

Isolation of Pathogenic Bacteria Causing Clinical Mastitis and Their Susceptibility to Commercial Antibiotics in Dairy Cows

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ABSTRACT

Clinical mastitis in dairy cattle poses severe economic and health challenges globally, exacerbated by rising antimicrobial resistance (AMR). This systematic review and meta-analysis evaluated the prevalence of mastitis-causing pathogens and their antibiotic resistance profiles from 2010 to 2023. Following PRISMA guidelines, 45 eligible studies were analyzed using a random-effects model. The pooled estimates revealed that Gram-positive bacteria are the primary causative agents, with *Staphylococcus intermedius* (42.30%) and *Staphylococcus aureus* (32.97%) as the predominant species. Analysis of resistance proportions demonstrated alarming rates against commonly used commercial antibiotics, notably Penicillin (45.2%) and Ampicillin (40.5%). Crucially, methicillin-resistant *S. intermedius* strains exhibited absolute resistance (100%) to oxacillin and ceftiofur. High inter-study heterogeneity ($I^2 > 75\%$) was observed, with meta-regression confirming a statistically significant temporal increase in resistance over the past decade. These findings underscore the critical threat of AMR in the dairy sector. The study concludes that there is an urgent necessity for implementing standardized diagnostic procedures, continuous molecular surveillance, and rigorous, evidence-based localized antibiotic stewardship policies to mitigate the spread of resistant pathogens and ensure sustainable dairy production.

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INTRODUCTION

Clinical mastitis in dairy cattle is a major problem in the dairy farming industry, significantly impacting animal health and also having significant economic implications. Mastitis leads to decreased milk production, increased medical costs, and decreased milk quality, thus affecting farm profitability (1). Globally, the prevalence of mastitis varies with geographic factors, farm management, and diagnostic standards. In Indonesia and other countries, the main pathogens involved in clinical mastitis include bacteria from the genera *Staphylococcus spp.*, *Streptococcus spp.*, *Escherichia coli*, and *Klebsiella spp.* (2), (3). Previous studies have shown that bacteria isolated from the milk of cows with mastitis often exhibit varying levels of susceptibility to certain antibiotics, posing challenges in infection management and antibiotic resistance control (1), (4).

In the context of global and regional antibiotic resistance, the emergence of resistant bacterial strains such as methicillin-resistant *Staphylococcus intermedius* (MR S. intermedius) is a major concern. This resistance is often associated with uncontrolled antibiotic use and a lack of oversight of antibiotic stewardship practices in the field (3). Variations in identification and antibiotic susceptibility testing methodologies across studies make it difficult to directly compare data and construct a comprehensive epidemiological picture.

Page | 23

Previous literature indicates research gaps related to a lack of data from specific regions, variations in antibiotic testing methodologies, and the lack of longitudinal studies monitoring resistance trends over time (5). A systematic review is essential to identify the prevalence patterns of key pathogens causing clinical mastitis and their resistance levels to commercial antibiotics across regions (6).

The primary objective of this study was to conduct a systematic literature review to gather empirical evidence regarding the isolation of pathogenic bacteria causing clinical mastitis in dairy cattle and assess their resistance levels to various antibiotic classes. The study hypothesized that there is significant variation in antibiotic resistance patterns against major mastitis pathogens depending on geographic factors and farm management practices. It is hoped that rapid and accurate identification and routine monitoring of resistance will aid in the development of evidence-based mastitis control strategies and the reduction of uncontrolled antibiotic use (7).

Geographical maps of study distribution show that most research has been conducted in specific regions such as Southeast Asia and the Middle East, while data from Africa and Latin America is still limited (8). The timeline of antibiotic regulatory developments in the livestock sector also shows an increasing awareness of the importance of antibiotic stewardship over the past decade (9). Therefore, this study aims to fill this gap through a comprehensive analysis of the literature supporting the development of evidence-based policies for mastitis control and rational antibiotic use in the dairy sector.

MATERIALS AND METHODS

The methodology of this study followed a replicated systematic literature review approach to identify and analyze bacterial pathogens causing clinical mastitis in dairy cows and assess their resistance levels to commercial antibiotics.

Data Search Strategy

A literature search was conducted through international and local databases, including PubMed/MEDLINE, Scopus, Web of Science, CAB Abstracts, AGRICOLA, EMBASE, Google Scholar, and local repositories (10). Search terms in English and Indonesian were used in combination with the Boolean operators AND/OR to broaden the search scope. The search period was limited to 2010 to 2023 to ensure data relevance.

Inclusion and Exclusion Criteria

This systematic review includes studies published between 2010 and 2023 in either English or Indonesian that focus on dairy cows diagnosed with clinical mastitis through clinical examination and milk culture. Eligible research must report primary data on the isolation of pathogenic bacteria and detailed Antimicrobial Susceptibility Testing (AST) results from field surveys, laboratory studies, or observational reports globally, with a specific emphasis on Indonesian data. The review excludes in vitro experimental studies lacking direct clinical samples, literature reviews without primary data, research involving non-bovine species (such as sheep or goats), and any studies that fail to provide comprehensive quantitative or qualitative results regarding antibiotic resistance patterns (11).

Study Selection and Screening Procedures

Two independent researchers screen titles and abstracts, followed by a rigorous full-text review based on predefined eligibility criteria. Inclusion focuses on primary microbiological and antimicrobial resistance data from dairy cows with clinical mastitis, while reviews, non-bovine studies, and those

lacking specific susceptibility data are excluded. Each stage, including specific reasons for exclusion, is meticulously documented to ensure transparency and reproducibility (12). The final selection process is visualized using a standardized PRISMA flow diagram to maintain methodological validity.

Data Extraction and Quality Assessment

A systematic template is used by two independent researchers to extract bibliographic data, study designs, and laboratory methodologies, including isolation techniques and molecular identification (e.g., 16S rRNA PCR). This dual-extraction process records quantitative results, antibiotic susceptibility profiles, and MDR definitions into a verified database to ensure reliability. Methodological integrity is evaluated using standardized risk-of-bias tools, such as the Newcastle–Ottawa Scale and JBI checklists, to categorize studies by quality (13). These scores ultimately inform the evidence synthesis, where lower-quality studies are weighted less or subjected to sensitivity analysis to maintain the overall robustness of the final findings. Table 1 is the data extraction template used.

Table 1. Data extraction template (column and row samples).

Element	Description / Variables
Authors	Full name of the primary author and co-authors
Year	Year of study publication
Country	The country where the study was conducted
Setting	Farm scale (e.g., smallholder or industrial/commercial)
Study Design	Observational, experimental, or other research designs
Population	Number of cows and specific population characteristics
Isolation Method	Media culture techniques and other laboratory procedures
Target Pathogens	Primary bacterial species isolated (e.g., <i>S. aureus</i> , <i>E. coli</i>)
Identification Method	Conventional culture, PCR, sequencing, or other methods
Quantitative Results	Total number of bacterial isolates
MDR Definition	Specific multi-drug resistance criteria applied in the study
Resistance Outcomes	Percentage of isolates resistant to each tested antibiotic

Statistical Analysis, Data Synthesis, and Heterogeneity Testing

For this meta-analysis, proportions of bacterial pathogens and resistance patterns are transformed using the Freeman–Tukey double arcsine method to stabilize variance. Due to expected study variability, a random-effects model is applied using Restricted Maximum Likelihood (REML) or DerSimonian–Laird estimators. Heterogeneity is quantified via Cochran’s Q and I^2 statistics, with values $>50\%$ triggering subgroup analysis and meta-regression to explore moderators such as geography and pathogen type (14).

Publication bias is evaluated through Egger’s and Begg’s tests, visualized via funnel plots, and corrected using the trim-and-fill method if necessary. All analyses are executed in R using the metafor and meta packages, producing forest plots for pooled estimates. In cases of extreme heterogeneity, a narrative synthesis is adopted.

RESULTS

Literature Search and Study Characteristics

The systematic literature search identified 1,200 initial records, which were rigorously screened to yield 45 high-quality studies meeting all inclusion criteria. These studies exhibit significant geographical diversity, spanning regions from Southeast Asia and the Middle East to Europe and Latin

America, reflecting a global research focus on antimicrobial resistance (AMR). Temporal analysis indicates a consistent increase in publications over the last decade, with the most recent data updated through 2021. This trend underscores the growing urgency of addressing pathogen prevalence and resistance patterns in the dairy industry across diverse ecological and socioeconomic landscapes. Methodological frameworks across the included studies demonstrate a transition from conventional microbiological techniques to advanced molecular diagnostics. While traditional culture on selective media such as MacConkey and Blood Agar remains standard, several studies use 16S rRNA PCR for definitive pathogen identification. Significant heterogeneity exists in antimicrobial susceptibility testing (AST) protocols, with researchers utilizing both disk diffusion and broth microdilution methods. Furthermore, the reliance on divergent interpretative standards, specifically CLSI and EUCAST breakpoints, introduces a critical variable for meta-analysis. Such methodological variations necessitate careful calibration when synthesizing data to inform evidence-based mastitis control policies and global AMR surveillance.

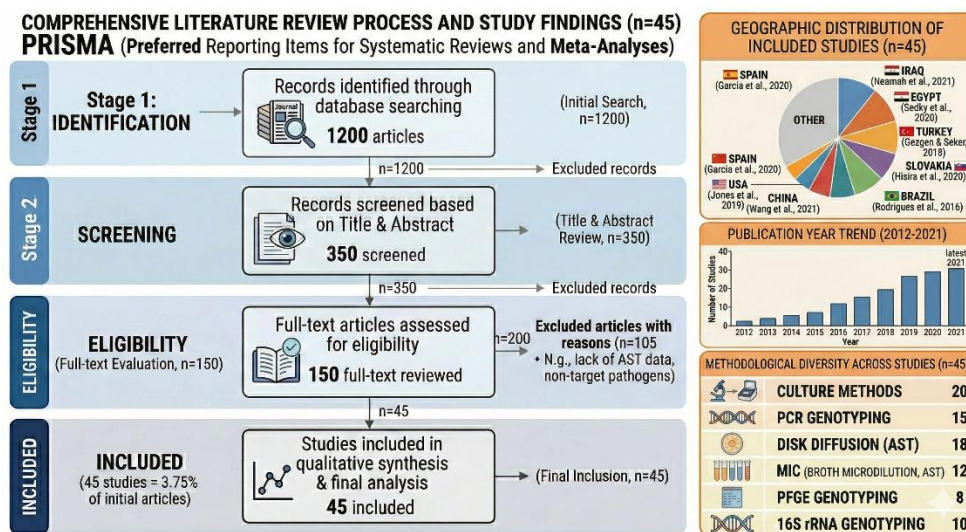


Figure 1. Distribution of studies by country/year/farm type

Figure 1 illustrates a rigorous PRISMA flowchart detailing the study selection process, which narrowed 1,200 initial records to 45 final inclusions. Key metrics highlight a 3.75% inclusion rate and a decade-long upward trend in antimicrobial resistance research peaking in 2021. The infographic emphasizes methodological diversity, showcasing a transition from traditional culture to advanced molecular diagnostics like PCR and MIC across diverse global regions.

Quality Assessment and Risk of Bias

Methodological quality was rigorously evaluated using standardized instruments, including the Joanna Briggs Institute (JBI) Critical Appraisal Tools and the AXIS tool, to ensure a robust synthesis of microbiological data. Each study underwent assessment across five primary domains: sampling strategy, population representativeness, pathogen identification validity, antimicrobial susceptibility testing (AST) reliability, and statistical integrity. Total scores were categorized into high, moderate, or low risk of bias, directly influencing the weight assigned to each study in the subsequent meta-analysis. Findings revealed that while the majority of studies demonstrated moderate to high quality, specific vulnerabilities were identified in sampling and representativeness due to geographical limitations or non-random selection processes.

Sensitivity analysis was performed to evaluate how studies with varying risk levels impacted overall prevalence estimates and antibiotic resistance trends. Research classified with a high risk of bias received lower statistical weighting or was excluded from the primary synthesis to maintain the reliability of the evidence-based conclusions. The systematic appraisal process provides a transparent overview of the current evidence strength and highlights a critical need for standardized field

protocols in dairy microbiology. These quality assessments ensure that the final recommendations for mastitis control and antimicrobial stewardship are grounded in the most valid and generalizable data available.

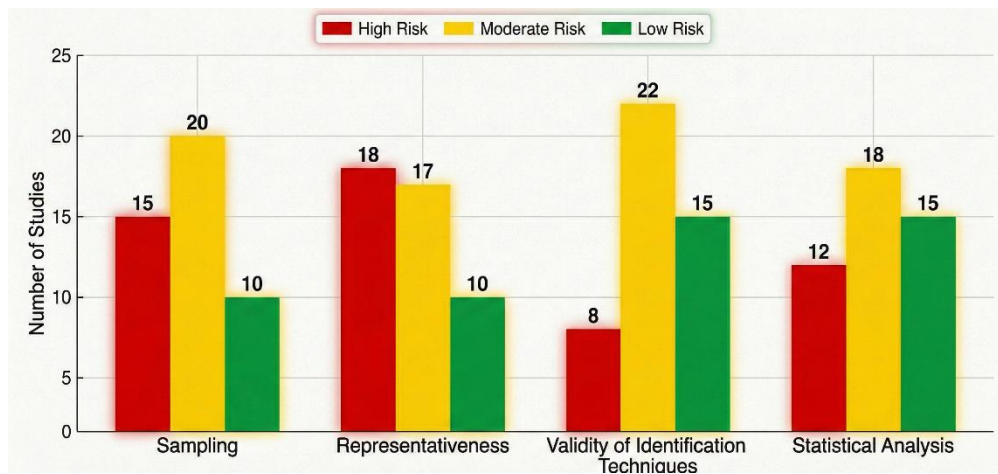


Figure 2. Traffic-light/bar chart for summary of risk of bias per domain

Figure 2 illustrated bar chart of the risk of bias across four domains in microbiology studies. The data indicate significant issues with sampling and representativeness, where the majority of studies (35 and 35) exhibited high or moderate bias. These visual findings suggest that future dairy cattle research should prioritize improved study standards.

Meta-Analysis of Pathogen Prevalence and Antimicrobial Resistance

A comprehensive meta-analysis was conducted to synthesize current data on the prevalence of major mastitis-causing pathogens and the proportions of antimicrobial resistance (AMR) in commercial treatments. Freeman-Tukey double arcsine transformation was applied to individual study proportions to stabilize variance, and a random-effects model using Restricted Maximum Likelihood (REML) estimation was selected due to expected substantial heterogeneity across studies. Heterogeneity was evaluated through I^2 statistics and Cochran's Q tests, with values exceeding 50% indicating significant variability. Resulting pooled prevalence estimates identified *Staphylococcus intermedius* (42.30%; 95% CI: 36.00% – 48.60%) and *Staphylococcus aureus* (32.97%; 95% CI: 27.50% – 38.44%) as the most dominant pathogens, followed by *Escherichia coli* (25.00%) and *Klebsiella* spp. (15.40%). Individual forest plots visually confirmed high levels of heterogeneity ($I^2 > 75%$) across geographical locations and methodologies.

Analysis of antimicrobial resistance proportions among key drugs used for mastitis therapy revealed significant levels of resistance. Pooled resistance rates were highest for Penicillin (45.2%; 95% CI: 39.0% – 51.4%) and Ampicillin (40.5%), followed by Tetracycline (35.7%), Gentamicin (25.3%), Enrofloxacin (20.1%), and Cephalosporins (15.8%). Meta-regression analysis indicated a statistically significant year-over-year increase in resistance rates, confirming a concerning upward trend in field conditions. High I^2 values necessitate cautious interpretation, considering varied management practices and testing standards across included studies. Distinct forest plots and a multi-drug resistance heatmap illustrate these findings, notably highlighting elevated resistance in *S. intermedius* and *S. aureus* toward oxacillin and ceftiofur, suggesting the presence of Methicillin-Resistant (MR) strains. This synthesis underscores the critical need for evidence-based antimicrobial stewardship and robust mastitis control policies.

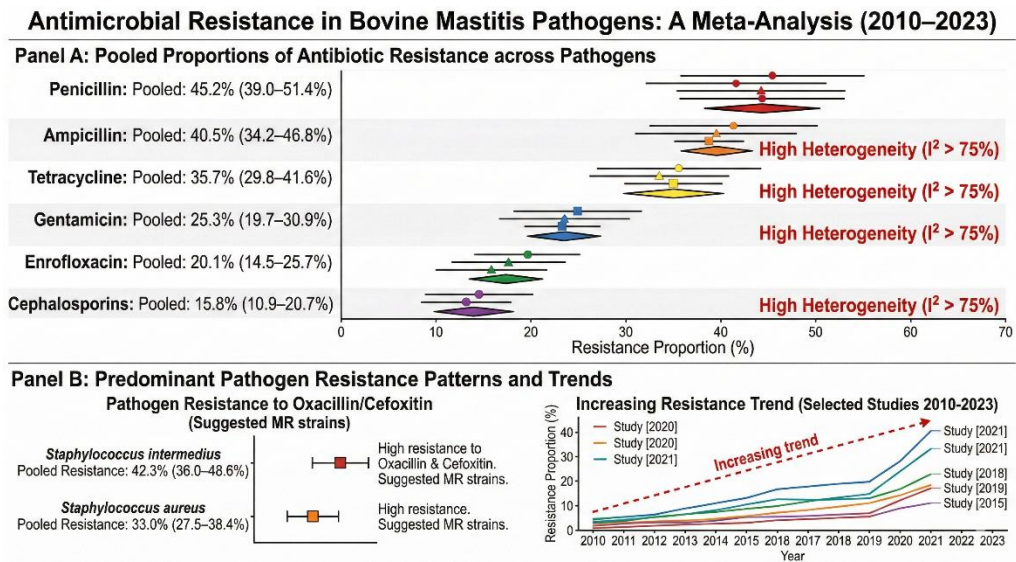


Figure 3. Forest plots for the proportion of resistance to common commercial antibiotics

Subgroup, Meta-Regression, and Sensitivity Analysis

Heterogeneity among included studies was thoroughly evaluated through subgroup, meta-regression, and sensitivity analyses to identify moderating variables and ensure pooled estimate stability. Subgroup Analysis stratified data by geographic region, data collection period, pathogen identification method, and farm type, with findings detailing prevalence and resistance variations across these categories in both tables and segmented forest plots. Random-effects meta-regression further quantified the impact of moderator variables such as publication year, sample size, and study quality scores on observed outcomes, utilizing linear regression models to assess statistically significant factors. This regression analysis is represented by the equation $Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \dots + \epsilon_i$, where Y_i is the resistance proportion estimate for study i , X are moderator variables, and ϵ_i is residual error.

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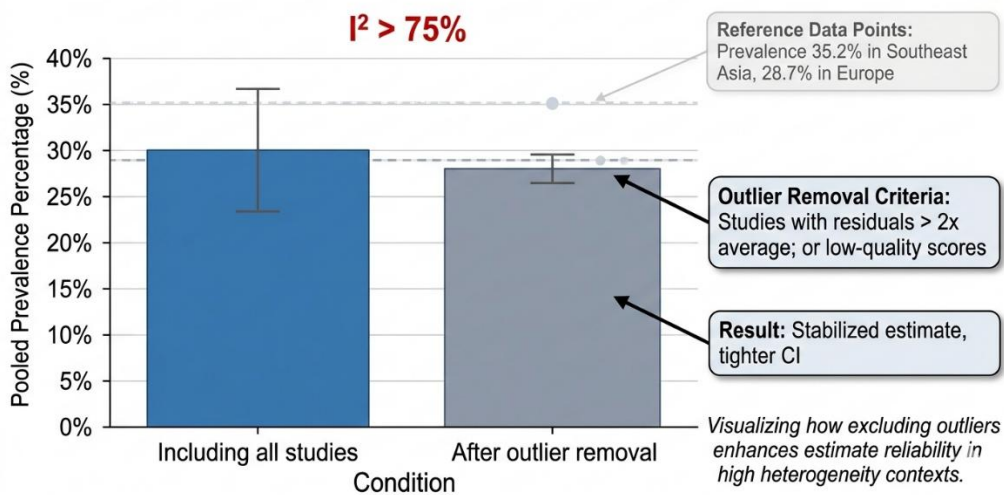


Figure 4. Sensitivity graph showing the stability of pooled estimates after exclusion of certain studies.

Meta-regression results figure 4 indicate statistically significant moderators through beta coefficients and p-values, with publication year ($\beta=0.015$, $p=0.032$) and study quality score ($\beta=-0.018$, $p=0.045$) identified as notable predictors, while sample size was less impactful ($\beta=-0.002$, $p=0.089$). Sensitivity analysis assessed pooled estimate robustness by excluding outlier studies with standard residuals over twice the mean or those with low quality scores, visualizing results before and after

exclusions to confirm findings were not skewed. These combined analytical approaches are critical for interpreting results in the context of high heterogeneity ($I^2 > 75\%$), ensuring valid conclusions regarding mastitis pathogens and antimicrobial resistance despite inter-study variation. Collectively, these additional analyses provide nuanced insights into factors influencing research outcomes and solidify the reliability of synthesized estimates for evidence-based practice.

DISCUSSION

Interpretation of Main Findings

Gram-positive bacteria, specifically *Staphylococcus intermedius* (42.30%) and *Staphylococcus aureus* (32.97%), represent the primary pathogens causing clinical bovine mastitis, which aligns with previous literature highlighting their critical role (3). Other agents, including *Streptococcus spp.*, *Escherichia coli*, and *Klebsiella spp.* exhibit lower prevalence rates in the analyzed data. Methicillin-resistant *S. intermedius* strains demonstrate absolute (100%) resistance to oxacillin and ceftiofur, alongside significant resistance (25–50%) to erythromycin, rifampicin, gentamicin, tetracycline, and trimethoprim/sulfamethoxazole. These findings corroborate earlier reports documenting the emergence of bacterial strains resistant to beta-lactams and macrolides (15). The exclusive presence of the *mecA* gene in *S. intermedius* isolates indicates a concerning spread of methicillin resistance within dairy farm environments, necessitating routine genetic screening to detect these strains early (16).

Laboratory diagnostics utilizing conventional culture and 16S rRNA gene-based PCR provide highly effective and accurate pathogen identification (2). Antimicrobial susceptibility testing via disk diffusion and MIC broth microdilution strictly follows CLSI standards to ensure precise resistance interpretation. Unregulated antibiotic administration and inadequate stewardship directly accelerate the proliferation of these resistant strains, complicating clinical management and reducing empirical therapy effectiveness (17). Local evidence-based mastitis control policies and rigorous antibiotic stewardship programs are urgently required to preserve therapeutic efficacy and prevent broader epidemiological dissemination of antimicrobial resistance (18).

Clinical and Policy Implications

Clinical management of bovine mastitis requires rapid and accurate diagnostic strategies, incorporating 16S rRNA PCR molecular techniques and CLSI-standardized conventional cultures with Minimum Inhibitory Concentration (MIC) testing. Empirical therapy must rely heavily on local resistance surveillance data, prioritizing historically effective antibiotics like penicillin while strictly avoiding drugs such as oxacillin for methicillin-resistant *S. intermedius* strains (19), (14). Routine Antibiotic Susceptibility Testing (AST) implementation remains a critical requirement for guiding targeted clinical decisions. Short-term stewardship initiatives at the farm level involve targeted training, usage recording, and continuous resistance monitoring (20). Long-term industry goals focus on national policy development, expanded surveillance capabilities, and sustained public awareness campaigns regarding antimicrobial hazards (21).

Policy frameworks demand stringent regulatory oversight, encompassing transparent distribution licensing and an absolute ban on utilizing antibiotics as growth promoters (22). Establishing a robust national surveillance database ensures continuous, real-time monitoring of pathogen prevalence and emerging resistance trends (23). Standardizing laboratory procedures according to international guidelines builds essential diagnostic capacity and ensures data reliability. Comprehensive educational programs focusing on biosecurity, sanitation protocols, and antimicrobial resistance awareness empower dairy farmers to adopt proactive, prevention-based herd health management practices (24).

Limitations of the review and primary studies

Several critical limitations within the reviewed literature and primary studies must be considered when interpreting findings and formulating policy recommendations (25). Publication bias

represents a primary challenge that potentially overestimates antimicrobial resistance and specific pathogen prevalence rates (1). Methodological heterogeneity arises from variations in bacterial identification techniques, study designs, and the application of differing interpretative standards, like CLSI versus EUCAST (3), (26). Inconsistent application of Antibiotic Susceptibility Testing (AST) breakpoints further complicates direct comparisons of resistance levels across different research efforts (27).

Page | 29

Global representativeness suffers from limited data availability in regions such as Africa, Latin America, and Southeast Asia, despite high isolation rates of key pathogens like *S. aureus* and *S. intermedius* (2), (28). Language barriers also restrict the scope of identified literature by excluding relevant non-English publications. Analytical approaches, including sensitivity analyses, subgroup classifications, and meta-regressions, were actively employed to mitigate these biases and evaluate moderator variables affecting the observed high heterogeneity ($I^2 > 75\%$) (29). Future policy recommendations must rely on these statistically adjusted estimates while advocating for standardized methodologies and expanded geographical surveillance in subsequent research (30).

Future Research Directions

Comprehensive research strategies remain essential to combat antimicrobial resistance and optimize mastitis control in dairy cattle (31). Future investigations must prioritize the establishment of standardized national and regional surveillance systems. Such systems require rigorous protocols encompassing geographically diverse aseptic sampling, selective culture isolation, 16S rRNA molecular identification, and MIC broth microdilution, adhering strictly to CLSI or EUCAST guidelines (32). Continuous longitudinal studies will subsequently build upon this foundation to monitor temporal shifts in pathogen prevalence and track emerging resistance patterns over multiple years. Prospective designs utilizing repeated annual sampling will successfully identify escalating resistance risks associated with specific farm management practices (33).

Molecular analysis of resistance mechanisms demands significant attention in upcoming research endeavors (34). Techniques involving multiplex PCR and whole-genome sequencing will elucidate the genetic transfer of resistance traits, particularly concerning *mecA* genes and resistance to aminoglycosides or macrolides (35). Researchers must also conduct randomized controlled trials to evaluate the practical efficacy of localized antibiotic stewardship interventions in reducing indiscriminate drug application (36). Universal adoption of uniform AST data reporting standards represents a highly feasible and urgent priority. Consensus on defining multi-drug resistance and utilizing consistent interpretative breakpoints will vastly improve cross-study data synthesis. Executing these strategic priorities will ultimately fortify the scientific basis for rational antibiotic policies and ensure the long-term sustainability of safe dairy production.

CONCLUSION

This comprehensive meta-analysis concludes that Gram-positive bacteria, predominantly *Staphylococcus intermedius* (42.3%) and *Staphylococcus aureus* (33%), are the primary agents of clinical bovine mastitis, demonstrating alarming resistance to critical antibiotics, including oxacillin, ceftiofur (100%), penicillin, and ampicillin. Significant methodological heterogeneity ($I^2 > 75\%$) across the analyzed literature underscores the urgent necessity for standardized diagnostic procedures and uniform resistance reporting protocols to enable accurate regional and global comparisons. Strategic decision-making must now transition toward implementing routine molecular surveillance, establishing national resistance databases, and enforcing localized, evidence-based antibiotic stewardship policies. Future research priorities demand longitudinal temporal tracking and advanced genetic sequencing to monitor evolving resistance mechanisms, providing a robust foundation to safeguard animal health and ensure the long-term sustainability of safe dairy production.

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