# Evolving Trends in Mastitis Causative Pathogens: A Fifteen-Year Retrospective Analysis study in Karnataka-India

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**ABSTRACT**

Mastitis remains one of the most prevalent and economically significant diseases affecting the dairy industry worldwide. Among the bacterial etiological agents, *Staphylococcus spp.*, *Escherichia coli spp.* and *Klebsiella spp.* are the predominant pathogens responsible for the majority of infections. The emergence of antimicrobial resistance (AMR) among these organisms has further compounded treatment challenges, posing a serious threat to both animal productivity and public health. This retrospective study evaluated the antimicrobial sensitivity patterns of major mastitis-causing pathogens isolated from cattle herds in Karnataka, India, over a fifteen-year period (2009–2023). A total of 580 milk samples were collected from milking cows, of which 368 (63.44%) yielded microbial growth and were subjected to antimicrobial susceptibility testing against fourteen antibiotics. *Staphylococcus spp.* emerged as the most frequent isolate (44.02%), followed by *E. coli spp.* (27.44%) and *Klebsiella spp.* (18.20%), collectively accounting for 89.67% of all mastitis cases. The occurrence of mastitis was highest during the monsoon season (41.30%), though seasonal variations did not significantly affect pathogen distribution. Analysis across three consecutive five-year intervals revealed a relatively consistent pattern in the prevalence of these pathogens. The persistence of these dominant bacterial species underscores the need for improved herd management, routine monitoring of antimicrobial susceptibility, and implementation of rational antibiotic use policies. Strengthening hygiene practices and adopting targeted control strategies are essential to mitigate mastitis and curb the growing threat of antimicrobial resistance in the dairy sector.

**Keywords:** Mastitis, antimicrobial resistance, *Staphylococcus spp.*, *E. coli spp.*, *Klebsiella spp.*, antibiotic susceptibility, dairy cattle, mitigation strategies

**1.0 Introduction:** Antimicrobial resistance (AMR) represents one of the most pressing global health threats and a rapidly expanding field of research, with a remarkable surge in scientific studies and global attention in recent years **(1).** Despite the growing interest in antimicrobial resistance (AMR), significant knowledge gaps persist, particularly in understanding long-term AMR trends across national, regional, and global levels, as well as across key sectors such as public health, veterinary medicine, and agriculture in the Indian context. These gaps hinder the development of effective policies. As the Global Antimicrobial Resistance Partnership states, Zero level of knowing the trends of AMR, it is impossible to make rational recommendations or monitor the effectiveness of interventions **(2).** Considering the extensive use of antimicrobials across various sectors, including veterinary medicine, conducting comprehensive studies on antimicrobial resistance (AMR) trends is essential for effective monitoring and control.

In Veterinary medicine and animal husbandry, mastitis is one of the most economically significant diseases affecting the dairy industry and is a major reason for the use of antimicrobials on dairy farms **(12).** The economic impact of mastitis goes beyond the cost of antimicrobial treatment and includes the premature culling of affected animals, discarded milk, penalties for high somatic cell counts (SCC), and reduced fertility, all of which collectively lower the overall profitability of dairy farmers **(3).** Although several pathogens are known to cause mastitis, *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Streptococcus agalactiae (S. agalactiae), and Klebsiella pneumoniae (K. pneumoniae)* are recognised as the major causative agents in dairy animals **(4).**

Numerous studies have determined the antibacterial susceptibility patterns of mastitis pathogens isolated from clinical studies or submissions to diagnostic laboratories **(5)**. Overall, the antimicrobial susceptibility patterns of different bacteria have shown similar results across various studies; however, only a few have examined long-term trends in these patterns over several years **(6)**reported no significant change in the proportion of *Staphylococcus aureus* isolates susceptible to antibacterial agents over year period. Conversely, mastitis caused by *Staphylococcus aureus* is highly contagious, chronic, and notoriously difficult to treat with antibiotics **(7)**. Literature is affluent with articles describing antibacterial resistance patterns among bovine mastitis pathogens either isolated from clinical studies or submitted to diagnostic laboratories **(8)**. Despite the presence of this numerous number of studies, convincing evidence is lacking to support a widespread, emerging resistance among mastitis pathogens to antibacterial drugs **(9)**.

Ultimately, definite conclusions on resistance patterns of mastitis pathogens are difficult given the limitations of the data presented in the literature.

The spontaneous cure rate of mastitis varies significantly among pathogens, antibiotic therapy must be tailored based on the specific mastitis aetiology **(10)**. In the present study comparisons were made to determine if the proportion of susceptible pathogens had changed during the time period. Antimicrobial susceptibility testing (AST) of mastitis pathogens is an essential diagnostic and monitoring tool for assessing antibiotic resistance trends in dairy herds across India. Although several studies have investigated antimicrobial resistance (AMR) in bovine mastitis pathogens, limited research has systematically analysed long-term resistance patterns under Indian conditions. Continuous surveillance of AMR is crucial for the early detection of emerging resistance, enabling evidence-based therapeutic decisions, effective mastitis management, and the formulation of region-specific control strategies. Such monitoring not only improves treatment efficacy and herd health but also contributes to reducing the economic losses associated with mastitis and antimicrobial misuse in the Indian dairy sector **(11).** The present study aimed to investigate trends in antimicrobial resistance (AMR) associated with the therapeutic use of antimicrobials in mastitis management, recognising AMR as a critical and escalating global public health concern.

**2.0 Materials and Methods:**

**2.1 Collection of Samples:** The bacteriological data analysed in this study were derived from milk samples collected from cows of seven districts in Karnataka which were suspected of intramammary infections and submitted to the SRDDL, IAH&VB, Bengaluru between 2009 and 2023. A total of 580 mastitis milk samples over a period of 15 years from 2009 to 2023 were analysed for Antibiotic sensitivity at SRDDL, IAH&VB, Bengaluru. A total of 14 antibiotics were tested for sensitivity. Of the 580 samples tested, 63 (10.86%) were collected from Chikkaballapur district, 22 (3.79%) from Belagavi, 240 (41.37%) from Bengaluru Urban, 102 (17.58%) from Bengaluru Rural, 27 (4.65%) from Ramanagara, 43 (7.41%) from Sirsi, and 83 (14.31%) from Tumkuru. Regarding seasonal distribution, the highest proportion of samples 230 (39.65%) were collected during Monsoon, followed by Summer 204 (35.17%) and winter 146 (25.17%) respectively.

**2.2Antimicrobial Susceptibility Testing:**

**2.2.1 Culturing and Identification of Bacteria:** The suspected mastitis milk samples were first inoculated into nutrient broth for enrichment, followed by streaking onto selective and differential media, including Mannitol Salt Agar, Edward's Medium, Eosin Methylene Blue (EMB) Agar, Blood Agar, and MacConkey's Agar, to isolate and obtain pure bacterial cultures. Identification of bacteria was done by grams staining as per the method of **(13).**

**2.2.2 Bio-chemical confirmation:** Following cultural identification of isolates, biochemical tests such as Catalase test, Coagulase test, Oxidase test, Indole test, Methyl red test, Voges Proskauer test and Citrate test were done as per the protocols mentioned in the text book of Clinical Veterinary Microbiology by **(14).**

**2.3 The antibiotics evaluated in this study included** Enrofloxacin, Ampicillin, Amikacin, Ciprofloxacin, Gentamicin, Ceftriaxone, Cefpodoxime, Ceftazidime, Oxacillin, Nitrofurantoin, Cefotaxime, Amoxiclav, Amoxicillin, and Norfloxacin.

**2.3.1 ABST was performed by disk diffusion method:** In-vitro antibiotic sensitivity test was carried out as per the Disc diffusion method described by **(15).** All the 580mastitis suspected milk samples were subjected for culturing and identification and then out of which 330 samples which have yielded the growth of *Staphylococcus spp., E.coli spp*. and *Klebsiella spp*. were subjected to antibiotic sensitivity test by standard disc diffusion technique as per the method of **(15)** using Muller Hinton agar media. Each bacterial isolate was uniformly swabbed onto the surface of MHA plates, and antibiotic discs were carefully placed on the inoculated surface using sterile forceps. The plates were then incubated at 37°C for 18–24 hours. After incubation, the diameters of the zones of inhibition surrounding each disc were measured in millimetres. The antibiotics discs were procured from Hi-Media laboratories private limited, Bombay, India. The sensitivity and resistance patterns were recorded with the zone of inhibition and interpreted based on the Manufacturers instruction.

**2.3.2 Interpretation:** The results were interpreted as Highly Sensitive (SSS), moderately sensitive (SS), Sensitive (S) and Resistant by comparing the measured inhibition zones with the standard interpretive criteria recommended by manufacturer.

**2.4 Antibiotic Cumulative Percentage:** Fourteen antibiotics were evaluated for their efficacy against the major three bovine mastitis pathogens in the present study. The cumulative sensitivity of each antibiotic against mastitis pathogens was calculated and ranked accordingly.

The cumulative antibiotic sensitivity for a particular region was determined using the following formula:

**Cumulative Sensitivity of an Antibiotic (%)** = **∑ (%Prevalence of Pathogen × %Antibiotic Sensitivity of that Pathogen) / 100**

**3.0 Results:**

**3.1 Bacterial Isolates and Susceptibility Testing:** Among the 580 milk samples analysed, 368 milk samples have shown the growth of the different bacterial isolates (Table No.1). Among them *Staphylococcus spp.* (44.02%; 162 isolates), *E. coli spp*. (27.44%; 101 isolates) and *Klebsiella spp*. (18.20%; 67 isolates) and remaining were *candida* (2.98%; 11 isolates), *Proteus* (0.54%; 2 isolates), *Pseudomonas* (2.98%; 11 isolates), *Streptococcus* (2.71%; 4 isolates) and Yeast has (1.086%; 4 isolates). Samples exhibited no bacterial growth. A total of 330 isolates consisting of *Staphylococcus spp*, *E.coli spp*. and *Klebsiella spp*. (89.67%) were subjected to antimicrobial susceptibility testing.

**Table No: 1 The Percentage growth of different isolates from suspected mastitis samples**

| **District** | **Total cases** | **Total Growth of Positive samples** | ***Candida*** | ***E coli*** | ***Klebsiella*** | ***Proteus*** | ***Pseudomonas*** | ***Staphylococcus*** | ***Streptococcus*** | ***Yeast*** |
|---|---|---|---|---|---|---|---|---|---|---|
| Chikkaballapur | 63 | 30 | (6.66) 2 | (20) 6 | (23.33)7 | 0 | (3.33)1 | (46.66) 14 | 0 | 0 |
| Belagavi | 22 | 15 | (13.33) 2 | (20) 3 | (20) 3 | 0 | 0 | (46.66)7 | 0 | 0 |
| Bengaluru Urban | 240 | 196 | (3.06) 6 | (28.57) 56 | (14.28) 28 | (0.51) 1 | (3.57) 7 | (43.36)85 | (4.59)9 | (2.04)4 |
| Bengaluru Rural | 102 | 31 | 0 | (25.80)8 | (25.80)8 | 0 | 0 | (45.16) 14 | (3.22)1 | 0 |
| Ramanagara | 27 | 17 | 0 | (29.41) 5 | (11.76)2 | 0 | (5.88) 1 | (52.94)9 | 0 | 0 |
| Sirsi | 43 | 41 | (2.43) 1 | (29.26) 12 | (24.39) 10 | 0 | (4.87) 2 | (39.02) 16 | 0 | 0 |
| Tumkuru | 83 | 38 | 0 | (28.94) 11 | (23.68) 9 | (2.63)1 | 0 | (44.73) 17 | 0 | 0 |
| **Total** | **580** | **368** | **(2.98) 11** | **(27.44) 101** | **(18.20) 67** | **(0.54) 2** | **(2.98) 11** | **(44.02) 162** | **(2.71) 10** | **(1.086) 4** |

**3.1.1 *Staphylococcus spp*.:** *Staphylococcus spp*. accounted for 44.02% (162/368) of the total mastitis-positive samples tested for antimicrobial resistance across seven districts of Karnataka. On assessing the temporal trends, of *Staphylococcus spp*. as a causative pathogen for mastitis over the past 15 years which was categorized into three consecutive five-year periods based on cumulative cases reported. i.e. From 2009–2013, *Staphylococcus spp*. represented 41.17% (35/85); during 2014–2018, it accounted for 37.19% (61/164); and during 2019–2023, the proportion increased to 49.57% (59/119). The distribution of *Staphylococcus spp*. across these periods is illustrated in Graph 1 and Table 2

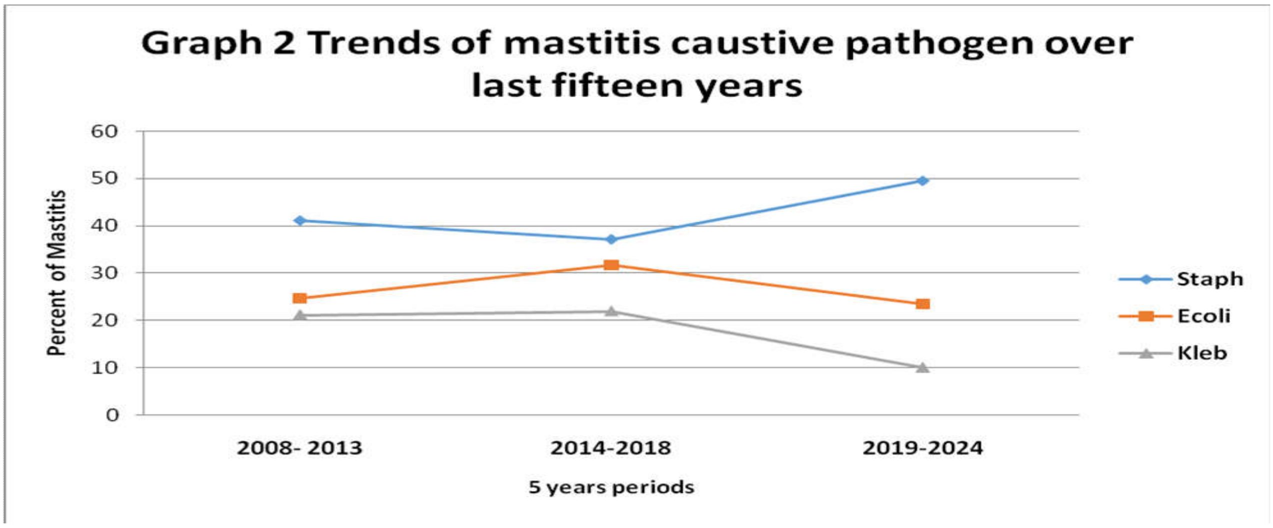
**3.1.2 *Escherichia coli spp*.:** *E. coli spp*. accounted for 27.44% (101/368) of the total mastitis-positive samples tested for antimicrobial resistance across seven districts of Karnataka. The temporal trends, of *E. coli* spp. as a causative pathogen for mastitis over the past 15 years which was categorized into three consecutive five-year periods based on cumulative cases reported was analysed. i.e From 2009–2013, *E. coli spp*. represented 24.70% (21/85); during 2014–2018, it accounted for 37.70% (52/164) and during 2019–2023, the proportion shows as 23.52% (28/119). The distribution of *E. coli spp*. across these periods is illustrated in Graph 1 and Table 2.

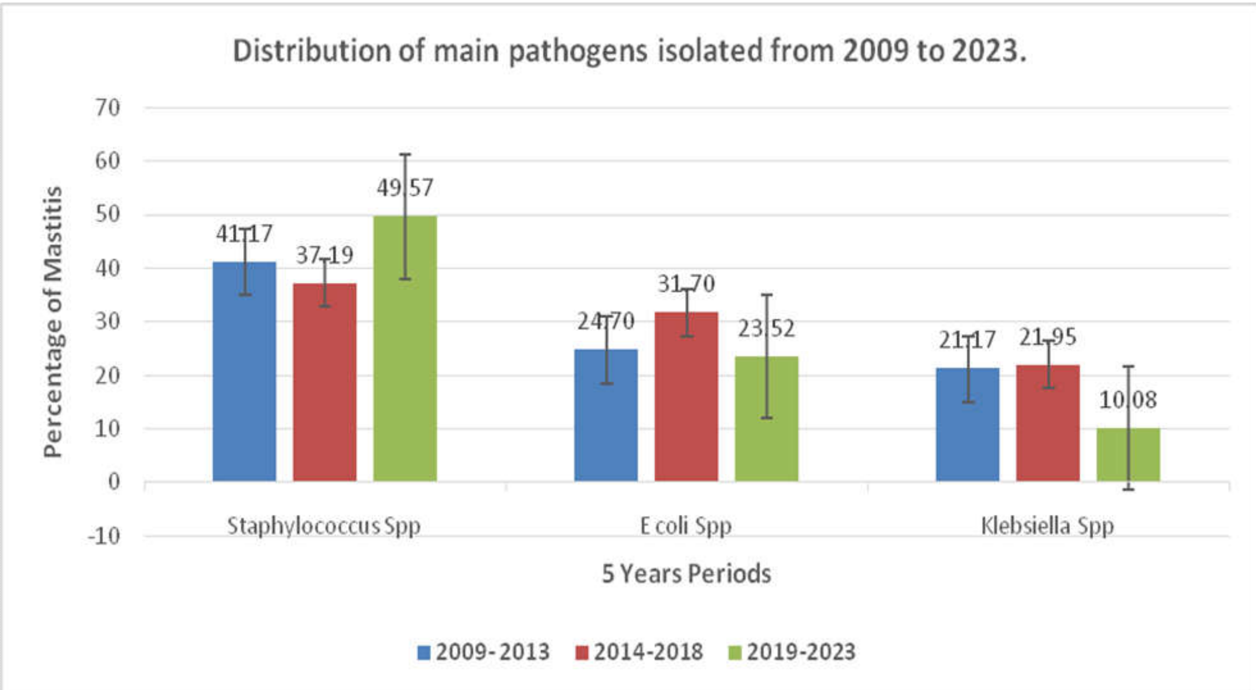
**3.1.3 *Klebsiella spp*.:** *Klebsiella spp*. accounted for 18.20% (67/368) of the total mastitis-positive samples tested for antimicrobial resistance across seven districts of Karnataka. On assessing the temporal trends, of *Klebsiella spp*. as a causative pathogen for mastitis over the past 15 years which was categorized into three consecutive five-year periods based on cumulative cases reported was analysed. i.e From 2009–2013, *Klebsiella spp*. represented 21.17% (18/85); during 2014–2018, it accounted for 21.95%

(36/164) and during 2019–2023, the proportion shows as 10.08% (12/119). The distribution of *Klebsiella spp.* across these periods is illustrated in Graph 1 and Table 2.

**TABLE 2 Trends of Mastitis Caustive Pathogen over Fifteen Years**

| Years | *Staphylococcus spp.* | | | *Escherichia coli spp.* | | | *Klebsiella spp.* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total Samples | No. of Growth (+Ve) sample | % of Growth sample | Total Samples | No. of Growth (+Ve) sample | % of Growth sample | Total Samples | No. of Growth (+Ve) sample | % of Growth sample |
| 2009- 2013 | 85 | 35 | 41.17 | 85 | 21 | 24.70 | 85 | 18 | 21.17 |
| 2014-2018 | 164 | 61 | 37.19 | 164 | 52 | 31.70 | 164 | 36 | 21.95 |
| 2019-2023 | 119 | 59 | 49.57 | 119 | 28 | 23.52 | 119 | 12 | 10.08 |



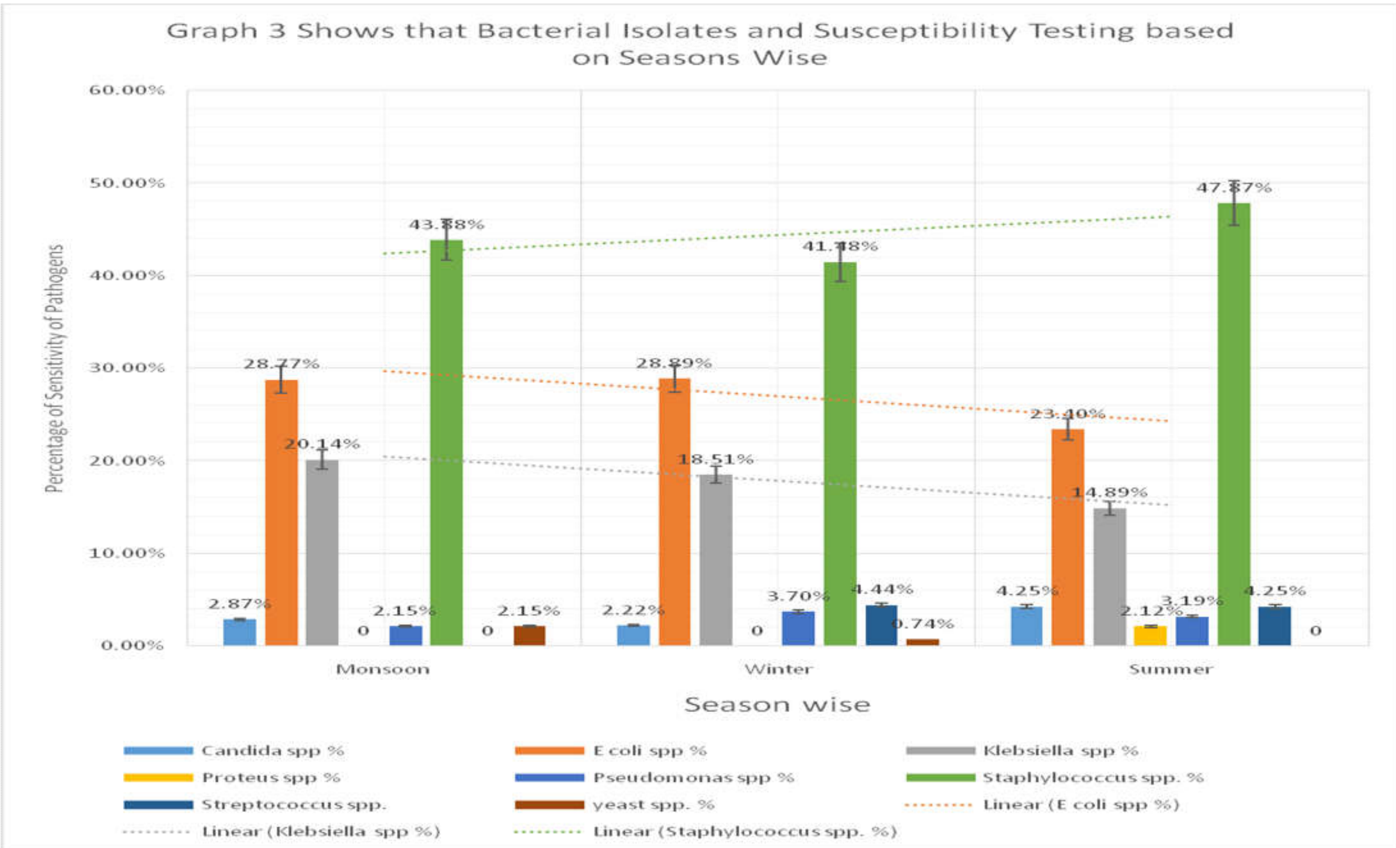




**3.2 Bacterial Isolates and Susceptibility Testing based on Seasons Wise:**

A total of 368 mastitis-positive milk samples were analysed in the present study. Among the isolates, *Staphylococcus spp*. was identified as the predominant pathogen across all seasons (44.02%; 162 isolates), followed by *E. coli spp*. (27.44%; 101 isolates) and *Klebsiella spp*. (18.20%; 67 isolates). Minor isolates included *Candida* (2.98%), *Pseudomonas* (2.98%), *Streptococcus* (2.71%), *Yeast* (1.08%), and Proteus (0.54%).Seasonal distribution revealed that the Monsoon (139 samples) and Winter (135 samples) seasons recorded the highest number of mastitis-positive cases, while the summer season had relatively fewer cases (94 samples). Staphylococcus spp. remained the dominant pathogen throughout the year, with the highest prevalence observed during summer (47.87%). In contrast, *E. coli spp*. infections were slightly more frequent during the Monsoon and winter seasons (approximately 29%) compared to summer (23.4%). Similarly, *Klebsiella spp*. occurrence peaked during the Monsoon (20.14%) and declined in summer (14.89%) were shown in the Table 3.

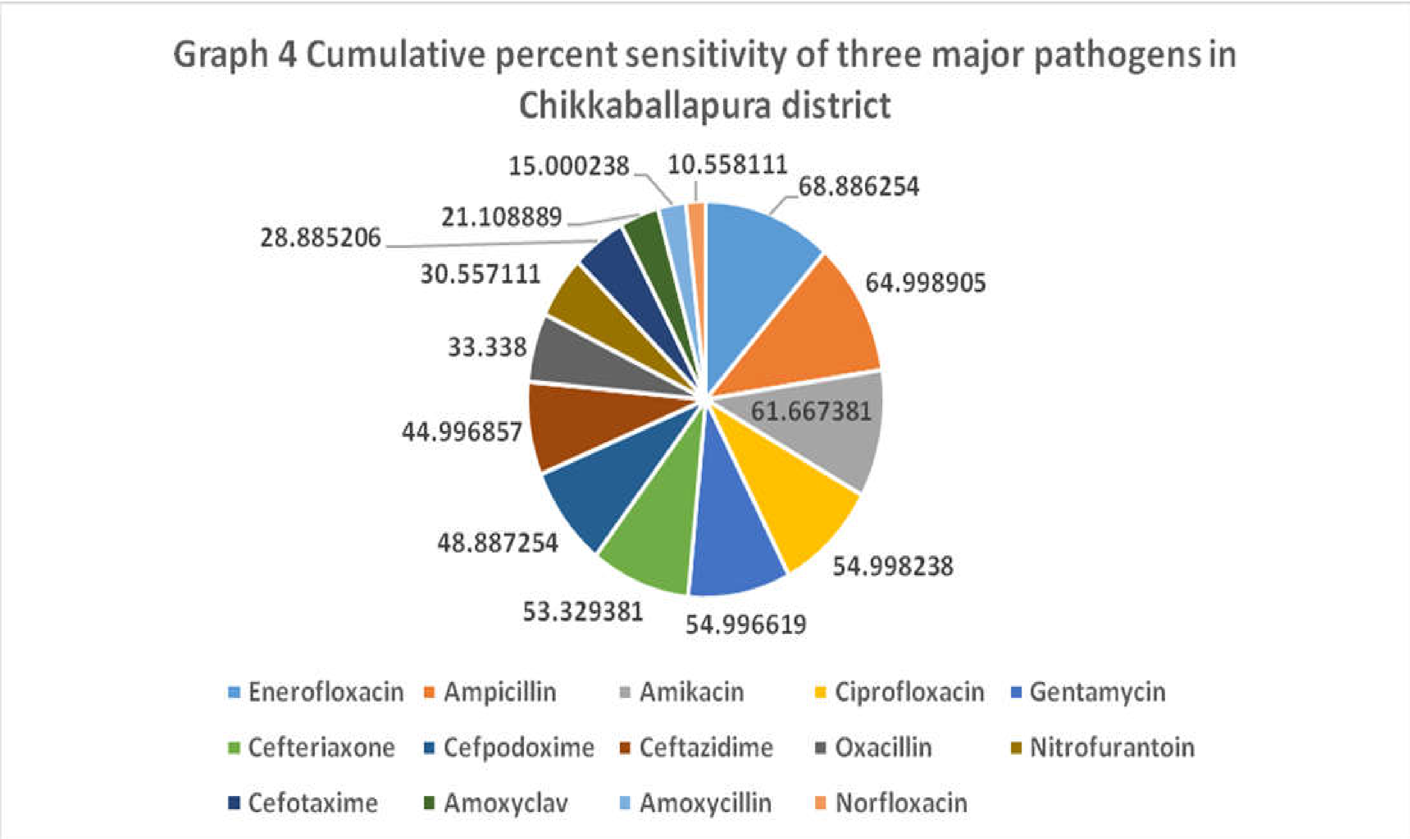
**Table 3 Seasons Wise growth of Bacterial Isolates from suspected Mastitis samples**

| Season | Total Growth of Positive samples | *Candida* | *E coli* | *Klebsiella* | *Proteus* | *Pseudomonas* | *Staphylococcus* | *Streptococcus* | *Yeast* |
|---|---|---|---|---|---|---|---|---|---|
| Monsoon | 139 | (2.87)4 | (28.77)40 | (20.14)28 | 0 | (2.15) 3 | (43.88) 61 | 0 | (2.15)3 |
| Winter | 135 | (2.22)3 | (28.89)39 | (18.51)25 | 0 | (3.70) 5 | (41.48) 56 | (4.44) 6 | (0.74) 1 |
| Summer | 94 | (4.25)4 | (23.40)22 | (14.89) 14 | (2.12) 2 | (3.19) 3 | (47.87) 45 | (4.25) 4 | 0 |
| **Total** | **368** | **(2.98)11** | **(27.44) 101** | **(18.20)67** | **(0.54) 2** | **(2.98) 11** | **(44.02) 162** | **(2.71) 10** | **(1.08)4** |



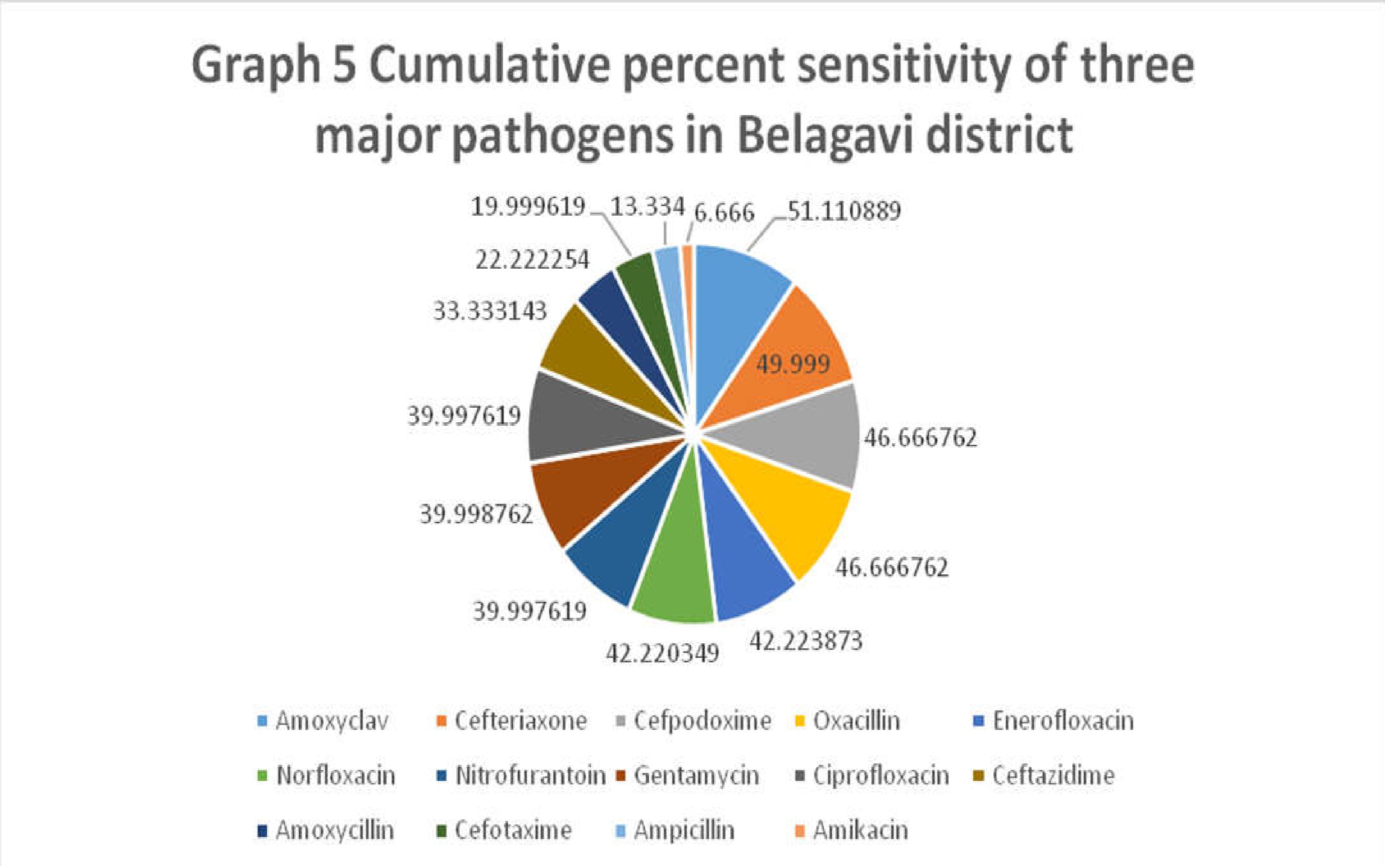

**3.2.1 Antibacterial Sensitivity**: A total of fourteen antibiotics were evaluated for their sensitivity against mastitis pathogens in the present study. The results revealed variable sensitivity patterns depending on both the bacterial species and the geographical area from which the samples were collected. Among the isolates, *Staphylococcus, Escherichia coli,* and *Klebsiella* emerged as the most prevalent causative agents of bovine mastitis. These pathogens exhibited distinct antimicrobial response profiles from the seven districts of Karnataka, reflecting differences in local antibiotic usage practices, environmental conditions, and management systems.

| Table 4 Cumulative percent sensitivity of three major pathogens in Chikkaballapura district | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **District** | ***Bacterial Isolates*** | **% of Pathogens** | **Enerofloxacin** | **Ampicillin** | **Amikacin** | **Ciprofloxacin** | **Gentamycin** | **Ceftriaxone** | **Cefpodoxime** | **Ceftazidime** | **Oxacillin** | **Nitrofurantoin** | **Cefotaxime** | **Amoxyclav** | **Amoxycillin** | **Norfloxacin** |
| Chikka ballapur | *Staphylococcus Spp.* | 46.67 | 100 | 92.86 | 92.86 | 78.57 | 85.71 | 78.57 | 71.43 | 57.14 | 64.29 | 42.86 | 35.71 | 28.57 | 21.43 | 14.29 |
| | *Klebsiella Spp.* | 23.33 | 85.71 | 85.71 | 71.43 | 71.43 | 57.14 | 57.14 | 57.14 | 71.43 | 14.29 | 42.86 | 42.86 | 28.57 | 14.29 | 14.29 |
| | *E.coli Spp.* | 3.33 | 66.67 | 50 | 50 | 50 | 50 | 100 | 66.67 | 50 | 0 | 16.67 | 66.67 | 33.33 | 50 | 16.67 |
| | **Cumulative percent sensitivity** | | **68.88** | **64.99** | **61.66** | **54.99** | **54.99** | **53.32** | **48.88** | **44.99** | **33.33** | **30.55** | **28.88** | **21.10** | **15.00** | **10.55** |




**3.2.2 Antibiotic Sensitivity Profile of Chikkaballapur District:** In the Chikkaballapur district, the overall antibiotic sensitivity of mastitis-causing bacteria varied significantly among the tested antibiotics (Table 4 and Graph 4). Enrofloxacin (68.88%) exhibited the highest cumulative sensitivity, indicating strong effectiveness against most isolates. Ampicillin (65%) and Amikacin (61.67%) also showed good antibacterial efficacy. Moderate sensitivity was observed with Ciprofloxacin (55%), Gentamycin (55%), and Ceftriaxone (53.33%). Lower sensitivity levels were recorded for Cefpodoxime (48.89%) and Ceftazidime (44.99%), suggesting reduced therapeutic potential. Oxacillin displayed the least sensitivity (33.34%), indicating a higher degree of resistance among the isolates to this antibiotic.

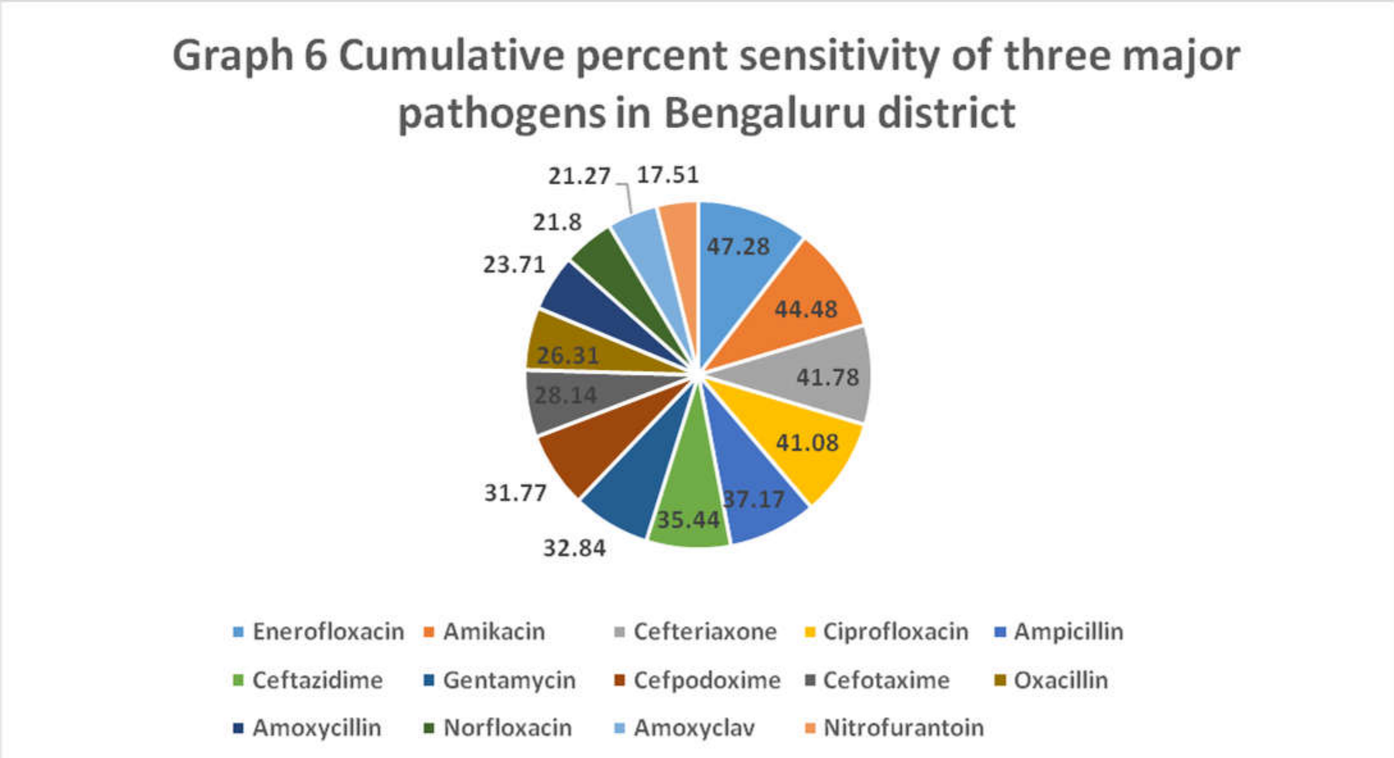
| Table 5 Cumulative Percent sensitivity of three major pathogens in Belagavi district | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **District** | **Bacterial isolates** | **%** | **Amoxyclav** | **Ceftriaxone** | **Cefpodoxime** | **Oxacillin** | **Enerofloxacin** | **Norfloxacin** | **Nitrofurantoin** | **Gentamycin** | **Ciprofloxacin** | **Ceftazidime** | **Amoxycillin** | **Cefotaxime** | **Ampicillin** | **Amikacin** |
| Belagavi | *Staphylococcus spp.* | 46.67 | 66.67 | 50 | 42.86 | 42.86 | 42.86 | 57.14 | 28.57 | 42.86 | 28.57 | 14.29 | 14.29 | 28.57 | 0 | 0 |
| | *Klebsiella spp.* | 20 | 33.33 | 66.67 | 66.67 | 66.67 | 66.67 | 33.33 | 66.67 | 33.33 | 66.67 | 66.67 | 33.33 | 33.33 | 66.67 | 33.33 |
| | *E.coli spp.* | 13.33 | 100 | 100 | 100 | 100 | 66.67 | 66.67 | 100 | 100 | 100 | 100 | 66.67 | 0 | 0 | 0 |
| | **Cumulative percent sensitivity** | | **51.11** | **49.99** | **46.66** | **46.66** | **42.22** | **42.22** | **39.99** | **39.99** | **39.99** | **33.33** | **22.22** | **19.99** | **13.33** | **6.66** |




**3.2.3 Antibiotic Sensitivity Profile of Belagavi District:** The antibiotic sensitivity profile of mastitis-causing bacterial isolates from the Belagavi district revealed notable variations across different antibiotics (Table 5 and Graph 5). Amoxyclav (51.11%) exhibited the highest cumulative sensitivity, indicating its relatively good efficacy against the isolates. Moderate sensitivity was recorded for Ceftriaxone (49.99%), Cefpodoxime (46.66%), and Oxacillin (46.66%), suggesting partial effectiveness. Lower sensitivity rates were observed for Enrofloxacin and Norfloxacin (42.22%), followed by Nitrofurantoin, Gentamycin, and Ciprofloxacin (39.99%), showing limited activity. The least sensitivity was recorded for Amikacin (6.66%), Ampicillin (13.33%), and Cefotaxime (19.99%), reflecting a higher level of resistance among the isolates to these antibiotics.

**Table 6 Cumulative Percent sensitivity for three major pathogens in Bengaluru district**

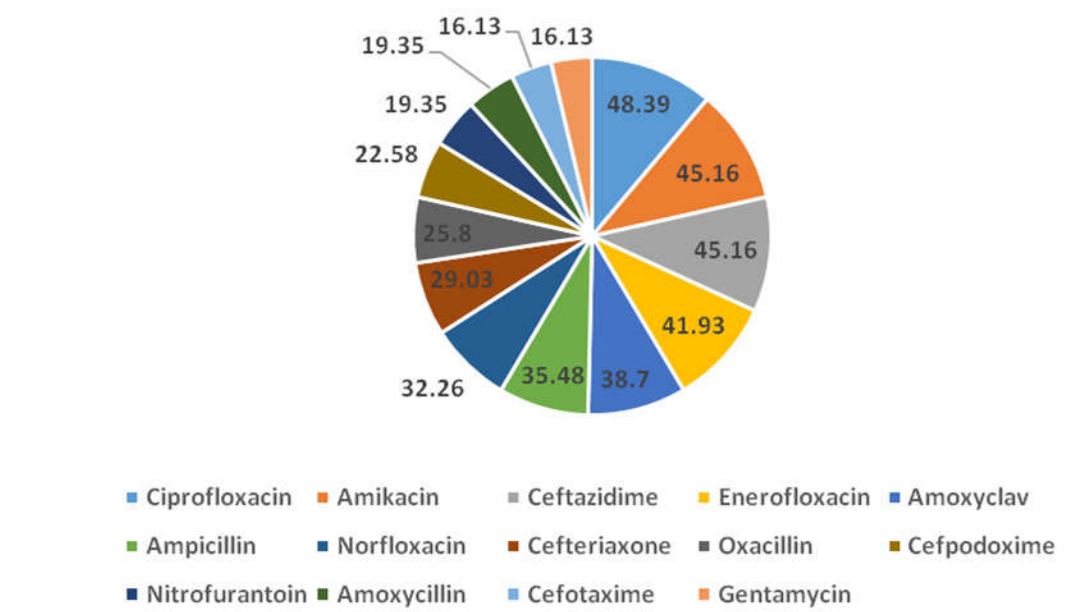
| District | Bacterial isolates | % | Enerofloxacin | Amikacin | Cefteriaxone | Ciprofloxacin | Ampicillin | Ceftazidime | Gentamycin | Cefpodoxime | Cefotaxime | Oxacillin | Amoxycillin | Norfloxacin | Amoxyclav | Nitrofurantoin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bengaluru | *Staphylococcus spp.* | 43.59 | 76.47 | 72.94 | 65.88 | 64.71 | 62.35 | 55.29 | 55.29 | 50.59 | 44.71 | 45.88 | 36.47 | 41.18 | 35.29 | 28.24 |
| | *Klebsiella spp.* | 14.36 | 85.71 | 75 | 78.57 | 78.57 | 60.71 | 67.86 | 53.57 | 60.71 | 53.57 | 35.71 | 50 | 21.43 | 32.14 | 32.14 |
| | *E.coli spp.* | 2.56 | 64.29 | 75 | 69.64 | 62.5 | 50 | 62.5 | 41.07 | 39.29 | 37.5 | 46.43 | 25 | 30.36 | 50 | 23.21 |
| | **Cumulative percent sensitivity** | | **47.28** | **44.48** | **41.78** | **41.08** | **37.17** | **35.44** | **32.84** | **31.77** | **28.14** | **26.31** | **23.71** | **21.80** | **21.27** | **17.51** |




**3.2.4 Antibiotic Sensitivity Profile of Bengaluru District:** The antibiotic sensitivity pattern of mastitis-causing bacterial isolates from the Bengaluru district revealed variable responses to the tested antibiotics(Table 6 and Graph 6). Enrofloxacin (47.28%) exhibited the highest cumulative sensitivity, suggesting it remains relatively effective against the bacterial isolates. Moderate sensitivity was observed for Amikacin (44.48%), Ceftriaxone (41.78%), and Ciprofloxacin (41.08%), indicating partial antibacterial efficacy. Reduced sensitivity was noted for Ampicillin (37.17%), Ceftazidime (35.44%), and Gentamycin (32.84%), while Cefpodoxime (31.77%) and Cefotaxime (28.14%) showed limited activity. The lowest sensitivity levels were recorded for Oxacillin (26.31%), Amoxycillin (23.71%), Norfloxacin (21.80%), Amoxyclav (21.27%), and Nitrofurantoin (17.51%), indicating a higher resistance trend among the isolates toward these antibiotics.

**Table 7 Cumulative Percent sensitivity for three major pathogens in Bengaluru R district**

| District | Bacterial isolates | % | Ciprofloxacin | Amikacin | Ceftazidime | Enerofloxacin | Amoxyclav | Ampicillin | Norfloxacin | Cefteriaxone | Oxacillin | Cefpodoxime | Nitrofurantoin | Amoxycillin | Cefotaxime | Gentamycin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bengalur u-R | *Staphylococcus spp.* | 45.16 | 64.29 | 64.29 | 50 | 50 | 57.14 | 50 | 42.86 | 35.71 | 35.71 | 28.57 | 28.57 | 35.71 | 14.29 | 14.29 |
| | *Klebsiella spp.* | 25.81 | 75 | 62.5 | 87.5 | 75 | 50 | 50 | 50 | 50 | 37.5 | 37.5 | 25 | 12.5 | 37.5 | 37.5 |
| | *E.coli spp.* | 0 | 87.5 | 100 | 62.5 | 87.5 | 25 | 62.5 | 37.5 | 62.5 | 37.5 | 62.5 | 25 | 25 | 25 | 50 |
| **Cumulative percent sensitivity** | | | **48.39** | **45.16** | **45.16** | **41.93** | **38.70** | **35.48** | **32.26** | **29.03** | **25.80** | **22.58** | **19.35** | **19.35** | **16.13** | **16.13** |

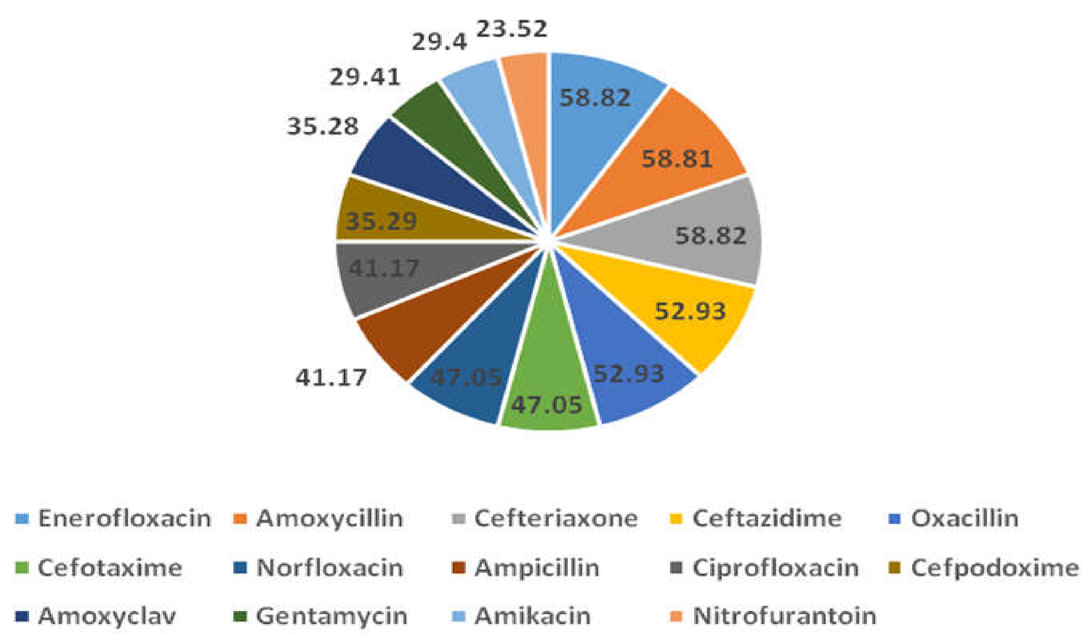



**Graph 7 Cumulative percent sensitivity of three major pathogens in Bengaluru -R district**

**3.2.5 Antibiotic Sensitivity Profile of Bengaluru-Rural District:** The antibiotic sensitivity analysis of mastitis-causing bacterial isolates from the Bengaluru-R district revealed considerable variation in response to different antibiotics(Table 7 and Graph 7). Ciprofloxacin (48.39%) exhibited the highest cumulative sensitivity, indicating relatively strong antibacterial efficacy against the isolates. Amikacin (45.16%) and Ceftazidime (45.16%) also demonstrated good activity, suggesting moderate effectiveness. Enrofloxacin (41.93%) and Amoxyclav (38.70%) showed fair sensitivity, whereas Ampicillin (35.48%) and Norfloxacin (32.26%) indicated reduced responsiveness. Lower sensitivity levels were recorded for Ceftriaxone (29.03%), Oxacillin (25.80%), and Cefpodoxime (22.58%), reflecting emerging resistance. The least sensitivity was observed for Nitrofurantoin (19.35%), Amoxycillin (19.35%), Cefotaxime (16.13%) and Gentamycin (16.13%), suggesting a high degree of resistance among isolates to these drugs.

**Table 8 Cumulative percent sensitivity for three major pathogens in Ramanagara district**

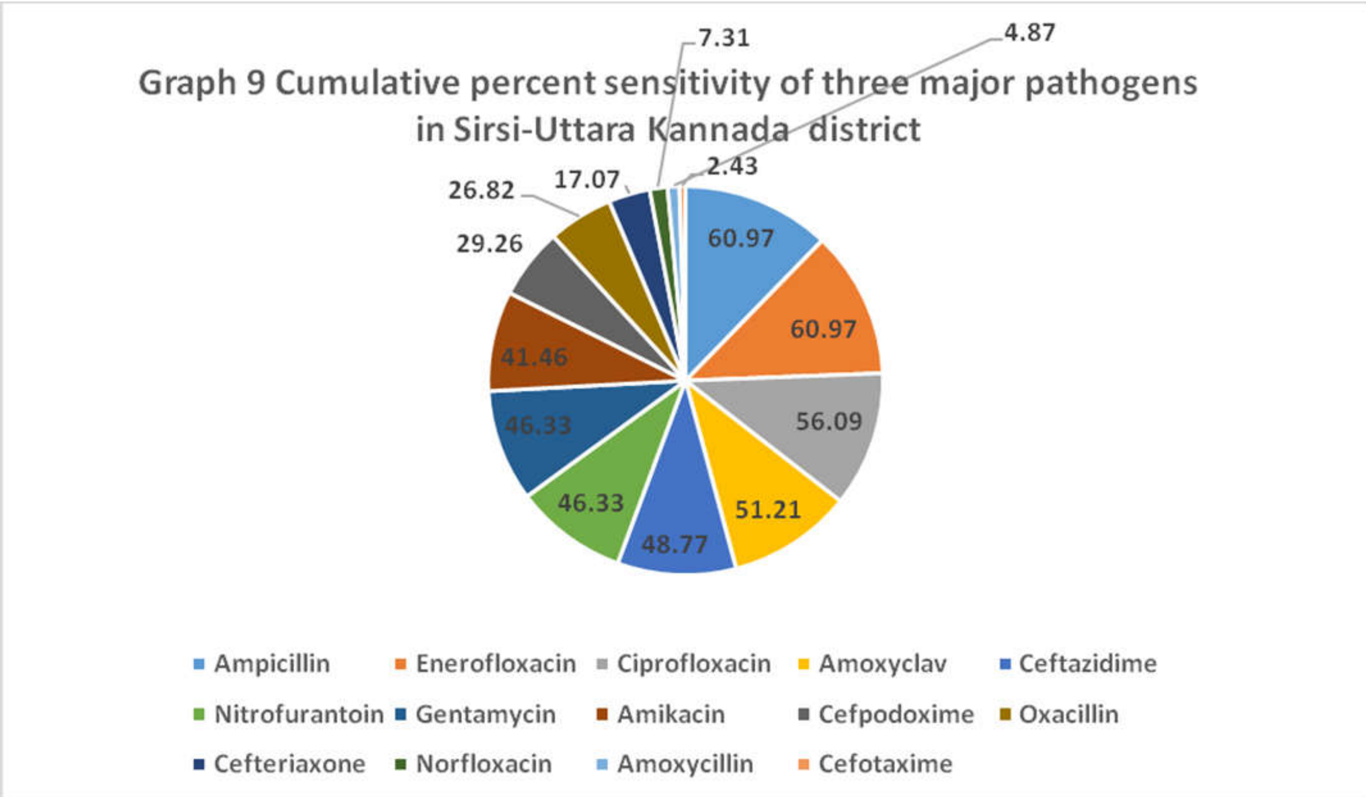
| District | Bacterial isolates | % | Enerofloxacin | Amoxycillin | Cefteriaxone | Ceftazidime | Oxacillin | Cefotaxime | Norfloxacin | Ampicillin | Ciprofloxacin | Cefpodoxime | Amoxyclav | Gentamycin | Amikacin | Nitrofurantoin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ramanagara | *Staphylococcus spp.* | 52.94 | 100 | 88.89 | 100 | 88.89 | 88.89 | 77.78 | 66.67 | 66.67 | 66.67 | 66.67 | 44.44 | 55.56 | 44.44 | 44.44 |
| | *Klebsiella spp.* | 11.76 | 50 | 100 | 50 | 50 | 50 | 50 | 100 | 50 | 50 | 0 | 100 | 0 | 50 | 0 |
| | *E.coli spp.* | 0 | 80 | 60 | 60 | 60 | 80 | 60 | 60 | 20 | 80 | 80 | 60 | 80 | 60 | 20 |
| **Cumulative % sensitivity** | | | **58.82** | **58.81** | **58.82** | **52.93** | **52.93** | **47.056** | **47.05** | **41.17** | **41.17** | **35.29** | **35.28** | **29.41** | **29.40** | **23.52** |



Graph 8 Cumulative percent sensitivity of three major pathogens in Ramanagara district

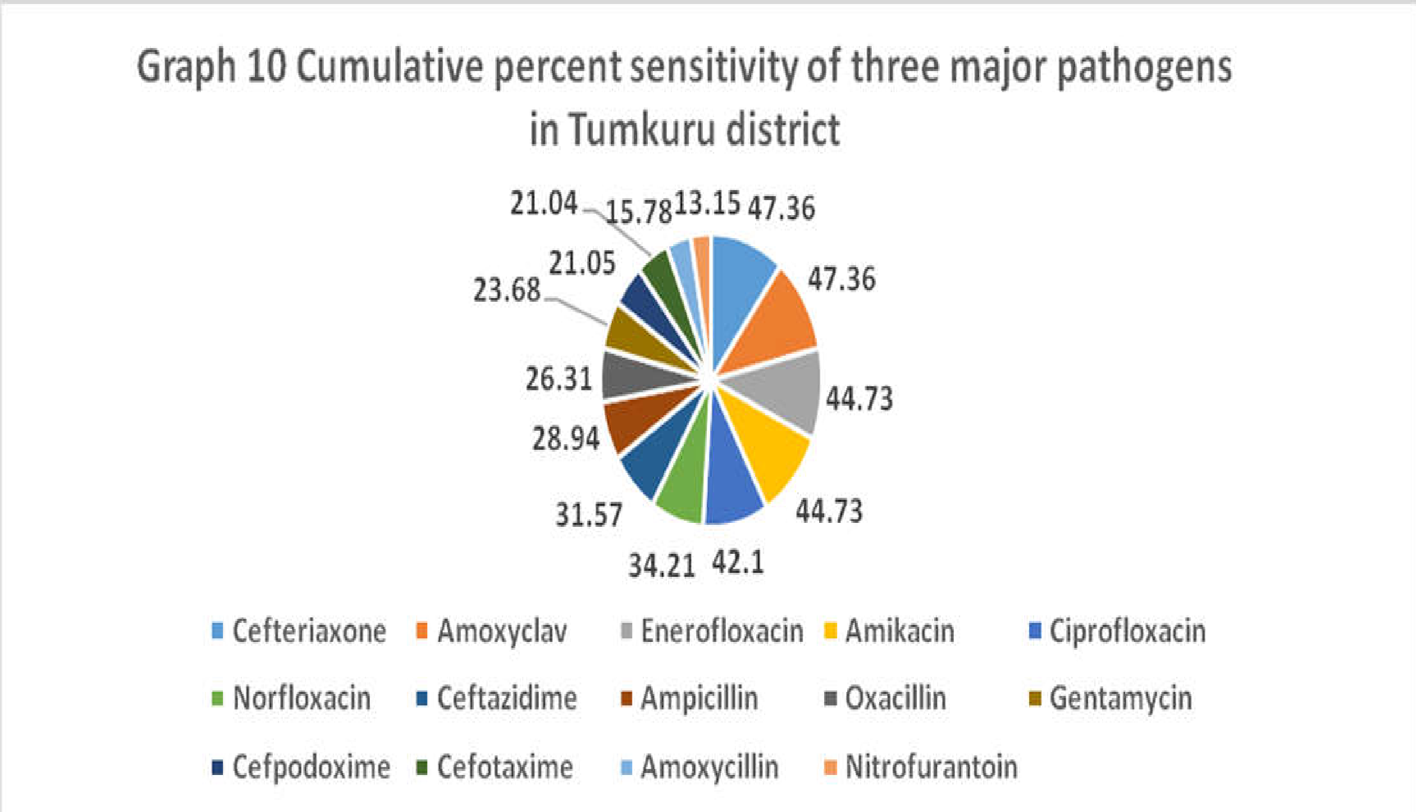
**3.2.6 Antibiotic Sensitivity Profile of Ramnagara District:** The antibiotic sensitivity profile of mastitis-causing bacterial isolates from the Ramanagara district demonstrated distinct variation across different antimicrobial agents( Table 8 and Graph 8). Enrofloxacin (58.82%), Amoxycillin (58.81%), and Ceftriaxone (58.82%) exhibited the highest cumulative sensitivity, indicating strong effectiveness against the majority of isolates. Moderate sensitivity was observed for Ceftazidime (52.93%) and Oxacillin (52.93%), suggesting satisfactory therapeutic potential. Cefotaxime (47.05%) and Norfloxacin (47.05%) also showed fair efficacy, while Ampicillin (41.17%) and Ciprofloxacin (41.17%) displayed moderate antibacterial activity. Reduced sensitivity was recorded for Cefpodoxime (35.29%) and Amoxyclav (35.28%), and the lowest responses were noted for Gentamycin (29.41%), Amikacin (29.40%), and Nitrofurantoin (23.52%), indicating emerging resistance trends among the isolates to these antibiotics.

| Table 9 Cumulative percent sensitivity for three major pathogens in Sirsi – Uttara Kannada district | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **District** | **Bacterial isolates** | **%** | **Ampicillin** | **Enerofloxacin** | **Ciprofloxacin** | **Amoxyclav** | **Ceftazidime** | **Nitrofurantoin** | **Gentamycin** | **Amikacin** | **Cefpodoxime** | **Oxacillin** | **Cefteriaxone** | **Norfloxacin** | **Amoxycillin** | **Cefotaxime** |
| Sirsi | *Staphylococcus spp* | 39.02 | 100 | 100 | 87.5 | 75 | 68.75 | 68.75 | 68.75 | 56.25 | 56.25 | 62.5 | 37.5 | 18.75 | 12.5 | 0 |
| | *Klebsiella spp.* | 24.39 | 90 | 90 | 90 | 90 | 90 | 80 | 80 | 80 | 30 | 10 | 10 | 0 | 0 | 10 |
| | *E.coli spp.* | 0 | 91.67 | 83.33 | 75 | 66.67 | 83.33 | 75 | 33.33 | 83.33 | 33.33 | 8.33 | 50 | 25 | 41.67 | 25 |
| **Cumulative percent sensitivity** | | | **60.97** | **60.97** | **56.09** | **51.21** | **48.77** | **46.33** | **46.33** | **41.46** | **29.26** | **26.82** | **17.07** | **7.31** | **4.87** | **2.43** |




**3.2.7 Antibiotic Sensitivity Profile of Sirsi - Uttar Kannada District** The antibiotic sensitivity profile of mastitis-causing bacterial isolates from the Sirsi - Uttara Kannada district revealed marked variability in drug response (Table 9 and Graph 9). Ampicillin (60.97%) and Enrofloxacin (60.97%) demonstrated the highest cumulative sensitivity, indicating strong antibacterial efficacy. Ciprofloxacin (56.09%) and Amoxyclav (51.21%) also showed good effectiveness against the isolates. Moderate sensitivity was recorded for Ceftazidime (48.77%), Nitrofurantoin (46.33%), and Gentamycin (46.33%), reflecting partial therapeutic potential. Amikacin (41.46%) exhibited reduced sensitivity, while Cefpodoxime (29.26%) and Oxacillin (26.82%) showed limited antibacterial activity. The lowest sensitivity was observed for Ceftriaxone (17.07%), Norfloxacin (7.31%), Amoxycillin (4.87%), and Cefotaxime (2.43%), indicating a high level of resistance among isolates to these antibiotics.

| Table 10 Cumulative percent sensitivity for three major pathogens in Tumkuru district | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| District | Bacterial isolates | % | Cefteriaxone | Amoxyclav | Enerofloxacin | Amikacin | Ciprofloxacin | Norfloxacin | Ceftazidime | Ampicilin | Oxacillin | Gentamycin | Cefpodoxime | Cefotaxime | Amoxycillin | Nitrofurantoin |
| Tumkuru | *Staphylococcus spp.* | 44.74 | 64.71 | 58.82 | 64.71 | 58.82 | 58.82 | 41.18 | 35.29 | 47.06 | 35.29 | 41.18 | 29.41 | 11.76 | 29.41 | 29.41 |
| | *Klebsiella spp.* | 23.68 | 77.78 | 88.89 | 66.67 | 77.78 | 66.67 | 66.67 | 66.67 | 33.33 | 44.44 | 22.22 | 33.33 | 66.67 | 11.11 | 0 |
| | *E.coli spp.* | 0 | 81.82 | 54.55 | 90.91 | 90.91 | 90.91 | 54.55 | 90.91 | 63.64 | 36.36 | 36.36 | 27.27 | 45.45 | 36.36 | 45.45 |
| **Cumulative percent sensitivity** | | | **47.36** | **47.36** | **44.73** | **44.73** | **42.10** | **34.21** | **31.57** | **28.94** | **26.31** | **23.68** | **21.05** | **21.04** | **15.78** | **13.15** |



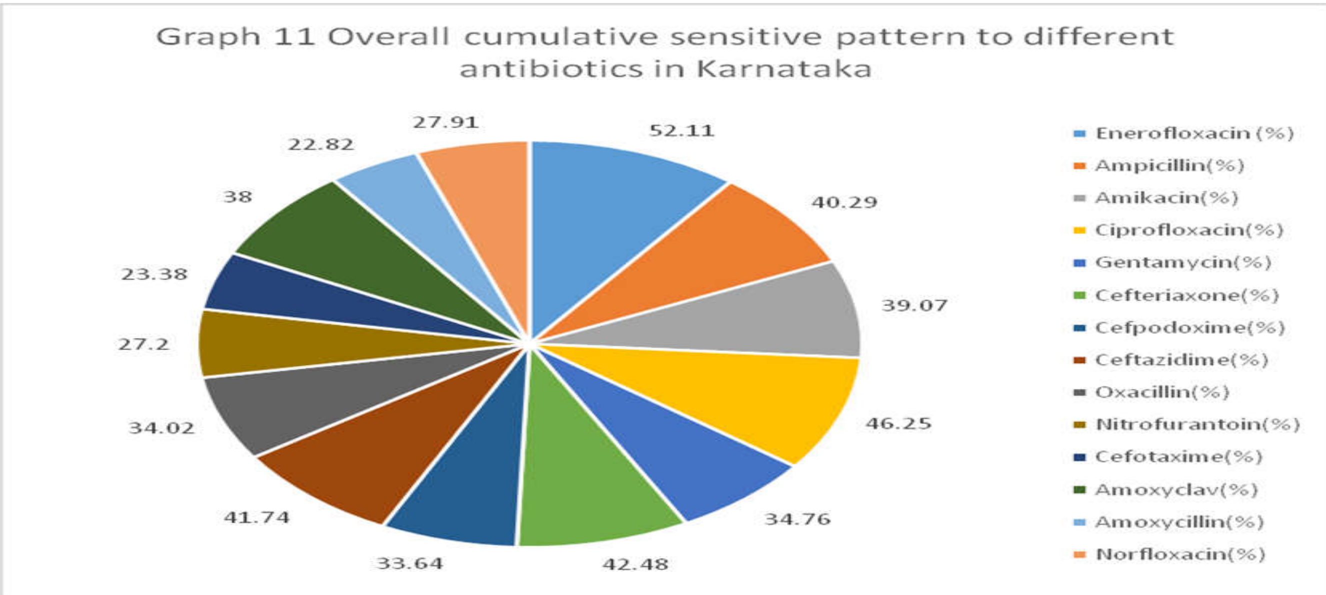
Graph 10 Cumulative percent sensitivity of three major pathogens in Tumkuru district

**3.2.8 Antibiotic Sensitivity Profile of Tumkuru District:** The antibiotic sensitivity pattern of mastitis-causing bacterial isolates from the Tumkuru district revealed distinct variations in susceptibility across different antibiotics(Table 10 and Graph 10). Ceftriaxone (47.36%) and Amoxyclav (47.36%) exhibited the highest cumulative sensitivity, indicating moderate to good efficacy against most bacterial isolates. Enrofloxacin (44.73%), Amikacin (44.73%), and Ciprofloxacin (42.10%) showed fair antibacterial activity, while Norfloxacin (34.21%) and Ceftazidime (31.57%) demonstrated reduced effectiveness. Lower sensitivity responses were observed for Ampicillin (28.94%), Oxacillin (26.31%), and Gentamycin (23.68%), suggesting partial resistance. The least activity was recorded for Cefpodoxime (21.05%), Cefotaxime (21.04%), Amoxycillin (15.78%), and Nitrofurantoin (13.15%), reflecting a high degree of resistance among the isolates toward these antibiotics.

**3.2.9** Overall cumulative sensitive pattern to different antibiotics in Karnataka.

**Overall cumulative sensitive pattern to different antibiotics in Karnataka: Table 11**

| Districts | Enerofloxacin (%) | Ampicillin (%) | Amikacin (%) | Ciprofloxacin (%) | Gentamycin (%) | Cefteriaxone (%) | Cefpodoxime (%) | Ceftazidime (%) | Oxacillin (%) | Nitrofurantoin (%) | Cefotaxime (%) | Amoxyclav (%) | Amoxycillin (%) | Norfloxacin (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chikkaballapur | 68.88 | 64.99 | 61.66 | 54.99 | 54.99 | 53.32 | 48.88 | 44.99 | 33.33 | 30.55 | 28.88 | 21.1 | 15 | 10.55 |
| Belagavi | 42.22 | 13.33 | 6.66 | 39.99 | 39.99 | 49.99 | 46.66 | 33.33 | 46.66 | 39.99 | 19.99 | 51.11 | 22.22 | 42.22 |
| Bengaluru | 47.28 | 37.17 | 44.48 | 41.08 | 32.84 | 41.78 | 31.77 | 35.44 | 26.31 | 17.51 | 28.14 | 21.27 | 23.71 | 21.8 |
| Bengaluru -R | 41.93 | 35.48 | 45.16 | 48.39 | 16.13 | 29.03 | 22.58 | 45.16 | 25.8 | 19.35 | 16.13 | 38.7 | 19.35 | 32.26 |
| Ramanagara | 58.82 | 41.17 | 29.4 | 41.17 | 29.41 | 58.82 | 35.29 | 52.93 | 52.93 | 23.52 | 47.05 | 35.28 | 58.81 | 47.05 |
| Sirsi | 60.97 | 60.97 | 41.46 | 56.09 | 46.33 | 17.07 | 29.26 | 48.77 | 26.82 | 46.33 | 2.43 | 51.21 | 4.87 | 7.31 |
| Tumkuru | 44.73 | 28.94 | 44.73 | 42.1 | 23.68 | 47.36 | 21.05 | 31.57 | 26.31 | 13.15 | 21.04 | 47.36 | 15.78 | 34.21 |
| **Average Grand Total (%)** | **52.11** | **40.29** | **39.07** | **46.25** | **34.76** | **42.48** | **33.64** | **41.74** | **34.02** | **27.2** | **23.38** | **38.00** | **22.82** | **27.91** |

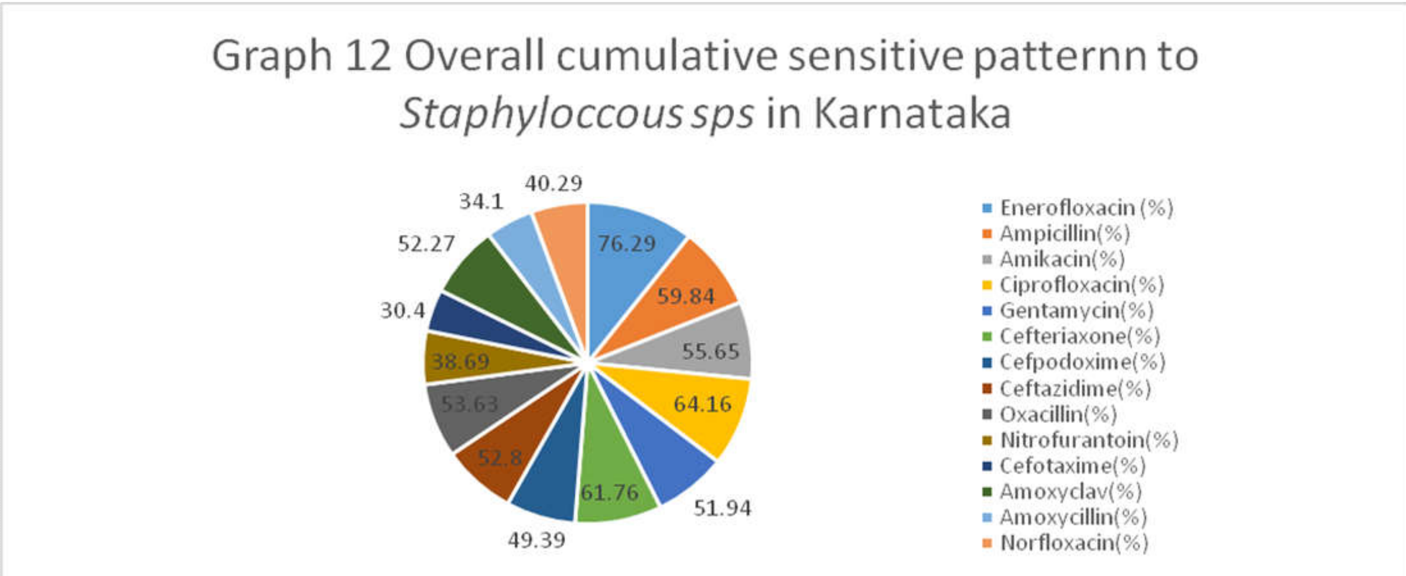



The table shows how sensitive *Staphylococcus* bacteria from mastitic milk are to different antibiotics in seven districts of Karnataka. Overall, **Enrofloxacin (52%)** was the most effective antibiotic, followed by **Ciprofloxacin (46%)** and **Ceftriaxone (42%)**, meaning these drugs still work fairly well. **Ampicillin (40%)**, **Amikacin (39%)**, and **Ceftazidime (42%)** had moderate effectiveness. Older or commonly used antibiotics like **Amoxycillin (23%)**, **Cefotaxime (23%)**, and **Norfloxacin (28%)** were less effective, showing higher resistance. **Oxacillin (34%)** results suggest some strains may be methicillin-resistant (*MRSA*). Among districts, **Chikkaballapur** had the highest overall sensitivity, indicating better response to antibiotics, while **Belagavi** and **Sirsi** showed lower sensitivity, possibly due to overuse or misuse of antibiotics. Overall, the results highlight the need for **careful antibiotic use** and **monitoring resistance** to treat mastitis effectively.

**3.2.10** Overall cumulative sensitive patternn to *Staphyloccous sps* in Karnataka.

**Overall cumulative sensitive patternn to *Staphyloccous sps* in Karnataka: Table 12**

| Districts | **Enerofloxacin (%)** | **Ampicillin (%)** | **Amikacin (%)** | **Ciprofloxacin (%)** | **Gentamycin (%)** | **Cefteriaxone (%)** | **Cefpodoxime (%)** | **Ceftazidime (%)** | **Oxacillin (%)** | **Nitrofurantoin (%)** | **Cefotaxime (%)** | **Amoxyclav (%)** | **Amoxycillin (%)** | **Norfloxacin (%)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chikkaballapur | 100 | 92.86 | 92.86 | 78.57 | 85.71 | 78.57 | 71.43 | 57.14 | 64.29 | 42.86 | 35.71 | 28.57 | 21.43 | 14.29 |
| Belagavi | 42.86 | 0 | 0 | 28.57 | 42.86 | 50 | 42.86 | 14.29 | 42.86 | 28.57 | 28.57 | 66.67 | 14.29 | 57.14 |
| Bengaluru | 76.47 | 62.35 | 72.94 | 64.71 | 55.29 | 65.88 | 50.59 | 55.29 | 45.88 | 28.24 | 44.71 | 35.29 | 36.47 | 41.18 |
| Bengaluru-R | 50 | 50 | 64.29 | 64.29 | 14.29 | 35.71 | 28.57 | 50 | 35.71 | 28.57 | 14.29 | 57.14 | 35.71 | 42.86 |
| Ramanagara | 100 | 66.67 | 44.44 | 66.67 | 55.56 | 100 | 66.67 | 88.89 | 88.89 | 44.44 | 77.78 | 44.44 | 88.89 | 66.67 |
| Sirsi | 100 | 100 | 56.25 | 87.5 | 68.75 | 37.5 | 56.25 | 68.75 | 62.5 | 68.75 | 0 | 75 | 12.5 | 18.75 |
| Tumkuru | 64.71 | 47.06 | 58.82 | 58.82 | 41.18 | 64.71 | 29.41 | 35.29 | 35.29 | 29.41 | 11.76 | 58.82 | 29.41 | 41.18 |
| **Average Grand Total %** | **76.29** | **59.84** | **55.65** | **64.16** | **51.94** | **61.76** | **49.39** | **52.8** | **53.63** | **38.69** | **30.4** | **52.27** | **34.1** | **40.29** |



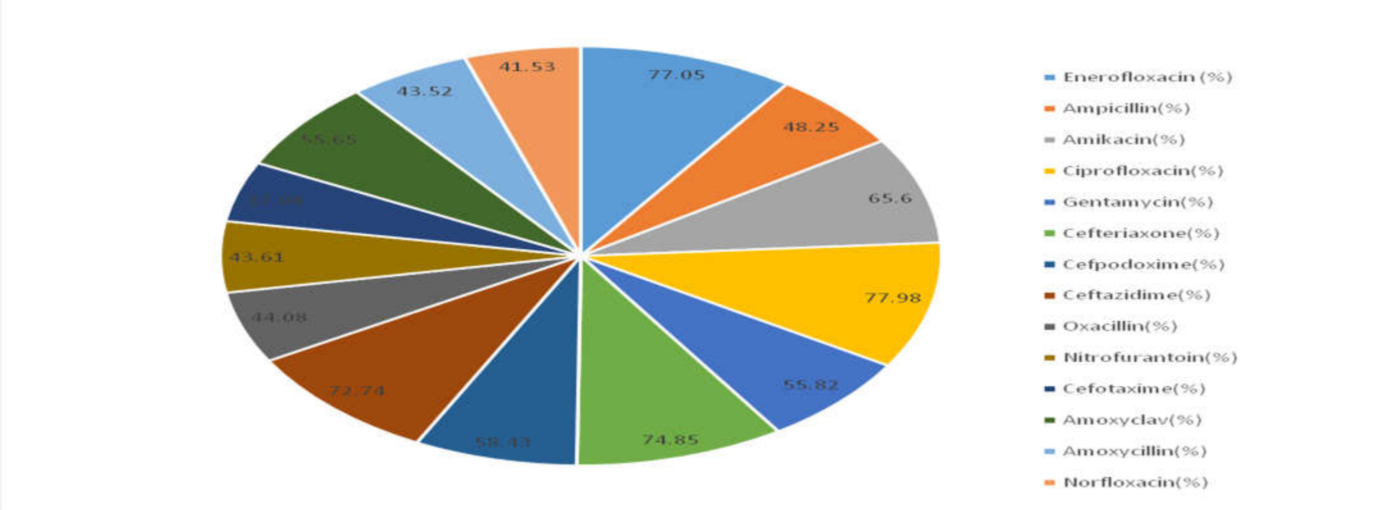

The table shows how sensitive *Staphylococcus* bacteria from mastitis milk are to different antibiotics across seven districts. Overall, **Enrofloxacin (76%)** was the most effective antibiotic, followed by **Ciprofloxacin (64%)** and **Ceftriaxone (62%)**, meaning these drugs work well against the bacteria. **Ampicillin (60%)** and **Amikacin (56%)** showed moderate effectiveness, while **Gentamycin (52%)** and **Ceftazidime (53%)** were fairly effective. Older or commonly used antibiotics like **Amoxycillin (34%)**, **Cefotaxime (30%)**, and **Norfloxacin (40%)** were less effective, indicating higher resistance. **Oxacillin (53.63%)** results suggest some bacteria may be **methicillin-resistant (MRSA)**. Among districts, **Chikkaballapur, Ramanagara, and Sirsi** had higher overall sensitivity, showing better antibiotic response, while **Belagavi and Tumkuru** showed lower sensitivity, possibly due to overuse or misuse of antibiotics. Overall, the results highlight the need for **careful and rational use of antibiotics** and **regular monitoring of resistance** to manage mastitis effectively.

**3.2.11** Overall cumulative sensitive pattern to *E.coli sps* in Karnataka:

**Overall cumulative sensitive pattern to *E.coli sps* in Karnataka: Table 13**

| District | Enerofloxacin (%) | Ampicillin (%) | Amikacin (%) | Ciprofloxacin (%) | Gentamycin (%) | Cefteriaxone (%) | Cefpodoxime (%) | Ceftazidime (%) | Oxacillin (%) | Nitrofurantoin (%) | Cefotaxime (%) | Amoxyclav (%) | Amoxycillin (%) | Norfloxacin (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chikkaballapur | 66.67 | 50 | 50 | 50 | 50 | 100 | 66.67 | 50 | 0 | 16.67 | 66.67 | 33.33 | 50 | 16.67 |
| Belagavi | 66.67 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 66.67 | 66.67 |
| Bengaluru | 64.29 | 50 | 75 | 62.5 | 41.07 | 69.64 | 39.29 | 62.5 | 46.43 | 23.21 | 37.5 | 50 | 25 | 30.36 |
| Bengaluru-R | 87.5 | 62.5 | 100 | 87.5 | 50 | 62.5 | 62.5 | 62.5 | 37.5 | 25 | 25 | 25 | 25 | 37.5 |
| Ramanagara | 80 | 20 | 60 | 80 | 80 | 60 | 80 | 60 | 80 | 20 | 60 | 60 | 60 | 60 |
| Sirsi | 83.33 | 91.67 | 83.33 | 75 | 33.33 | 50 | 33.33 | 83.33 | 8.33 | 75 | 25 | 66.67 | 41.67 | 25 |
| Tumkuru | 90.91 | 63.64 | 90.91 | 90.91 | 36.36 | 81.82 | 27.27 | 90.91 | 36.36 | 45.45 | 45.45 | 54.55 | 36.36 | 54.55 |
| **Average Grand Total %** | **77.05** | **48.25** | **65.6** | **77.98** | **55.82** | **74.85** | **58.43** | **72.74** | **44.08** | **43.61** | **37.08** | **55.65** | **43.52** | **41.53** |

Graph 13 Overall cumulative sensitive pattern to *E.coli sps* in Karnataka:



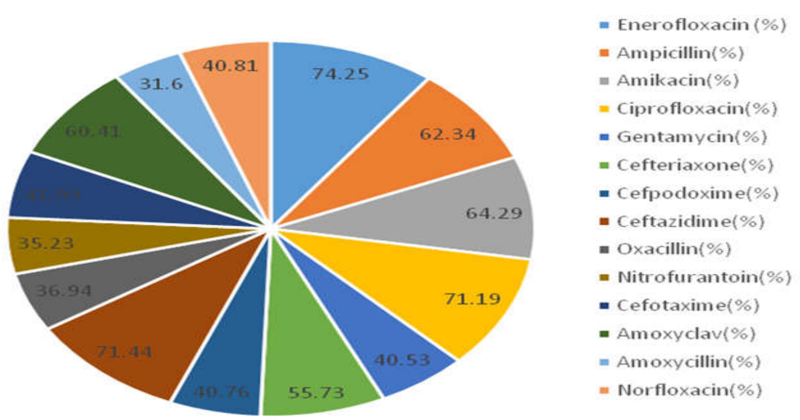

The table shows how sensitive *E. coli* bacteria from mastitic milk are to different antibiotics across seven districts. Overall, **Ciprofloxacin (78%)** and **Enrofloxacin (77%)** were the most effective antibiotics, showing that fluoroquinolones work well against *E. coli*. **Ceftriaxone (75%)**, **Ceftazidime (73%)**, and **Amikacin (66%)** also showed good effectiveness. Moderate sensitivity was observed for **Gentamycin (56%)**, **Cefpodoxime (58%)**, and **Amoxyclav (56%)**. Low sensitivity was seen for **Oxacillin (44%)**, **Nitrofurantoin (44%)**, **Amoxycillin (44%)**, and **Cefotaxime (37%)**, suggesting resistance to these drugs. Among districts, **Chikkaballapur, Ramanagara, and Tumkuru** showed high antibiotic sensitivity, while **Belagavi and Sirsi** had more variable responses. The results indicate that **fluoroquinolones and certain cephalosporins remain effective**, but **careful use of antibiotics and monitoring resistance** are necessary to control *E. coli* mastitis.

**3.2.12** Overall cumulative sensitive pattern to *Klebsiella sps* in Karnataka:

**Overall cumulative sensitive pattern to *Klebsiella sps* in Karnataka: Table 14**

| District | Enerofloxacin (%) | Ampicillin (%) | Amikacin (%) | Ciprofloxacin (%) | Gentamycin (%) | Cefteriaxone (%) | Cefpodoxime (%) | Ceftazidime (%) | Oxacillin (%) | Nitrofurantoin (%) | Cefotaxime (%) | Amoxyclav (%) | Amoxycillin (%) | Norfloxacin (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chikkaballapur | 85.71 | 85.71 | 71.43 | 71.43 | 57.14 | 57.14 | 57.14 | 71.43 | 14.29 | 42.86 | 42.86 | 28.57 | 14.29 | 14.29 |
| Belagavi | 66.67 | 66.67 | 33.33 | 66.67 | 33.33 | 66.67 | 66.67 | 66.67 | 66.67 | 66.67 | 33.33 | 33.33 | 33.33 | 33.33 |
| Bengaluru | 85.71 | 60.71 | 75 | 78.57 | 53.57 | 78.57 | 60.71 | 67.86 | 35.71 | 32.14 | 53.57 | 32.14 | 50 | 21.43 |
| Bengaluru-R | 75 | 50 | 62.5 | 75 | 37.5 | 50 | 37.5 | 87.5 | 37.5 | 25 | 37.5 | 50 | 12.5 | 50 |
| Ramanagara | 50 | 50 | 50 | 50 | 0 | 50 | 0 | 50 | 50 | 0 | 50 | 100 | 100 | 100 |
| Sirsi | 90 | 90 | 80 | 90 | 80 | 10 | 30 | 90 | 10 | 80 | 10 | 90 | 0 | 0 |
| Tumkuru | 66.67 | 33.33 | 77.78 | 66.67 | 22.22 | 77.78 | 33.33 | 66.67 | 44.44 | 0 | 66.67 | 88.89 | 11.11 | 66.67 |
| Average Grand Total % | 74.25 | 62.34 | 64.29 | 71.19 | 40.53 | 55.73 | 40.76 | 71.44 | 36.94 | 35.23 | 41.99 | 60.41 | 31.6 | 40.81 |

Graph 14: Overall cumulative sensitive pattern to *Klebsiella sps* in Karnataka




The table shows how sensitive *Klebsiella* bacteria from mastitic milk are to different antibiotics across seven districts. Overall, **Ciprofloxacin (71%)** and **Enrofloxacin (74%)** were the most effective antibiotics, showing fluoroquinolones work well against *Klebsiella*. **Amikacin (64%)** and **Ceftazidime (71%)** also showed good effectiveness. Moderate sensitivity was seen for **Ampicillin (62%)**, **Ceftriaxone (56%)**, and **Amoxyclav (60%)**. Low sensitivity was observed for **Gentamycin (41%)**, **Cefpodoxime (41%)**, **Oxacillin (37%)**, **Nitrofurantoin (35%)** and **Amoxycillin (32%)**, indicating resistance to these drugs. Among districts, **Chikkaballapur, Bengaluru, and Sirsi** showed higher sensitivity, while **Ramanagara and Tumkuru** had lower responses. The results suggest that **fluoroquinolones and certain cephalosporins are effective**, but **careful antibiotic use and regular resistance monitoring** are needed to manage *Klebsiella*spp. mastitis effectively.

**4.0 Discussion:**

The present study revealed that out of 580 mastitis-positive milk samples analysed, 368 (63.44%) showed bacterial growth, indicating a high prevalence of infectious mastitis among the sampled dairy cows. Remaining 212 samples didn't show growth of any bacteria, indicating that the samples were collected during the intermediate stage of antibiotic usage or treatment or sufficient withdrawal period was not followed leading to the residual effect of the previously used antibiotics. Apart from this few mastitis samples were due to some other etiological agents like Mycoplasma or other agents which might not have grown in the normal bacterial culture media. However, nearly the percentage of the samples which have not yielded any bacterial growth is an alarming sign or proof for the indiscriminate usage of the routinely available antibiotics by the field practitioners without following any planned antibacterial usage regime or ABST tests. *Staphylococcus spp*. (44.02%) was identified as the predominant pathogen, followed by *Escherichia coli spp*. (27.44%) and *Klebsiella spp*. (18.20%), while minor isolates included *Candida, Proteus, Pseudomonas, Streptococcus* and *Yeast* species. The predominance of *Staphylococcus spp*. aligns with previous reports **(19), (20), (24)** and **(25)** emphasizing its strong pathogenic potential due to its ability to adhere to mammary epithelial cells and form biofilms, which promote persistence and resistance to host immune defence. The resistance to antimicrobial agents against *Staphylococcus sps* in many regions of the world vary from region to region or herd to herd **(18)**.

Apart from this the diversity of Gram-negative bacteria (GNB) such as *E. coli spp.*, *Klebsiella spp.*, has also been well documented, highlighting their significant role and antimicrobial resistance in mastitis. Studies by **(21)** and **(22)** emphasized GNB diversity in mastitis and bulk tank milk, indicating environmental contamination sources. The indiscriminate use of antibiotics has directly contributed to the increased prevalence of antimicrobial resistance **(16)**. The overall use of antimicrobials within a herd for a prolonged period can influence the resistance patterns of *E. coli* isolates by elevating the concentration of these agents in the cow's environment. The findings of the aforementioned studies are in agreement with the results of the present investigation.

**4.1 *Staphylococcus spp*.:** Staphylococcus *spp*. accounted for 44.02% of mastitis isolates in the study area of Karnataka across the period of study, rising from 41.17% (2009–2013) to 49.57% (2019–2023), confirming its persistent dominance, Similar observations was reported by **(26)** and **(27)** who also identified *Staphylococcus sps* as the leading mastitis causing pathogen in India. Biofilm formation and resistance mechanisms enhance its persistence and recurrence more likely due to poor milking hygiene, chronic carriers, and antimicrobial misuse **(28)** and **(29)**. These findings stress the urgent need for better hygiene, culture-based therapy, and strong antimicrobial stewardship in dairy management.

**4.2 *Escherichia coli* spp:** *Escherichia coli* accounted for 27.44% of mastitis isolates in the study area of Karnataka, with fluctuating trends of 24.70% (2009–2013), 37.70% (2014–2018), and 23.52% (2019–2023). The environmental contamination and poor hygiene as major causes of coliform mastitis. Similar findings were reported by **(30), (31)** and **(32)**. Therefore, strengthened sanitation, regular herd screening, and judicious antibiotic use are essential for controlling *E. coli*-associated mastitis.

**4.3 *Klebsiella spp*.:** *Klebsiella spp.* accounted for 18.20% of total mastitis-positive samples across the study area of Karnataka, showing a clear decline from 21.17% (2009–2013) and 21.95% (2014–2018) to 10.08% (2019–2023).This downward trend reflects improved udder hygiene, housing, and milking practices **(33)**. Similar reductions in *Klebsiella* prevalence (10–20%) have been reported in other Indian studies over a period of time **(34)** and **(35).** Despite the decline, its persistence highlights the need for continued hygiene monitoring and antimicrobial stewardship **(36).**

**4.4 Bacterial Isolates and Susceptibility Testing based on Seasons Wise:** *Staphylococcus spp.* was the predominant mastitis pathogen (44.02%), followed by *E. coli* (27.44%) and *Klebsiella spp.* (18.20%) across seven districts of Karnataka. Its dominance throughout all seasons, especially in summer (47.87%), reflects its strong adaptability to dry and warm conditions **(35)**. *E. coli* and *Klebsiella spp.* showed higher prevalence during monsoon and winter, linked to contaminated environments **(34)**. Minor isolates like *Candida*, *Pseudomonas*, and *Streptococcus* occurred at lower frequencies, aligning with previous Indian reports **(36)**. Seasonal variation underscores the role of environmental hygiene and management in reducing mastitis incidence **(33)**.The number of Mastitis cases were more in rainy season compared to the other seasons might be due to the frequent exposure to the unhygienic conditions in the milk shed due to the overflow of drainage and mixing of rain water in the shed with dung leading to the growth of multiple bacterial contaminants which gain entry into the teats in the milking sheds when the dairy cows lies down in the sheds for resting.

**4.5 Antibacterial Sensitivity**: Fourteen antibiotics were tested against mastitis pathogens, showing variable sensitivity across bacterial species and regions. *Staphylococcus spp., E. coli, and Klebsiella spp*. were the predominant isolates with distinct antimicrobial profiles. Regional variations reflected differences in antibiotic use, hygiene, and environmental conditions **(33)**.Similar trends of antimicrobial resistance have been documented in other Indian studies **(37)** and **(38)**. The findings highlight the need for region-specific antibiotic surveillance and rational drug use to curb resistance **(35)**.

**4.6 Cumulative Percentage of Antibiotics sensitivity in Chikkaballapur District**In the Chikkaballpur district Enrofloxacin (68.88%), Ampicillin (64.99%), and Amikacin (61.66%) has shown higher sensitivity followed by Ciproflooxacin (54.99%) Gentamicin (54.99%) and Ceftrioxone (53.32%).Lower responses were noted for the remaining antibiotics. Reduced efficacy of β-lactam drugs suggests rising resistance due to overuse **(37)**.

**4.7 Cumulative Percentage of Antibiotics sensitivity in Belagavi District:**In the Belagavi district, Amoxyclav showed the highest overall sensitivity (51.11%), indicating moderate effectiveness against mastitis pathogens. Ceftriaxone (49.99%), Cefpodoxime (46.66%) and Oxacillin (46.66%) also displayed fair activity (~47%), suggesting modium susceptibility. Lower responses were noted for Enrofloxacin (42.22%), Norfloxacin (42.22%) and Ciprofloxacin (39.99%), while Amikacin (13.33%) and Ampicillin (6.66%) showed poor efficacy (<15%). This reduced sensitivity aligns with the earlier Indian reports of increasing β-lactam and aminoglycoside resistance in mastitis isolates **(37)** and **(38).**

**4.8 Cumulative Percentage of Antibiotics sensitivity in Bengaluru District:** In the Bengaluru district, Enrofloxacin showed the highest sensitivity (47.28%), indicating moderate efficacy against mastitis pathogens. Amikacin (44.48%), Ceftriaxone (41.78%) and Ciprofloxacin (41.08%) exhibited fair responses (~42%), while Ampicillin (37.17%) and Gentamycin (32.84%) showed reduced activity (<40%). Cephalosporins like Cefpodoxime (31.77%) and Cefotaxime (28.14%) displayed limited effectiveness, and β-lactam drugs such as Oxacillin (26.31%) and Amoxyclav (21.27%) showed poor responses (<25%). These findings align with Indian studies reporting declining efficacy of β-lactams and cephalosporins due to rising resistance **(37)** and **(38).**

**4.9 Cumulative Percentage of Antibiotics sensitivity in Bengaluru-R District:** In the Bengaluru-R district, Ciprofloxacin showed the highest sensitivity (48.39%), followed by Amikacin (45.16%) and Ceftazidime (45.16%), indicating moderate antibacterial efficacy. Enrofloxacin (41.93%) and Amoxyclav (38.70%) displayed fair activity, while Ampicillin (35.48%) and Norfloxacin (32.26%) showed reduced effectiveness (<36%). Cephalosporins like Ceftriaxone (29.03%) and Cefpodoxime (22.58%) exhibited low sensitivity (<30%), suggesting emerging resistance. Poor responses to Nitrofurantoin (19.35%), Amoxycillin (19.35%)and Gentamycin (16.13%)reflect widespread resistance among isolates. These results align with Indian reports highlighting growing resistance to β-lactams and aminoglycosides, emphasizing the need for rational antibiotic use **(37)** and **(38).**

**4.10 Cumulative Percentage of Antibiotics sensitivity in Ramnagara District:** In the Ramanagara district, Enrofloxacin, Amoxycillin, and Ceftriaxone showed the highest sensitivity (~59%), indicating strong efficacy against mastitis pathogens. Moderate responses to Ceftazidime, Oxacillin, and Cefotaxime suggest partial therapeutic potential. Lower sensitivity to Amoxyclav and Cefpodoxime reflects early resistance to β-lactam antibiotics. Poor responses to Gentamycin, Amikacin and Nitrofurantoin highlight increasing resistance among isolates. Similar resistance trends to aminoglycosides and β-lactams have been documented in Indian studies **(37)** and **(38),** emphasizing the need for region-specific antibiotic stewardship.

**4.11 Cumulative Percentage of Antibiotics sensitivity in Sirsi-Uttara Kannada District:** In the Sirsi district, Ampicillin and Enrofloxacin (60.97%) exhibited the highest sensitivity, indicating good efficacy against mastitis pathogens. Moderate responses to Ciprofloxacin, Amoxyclav, and Ceftazidime suggest partial therapeutic potential. Reduced sensitivity to Amikacin and Oxacillinreflects emerging resistance among both Gram-positive and Gram-negative isolates. Very low responses to Ceftriaxone, Norfloxacin, and Amoxycillin indicate extensive β-lactam resistance. Comparable resistance trends have been documented in Indian studies, emphasizing misuse-driven resistance and the need for rational antibiotic use **(37) & (38).**

**4.12 Cumulative Percentage of Antibiotics sensitivity in Tumkuru District:** In the Tumkuru district, Ceftriaxone and Amoxyclav (47.36%) showed the highest sensitivity, indicating moderate efficacy against mastitis pathogens. Fluoroquinolones such as Enrofloxacin and Ciprofloxacin exhibited fair activity, consistent with their broad-spectrum use in dairy infections. Reduced sensitivity to β-lactams (Ampicillin, Oxacillin) and aminoglycosides (Gentamycin) suggests rising resistance due to frequent therapeutic use. The lowest responses to Cefpodoxime and Nitrofurantoin highlight declining drug efficacy in field isolates. Similar resistance trends have been reported in Indian studies, underscoring the impact of empirical antibiotic use and the need for region-specific treatment strategies **(37)** and **(38).**

**5.0 Summary:** This retrospective study evaluated the antimicrobial sensitivity patterns of mastitis-causing pathogens in dairy cattle across Karnataka, India, over a 15-year period (2009–2023). Out of 580 milk samples collected from farms of varying sizes, 368 (63.44%) showed positive bacterial growth and were subjected to antimicrobial susceptibility testing against fourteen antibiotics.

*Staphylococcus spp.* emerged as the predominant pathogen, accounting for 44.02% of all cases (range 39.02–52.94%), followed by *Escherichia coli spp.* (27.44%) and *Klebsiella spp.* (18.20%). Together, these three major pathogens were responsible for 89.67% of all mastitis infections, underscoring their continued dominance in the region **(37).** The seasonal analysis revealed a higher incidence of mastitis during the monsoon (41.30%), compared to winter (35.61%) and summer (31.86%), although the distribution of specific pathogens remained largely unchanged across seasons, consistent with observations by **(33).**

When examined over three consecutive five-year intervals, the causative pattern remained relatively stable, indicating that *Staphylococcus spp.*, *E. coli spp.* and *Klebsiella spp.* have persistently dominated the mastitis landscape for over a decade. The antibiotic sensitivity profiles demonstrated variable resistance trends, emphasizing the ongoing need for judicious antimicrobial use and continuous monitoring **(38)**.

Overall, the study highlights the enduring prevalence of *Staphylococcus spp.* as the primary etiological agent, the stable pathogen pattern over time, and the importance of implementing targeted control strategies, improved farm hygiene, and rational antibiotic stewardship to effectively manage bovine mastitis and mitigate antimicrobial resistance **(39)**.

**6.0 Conclusion:** This 15-year retrospective study provides a comprehensive overview of the antimicrobial sensitivity trends and etiological patterns of bovine mastitis in Karnataka, India. The findings reveal that *Staphylococcus spp.* (44.02%) continues to be the predominant pathogen, followed by *Escherichia coli* (27.44%) and *Klebsiella spp.* (18.20%), together accounting for nearly 90% of all mastitis cases. The overall incidence was notably higher during the monsoon season, although the distribution of pathogens remained consistent across different seasons.

Across three consecutive five-year intervals (2009–2023), the dominance of these major pathogens showed remarkable stability, indicating a persistent infection trend within the region. The antimicrobial susceptibility results demonstrated variable resistance patterns among isolates, reflecting the emerging challenge of antimicrobial resistance.

Overall, the study underscores the continued dominance of *Staphylococcus spp*, *E. coli spp.* and *Klebsiella spp.* as key mastitis pathogens, the seasonal influence on disease occurrence, and the need for judicious antibiotic use, improved milking hygiene, and sustained surveillance programs. Strengthening farm-level biosecurity and promoting targeted therapy based on regular sensitivity testing are crucial to reduce mastitis burden, prevent antimicrobial resistance, and ensure sustainable dairy productivity in Karnataka and similar agro-climatic regions.

The growing antibiotic resistance and limited development of new drugs pose a serious public health threat. It is crucial to regulate antibiotic use in livestock and promote the integration of ethno-medicines as alternative therapies. Enhancing awareness among farmers and veterinarians on responsible antibiotic use, routine milk testing for early infection detection, and adopting clean milk production practices can effectively reduce infection risks, curb antibiotic resistance, and protect public health.

**Conflicts of Interest:** The authors declare that they have no conflicting interests.

**Acknowledgement:** Authors are thankful to SRDDL (GOI), IAH&VB, KVAFSU for providing funding and all required facilities for carrying out this work.

**References:**

1. Luz, C. F., J. M. van Niekerk, J. Keizer, et al. 2022. “Mapping Twenty Years of Antimicrobial Resistance Research Trends.” Artificial Intelligence Medical [Internet] 123, no.November 2021: 102216. https://doi.org/10.1016/j.artmed.2021.102216.
2. Balkhy,H., S. A. Al, N. Dagunton, and N Dagunton. 2015. “AMultifaceted Approach in Reducing Central Line Associated Bloodstream Infections (CLABSI) in Pediatric icus at a Tertiary Hospital.” Antimicrobial Resistance and Infection Control 4, no. S1: 2994.
3. Fadul-pacheco, L., H. Delgado, and V. E Cabrera. 2021. “Exploring Machine Learning Algorithms for Early Prediction of Clinical Mastitis.” International Dairy Journal [Internet] 119: 105051. Available From: https://doi.org/10.1016/j.idairyj.2021.105051.
4. Cobirka, M., V. Tancin, and P Slama. 2020. “Epidemiology and Classification of Mastitis.” Animals 10, no. 12: 1–17.
5. De Oliveira, A. P., J. L. Watts, S. A. Salmon, and F. M. Aarestrup. 2000. Antimicrobial susceptibility of Staphylococcus aureus isolated from bovine mastitis in Europe and the United States. J.Dairy Sci. 83:855–862.
6. Mackie, D. P., E. F. Logan, D. A. Pollock, and S. P. Rodgers. 1988. Antibiotic sensitivity of bovine staphylococcal and coliform mastitis isolates over four years. Vet. Rec. 123:515–517.
7. Petersson-Wolfe, C., I.K.Mullarky, andG. M. Jones. 2010. “Staphylococcus aureus Mastitis: Cause, Detection, and Control.” Virginia Cooperative Extention [Internet] 404, no. 229: 7. Available From: https://pubs.ext.vt. edu/404/404-229/404-229_pdf.pdf.
8. BROWN MB, SCASSERRA AE: Antimicrobial resistance in streptococcal species isolated from bovine mammary glands. Am. J. Vet. Res., 1990, 51, 2015-2018.

9. HILLERTON JE, BERRY EA: Treating mastitis in the cow–a tradition or an archaism. J. Appl. Microbiol., 2005, 98, 1250–1255.
10. Ruegg, P. L. 2018. "Making Antibiotic Treatment Decisions for Clinical Mastitis." The Veterinary Clinics of North America. Food Animal Practice 34, no. 3: 413–425..
11. Mushtaq M., Agrawal R., Bhat M.A., Singh R., Pande N. (2019). Antibiotic resistance gene typing in Staphylococcus aureus isolated from bovine mastitis. Indian Journal of Animal Sciences, 89(11): 1188-1191.
12. Elias, L., A. S. Balasubramanyam, O. Y. Ayshpur, et al. 2020. "Antimicrobial Susceptibility of Staphylococcus aureus, Streptococcus agalactiae, and Escherichia coli Isolated From Mastitic Dairy Cattle in Ukraine." Antibiotics 9, no. 8: 1–7.
13. Cruickshank, R., Duguid, J. P., Marmion, B. P., & Swain, R. H. A. (1970). Medical microbiology: A guide to the laboratory diagnosis and control of infection (11th ed.). E. & S. Livingstone
14. Markey, B.K., Leonard, F.C., Archambault, M., Cullinane, A., and Maguire, D.2013. Text book of Clinical Veterinary Microbiology, 2nd edition.Elsevier
15. Bauer, A.W., Kirby, W.M.M., Sherris, J.C.,and Turck,M. 1966. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clini.Path. 45:493–496.
16. DANMAP (2001). Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme, Copenhagen, Denmark.
17. Sori, T., Hussein, J. and Bitew, M.(2011). Prevalence and susceptibility assay of Staphylococcus aureus isolated from bovine mastitis in dairy farms of Jimma town South west Ethiopia. Journal of Animal and Veterinary Advances, 10(6):745-749.
18. Waage, S.B., Bjorland, J., Caugant, A., Oppegaard, H., Tollersrud, T., Mork, T. and Aarestrup, F. (2002). Spread of Staphylococcus aureus resistant to penicillin and tetracycline within and between dairy herds. Epidemiology of infection, 129: 193- 202
19. Bedada, B.A. and Hiko, A. 2011. Mastitis and antimicrobial susceptibility test at Asella,Oromia Regional state. Ethiopia. J. Microbiol. Antimic., 3(9): 228-232.
20. Sumathi, B.R., Veeregowda, B.M. and Amitha, R.G. 2008. Prevalence and antibiogram profi le of bacterial isolates from clinical bovine mastitis. Vet. World., 1(8): 237-238.
21. Malinowski, E., H. Lassa, A. Kłlossowska, S. Smulski, H. Markiewicz, and M. Kaczmarowski. 2006. Etiological agents of dairy cows' mastitis in western part of Poland. Pol. J. Vet. Sci. 9:191–194.
22. Jayarao, B. M., and L. Wang. 1999. A study on the prevalence of gram negative bacteria in bulk tank milk. J. Dairy Sci. 82:2620–2624.
23. Shinde SS, Kulkarni GB, Gangane GK, Degloorkar NM. Incidence of mastitis in parbhani district of Maharashtra. Proceedings of the Round Table Conference on Mastitis, Ludhiana, India, 2001
24. Barkema, H. W., Schukken, Y. H., & Zadoks, R. N. (2006). The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. *Journal of Dairy Science*, 89, 1877–1895.
25. Pitkälä, A., Haveri, M., Pyörälä, S., Myllys, V., & Honkanen-Buzalski, T. (2004). Bovine mastitis in Finland 2001—Prevalence, distribution of bacteria, and antimicrobial resistance. *Journal of Dairy Science*, 87, 2433–2441.
26. Kumar, S., Sharma, A., & Singh, R. (2023). Prevalence and antibiogram of *Staphylococcus aureus* isolated from bovine mastitis milk in South India. *Indian Journal of Veterinary Sciences & Biotechnology*, 19(1), 45–50.
27. Rani, R., Gupta, R., & Patel, D. (2022). Epidemiological investigation of *Staphylococcus aureus* associated with subclinical mastitis in Indian dairy cows. *Indian Journal of Animal Research*, 56(9), 1023–1028.
28. Khairullah, A. R., et al. (2024). Molecular detection and sequence analysis of the *mecA* gene from milk and farm worker hand swabs in India. *Veterinary World*, 17(4), 765–772.
29. Bhat, S. A., Kumar, A., & Shukla, S. K. (2023). Methicillin-resistant *Staphylococcus aureus* (MRSA) from bovine mastitis milk: prevalence, resistance pattern, and public health implications. *Journal of Entomology and Zoology Studies*, 11(2), 186–191.
30. Sharma, R., Singh, M., & Yadav, A. (2023). Prevalence and antibiotic resistance profile of *Escherichia coli* isolated from bovine mastitis in North India. *Indian Journal of Animal Research*, 57(5), 621–626.
31. Karthikeyan, R., Kumar, S., & Devi, R. (2022). Isolation and antibiogram of *E. coli* from mastitic milk samples in Tamil Nadu. *Indian Journal of Veterinary Sciences & Biotechnology*, 18(3), 92–97.
32. Patel, D. M., Joshi, R. K., & Mehta, S. R. (2024). Molecular characterization and antimicrobial resistance of *Escherichia coli* from bovine mastitis milk in Gujarat. *Veterinary World*, 17(6), 1054–1060.

33. Singh, S., Sharma, R., & Gupta, A. (2023). Temporal dynamics of environmental mastitis pathogens in dairy cattle: a multi-regional study from India.*Veterinary World*, 16(2), 312–320.
34. Devi, R., Kumar, A., & Singh, P. (2022). Epidemiological insights into bovine mastitis and antibiotic resistance patterns of major pathogens in India.*Indian Journal of Dairy Science*, 75(4), 450–457.
35. Kumar, R., Bhatt, V. D., & Patel, S. J. (2021). Environmental reservoirs and antimicrobial resistance of *Klebsiella* species in dairy environments.*Indian Veterinary Journal*, 98(7), 25–29.
36. Patel, D., Mehta, D., & Chauhan, H. (2020). Role of environmental hygiene in controlling mastitis-associated *Klebsiella* infections in Indian dairy farms.*Journal of Animal Research*, 10(5), 789–795.
37. Sharma, N., Singh, N. K., & Bhadwal, M. S. (2021). *Epidemiology and control of bovine mastitis in India: A review.*Asian Journal of Dairy and Food Research, 40(1), 1–8.
38. Devi, S., Das, R., & Roy, S. (2022). *Antibiotic resistance profile of mastitis pathogens in Indian dairy herds.*Veterinary World, 15(2), 347–354.
39. Tripathi, P., Chaudhary, S., & Ghosh, S. (2023). *Emerging resistance trends among Gram-negative mastitis pathogens in dairy cattle.*Indian Journal of Veterinary Microbiology, 33(2), 85–93.