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Uncovering Extended-Spectrum Beta-Lactamase (ESBL)-Producing Enterobacteriaceae: A Review of Mastitic Milk in Dairy Farming

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Abstract

The global rise of antimicrobial resistance has attracted urgent attention, as further research is needed to understand the transmission mechanisms of ESBL genes in Dairy Farming. This systematic review compiles recent evidence on the prevalence, predisposing factors, genetic characteristics, and antimicrobial resistance patterns of ESBL-producing bacteria discovered from livestock, particularly on dairy cattle and infections related to mastitis. The meta-analysis indicates that ESBL-producing Enterobacteriaceae, *Escherichia coli*, was recorded in 54.1% of milk samples (95% CI: 44.1- 63.9%), and *Klebsiella pneumoniae* was recorded at 24.1-53.3%. This review focused only on Enterobacteriaceae organisms in Sub-Saharan Africa, which had the highest rates, especially in Nigeria (66.4%). Presence of blaTEM and blaCTX-M genes in both humans and animals indicates potential transmission between species. Findings suggest a widespread occurrence of ESBL-Producing Enterobacteriaceae in livestock populations, with considerable genetic diversity and resistance profiles that improve therapeutic management. Several studies highlighted the potential for zoonotic spread through direct animal contact and consumption of contaminated milk. These findings underscore the urgent need for harmonized surveillance systems, standard prevention strategies, and a One Health approach to reduce antimicrobial resistance. Future research should focus on longitudinal surveillance and consider the role of environmental and farm management factors to elucidate antimicrobial resistance dynamics.

INTRODUCTION

The spread of Extended-Spectrum Beta-lactamase (ESBL)-producing Enterobacteriaceae has increased, emphasizing the need to understand its prevalence and risk factors associated with mastitic cattle [1]. In Nigeria, this is of significant concern because not only does it affect the health and well-being of cattle but has economic implications [2]. Previous studies suggest that 70% of raw milk samples contain *Escherichia coli*, which is one of the family of Enterobacteriaceae [3]. Isolates were separated with ethidium bromide and visualized using gel electrophoresis, and staining reveals Extended Spectrum Beta-lactamase genes [4]. The emergence of ESBL-producing Enterobacteriaceae has indeed added a layer of complexity to this issue, as Cattle are considered key carriers of *E. coli* [6]. These bacteria are increasingly prevalent, and this lack of understanding makes it more difficult to conduct research to support surveillance efforts to mitigate the spread of Antimicrobial Resistance in household settings where both humans and Animals intersect, which is why this review is crucial. The global rise of milk contamination caused by Enterobacteriaceae in the farming sector demands urgent attention [7].

Further review is needed to understand the total pooled prevalence and the transmission mechanisms of AMR, which is of critical public health concern. In some countries located in regions like Asia and other selected countries in sub-Saharan Africa, there have been policy implementations on the regulation of antimicrobial use, but ESBL-producing Enterobacteriaceae still continue to increase [8]. By reviewing the prevalence, predisposing factors, antimicrobial resistance mechanisms, and molecular basis underlying ESBL-producing Enterobacteriaceae in mastitic cattle, we take an important step toward unveiling this hidden threat. Dairy farming is the focus of the review, aimed at synthesizing the existing knowledge and providing evidence to enhance good clinical practice for farmers [8,9]. There is a need to provide data from the above-stated region to provide good intervention strategies to prevent the spread of Extended Spectrum Beta-lactamase genes in Nigerian dairy [10]. The aim is to determine the trends, predisposed factors, identify knowledge and regional gaps, as well as develop strategies to mitigate the spread and reduce public health risk [11].

Mastitis in dairy cattle worsens the issue, increasing antibiotic use and creating conditions for resistant bacteria to thrive [12–14]. Infections caused by ESBL-producing organisms lead to economic losses, reduced milk production, and animal welfare concerns [15,16]. Humans are also at risk through contact with infected animals or contaminated milk, posing a zoonotic threat [17–22]. Cattle are a milk source and also carriers of AMR Enterobacteriaceae, which requires a one-health approach [23–25]. Exploring the molecular nature underlying the ESBL-producing Enterobacteriaceae and antimicrobial resistance patterns in mastitic cattle will help us understand the transmission mechanisms to develop good mitigating strategies to control the spread [26–28]. Mastitis cases pose a significant global health threat [29,30]. The emergence of new ESBL variants has worsened the issue, but data on their molecular epidemiology is limited, especially at the farm level [31–33].

This study compares epidemiological patterns across regions, providing insights into the global burden of ESBL-producing bacteria in cattle [37,38]. The findings can inform policy decisions, improve antimicrobial stewardship, and guide veterinary practices [39–46]. By addressing antimicrobial resistance across human, animal, and environmental domains, this review aligns with One Health principles [52–54].

METHODS

Search Strategy

In this study, we examined how writers express their thoughts in a microbiological context investigating ESBL-producing Enterobacteriaceae in mastitic cattle, which was carried out using a region-specific approach to synthesize existing knowledge and evidence-based practice. Literature research was carried out using PRISMA guidelines to identify relevant studies up to 2025 [55]. Search was conducted using several databases, including PubMed, Google Scholar, ScienceDirect, and Scopus; the Scopus database was not used due to restrictions imposed by some institutions. Database search was done using keywords like Mastitis, Molecular nature, OR ESBL-producing Enterobacteriaceae, "OR" Bovine Mastitis " AND" "ESBL-producing Enterobacteriaceae" OR "mastitic milk "mastitic cattle". With the aim of synthesizing findings on the prevalence, Predisposing factors, and Molecular nature underlying ESBL production in mastitic cattle.

In order to develop an effective control strategy to support surveillance systems against the spread of ESBL-producing Enterobacteriaceae raised in various farms. With the limited evidence given, the final search was done on April 12, 2025. Studies were screened for relevance, and those not having a focus on ESBL-producing Enterobacteriaceae were excluded from this study. A meta-analytical approach was used by combining data from several studies to give an estimated prevalence of ESBL-producing Enterobacteriaceae in mastitic cattle. Studies were screened and selected based on the association between ESBL-producing Enterobacteriaceae species; peer-reviewed publications in English were included, and case reports, reviews, and conference papers were excluded when conducting this review. Data management was done using bibliographic details in the PRISMA checklist items.

Data Extraction

Extracted data from various studies included variables like study ID, authors, year of publication, and sample size. Studies carried out from 2010 to 2025 were included in this study. RevMan software was used to estimate the pooled prevalence, with the application of a random-effects model to solve the issue of heterogeneity. A meta-analysis using RevMan software estimated the pooled prevalence, applying a random-effects model to address heterogeneity (I^2 statistic). Quality of studies was evaluated with the Newcastle-Ottawa scale, providing a comprehensive overview of antimicrobial resistance in mastitic cattle. Important data on the study design, characteristics of the participants, and related outcomes of ESBL-producing Enterobacteriaceae in mastitic cattle were extracted. The risk of bias assessment was done for studies included in the review, evaluating items like Study ID, title, authors, year of publication, study methodology, and outcomes.

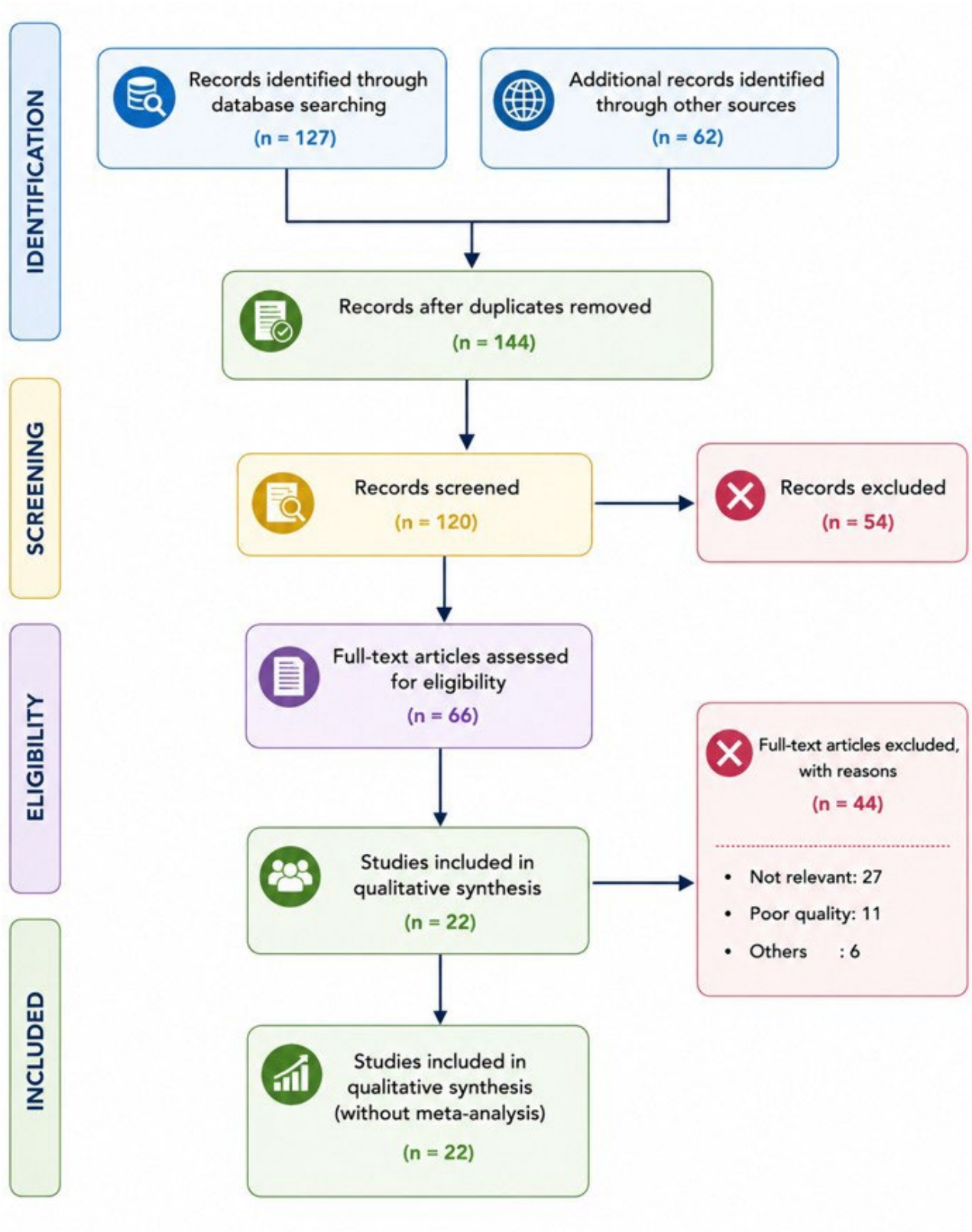


Figure 1. PRISMA flow chart of selected studies.

Table 1. Summary of some selected articles, names of journals, and years of publication included in this study.

Authors	Title	Journal	Results (Rephrased)	Outcomes (Rephrased)	Conclusion (Rephrased)	Risk of Bias
[2]	Prevalence and antimicrobial resistance of bovine subclinical mastitis pathogens in Bangladesh	Heliyon	SCM occurred in 64.9% of cows and 82.7% of herds.	ESBL genes detected included blaTEM, blaOXA, blaCTX-M1, blaCTX-M2, DHA.	Isolates showed resistance to penicillins, cephalosporins, and tetracycline.	2/5
[4]	ESBL-producing <i>E. coli</i> in dairy cattle with endometritis in China	BMC Veterinary Research	69.8% pathogen positivity with <i>E. coli</i> dominating isolates.	TEM (78%) and CTX-M (32%) ESBL genes found; many isolates were MDR and integron-positive.	High occurrence of drug-resistant ESBL <i>E. coli</i> in infected dairy cows.	2/5
[5]	AMR patterns of <i>E. coli</i> from dairy farm environment and milk	Antibiotics	66.1% prevalence of <i>E. coli</i> across farms.	Few isolates confirmed as ESBL producers.	Dairy environments may act as reservoirs for antimicrobial resistance.	2/5
[8]	Phylogeny and antibiogram of ESBL <i>E. coli</i> from bovine mastitis	Tropical Animal Science Journal	Isolates verified by 16S rRNA PCR.	Most strains belonged to phylogroup A, followed by D, B1, B2.	Mastitis isolates showed frequent MDR ESBL <i>E. coli</i> .	3/5
[9]	Phylogenetic characterization of ESBL <i>E. coli</i> from animals and humans	Microbial Drug Resistance	60 isolates confirmed as ESBL producers.	Major genes: TEM, CTX-M-15, CTX-M-1, CTX-M-9; ST131 dominant sequence type.	Evidence suggests cross-transmission between animals and humans.	2/5
[10]	Diversity of blaTEM and blaCTX-M genes in mastitis coliforms	Journal of Animal Science and Veterinary Medicine	<i>E. coli</i> showed highest prevalence (44%) among isolates.	Regional variation observed, with Plateau South highest prevalence.	Genetic similarity across regions indicates potential zoonotic spread.	3/5
[11]	Phenotypic and genotypic study of ESBL <i>E. coli</i> in mastitis cows	Open Access Research Article	<i>E. coli</i> detected in 80–86% of mastitis cases.	Many isolates remained susceptible to fluoroquinolones and gentamicin.	Certain antibiotics remain effective against ESBL isolates.	3/5
[12]	ESBL <i>E. coli</i> occurrence in livestock and farm workers	PLOS ONE	Isolates analyzed using MLST typing.	CTX-M enzymes were the most common ESBLs.	ESBL <i>E. coli</i> circulated among animals and farm workers.	2/5
[13]	MDR ESBL <i>E. coli</i> distribution in a dairy herd	Antibiotics	ESBL <i>E. coli</i> found in calves, cows, environment, and waste milk.	All isolates classified as multidrug resistant.	Multiple farm sources contribute to spread of MDR ESBL strains.	2/5
[14]	MDR ESBL <i>E. coli</i> from cattle farms in Ibadan	Medical Sciences Forum	36.4% ESBL producers and 60.7% MDR isolates detected.	Highest resistance observed to amoxicillin-clavulanate and pefloxacin.	ESBL <i>E. coli</i> in cattle represents public health risk due to cephalosporin resistance.	2/5
[15]	Molecular characterization of MDR <i>E. coli</i> from mastitis milk	Journal of Food Protection	Susceptibility testing done via disk diffusion.	38.4% isolates MDR; tetA and blaTEM most frequent genes.	Multiple resistance determinants detected in mastitis isolates.	3/5
[16]	ESBL <i>E. coli</i> in raw milk from smallholder dairy farms	Veterinary World	High resistance to ampicillin, cefotaxime, gentamicin.	Many isolates exhibited extensive MDR.	Over half of isolates carried ESBL traits, posing health risks.	2/5
[21]	Detection of blaTEM and blaCTX-M in dairy milk <i>E. coli</i>	Journal of Veterinary Research	91.8% <i>E. coli</i> isolation rate from samples.	Few isolates showed multidrug resistance.	ESBL gene detection highlights emerging AMR concerns.	3/5
[22]	ESBL and cefotaxime-resistant bacteria in cow milk	Israel Journal of Veterinary Medicine	23.8% of cows carried cefotaxime-resistant bacteria.	Species identified included <i>E. coli</i> , Enterobacter, and Pseudomonas.	Resistant bacteria are present in dairy milk samples.	3/5
[31]	Enteropathogenic <i>E. coli</i> in mastitis cows	Journal of Veterinary Research	198 mastitis milk samples analyzed.	Tested virulence genes typical of diarrheagenic strains were absent.	Mastitis isolates differed from known diarrheagenic <i>E. coli</i> types.	3/5
[37]	ESBL Enterobacteriaceae in cattle production worldwide	Heliyon	Resistant bacteria circulate through animal production systems.	Food chain acts as a vehicle for AMR gene dissemination.	ESBL organisms in livestock represent a global public health concern.	2/5
[45]	Effect of ESBL <i>E. coli</i> on bovine mammary epithelial cells	Pakistan Veterinary Journal	Infection increased ROS production and mitochondrial damage.	Apoptosis markers (bax/bcl-2, caspase-3) were elevated.	ESBL <i>E. coli</i> can induce apoptosis in mammary cells.	3/5
[47]	ESBL <i>E. coli</i> from cattle feces in Nigeria	International Journal of Pathogen Research	55.6% screening positive and 44% confirmed ESBL producers.	Imipenem showed highest effectiveness.	ESBL-producing <i>E. coli</i> are present in cattle fecal samples.	3/5

Data Synthesis

Narration of the synthesized data was done, including details of studies from research and meta-analyses explored among studies. Items such as variables, titles, results or findings, measurements, study ID, authors, publication year, populations, exposure, selected (NOS), study design, age range, sample size, ESBL-producing Enterobacteriaceae, and outcome(s) reported were data that were assessed in the included studies. An assessment of the prevalence of ESBL-producing Enterobacteriaceae was conducted, with several studies suggesting that *E. coli* and *Klebsiella pneumoniae* were discovered across regions.

RESULTS

After conducting this review, we discovered 189 entries, and 66 were only screened when other studies were excluded from this review due to massive amounts of research in relevant records and unrelated articles. We further evaluated 20 studies in qualitative synthesis and 20 studies in Quantitative synthesis. The small number of papers recorded indicates expansion of our selection criteria. 22 papers on ESBL-producing Enterobacteriaceae in mastitic cattle covered only Asia, Sub-Saharan Africa, and South America. Summarized findings on ESBL-producing Enterobacteriaceae suggest that *Escherichia coli* and *Klebsiella pneumoniae* are the only organisms recorded in most of the articles reviewed between 2010 and 2025. The meta-analysis indicates that ESBL-producing *Escherichia coli* recorded 54.1% of milk samples (95% CI: 44.1-63.9%) and *Klebsiella pneumoniae* recorded 24.1-53.3%. Other percentages of organisms could be transient or visiting organisms' infection, which might be due to cross-contamination. In summing all the prevalence, Enterobacteriaceae Sub-Saharan African studies had the highest rates, especially in Nigeria (66.4%) [56].

European rates varied from 1% in Scandinavia to 54% in southern America [57]. The I-squared score of 94.5% showed high heterogeneity between studies, meaning they differed significantly in design, demographics, and other factors [59]. Since the focus was on prevalence, the pooled prevalence was estimated using prevalence ratio and CI. The meta-analysis found a pooled prevalence of 54.1% for ESBL-producing *E. coli* and *k. pneumonia* in cattle suffering from Mastitis, indicating a serious threat [60]. Prevalence varied across locations, highlighting the need for region-specific strategies to curb antibiotic resistance [61]. The high heterogeneity suggests factors like study design, demographics, and geography influence ESBL-producing *E. coli* and *k. pneumonia* prevalence, requiring further research to identify these mechanisms.

DISCUSSION

Findings of this review suggest sporadic transmission mechanisms of Extended Spectrum Beta-lactamase lactamase genes from organisms belong to Enterobacteriaceae in Dairy Farming indicating high contamination in mastitic milk, transmissible to human through food chain in household settings emphasizing the need to support surveillance efforts in developing mitigating strategies by conducting research to investigate and focus on the prevalence, associated risks antimicrobial resistance profile and also determine the molecular biology of this Enterobacteriaceae to understand the targeted therapeutics areas at the end of this study the prevalence of species belonging to the family of Enterobacteriaceae. Summarized findings on ESBL-producing Enterobacteriaceae suggest that *Escherichia coli* and *Klebsiella pneumoniae* are the only organisms recorded in most of the articles reviewed between 2010 and 2025. The meta-analysis indicates that ESBL-

producing *Escherichia coli* was recorded in 54.1% of milk samples (95% CI: 44.1-63.9%), and *Klebsiella pneumoniae* was recorded in 24.1-53.3%. Other percentages of organisms could be transient or visiting organisms' infection, which might be due to cross-contamination. In summing all the prevalence, Enterobacteriaceae sub-Saharan African studies had the highest rates, especially in Nigeria (66.4%) [62]. The presence of these genes, such as blaTEM and blaCTX-M, in both humans and animals, specifically cattle, indicates that these Enterobacteria can be transmitted between species, making consumers of mastitic milk vulnerable to acquiring the genes [63, 64]. Studies from Bangladesh, Indonesia, and Nigeria suggest variability in resistance due to the ongoing evolution and diversity of resistant Enterobacteria in livestock farming [65].

Multiple drug resistance makes it difficult to develop an effective control strategy against infection caused by my Enterobacteriaceae, leading to mastitis in cattle, which has suggested physical resistance profiles [66]. The importance to public health is significant, including the risk of transmission between animals and humans, most especially among dairy farmers [67, 68]. In order to prevent the spread of ESBL-producing Enterobacteriaceae, there is a need for a comprehensive one-health approach, including responsible antimicrobial use, good clinical practice, and proper farm management systems [69].

Evidence from several studies is a bit patchy, affecting the reliability of evidence-based practice [70]. Studies that utilize robust molecular methods and transparent sampling techniques are more reliable. Likewise, research containing mixed samples and unclear study design is prone to bias and less applicable [71]. Before reporting clarity on sample size, the criteria for selection or laboratory procedures reduce confidence in the result [72]. There is a need to carry out more research with bigger sample representation, with a clear study design and a standardized approach [73]. Longitudinal studies would be great to better understand the antimicrobial resistance mechanisms over time, with priority given to environmental and management factors, which could also help us to discover how antimicrobial resistance is developed in livestock farms [74].

The Quantitative analysis indicates that half of the cattle tested harbor ESBL-producing Enterobacteriaceae with a pooled prevalence of 54.1% [75]. There is also a regional difference, with Sub-Saharan Africa recording the highest rates. The huge difference between studies indicates that prevalence is influenced by research methods, populations, and geographical location [75]. In order to reduce the burden of this complex issue, there is a need to understand these variations and inform strategies to reduce ESBL-producing Enterobacteriaceae in cattle [76, 77].

However, the approach to monitoring varies, with some studies taking a broad view and others focusing on specific locations, like Tulungagung, Indonesia, or Ibadan, Nigeria. This diversity is crucial for understanding the global impact of ESBL-producing *E. coli* in dairy production, but it also raises concerns about data consistency and generalizability [78]. The studies have varying risks of bias, with some having methodological flaws or small sample sizes. To improve comparability and reliability, future research should aim for larger, more diverse samples and standardized procedures. Studies with clear sample frames and molecular methods tend to be more trustworthy [8,12,14,16]. On the other hand, research with vague sampling techniques is more likely to be biased, as heterogeneity and lack of specificity can undermine the validity of the findings [10,18,20,78].

Table 4. PRISMA checklist items.

Section and topic	Item	Checklist item	Page number
Title and Abstract	1	Uncovering ESBL-Producing <i>Escherichia coli</i> and <i>Klebsiella pneumoniae</i> in Mastitic Cattle: Systematic Review and Meta-analysis	1
Abstract	2	(ESBL)-producing <i>Escherichia coli</i> and <i>Klebsiella pneumoniae</i> pose a significant threat to animal and public health. Findings reveal widespread distribution of ESBL producers in livestock, with considerable genetic heterogeneity and multidrug resistance complicating treatment strategies.	2
Introduction	3		
Rationale		The prevalence of ESBL-producing bacteria in cattle poses a significant concern in veterinary medicine and public health.	2
Objective		To synthesize data on the prevalence of ESBL-producing <i>E. coli</i> and <i>Klebsiella pneumoniae</i> in cattle globally.	2
Methods	4		8
Protocol registration	4	Protocol was prepare but not registered	
Eligibility criteria		Studies that reported the prevalence of ESBL-producing <i>E. coli</i> or <i>Klebsiella pneumoniae</i> in cattle.	9
Information sources	4	Various databases were searched for relevant studies. Including PubMed, in both text word and MeSH term format, Google Scholar, Semantic Scholar, Cochrane Library, and ScienceDirect.	9
Search strategy	4	Keywords likely included ('ESBL-producing <i>E. coli</i> ' OR 'ESBL-producing <i>Escherichia coli</i> ' OR ' <i>E. coli</i> ' AND ' <i>Klebsiella pneumoniae</i> ') OR 'extended-spectrum beta-lactamase-producing <i>E. coli</i> ' AND ' <i>Klebsiella pneumoniae</i> ') AND ('cattle' OR 'bovine' OR 'livestock') AND ('prevalence' OR 'risk factors' OR 'molecular characteristics').	9
Study selection	4	Studies were selected if they studied the association between ESBL-producing <i>E. coli</i> , <i>Klebsiella pneumoniae</i> , udder mastitis, and cattle. Only peer-reviewed works published in English were examined.	10
Data item	4	Study ID, sample size, number positive for ESBL, prevalence, and other relevant variables.	10
Risk of bias assessment	4	The risk of bias in the included studies varies, with some studies having smaller sample sizes or methodological limitations.	35
Results	5		26
Study selection		22 independent studies were included in the meta-analysis.	
Study characteristics	5	Studies were conducted in various regions and reported the prevalence of ESBL-producing <i>E. coli</i> and <i>Klebsiella pneumoniae</i> in mastitic cattle.	27
Synthesis of results	5	The pooled prevalence of ESBL-producing <i>E. coli</i> in milk samples was 54.1% (95% CI: 44.1-63.9%) and <i>Klebsiella pneumoniae</i> (95% CI: 24.1-53.3%)	28
Summary of findings		The meta-analysis found a high pooled prevalence of ESBL-producing <i>E. coli</i> but low ESBL-producing <i>K. pneumonia</i> in mastitic cattle, with significant implications for public health.	
Limitation	6	High heterogeneity between studies, Limited data on the prevalence of ESBL-producing <i>Klebsiella pneumoniae</i> in mastitic cattle, and Potential biases in the included studies	36
Conclusion	7	The findings highlight the need for effective strategies to reduce the prevalence of ESBL-producing <i>E. coli</i> in cattle and mitigate the risk of transmission to humans.	38

CONCLUSION

Spread of Extended Spectrum Beta-lactamase (ESBL)-producing Enterobacteriaceae has increased, emphasizing the need to understand its prevalence and risk factors associated with mastitic cattle. In Nigeria, this is of significant concern because not only does it affect the health and well-being of cattle but has economic implications. A previous study suggests that 70% of raw milk samples contain *Escherichia coli*, which is one of the family of Enterobacteriaceae. After a comprehensive literature search, this systematic review and meta-analysis suggest that *Escherichia coli* and *Klebsiella pneumoniae* are prevalent in mastitic cattle particularly in Asia and Sub Sahara Africa. Further research is needed to understand the rise of multiple drug resistance strains of Enterobacteriaceae in cattle, with dominance of blaTEM and blaCTX-M genes suggesting an urgent need for coordinated surveillance, stricter antibiotic regulations, and global AMR management strategies. If left unchecked, the spread of resistant genes from animals to humans will continue to threaten treatment effectiveness and public health. The pooled prevalence of ESBL-producing *E. coli* in cattle is 54.1% (95% CI: 44.1-63.9%), while for *Klebsiella pneumoniae*, it's between 24.1-53.3%. Sub-Saharan Africa has the highest prevalence of ESBL-producing *E. coli*, at 32.5%, for *Klebsiella pneumoniae*, though rates vary by location. The significant variation among studies suggests that factors like study design, demographics, and geography influence the prevalence of ESBL-producing *E. coli*.

DECLARATION OF COMPETING INTEREST

The authors declare no competing financial interests or personal relationships.

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AUTHORS' CONTRIBUTION

The conceptualization of the study was led by Garba Jamilu and Sulaiman Y.M. Data analysis was conducted by Garba Jamilu and Abdullahi M.S. The initial draft of the manuscript was prepared by Jamilu Garba, Ismaila A.A., and Maina B.B., with the remaining authors contributing to the review and editing.

DATA AVAILABILITY STATEMENT

Data available on request

AI USAGE DECLARATION

During the preparation of this work, the authors used generative AI technologies solely for language editing and refinement. All of the authors reviewed and edited the content. The authors take full responsibility for the final content of the publication.

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