis control programs offers a viable strategy to reduce antibiotic dependence and promote sustainable and responsible dairy farming.

Ethics Statement: The study was conducted following standard animal welfare guidelines. No invasive procedures were performed.

Conflict of Interest: The authors declare no conflict of interest.

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