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## Comparative analysis of the frequency of the occurrence of mastitis-causing pathogens and assessing their importance in the pathogenesis of mastitis

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### Abstract

Mastitis, involves changes in the physical, chemical, and microbiological properties of milk, accompanied by structural alterations in the mammary gland. A total of 300 isolates underwent screening using the NCBI National Database of Antibiotic Resistant Organisms (NDARO) for mastitis as isolation source. Out of the 300 isolates screened, the prevalence of each isolate was documented as follows *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Klebsiella oxytoca*, *Enterococcus faecalis*, *Bacillus cereus*, *Enterobacter cloacae*. Notably, there was a high prevalence of beta-lactamase genes, including blaTEM (3), blaSHV (177), blaCTXM (6), blaEC (69), among the coliforms. This data suggests a significant presence of antibiotic resistance genes within the isolated bacterial strains.

**Keywords:** Frequency, mastitis-causing pathogens, pathogenesis, mastitis

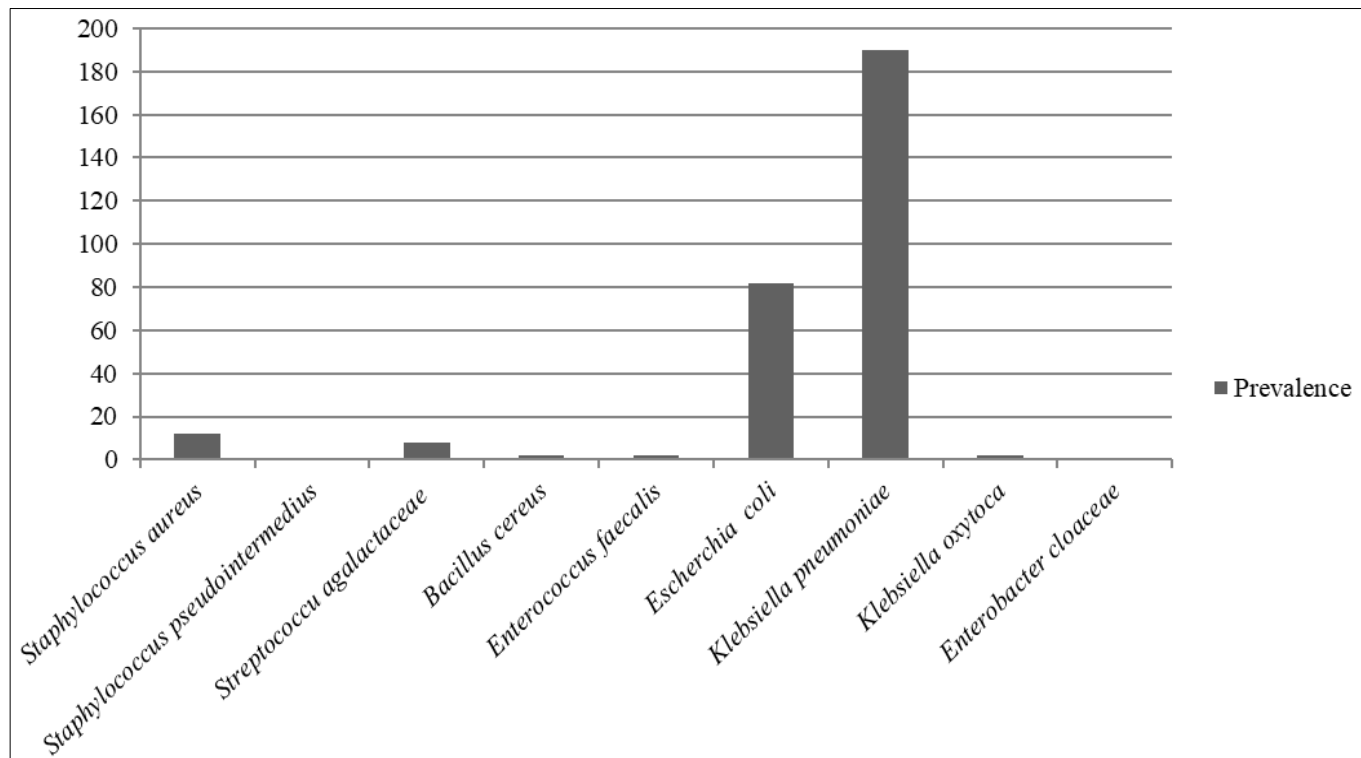
### Introduction

Mastitis, originating from the Greek words "Mastos" for breast and "itis" indicating inflammation, denotes the inflammation of mammary glands. This condition manifests through alterations in the physical, chemical, and microbiological aspects of milk, coupled with changes in the mammary gland's structure. In dairy ruminants, *E. coli*, streptococci, and *Staphylococcus aureus* are primary causative agents (Sharma *et al.*, 2007) [14]. Despite significant strides in comprehending its pathogenesis and adopting comprehensive management practices, mastitis continues to be a prominent ailment in dairy animals, causing substantial economic losses. Recent estimations indicate a 70% reduction in milk production, translating to an annual cost exceeding Rs 7,165 crores (Maity *et al.*, 2020) [9]. Moreover, mastitis presents a significant challenge in humans also, impacting up to 33% of lactating women (Angelopoulou *et al.*, 2018) [1]. Identifying mastitis pathogens is crucial for implementing effective control measures, reducing the risk of chronic infections, and optimizing antimicrobial therapy. Methods for Detecting Suspected Mastitis Milk Samples encompass the traditional Somatic Cell Count (SCC), evaluating milk quality and pricing with  $SCC \geq 200,000$  cells/ml. This can be measured using direct or indirect methods, such as automatic cell counters or the California Mastitis Test (CMT), a quick and cost-effective "cow-side" test. Phenotypic methods, including bacterial culture, assess characteristics like morphology, growth, and antimicrobial resistance. On-farm culture systems and Mass Spectroscopy (MALDI-TOF MS) further contribute to pathogen identification. Genotypic methods, utilizing DNA for identification, involve Polymerase Chain Reaction (PCR), a popular method for direct detection of nucleic acids. Other molecular typing methods like AFLP, ribotyping, RFLP, and PFGE provide species and strain-level information. Microarray Technology, capable of rapid pathogen detection, relies on PCR amplification and hybridization. Immunoassays, notably ELISA, are commonly used but may lack sensitivity for low-concentration antigens.

Transcriptome and Proteome Analysis, employing advanced techniques like 2-dimensional gel electrophoresis and mass spectroscopy, have uncovered biomarkers for early mastitis detection and treatment efficacy. Mastitis-specific biomarkers, including acute phase proteins like haptoglobin, CRP, and mammary-associated serum amyloid A3, offer molecular-level diagnostic insights.

### Materials and Methods

The isolates underwent screening using the NCBI National Database of Antibiotic Resistant Organisms (NDARO) at <https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/>. The screening process involved utilizing the "isolation source" search option to specifically target isolates of mastitis origin. Thereafter the isolates were arranged in order of their prevalence in the database and represented in the form of a graph (Figure 1).



**Fig 1:** Prevalence of mastitis causing organisms deposited at NCBI database

### Results

The organisms recorded were *Staphylococcus aureus*, *Staphylococcus pseudintermedius*, *Streptococcus agalactiae*, *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*. Among 300 isolates screened, the prevalence of each isolate was recorded in order, *Klebsiella pneumoniae* (190), *Escherichia coli* (82), *Staphylococcus aureus* (12), *Streptococcus agalactiae* (8), *Klebsiella oxytoca* (2), *Enterococcus faecalis* (2), *Bacillus cereus* (2), *Enterobacter cloacae* (1). A very high prevalence of beta lactamase genes such as *blaTEM* (3), *blaSHV* (177), *blaCTXM* (6), *blaEC* (69) etc were recorded among the coliforms.

### Discussion

Highest prevalence was recorded for *Klebsiella pneumoniae* followed by *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella oxytoca*, *Enterococcus faecalis*, *Bacillus cereus*, *Enterobacter cloacae*. Hence it can be seen that mastitis is mostly caused by coliform organisms. Mastitis continues to be a significant infection affecting dairy cows, posing a crucial concern for both dairy farmers and the broader dairy industry. *Escherichia coli* strains, in particular, contribute substantially to the prevalence of these infections (Roussel *et al.*, 2017) [13]. This underscores the ongoing challenge in managing and preventing mastitis, emphasizing the need for

effective strategies and interventions within the dairy sector. A very high prevalence of beta lactam genes in the isolates indicate a high presence antimicrobial resistance against majority of the coliforms. Mastitis ranks among the top three reasons for culling dairy cows, alongside lameness and failure to conceive (Esslemont and Kossaibati, 1997) [11]. The consequences include reduced conception rates (Hertl *et al.*, 2010) [6], lower milk yields (Schukken *et al.*, 2009), increased mortality risk (Bar *et al.*, 2008a; Hertl *et al.*, 2011) [3, 5], and significant treatment costs. Dairy farmers consider factors such as disease presence, milk yield, conception status, lactation stage, and parity when deciding on cow management and culling.

### Conclusion

Hence it can be concluded that mastitis has considerable economic implications for dairy farmers, leading to decreased milk yield, increased treatment costs, and being a major reason for culling dairy cows. Effective management strategies, including early detection and appropriate treatment, are crucial to mitigate the impact of mastitis on both the individual cow and the dairy herd as a whole. Moreover, rampant use of antimicrobials has resulted in increased incidence of antimicrobial resistance worldwide. This further complicates the case by increase in the burden of treatment cost. Thus, judicious use of antibiotics helps to mitigate the problem of antimicrobial resistance.

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