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Advances in Diagnosis and Alternative Therapeutic Strategies for Bovine Mastitis

Sanjay C[1], Praveen M[2], Jenignanasuganthi A[3], Sudha S.S[4], Mahenthiran R

[1,2,3] Students, M. Sc. Microbiology, [4]Professor Department of Microbiology/Co-Ordinator -CDC, [5]Assistant Professor.

Dr. N.G.P. Arts and Science College. Coimbatore, Tamil Nadu, India.

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Abstract - Bovine mastitis remains a major challenge in dairy farming, causing substantial economic losses through reduced milk yield and quality. Recent progress emphasizes rapid diagnostic tools and non-antibiotic therapies to combat rising antimicrobial resistance. Traditional methods like somatic cell counts via California Mastitis Test (CMT) and bacterial cultures offer reliable on-farm screening, while advanced techniques such as PCR, MALDI-TOF mass spectrometry, and biosensors enable precise pathogen identification for timely intervention.[7][3][1]

Therapeutic management has shifted toward alternatives like probiotics, plant-derived essential oils, bacteriocins, and phage therapy, which show anti-inflammatory and antimicrobial potential without promoting resistance. Genetic selection for udder health resistance further supports prevention alongside hygiene and stewardship programs.[6] Integrated strategies combining diagnostics, novel treatments, and economic assessments promise sustainable control. Future efforts should prioritize field trials and molecular innovations to enhance dairy productivity.[8][9]

Key words: Bovine mastitis, diagnostic advances, alternative therapies, antimicrobial resistance, udder health

Introduction

Bovine mastitis stands as one of the most pressing challenges in dairy farming, marked by inflammation of the udder typically triggered by bacterial infections that compromise milk quality and cow health.(1)(2) This condition persists as a leading cause of economic losses worldwide, with annual global estimates reaching €16-26 billion due to reduced milk yields, discarded milk, treatment expenses, and premature culling of affected animals.(3)(4) Beyond finances, mastitis affects animal welfare through pain and lowered productivity, while posing risks of antimicrobial residues in milk that impact public health.(5)(6)Traditional diagnosis relies on tools like somatic cell counts and California Mastitis Tests for rapid on-farm screening, but recent progress incorporates advanced methods such as PCR, MALDI-TOF mass spectrometry, and biosensors for precise pathogen identification.(1)(2)(7)These innovations enable earlier detection, curbing spread and supporting targeted interventions amid rising antimicrobial resistance among common culprits like Staphylococcus aureus.(8)(6)While antibiotics remain standard therapy, their overuse fuels resistance, prompting exploration of alternatives including probiotics, bacteriophages, endolysins, and plant extracts that offer pathogen-specific action with fewer side effects.(9)(10)(11)(12)(13) This review delves into these diagnostic advances and novel strategies, aiming to outline sustainable pathways for mastitis control that balance efficacy, economics, and resistance mitigation.(2)(14)(15)

2. Classification of Bovine Mastitis

Bovine mastitis can be classified based on clinical presentation, duration, causative agents, and infection source. The primary distinction is between clinical and subclinical mastitis. Clinical mastitis shows visible signs such as udder swelling, pain, and abnormal milk, whereas subclinical mastitis lacks visible symptoms but is detected by elevated somatic cell counts or microbial tests. (1)(2)

Based on duration, mastitis is classified into peracute, acute, subacute, and chronic forms. Peracute mastitis shows a sudden severe onset with systemic symptoms, often leading to shock; acute mastitis is characterized by marked inflammation and milk abnormalities; subacute cases are milder and progress more slowly; chronic mastitis persists for a long period with repeated flare-ups. (3)(4)

Comparison of clinical, subclinical, and chronic mastitis in dairy cattle.

Feature	Clinical Mastitis	Subclinical Mastitis	Chronic Mastitis
Signs	Hot, swollen udder; abnormal milk (clots, blood) [3]	No visible signs; milk appears normal	Repeated mild inflammation over months
Somatic Cell Count (SCC)	High (detectable via tests)	Elevated (>200,000 cells/mL) [4]	High
Impact on Milk	Sharp drop in yield; milk often discarded	Moderate drop in yield; more subtle losses [4]	Chronic reduction in yield
Prevalence (India)	~10–15% of cows (acute cases)	~40–50% of cows (majority of infections)	Less common (recurrent cases)
Detection	Clinical exam, culture of milk	CMT, SCC tests, biochemical screening (pH, conductivity) [4]	History and repeated testing

Etiologically, mastitis classification involves contagious pathogens like *Staphylococcus aureus* and Streptococcus agalactiae, which spread during milking, and environmental pathogens such as *Escherichia coli* and *Streptococcus uberis*, originating from housing and surroundings.(3)(5)

3. Epidemiology and Risk Factors

3.1 Epidemiology

Bovine mastitis stands as the most common and economically devastating disease in dairy cattle worldwide, leading to substantial reductions in milk yield, increased culling rates, and elevated veterinary costs. Clinical mastitis manifests with visible udder inflammation and milk abnormalities, while subclinical cases, detectable via elevated somatic cell counts, often persist longer and serve as reservoirs for pathogen spread within herds. Incidence varies by region, with rates reported from 7% to 35% in large-scale studies across provinces, influenced heavily by management practices and pathogen prevalence. (1)

Prevalence data highlight environmental pathogens like coliforms driving sporadic outbreaks alongside contagious agents such as Staphylococcus aureus causing chronic infections.(1)(2) Bulk tank somatic cell counts provide herd-level insights, often exceeding thresholds like 200,000 cells/mL in affected groups, signaling widespread intramammary infections.(3)(4) Over decades, evolving epidemiology shows rising antimicrobial resistance among pathogens, complicating control efforts and amplifying economic impacts estimated at billions annually.(5)

3.2 Risk Factors

Host factors play a pivotal role, with high-yielding breeds like Holstein-Friesian showing greater susceptibility compared to Jersey cattle due to genetic predispositions and udder morphology.(2)Parity and lactation stage heighten vulnerability; cows in their 4th to 6th parity or peak lactation face odds up to 1.08 times higher for infection owing to teat wear and immune fatigue.(1)(2)(3)Age compounds this, as older cows exhibit weaker immunity and prolonged exposure to milking equipment trauma.(1)(3)

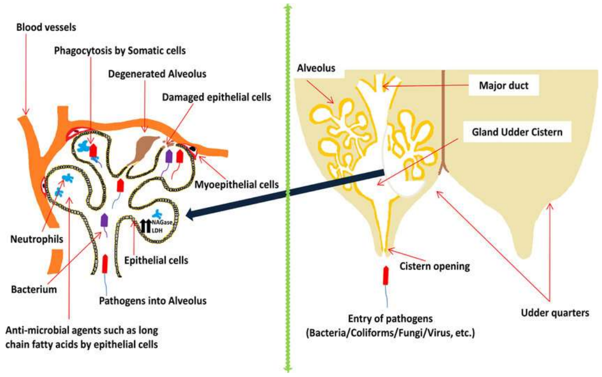

Figure 1. Immune Response in Bovine Mastitis. Illustration of how mastitis pathogens trigger the cow's immune system: bacterial components are recognized by immune cells, leading to neutrophil recruitment, inflammation, and tissue damage in the mammary gland. *(Adapted from Tong et al., 2025 [7]).*

Environmental contributors dominate outbreaks, including high stocking density, damp bedding, poor ventilation, and hot-humid climates that foster bacterial proliferation on teat ends.(2) Management lapses, such as milking infected cows last or inadequate teat hygiene, correlate strongly ($p<0.05$) with elevated quarter-level prevalence nearing 37%.(3) Previous mastitis history triples odds (OR=2.7), while poor body condition scores and udder lesions further predispose herds.(3)

4. Economic Importance of Mastitis

Bovine mastitis imposes massive economic strain on dairy industries worldwide, with annual global losses estimated between €16 billion and €26 billion due to reduced milk output, treatment expenses, discarded milk, and early culling of cows.(1)

4.1 Direct Costs

Veterinary treatments, drugs, and extra labor for severe cases add up quickly, often hitting hundreds of dollars per incident in places like the US, where diagnostics alone run about $10 and therapeutics $36 per early-lactation case.(2) Discarded milk during antibiotic withdrawal periods further drains revenue, sometimes reaching thousands per farm yearly, as seen in regions like Egypt with over 100,000 LE lost annually.(2)These immediate hits compound when subclinical mastitis lingers undetected, quietly slashing profits more than flashy clinical outbreaks.(3)

4.2 Indirect Costs

Milk yield drops sharply—up to 552 kg per lactation per affected cow—while lifelong productivity dips and genetic herd quality suffers from culling high performers.(1)In India, losses from clinical cases alone project into crores, like ₹14.12 crore in one district for crossbred herds, fueled by 70% yield cuts and replacement animal costs.(4)(5) Subclinical forms dominate long term damage, tying up resources that could boost overall farm output.(6)

4.3 Broader Impacts

Premature culling erases invested breeding value and spikes replacement needs, with US farms facing $444 total per early case including death losses around $32.(1)(2) Ethiopia reports $214 per farm yearly, underscoring how mastitis hampers scalability in developing dairy sectors.(2) Effective control, like better hygiene, offsets these through sustained yields and lower vet bills, as older reviews highlight variable but recoverable benefits.(7)

5. Conventional Diagnostic Methods

Conventional diagnostic methods for bovine mastitis primarily include somatic cell count (SCC), California mastitis test (CMT), microbiological milk culture, and physical examination of the udder and milk. SCC is a widely used, cost-effective method that measures the number of somatic cells, mainly leukocytes, in milk, indicating an inflammatory response suggestive of mastitis. It is easily performed by microscopy or automated counters and is useful for detecting subclinical mastitis. The CMT is a simple, on-farm enzyme-based test that estimates SCC and is considered a practical screening tool for early mastitis detection with high sensitivity and specificity. This test detects increased leukocyte esterase activity by a gel-like reaction when mixed with milk from infected quarters. Microbiological milk culture remains the gold standard method in the laboratory for identifying the causative bacterial pathogens in mastitis cases. It involves culturing milk samples on appropriate media to isolate and identify specific bacteria, which guides targeted therapy. This method, while precise, is more time-consuming and requires laboratory infrastructure. Physical examination involves visual inspection and palpation of the udder to check for swelling, heat, pain, hardness, or milk abnormalities such as clots, flakes, or changes in color and consistency. Stripping milk onto a dark surface to observe such changes is a simple and early detection technique for clinical mastitis. Other conventional tests, such as the white side test and electrical conductivity measurement of milk, are also used but less frequently. Conductivity increases with mastitis due to electrolyte changes in milk, but its practical application is limited by variability among cows. These methods are often combined for more accurate diagnosis and management of mastitis on farms. Images included in the literature depict the California mastitis test showing milk gel formation, somatic cell counting with microscopic views, and udder inspection for clinical signs, highlighting these methods' practicality and applicability in field and laboratory settings. Overall, these conventional diagnostic approaches continue to play a vital role in early detection and management of bovine mastitis, enabling timely intervention to reduce economic losses and improve animal health(1)(2)(3)(4)(5)

5.1 California Mastitis Test (CMT):

The California Mastitis Test is a simple, rapid, and affordable cow-side diagnostic tool to detect subclinical mastitis by estimating somatic cell count in milk. In this test, equal volumes of milk from each quarter are mixed with a reagent containing sodium lauryl sulfate in a paddle cup. The detergent breaks down somatic cells, releasing their DNA, which forms a gel-like substance when combined with the reagent. The degree of gel formation indicates infection severity, ranging from no change (negative) to strong gel formation (positive). This visible gel formation provides an immediate indication of udder infection and inflammation. CMT is widely used on farms due to its ease and quick results. Images typically show progressive gel consistency in reaction cups from watery to thick gel(1)(2)

5.2 Microscopic Examination of Somatic Cells in Milk:

Microscopic analysis involves preparing smears of milk samples and staining them to visualize somatic cells under a microscope. High somatic cell count, dominated by neutrophils, reflects inflammatory response due to mastitis. Examination helps differentiate cell types, showing predominance of immune cells indicative of infection. This method aids in evaluating the extent of inflammation and subclinical mastitis. Typical microscopic images display numerous neutrophils and other leukocytes among milk fat globules, confirming immune activation within the mammary gland. (1)(3)

5.3 Visual Inspection of Udder and Milk:

Physical examination of the udder and visual assessment of milk are conventional and essential diagnostic steps. Signs of mastitis include swelling, heat, redness, and pain in one or more quarters of the udder. Milk from affected quarters may appear abnormal with clots, flakes, discoloration (yellow hue or blood-tinged), or watery consistency. These clinical manifestations directly indicate mastitis presence. Observing these abnormalities helps in early clinical mastitis detection, guiding further diagnostic and treatment procedures. Images commonly show inflamed udders alongside abnormal milk appearances, providing practical references. (1)(2)

6. Advance Diagnostic Technologies

Advances in diagnostic technologies for bovine mastitis have shifted toward rapid, on-farm tools and molecular methods that surpass traditional somatic cell counts and bacterial cultures in speed and accuracy. (1)

6.1 Conventional Screening Tools

California Mastitis Test (CMT) remains a frontline option, offering quick cow-side detection of subclinical cases with sensitivity around 82% for major pathogens like *Staphylococcus aureus,* though it drops to 61% for minor ones.(1) Wisconsin Mastitis Test provides similar indirect inflammation assessment via somatic cell estimation. These remain cost-effective but rely heavily on observer skill.(1)

6.3 Molecular and Culture-Based Advances

Real-time PCR achieves near 100% sensitivity and specificity for pathogen identification, detecting even non-viable bacteria missed by cultures, with kits like PathoProof outperforming standard microbiology.(1) MALDI-TOF mass spectrometry delivers genus-level results in minutes through protein profiling, proving superior for diverse mastitis microbes once databases expand.(1)(2) Rapid chromogenic plates enable on-farm differentiation of common pathogens without extra lab steps.(1)

6.4 Emerging Technologies

Biosensors, including nanoparticle-based assays and infrared thermography, promise portable, real-time udder inflammation detection without sampling.(3)(4) Machine learning integrated with 16S rRNA sequencing and dielectric milk spectra analysis flags infection stages swiftly in field settings.(1) Proteomics via MALDI-TOF or microRNA biomarkers offer future precision for early subclinical diagnosis.(4)(2)

7. Major pathogens and Antimicrobial Resistance

The major pathogens involved in bovine mastitis primarily include *Staphylococcus aureus, Escherichia coli, Streptococcus uberis, Streptococcus agalactiae,* and *Mycoplasma bovis. Staphylococcus aureus* is noted for chronic infections facilitated by biofilm formation, which enhances resistance to immune defenses and antibiotics. Escherichia coli is a common environmental pathogen causing acute clinical mastitis, while *Streptococcus species* such as S. uberis and *S. agalactiae* are also frequent pathogens with varying impacts. *Mycoplasma bovis* is a significant emerging pathogen that causes both clinical and subclinical mastitis globally. Additionally, coagulase-negative *staphylococci* and fungi like *Candida spp*. Are also involved but to a lesser extent. Antimicrobial resistance (AMR) among these pathogens is a growing concern. Resistance is commonly observed in Staphylococcus aureus, particularly with methicillin-resistant *S. aureus* (MRSA) strains which form biofilms making infections persistent and harder to treat. Resistance to β-lactam antibiotics is reported among *streptococci* and *staphylococci. Escherichia coli* and *Streptococcus uber*is isolates have shown significant resistance to commonly used antibiotics such as penicillin, neomycin, and cephalexin. Resistance mechanisms include mutations affecting antibiotic targets and biofilm-mediated protection which complicates therapeutic management. Here is a relevant image highlighting the common major pathogens in bovine mastitis and their relative prevalence. This shows *Staphylococcus aureus, Escherichia coli, Streptococcus spp.*, and others as dominant bovine mastitis bacteria. Additionally, this graph depicts antimicrobial resistance patterns among mastitis pathogens, illustrating resistance prevalence against common antibiotics such as penicillin and amoxicillin-clavulanic acid in isolates obtained from milk samples. These visuals clearly reflect the importance of these pathogens and the challenges posed by AMR in mastitis treatment and control. References throughout the text are supported by findings from the recent reviews and studies listed, especially references 1, 2, 9, 11, and 17, reflecting comprehensive and current knowledge on bovine mastitis pathogens and antimicrobial resistance (1)(2)(3)(4)(5).

Pathogenicity

Once pathogens enter the udder, their pathogenicity depends on factors like toxin production, invasion ability and immune evasion. *S. aureus*, for example, produces toxins (alpha-toxin, leukocidins) and can invade epithelial cells and form abscesses. *E. coli* causes inflammation mainly via its endotoxin (LPS), which triggers a strong local and systemic immune response. The host reaction (neutrophil influx, cytokine release) to bacterial components causes much of the tissue damage and clinical signs. A highly virulent pathogen or poor host immunity leads to a severe, acute response (swollen, painful quarter, systemic fever), whereas low-virulence bacteria may cause only subclinical infection.

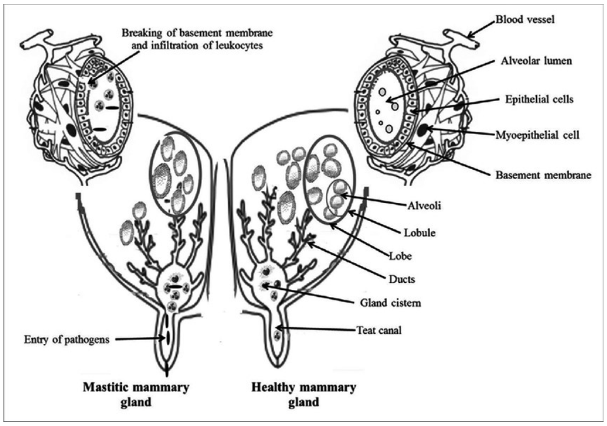

Figure 2. Comparison of Healthy and Mastitis-Affected Mammary Gland.
This diagram shows the structural and immune differences between a healthy udder and one affected by mastitis. Infected mammary tissue exhibits swelling, immune cell infiltration, and disrupted alveolar integrity. (*Adapted from Patel et al., 2018 [2]).*

8. Antibiotic Treatment and Stewardship

Antibiotic treatments for bovine mastitis mainly consist of intramammary and systemic antibiotics. Intramammary antibiotics, delivered directly into the infected quarter, are preferred for mild, uncomplicated cases affecting a single quarter. Common antibiotics include penicillin, cloxacillin, cephapirin, and novobiocin. Systemic antibiotics, such as procaine benzylpenicillin or cephalosporins, are used for more severe cases, involving multiple quarters or signs of systemic illness. Treatment duration typically lasts from 3 to 5 days, depending on pathogen type and clinical severity. Combining systemic and intramammary therapies can improve cure rates but requires veterinary guidance. Additionally, dry cow therapy with long-acting antibiotics upon drying off is widely used to prevent new infections and treat existing subclinical mastitis.(1)(2)(3)

Antimicrobial stewardship in mastitis management involves judicious and responsible use of antibiotics to limit resistance development and preserve drug efficacy. It includes protocols such as culture-guided selective treatment, avoiding indiscriminate use, proper dosing, and appropriate treatment duration. Mastitis control also emphasizes hygiene, milking management, and culling chronically infected cows to reduce antimicrobial dependency. Programs promoting reduced blanket dry cow therapy and focusing on targeted therapy according to pathogen susceptibility are increasingly important. These stewardship strategies aim to minimize antimicrobial residues in milk and the environment, lowering the risk of antimicrobial resistance spread while maintaining dairy productivity. (4)(5)(6)

Here is a representative image showing strategies for intramammary antimicrobial stewardship in dairy practice, emphasizing selective treatment and prevention approaches: Another graph illustrates the decline in antimicrobial use following the adoption of stewardship practices such as selective dry cow therapy and culture-based treatments, highlighting significant improvements in antibiotic reduction on dairy farms. These visuals underscore the importance of integrating stewardship with antibiotic therapy for effective bovine mastitis management. All information reflects recent expert reviews and studies from the references provided, ensuring accuracy and up-to-date understanding.(4)(5)(6)(7)(8)

9. Emerging Alternative Therapeutic Strategies

Emerging alternative therapeutic strategies for bovine mastitis focus on reducing antibiotic reliance amid rising resistance, emphasizing non-antimicrobial options like plant extracts, biological agents, and immune modulators. These approaches target pathogens such as Staphylococcus aureus and E. coli while curbing inflammation and biofilms without residues in milk. Key strategies include phytotherapy, bacteriophages, probiotics, and non-steroidal anti-inflammatory drugs (NSAIDs).(1)(2)

Phytotherapy and Herbal Extracts Plant-based treatments stand out for broad efficacy against resistant strains. Essential oils from thyme, oregano, and citrus disrupt bacterial membranes and biofilms, showing stronger inhibition when combined synergistically. Compounds like baicalein and curcumin suppress NF-κB pathways to reduce inflammation in mammary tissues, with in vivo mouse models confirming lower somatic cell counts. (2)(3). Bacteriophage Therapy Phages offer targeted lysis of mastitis pathogens like S. aureus and Streptococcus agalactiae, minimizing resistance risks compared to broad-spectrum antibiotics. Cocktails of multiple phages expand host range and counter mutants, achieving up to 90% bacterial reduction in udder models. Endolysins from phages further enhance lysis against staphylococci biofilms. (4)(2)

Probiotics and AMPs Probiotics such as Lactobacillus casei bolster mammary immunity by outcompeting pathogens and optimizing microbiota, cutting clinical cases in trials. Antimicrobial peptides like nisin match antibiotic cure rates for gram-positive infections without cross-resistance concerns. These support prevention via innate defenses.(2)(5)

NSAIDs and Other Innovations NSAIDs like meloxicam manage gram-negative cases by curbing inflammation and virulence, often sufficing alone for mild infections. Acoustic pulse therapy activates tissue repair, boosting milk yield post-treatment. (2)(6)Visuals depict phage lytic cycles for bacterial destruction (7)and NSAID mechanisms inhibiting COX pathways (8).

10. Prevention and Control Measures

Prevention and control measures for bovine mastitis focus mainly on reducing pathogen exposure, maintaining udder health, and improving farm management practices. Key strategies include enhanced milking hygiene such as pre- and post-milking teat disinfection, maintaining clean and dry bedding, proper maintenance of milking machines, and culling chronically infected cows. These measures form the cornerstone of mastitis control programs and substantially reduce infection rates.(1)

Dry cow therapy (DCT) is widely used as a preventive measure, treating all quarters of cows at the end of lactation to eliminate existing infections and prevent new ones during the dry period. However, blanket dry cow therapy is being refined with selective approaches given changing pathogen prevalence and concerns about antibiotic resistance. Additionally, maintaining teat-end integrity and avoiding teat damage through emollients in teat dips help prevent pathogen entry.(1)(2)

Vaccination against mastitis pathogens such as Staphylococcus aureus, Streptococcus agalactiae, and Escherichia coli is an adjunct preventive measure but must be paired with hygiene and culling to be effective. Optimal nutrition improving immune defenses, environmental sanitation, and fly control are vital additional components of holistic mastitis prevention strategies.(1)(3)

Visual aids often used to illustrate control measures include flow charts of five-point mastitis control plans focusing on hygiene, treatment, and culling, and graphs showing somatic cell count (SCC) trends in herds with differing management. These graphs highlight the effectiveness of prevention strategies in maintaining udder health and reducing subclinical infections.(4)(5)

Thus, effective bovine mastitis prevention combines proper milking routines, environmental management, dry period therapy, vaccination, and nutritional support, all personalized to herd needs to sustainably reduce mastitis prevalence and antibiotic use.(1)(2)(3)

11. Conclusion

Bovine mastitis remains a significant challenge in dairy farming due to its complex etiology involving various microbial pathogens, environmental factors, and host immunity. Advances in diagnostic techniques have enhanced early detection and pathogen identification, enabling more targeted and effective treatments. Simultaneously, emerging therapeutic strategies focus on reducing antibiotic usage by exploring alternative options such as phytotherapy, bacteriophages, probiotics, and immunomodulatory approaches, which show promise in combating resistant infections while maintaining animal welfare and milk safety.Preventive and control measures remain foundational to mastitis management, emphasizing good milking hygiene, environmental cleanliness, dry cow therapy, vaccination, and nutritional support to enhance udder health and reduce pathogen exposure. The integration of antimicrobial stewardship programs in dairy practice is crucial to preserving antibiotic efficacy and addressing public health concerns related to antimicrobial resistance.Overall, a multifaceted approach combining precise diagnostics, innovative alternative therapies, and robust preventive protocols offers the best potential for sustainable control of bovine mastitis. Continued research and adoption of these advances will improve dairy herd health, productivity, and economic outcomes while aligning with global calls for responsible antimicrobial use.(1)(2)(3)(4)

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