

Research Article

## PREVALENCE OF *KLEBSIELLA PNEUMONIAE* AND ANTIMICROBIAL RESISTANCE GENES IN BOVINE MASTITIS IN CAUVERY DELTA REGION OF TAMIL NADU

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**ABSTRACT:** *Klebsiella pneumoniae* is emerging as a major environmental mastitis pathogen, often linked with poor cure rates. The studies of *K. pneumoniae* associated mastitis in dairy cattle are very limited. The study was carried out to assess the prevalence, antimicrobial resistance pattern and biofilm forming capacity of *K. pneumoniae* in bovine mastitis in the Cauvery delta region of Tamil Nadu, India. A total of 155 milk samples were collected from mastitis cases and bacterial culture was performed. The bacteria was identified based on colony characters and confirmed by PCR. Further, the antimicrobial sensitivity assay, minimum inhibitory concentration and biofilm assay was performed in *K. pneumoniae* isolates to determine the antimicrobial resistance, and its resistance genes were confirmed by PCR. Among 155 milk samples, bacterial growth was observed in 123 samples, of which 78 (63%) samples had *K. pneumoniae*. The antimicrobial sensitivity assay revealed that the 91.25% of *K. pneumoniae* isolates were sensitive to enrofloxacin, ciprofloxacin, ceftriaxone and co-trimoxazole and 62.50% were sensitive to oxytetracycline and 100% were resistant to ampicillin and methicillin. The MIC of amoxicillin-cloxacillin, amoxicillin-sulbactam, gentamicin and oxytetracycline against *K. pneumoniae* were ranged between 125-500, 25-220, 200-400 and 75-500 µg/ml, respectively. All *K. pneumoniae* isolates from mastitis cases were moderate to strong biofilm formers. All *K. pneumoniae* isolates contained ESBL genes, specifically *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>. Among these, 66% of isolates had a combination of both *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes, 24% of isolates had *bla*<sub>SHV</sub> and 10% had the *bla*<sub>TEM</sub>. The aminoglycoside resistant gene was detected in two isolates only. None of the isolates had the fluoroquinolone resistance gene (*gyrA* and *gyrB*), cefotaxime resistance gene (*bla*<sub>CTX-M-G1</sub> and *bla*<sub>CTX-M-G2</sub>), sulfonamide resistance gene (*sulI*), tetracycline resistance gene (*tetA* and *tetM*) and the multidrug efflux pump gene (*acrAKp*). The *K. pneumoniae* was highly prevalent in clinical mastitis cases, and the isolates exhibited high MIC, biofilm forming ability and carriage of AMR genes. An integrated AMR surveillance to be implemented in future.

**Keywords:** AMR, Biofilm, ESBLs, *Klebsiella pneumoniae*, Mastitis, MIC

### INTRODUCTION

Mastitis is one of the major production losses causing disease in the dairy animals throughout the world. It is most commonly caused by contagious and environmental pathogens. Nowadays, the incidence of environmental mastitis gradually increases with the decline of contagious mastitis [1]. The large proportion of environmental mastitis in cattle is caused

by *Escherichia coli*, *Klebsiella* spp and *Enterobacter* sp [2, 3], and which are found in soil, feed, drinking water, faeces, rumen contents, bedding material, sewage, surface water and plants, which is transmitted at any time, during milking, between milking, dry period and prior to calving in heifers [4, 5]. The intramammary infection of *Klebsiella pneumoniae* commences as subclinical in the early dry period and progresses to

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clinical mastitis during lactation [6]. The increased production of inflammatory cytokines (IL-1, IL-6, IL-8 and TNF $\alpha$ ) during an infection induces a severe systemic inflammatory reaction and strong immune response, the damaged mammary epithelial cells undergoes apoptosis [5]. The *Klebsiella* spp more often causes severe long lasting intramammary infection and persists for more than 200 days and more often detrimental effects on milk production and increased premature culling in dairy cattle [3,4,7]

The *K. pneumoniae* is a large capsulated gram negative bacilli that encodes major virulence factors, viz., fimbriae, capsules, lipopolysaccharides and siderophores [5]. The *K. pneumoniae* is classified as classical and hypervirulent based on virulence [8]. The capsular serotype is usually associated with hypervirulence and emerging as multidrug resistance [9, 10]. The biofilm formation is a key virulence mechanism responsible for antimicrobial resistance and poor response to treatment [11]. The *K. pneumoniae* inherently resistant to penicillin and ampicillin through the production of extended spectrum  $\beta$ -lactamase (ESBL) [5]. The rate of antimicrobial resistance (AMR) among *K. pneumoniae* isolates in dairy herds varied with the regions [12]. The documentation of region-wise AMR pattern of *K. pneumoniae* is utmost importance to select the choice of antibiotics for treatment to improve the recovery rate. The present article described the prevalence and antimicrobial resistance patterns of *K. pneumoniae* isolated from bovine mastitis cases in the Cauvery delta region of Tamil Nadu.

## MATERIALS AND METHODS

A total of 155 milk samples were collected from dairy cows affected with clinical mastitis, which was presented to the large animal medicine unit of the Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu. The colour and consistency of milk varied from yellow serous fluid with fibrin clots, yellowish milk with flakes, watery milk with flakes and brown coloured fluid.

### Isolation of bacteria

The isolation of bacteria in milk samples was performed as per standard procedures [13]. The milk samples were inoculated into sterile nutrient broth and incubated at 37°C for 12-24 hrs. The broth culture was streaked into MacConkey agar (MA) and Eosin and Methylene Blue (EMB) agar by using a sterile inoculation loop and incubated at 37°C. The colony characters of bacteria were examined after 24 hrs. The

biochemical tests, like indole, methyl red, citrate and Voges-Proskauer test were done to identify the bacteria.

### Antibacterial sensitivity test

The antimicrobial sensitivity pattern of *K. pneumoniae* isolates were performed as per the Kirby Bauer disc diffusion method [14]. A single *K. pneumoniae* colony from each isolate was inoculated into nutrient broth and incubated at 37°C for 12 hrs. The broth culture was swabbed in Muller Hinton agar and antibiotic impregnated disc such as ciprofloxacin (5  $\mu$ g), enrofloxacin (5  $\mu$ g), gentamicin (10  $\mu$ g), amikacin (30  $\mu$ g), ampicillin (10  $\mu$ g), ceftriaxone (30  $\mu$ g), ampicillin/sulbactam (10/10  $\mu$ g), methicillin (5  $\mu$ g), co-trimoxazole (25  $\mu$ g) and oxytetracycline (30  $\mu$ g) were placed at equal distance and incubated at 37°C for 24 hrs. The zone of inhibition for each antibiotic was measured and recorded.

### Determination of minimum inhibitory concentration (MIC)

The MIC of antibiotics were carried out as per the method described by Eroglu *et al.* (2024) [15]. Frozen cultures of *K. pneumoniae* isolates were thawed to room temperature and inoculated 100  $\mu$ l in sterile nutrient broth (5ml), incubated at 37°C for 24 hrs and observed for growth based on turbidity appearance. This culture (100  $\mu$ l) again inoculated in sterile nutrient broth and incubated at 37°C for 24 hrs. Thereafter, bacterial culture was centrifuged at 3000 rpm for 10 minutes, the supernatant was discarded and the bacterial pellet was washed twice with phosphate buffered saline (pH 7.2). Then bacterial pellet was reconstituted in sterile nutrient broth and 200  $\mu$ l of culture transferred to a 96 well sterile microtitre plate and antibiotics (amoxicillin cloxacillin, amoxicillin sulbactam, gentamicin and oxytetracycline) were added in different concentrations separately for each isolates and incubated at 37°C for 24 hrs along with a negative control (nutrient broth culture without antibiotics). The absorbance was measured on 0 and 24 hrs at 570 nm to determine the inhibition of growth using a spectrophotometer. The MIC of antibiotics (amoxicillin-cloxacillin, amoxicillin-sulbactam, gentamicin and oxytetracycline) were tested in the range of 0.1-1000  $\mu$ g/ml.

### Biofilm assay

The overnight nutrient broth culture (100  $\mu$ l) of *K. pneumoniae* isolates were inoculated in 10 ml of brain heart infusion (BHI) broth enriched with sucrose (2%) and incubated at 37°C for 6-8 hrs. The log phase BHI enriched

broth culture (1:100 dilution) 200 µl was transferred to a sterile 96 well microtitre plate and incubated at 37°C for 48 hrs, the liquid phase was decanted and plate was washed with 200 µl of phosphate buffered saline for three times. Thereafter, 200 µl of 0.1% crystal violet dye was added and incubated at room temperature for 15 minutes, then the contents were decanted and washed with distilled water for three times, the plate was dried by blotting on tissue paper. The glacial acetic acid (30%) 100 µl was added to each well to solubilize the biofilm, allowed to stand for 15 minutes and measured the absorbance at 570 nm using a spectrophotometer. The biofilm was graded as weak, moderate and strong based on biofilm forming capacity [16].

#### Extraction of bacterial DNA

The bacterial DNA was extracted by the boiling and lysis method [17]. A single pure colony was transferred into a 1.5 ml microcentrifuge tube containing 500 µl of sterile distilled water, mixed well, boiled for 10 minutes and cooled at room temperature. The sample was centrifuged at 8000 rpm for 6 minutes, and the clear supernatant was carefully aspirated and stored at -20°C for PCR analysis.

#### Molecular detection of bacteria and AMR genes

The genotypic identification of *Klebsiella* spp was done by PCR. The primer sequences of target bacteria and antimicrobial resistance genes are given in table 1 [18, 19] and table 2 [20, 21, 22, 23]. The PCR was carried out with optimized thermal cycling conditions of initial denaturation at 94°C for 10 minutes followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, extension for 1 minute and final extension at 72°C for 10 minutes. The PCR confirmed field isolate of *K. pneumoniae* was used as a positive control, and the PCR reaction without a DNA template was used as a negative control. The amplified PCR products were electrophoresed in 1.5 % agarose with 1X TAE buffer at 60 V for 45 minutes, visualized under a UV transilluminator and documented in the Gel Doc system.

## RESULTS AND DISCUSSION

In bacterial culture, out of 155 milk samples, the growth was observed in 123 samples, of which 78 samples were positive for *Klebsiella* spp (63%). In MacConkey agar, the *Klebsiella* spp exhibited lactose-fermenting small to large dome shaped glistening pink to yellow mucoid colonies with smooth edges. The colonies had a pink centre and pink edges or yellow centre with pink edges (Fig. 1). In the biochemical test, the *Klebsiella* spp showed a positive reaction to citrate, acid production with gas in triple sugar iron agar and a negative reaction to indole. The PCR amplification confirmed the presence of *Klebsiella* spp and *K. pneumoniae* and its expected amplicon sizes were 441 and 130 bp, respectively (Fig. 2).

In this study, the prevalence of *K. pneumoniae* was 63%. This finding was in concordance with the report of Salauddin *et al.* (2019) [24], who reported that 62.5% of bovine mastitis in Bangladesh due to *Klebsiella* spp. The country-wise reported prevalence of *K. pneumoniae* in clinical mastitis cases of dairy cows in Japan (38.8%), Asia (14.26%), China (7.49%), sub-saharian Africa (8.6%), South Africa (18.5%), Kenya (23%) and Nigeria (11.1%), Northwestern Iran (40%) and European countries (0.73%). Among Asian countries, the higher prevalence of *K. pneumoniae* was reported in Bangladesh followed by Iran and Japan. The least prevalence was reported in European countries [3, 25, 26, 27].

In ABST, the *K. pneumoniae* isolates of bovine mastitis showed 91.25% sensitivity to enrofloxacin, ciprofloxacin, ceftriaxone and co-trimoxazole, 62.5% sensitivity to oxytetracycline and 100% resistance to ampicillin and methicillin (Fig. 3). These finding were in concordance with the report of Fu *et al.* (2022) [5], who reported that the *K. pneumoniae* was highly sensitive to enrofloxacin (92.31%) followed by tobramycin and ceftiofur (73.85%). Arya *et al.* (2020) [28] reported that the *K. pneumoniae* isolates in bovine milk samples were highly susceptible to the quinolone group of antibiotics and resistant to ampicillin. Yadav *et al.* (2021) [29] reported that *K.*

**Table 1. Primer sequence of *Klebsiella* spp.**

S. No.	Name of the bacteria	Target gene	Primer sequence	Amplicon size (bp)	Reference
1.	<i>Klebsiella</i> spp	<i>gyrA</i>	F-5'-CGCGTACTATACGCCATGAACGTA-3' R-5'-ACCGTTGATCACTTCGGTCAGG-3'	441	18
2.	<i>K. pneumoniae</i>	<i>16S-23S</i> rDNA	Pf 5'-ATTTGAAGAGGTTGCAAACGAT-3' Pr1 5'-TTCACCTCTGAAGTTTTCTTGTGTTC-3'	130	19

**Table 2. Primer sequence of antimicrobial resistance genes.**

S. No.	Antibiotics	Target gene	Primer sequence	Amplicon size (bp)	Reference
1.	Ampicillin	<i>bla</i> <sub>TEM</sub>	F- 5' TCCGCTCATGAGACAATAACC-3' R 3' ATAATACCGCACCACATAGCAG-5'	296	20
2.	Ampicillin	<i>bla</i> <sub>SHV</sub>	F 5' -TACCATGAGCGATAACAGCG-3' R 3' - GATTTGCTGATTTGCTCGG-5'	450	20
3.	Cefotaxime	<i>bla</i> <sub>CTX-M-G1</sub>	F- 5'- GTTACAATGTGTGAGAAGCAG-3' R-5'- CCGTTTCCGCTATTACAAAC-3'	1000	21
4.	Cefotaxime	<i>bla</i> <sub>CTX-M-G2</sub>	F-5'-CGTTAACGGCAGATGAC-3' R-5'-CGATATCGTTGGTGGTRCCA-3'	404	21
5.	Aminoglycoside	<i>aac</i> (6')- <i>aph</i> (2'')	F- 5'-TTGGGAAGATGAAGTTTTTAGA-3' R- 5'-CCTTTACTCCAATAATTTGGCT-3'	174	22
6.	Quinolones	<i>qnrA</i>	F-5'-ATTTCTCACGCCAGGATTTG-3' R- 5'-GATCGGCAAAGGTTAGGTCA-3'	516	18
7.	Tetracycline	<i>tetA</i>	F-5'-GGTTCACTCGAACGACGTC-3' R-5'-CTGTCCGACAAGTTGCATGA-3'	577	18
8.	Tetracycline	<i>tetM</i>	F-5'-GTGGACAAAGGTACAACGAG-3' R-5'-CGGTAAAGTTCGTCACACAC-3'	403	23
9.	Sulfonamide	<i>sulI</i>	F-5'-TTCGGCATTCTGAATCTCAC-3' R-5'-ATGATCTAACCCCTCGGTCTC-3'	822	18
10.	Multi-drug efflux pump	<i>acrAKp</i>	F-5'-ATTTCTCACGCCAGGATTTG-3' R-5-GATCGGCAAAGGTTAGGTCA-3'	940	18

**Table 3. Minimum inhibitory concentration (MIC) of antibiotics for *Klebsiella pneumoniae* isolated from mastitis cases.**

Mastitis conditions	No. of isolates	Minimum inhibitory concentration of antibiotics (µg/ml)			
		Amoxicillin-cloxacillin	Amoxicillin-sulbactam	Gentamicin	Oxytetracycline
Mastitis without systemic involvement	11	125	25	200	50
Mastitis with systemic involvement	35	500	50-100	240	75-125
Mastitis with severe systemic involvement	32	>1000	220	400	500

*pneumoniae* isolates of bovine mastitis milk were 100% susceptible to chloramphenicol, 83.30% to enrofloxacin, 72.20% to oxytetracycline and 55.60% to gentamicin and all isolates were resistant to the penicillin group of antibiotics. The *K. pneumoniae* isolates from mastitis milk samples in China showed resistant to sulphonamides (45.07%), tetracycline (36.18%), aminoglycosides (27.47%), β-lactams (27.35%) amphenicols (26.82%), macrolides (20.98%), lincosomides (21.24%), polypeptides (15.31%) and quinolones (7.80%) [25]. The low level of resistance has been reported to tetracycline (5.6-19.5%), and β-lactams (0-6.9%) in Europe and the United States [12].

The MIC of *K. pneumoniae* isolates against

amoxicillin-cloxacillin, amoxicillin-sulbactam, gentamicin and oxytetracycline were ranged between 125- 500 (isolates of mild to moderate cases), 25-220, 200-400 and 75-500 µg/ml, respectively (Table 3). *K. pneumoniae* isolates (11) from animals without systemic involvement showed MICs of 125, 25, 200 and 50 µg/ml, respectively for amoxicillin-cloxacillin, amoxicillin-sulbactam, gentamicin and oxytetracycline. The *K. pneumoniae* isolates (35) from systemic involvement like anorexia, reduced feed intake and yellowish milk with flakes showed MICs of 500, 50-100, 240 and 75-125 µg/ml, respectively for amoxicillin-cloxacillin, amoxicillin-sulbactam, gentamicin and oxytetracycline, respectively. The *K. pneumoniae* isolates (32) from

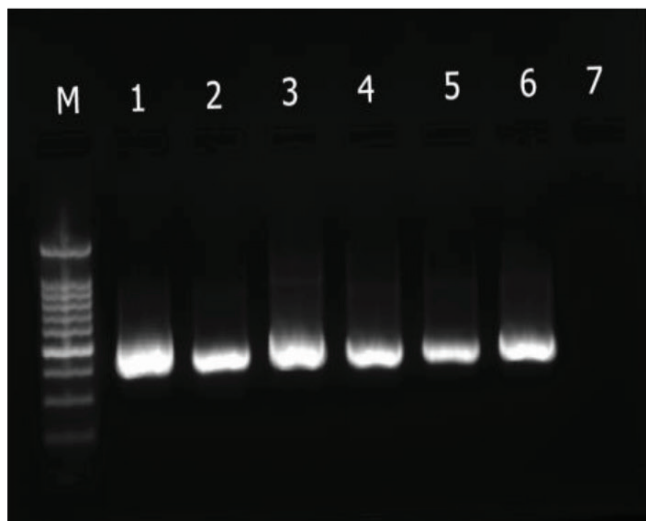
animals with severe systemic involvement like pyrexia, lameness, anorexia, and reduced feed intake and yellow serous fluid with fibrin clots from affected mammary quarters had MICs of 220, 400 and 500 µg/ml, respectively for amoxicillin-sulbactam, gentamicin and oxytetracycline, whereas, no MIC was observed for amoxicillin-cloxacillin @ 1000 µg/ml. (Fig. 4). The MIC of amoxicillin-cloxacillin for *K. pneumoniae* was

similar to the report of CLSI (2020) [14], reported that the MIC of amoxicillin for *K. pneumoniae* was >128 µg/ml. The result of MIC of amoxicillin-sulbactam was corroborates with the report of Mukherjee *et al.* (2013) [30], who opined that the MIC of sulbactam against *K. pneumoniae* was 25 µg/ml. In this study, the MIC of gentamicin (200-400 µg/ml) was higher than the report of Eroglu *et al.* (2024) [15], who reported that the MIC of gentamicin against *K. pneumoniae* was 0.09 µg/ml. In this study, the MIC of oxytetracycline for *K. pneumoniae* was similar to the report of Mosallam and Elshimy, (2024) [31], who reported that the MIC of oxytetracycline against *K. pneumoniae* was more than 100 µg/ml. The high MIC of amoxicillin-cloxacillin and gentamicin indicated the resistance status of *K. pneumoniae* in bovine mastitis.

Out of 78 isolates, 23 and 55 isolates had moderate (OD value - 0.175-0.230) and strong biofilm formation (OD value - 0.421-0.658), respectively (Fig.5). The *K. pneumoniae* isolates from clinical mastitis cases were 100% biofilm formers. The virulent *K. pneumoniae* (isolates of milk from yellow serous fluid with fibrin clots) strongly produced biofilm and exhibited a high MIC value and poorly responded to treatment. This finding indicated that a strong correlation exists between biofilm forming *K. pneumoniae* and antimicrobial resistance. The similar results were reported by

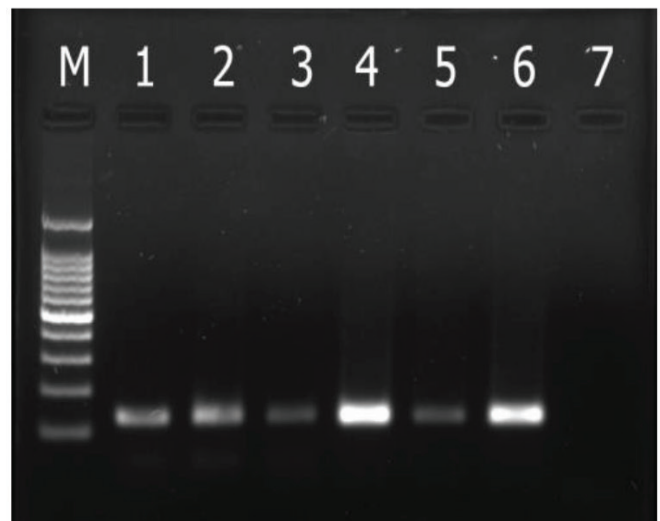


Fig. 1. Colony character of *K. pneumoniae* in MacConkey agar- pink to yellow mucoid colonies.



(a)

M - DNA marker (100 bp)  
 Lane 1 - Positive control  
 Lane 2-6 - Positive field samples  
 Lane 7 - Negative control



(b)

M - DNA marker (100 bp)  
 Lane 1 - Positive control  
 Lane 2-6 - Positive field samples  
 Lane 7 - Negative control

Fig. 2. Molecular confirmation of bacteria isolated from mastitis milk sample (a) *gyr A* of *Klebsiella* spp (441 bp) and (b) *16S-23SrDNA* gene of *Klebsiella pneumoniae* (130 bp).

Shadkam *et al.* (2025) [32], who opined that the antimicrobial resistance was higher in biofilm forming *K. pneumoniae* than non-biofilm formers.

In this study, the *K. pneumoniae* isolates harboured 100% ESBLs genes,  $bla_{TEM}$  (Fig. 6a) and  $bla_{SHV}$  genes (Fig. 6b). Among these 66% of isolates had both  $bla_{TEM}$  and  $bla_{SHV}$  genes, 24% isolates had  $bla_{SHV}$  and 10% had the  $bla_{TEM}$  gene alone. The similar findings were reported by Nahar *et al.* (2021) [33], who opined that the *K. pneumoniae* isolates exhibited 50%  $bla_{TEM}$ , 15%  $bla_{SHV}$  and 35% combination of  $bla_{TEM}$  and  $bla_{SHV}$  genes. The extended spectrum  $\beta$ -lactamase (ESBL) and carbapenemase were most common resistant

mechanisms in Enterobacteriaceae bacteria and confer resistance against penicillin, cephalosporins, monobactams, meropenem and imipenem antibiotics. The most frequently reported variants of ESBL were TEM (Temoneira), SHV (sulfhydryl reagent variable) and CTX-M (cefotaxime-hydrolysing beta-lactamase) [34]. The carbapenemases encoded by the  $bla_{KPC}$ ,  $bla_{NDM}$  and  $bla_{OXA-48}$  genes, as well as the plasmidic AmpC usually associated with the  $bla_{CMY}$  genes [35, 36].

In the present study, the aminoglycoside resistant gene was detected in two isolates only. None of the isolates carried the quinolone resistance gene (*qnrA*), cefotaxime resistance gene ( $bla_{CTX-M-G1}$  and  $bla_{CTX-M-G2}$ ), sulfonamide resistance gene (*sulI*), tetracycline resistance gene (*tetA* and *tetM*) and the multidrug efflux pump gene (*acrAKp*), whereas Tran *et al.* (2023) [18] reported that the *K. pneumoniae* isolates in Vietnam carried 100% multidrug efflux pumps and the *tetA* gene, 94% had  $bla_{SHV}$  and 8% harboured  $bla_{NDM}$  and none of the isolates carrying the *tetB* and *qnrA* genes. This variation indicated the presence or absence of antimicrobial resistance in bacteria varied with geographical area and antimicrobial usage [34]. The distribution of AMR genes in *K. pneumoniae* isolates of China had  $bla_{SHV}$  (100%) and  $bla_{TEM}$  (64.7%),  $bla_{NDM-1}$  (36.8%),  $bla_{VIM}$  (17.6%) and  $bla_{OXA-10}$  genes (19.1%) and the presence of  $bla_{OXA-10}$  was positively associated with tetracycline resistance [37]. *K. pneumoniae* isolates that carrying the CTX-M-15 and SHV-12  $\beta$ -lactamases exhibit resistance to both ceftiofur and cefquinome [38]. *K. pneumoniae* isolates from bovine mastitis were multidrug resistance and carried  $bla_{CTX-M}$ ,  $bla_{SHV}$  and  $bla_{TEM}$ , quinolone and tetracyclines resistance genes [12]



Fig. 3. Antimicrobial susceptibility test of *K. pneumoniae* on Muller Hinton agar.

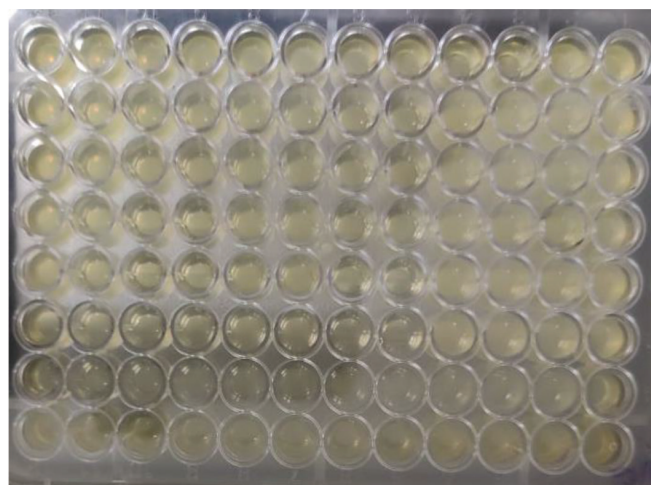


Fig. 4. Determination of minimum inhibitory concentration of *K. pneumoniae* by microtitre plate method.

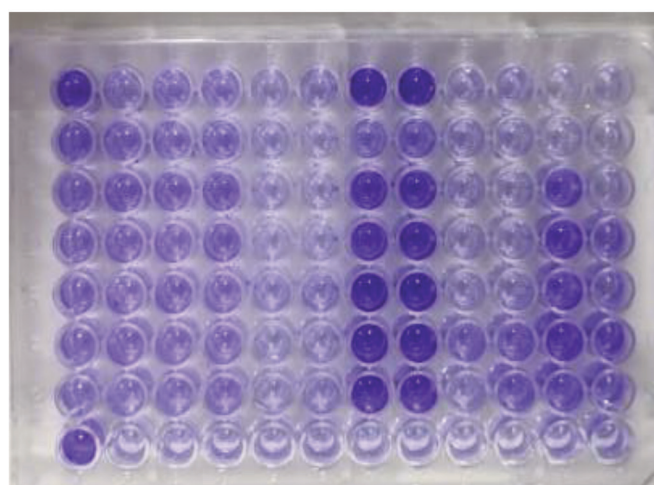
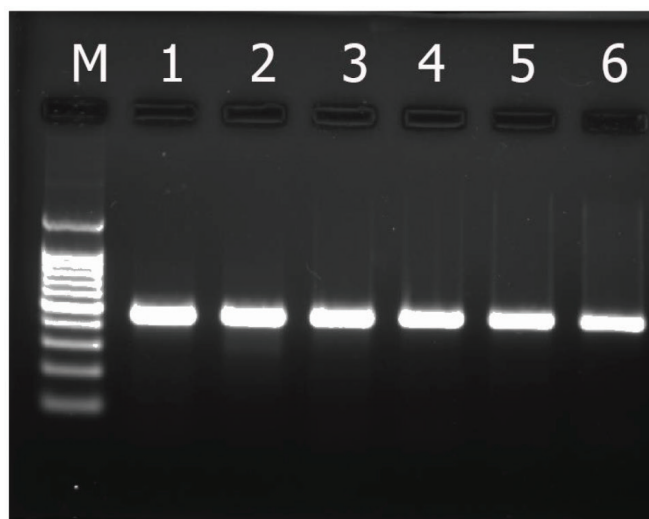


Fig. 5. Determination of biofilm assay of *K. pneumoniae* by microtitre plate method.



(a)

M- DNA marker (100 bp), Lane 1- 7 *bla*<sub>TEM</sub> gene



(b)

M- DNA marker (100 bp), Lane 1- 6 *bla*<sub>SHV</sub> gene

**Fig. 6. Molecular detection of antimicrobial resistance gene in *K. pneumoniae* (a) *bla*<sub>TEM</sub>- 296 bp and (b) *bla*<sub>SHV</sub>-450 bp.**

and a higher prevalence of aminoglycoside resistance gene *aac*(3)-iv (77.4%) and tetracycline genes *tetA* (76.7%) was reported in Bangladesh [39].

Based on observation, enrofloxacin could be used as the choice of antibiotic for *klebsiella* mastitis in dairy cows. The similar finding was reported by Fu *et al.* (2022) [5], who opined that *K. pneumoniae* showed 92.31% sensitive to enrofloxacin. In this study, the animals were treated with Inj. enrofloxacin @ 5 mg/Kg IM along with supportive therapy. Out of 78 animals, 23 (29.48%) animals were completely recovered, 47 (60.25%) animals infected quarters progressed to fibrosis and 8 (10.25%) animals quarter were progressed to gangrene. The gangrenous changes (3-6 days of initial symptoms) were observed in the affected quarter of some of the animals with yellow serous fluid with fibrin clots and anorexia.

The clinical outcome of enrofloxacin was 29.48%. These results were contradictory to the report of Sugiyama *et al.* (2022) [3], who reported that the enrofloxacin was effective in 76.70% of *K. pneumoniae* mastitis cases. The lower clinical outcome of enrofloxacin in this study might be due to the strong biofilm forming *K. pneumoniae* limiting the efficacy of the antibiotic.

The *K. pneumoniae* poses challenges for veterinarians and physicians because of treatment failure, which is a major transporter of resistance genes from the environment to clinically important bacteria through plasmids/horizontal gene transfer mechanisms. The community acquired *K. pneumoniae*

was hypervirulent and multidrug resistance led to serious clinical invasion in animals and humans [40]. The integrated AMR surveillance and control across animals, humans and the environment are needed to curb its spread and resistance.

### CONCLUSION

The *Klebsiella pneumoniae* isolates from bovine mastitis had high MIC, biofilm former and carried AMR genes (*bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes). The intramammary infection with *K. pneumoniae* not only caused the loss of milk yield in the affected quarter, but it resulted in generalized loss of milk secretion in all quarters and also increased culling percentage. Improving the health and immune status of the animal during the transition period could help to reduce the economic losses associated with mastitis.

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### ETHICS APPROVAL

Not applicable

## REFERENCES

1. Cobirka M, Tancin V, Slama P. Epidemiology and classification of mastitis. *Animals (Basel)*. 2020; 10(12):2212, DOI: 10.3390/ani10122212.
2. Wenz JR, Barrington GM, Garry FB, McSweeney KD, Dinsmore RP, Goodell G, Callan RJ. Bacteremia associated with naturally occurring acute coliform mastitis in dairy cows. *J Am Vet Med Assoc*. 2001; 219: 976- 981, DOI:10.2460/javma.2001.219.976.
3. Sugiyama M, Watanabe M, Sonobe T, Kibe R, Koyama S, Kataoka Y. Efficacy of antimicrobial therapy for bovine acute *Klebsiella pneumoniae* mastitis. *J Vet Med Sci*. 2022; 84(7): 1023-1028, DOI: 10.1292/jvms.21-0617.
4. Smith KL, Hogan JS. Environmental mastitis. *Vet Clin North Am Food Anim Pract*. 1993; 9(3):489-498
5. Fu S, Wen C, Wang Z, Qiu Y, Zhang Y, Zuo J, Xu Y, Han X, Luo Z, Chen W, Miao J. Molecular epidemiology and antimicrobial resistance of outbreaks of *Klebsiella pneumoniae* clinical mastitis in Chinese dairy farms. *Microbiol Spectr*. 2022; 10(6), DOI: 10.1128/spectrum.02997-22.
6. Bradley AJ, Green MJ. A study of the incidence and significance of intramammary enterobacterial infections acquired during the dry period. *J Dairy Sci*. 2000; 83(9):1957-1965, DOI: 10.3168/jds.S0022-0302(00)75072-7.
7. Grohn YT, Wilson DJ, Gonzalez RN, Hertl JA, Schulte H, Bennett G. Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. *J Dairy Sci*. 2004; 87:3358-3374, DOI: 10.3168/jds.S0022-0302(04)73472-4.
8. Guerra MES, Destro G, Vieira B, Lima AS, Ferraz LFC, Hakansson AP, Darrieux M, Converso TR. *Klebsiella pneumoniae* biofilms and their role in disease Pathogenesis. *Front Cell Infect Microbiol*. 2022; 12:877995, DOI: 10.3389/fcimb.2022.877995.
9. AL-Busaidi B, AL-Muzahmi M, AL-Shabibi Z, Rizvi M, AL-Rashdi A, AL-Jardani A, Farzand R, AL-Jabri Z. Hypervirulent capsular serotypes K1 and K2 *Klebsiella pneumoniae* strains demonstrate resistance to serum bactericidal activity and *Galleria mellonella* lethality. *Int J Mol Sci*. 2024; 25, DOI: 10.3390/ijms25031944.
10. Cai K, Xu M, Liu L, Zhao, H. Molecular epidemiology and antimicrobial resistance of *Klebsiella pneumoniae* strains isolated from dairy cows in Xinjiang, China. *Vet Med Sci*. 2025; 11(1):e70120, DOI: 10.1002/vms3.70120.
11. Kang, HJ, You JY, Kim SH, Moon JS, Kim HY, Kim JM, Kang HM. Genetic diversity, virulence genes, antimicrobial resistance, and biofilm formation of *Klebsiella pneumoniae* isolated from bovine mastitis milk in South Korea *Microbiol Spectr*. 2025; 13(11):e0134325, DOI: 10.1128/spectrum.01343-25.
12. Song S, He W, Yang D, Benmouffok M, Wang Y, Li J, Sun C, Song X, Ma S, Cai C, Dang S, Wu C, Shen Z, Wang Y. Molecular Epidemiology of *Klebsiella pneumoniae* from Clinical Bovine Mastitis in Northern Area of China (2018-2019). *Engineering*, 2022; 10: 146-154, DOI:10.1016/j.eng.2021.01.015
13. Hogan JS, Gonzalez RN, Harmon RJ, Nickerson SC, Oliver SP, Pankey JW, Smith KL. *Laboratory Handbook on Bovine Mastitis*. Third Edn. 2017, National Mastitis Council, USA.
14. Clinical and Laboratory Standard Institute (CLSI). *Performance Standards for Anti-Microbial Susceptibility Testing*. 30th Edition, M100. 2020. Available from: [https://clsi.org/media/3481/m100ed30\\_sample.pdf](https://clsi.org/media/3481/m100ed30_sample.pdf).
15. Eroglu B, Delik E, Tefon-Ozturk ET. Antibiotic concentrations affect the virulence of *Klebsiella quasipneumoniae* subsp. *similipneumoniae* isolates. *Cell Microbiol*. 2024; 920468, DOI: 10.1155/2024/5920468.
16. Triveni AG, Suresh Kumar M, Manjunath, C, Shivnavar CT, Gaddad SM. Biofilm formation by clinically isolated *Staphylococcus aureus* from India. *J Infect Dev Ctries*. 2018; 12(12): 1062-1066, DOI:10.3855/jidc.10671.
17. Munoz MA, Francis L, Welcome FL, Schukken YH, Zadoks RN. Molecular epidemiology of two *Klebsiella pneumoniae* mastitis outbreaks on a dairy farm in New York State. *J Clin Microbiol*. 2007; 45(12): 3964-3971, DOI: 10.1128/JCM.00795-07.
18. Tran MT, Vu DM, Vu MD, Bui TP, Dang BX, Dang LTM, Le TV. Antimicrobial resistance and molecular characterization of *Klebsiella* species causing bovine mastitis in Nghe An province, Vietnam. *J Adv Vet Anim Res*. 2023; 10(1): 132-143, DOI: 10.5455/javar.2023.j662.
19. Osman EA, El-Amin N, Adrees EAE, Al-Hassan L, Maowia Mukhtar M. Comparing conventional, biochemical and genotypic methods for accurate identification of *Klebsiella pneumoniae* in Sudan. *Access Microbiol*. 2020; 10;2(3), DOI: 10.1099/acmi.0.000096.
20. Doosti A, Pourabbas M, Arshi A, Chehelgerdi M, Kabiri H. TEM and SHV Genes in *Klebsiella pneumoniae* isolated from cockroaches and their antimicrobial resistance pattern. *Osong Public Health Res Perspect*. 2014; 6(1): 3-8, DOI: 10.1016/j.phrp.2014.10.011.
21. Diagbouga S, Salah FD, Sadjji AY, Dabire AM, Nadembega C, Kere AB, Soubeiga ST, Ouattara AK, Zohoncon T, Belemnegre M, Karou S, Simpore J. Detection of high prevalence of TEM/SHV/CTX-M Genes in ESBL producing and multidrug resistant *Klebsiella Pneumoniae* and *Klebsiella Oxytoca*. *J Clin Diagn Res*, 2016; 4:1, DOI: 10.4172/2376-0311.1000130.
22. Martineau F, Picard FJ, Lansac, N, Menard C, Roy PH, Ouellette M, Bergeron MG. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus*

and *Staphylococcus epidermidis*. *Antimicrob Agents Chemother.* 2000; 44(2): 231-238, DOI: 10.1128/AAC.44.2.231-238.2000.

23. Jose KR, Vijayakumar K. Antimicrobial resistance profiling of coagulase negative staphylococci isolated from bovine mastitis. *Indian J Anim Sci.* 2024; 94 (4): 308-314, DOI: 10.56093/ijans.v94i4.129868.

24. Salauddin M, Akter MR, Hossain, MK, Rahman MM. Isolation of multi-drug resistant *Klebsiella* sp. from bovine mastitis samples in Rangpur, Bangladesh. *J Adv Vet Anim Res.* 2019; 6(3): 362-365, DOI: 10.5455/javar.2019.f355.

25. Liu K, Zhang L, Gu X, Qu W. The prevalence of *Klebsiella* spp. associated with bovine mastitis in china and its antimicrobial resistance rate: A Meta-analysis. *Front Vet Sci.* 2022; 9:75750, DOI: 10.3389/fvets.2022.757504.

26. Song J, Xiang W, Wang Q, Yin J, Tian T, Yang Q, Zhang M, Ge G, Li J, Diao N, Liu F, Shi K, Cai R, Du R, Gong Q. Prevalence and risk factors of *Klebsiella* spp. in milk samples from dairy cows with mastitis-A global systematic review. *Front Vet Sci.* 2023; 10:1143257, DOI:10.3389/fvets.2023.1143257.

27. Olaitan MO, Orababa OQ, Shittu RB, Oyediran AA, Obunukwu GM, Arowolo MT, Kade AE, Yahaya KI, Yusuff RA. Extended-spectrum beta-lactam-resistant *Klebsiella pneumoniae* in sub-Saharan Africa: A systematic review and meta-analysis from a One Health perspective. *BMC Infect Dis.* 2025; 25(1):843, DOI: 10.1186/s12879-025-11276-9.

28. Arya LK, Manoj Kumar, Priya P, Saurabh K, Kumari N. Isolation and Identification of *Klebsiella pneumoniae* from a milk sample. *Indian Vet J.* 2020; 97 (01): 15-17.

29. Yadav R, Chhabra R, Singh M, Shrinet G, Talukdar SJ. Antibioqram of *Klebsiella pneumoniae* isolated from mastitic milk samples of cattle and buffalo. *Haryana Vet.* 2021; 60(2): 195-197.

30. Mukherjee S, Chaki, S, Pal TK, Mookerjee M, Das S, Dastidar SG. Experimental studies on synergism between meropenem and sulbactam. *Afr J Microbiol Res.* 2013; 7(27): 3461-3465, DOI: 10.5897/AJMR12.497.

31. Mosallam FM, Elshimy, R. Eradication of *Klebsiella pneumoniae* pulmonary infection by silver oxytetracycline nano-structure. *AMB Expr.* 2024; 14(1):62, DOI:10.1186/s13568-024-01720.

32. Shadkam S, Goli HR, Mirzaei B, Gholami M, Ahanjan M. Correlation between antimicrobial resistance and biofilm

formation capability among *Klebsiella pneumoniae* strains isolated from hospitalized patients in Iran. *Ann Clin Microbiol Antimicrob.* 2021; 20(1): 13, DOI: 10.1186/s12941-021-00418-x.

33. Nahar AK, Islam MA, Islam MN, Alam MM. ESBL-producing *Klebsiella* sp. isolated from raw milk of healthy cow in small holder dairy farms of Mymensingh district in Bangladesh. *Bangladesh J Vet Med.* 2021;19 (2): 141-150, DOI: 10.33109/bjvmjd2021amrt1.

34. Gelalcha BD, Dego OK. Extended-spectrum beta-lactamases producing *Enterobacteriaceae* in the USA dairy cattle farms and implications for public health. *Antibiotics (Basel).* 2022; 11(10), DOI: 10.3390/antibiotics11101313.

35. Lalak A, Wasyl D, Zajac M, Skarzynska, M, Hoszowski A, Samcik I, Wozniakowski G, Szulowski K. Mechanisms of cephalosporin resistance in indicator *Escherichia coli* isolated from food animals. *Vet Microbiol.* 2016; 194: 69-73, DOI: 10.1016/j.vetmic.2016.01.023.

36. King DT, Sobhanifar S, Strynadka, NCJ. The Mechanisms of resistance to  $\beta$ -Lactam antibiotics. In *Handbook of Antimicrobial Resistance*, 2017;1-22pp, DOI:10.1007/978-1-4939-0667-3-10-1.

37. Cao W, Xu Y, Huang Y, Xu T. Isolation of pathogenic bacteria from dairy cow mastitis and correlation of biofilm formation and drug resistance of *Klebsiella pneumoniae* in Jiangsu, China. *Agriculture.* 2023; 13, DOI:10.3390/agriculture13101984.

38. Timofte D, Maciucă IE, Evans NJ, Williams H, Wattret A, Fick JC, Williams NJ. Detection and molecular characterization of *Escherichia coli* CTX-M-15 and *Klebsiella pneumoniae* SHV-12  $\beta$ -lactamases from bovine mastitis isolates in the United Kingdom. *Antimicrob Agents Chemother.* 2014; 58(2): 789-794, DOI: 10.1128/AAC.00752-13.

39. Chowdhury MSR, Hossain H, Rahman MN, Rahman A, Ghosh PK, Uddin MB, Hoque MN, Hossain MM, Rahman MM. Emergence of highly virulent multidrug and extensively drug resistant *Escherichia coli* and *Klebsiella pneumoniae* in buffalo subclinical mastitis cases. *Sci Rep.* 2025; 15:11704, DOI:10.1038/s41598-025-95914-x.

40. Wyres KL, Holt KE. *Klebsiella pneumoniae* as a key trafficker of drug resistance genes from environmental to clinically important bacteria. *Curr Opin Microbiol.* 2018; 45:131-139, DOI: 10.1016/j.mib.2018.04.004.

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