B-5551
[1-8]

RESEARCH ARTICLE Indian Journal of Animal Research

Molecular Characterization and Antimicrobial Resistance Profiling of Carbapenemase Producing *E. coli* and *Klebsiella* spp. Isolates of Bovine Origin in Awadh Region of Uttar Pradesh

V. Yadav[1], R.K. Joshi[1], Namita Joshi[2], S.V. Singh[3], D.P. Srivastva[4], D. Yadav[3]

10.18805/IJAR.B-5551

ABSTRACT

Background: Carbapenems are used as last resort of antibiotics to treat infection caused by multi-drug resistant pathogens of human, hence resulting into emergence and dissemination of Carbapenem-resistant *Enterobacteriaceae* (CRE). The occurrence of CRE among livestock is of great concern as these can be transferred to human and animals through adulterated food, water and environment. Therefore a specific study on the occurrence of CRE and their AMR profiling is warranted with regard to therapeutic options.

Methods: Total 240 faecal samples were collected from Ayodhya and Sultanpur districts of Awadh region of Uttar Pradesh (India). The *E. coli* and *Klebsiella* spp. isolates were confirmed by PCR analysis using species specific uidA and 16S rRNA genes respectively. Carbapenemase producing isolates were confirmed by DDST, MBL E-strip and PCR analysis by targeting *bla*-NDM, *bla*-KPC and *bla*-OXA-48 genes. Antibiotic resistance profiling was performed against 20 antibiotics of 12 different classes.

Result: In this study, PCR analysis revealed 90.80% confirmed isolates including 71.67% *E. coli* and 19.16% *Klebsiella* spp., out of which 36/218 (16.51%) and 28/218 (12.84%) isolates were confirmed as carbapenemase producer by DDST and MBL E-strip test respectively. Carbapenemase genes were detected in 17/218 (7.80%) isolates and among them *bla*-KPC gene was detected in both isolates while *bla*-NDM and *bla*-OXA-48 were detected only in *E. coli* and *Klebsiella* spp. respectively. All phenotypically confirmed carbapenemase positive isolates of *E. coli* and *Klebsiella* spp. were found 100% sensitive to aminoglycosides, polypeptides and 85-100% resistance against carbapenem, 3rd and 4th generation cephalosporines, monobactams and penicillin class of antibiotics.

Key words: Bovine, Carnapenemase, *E. coli*, *Klebsiella* spp., MBL E-strip.

INTRODUCTION

The emergence of antimicrobial resistance (AMR) particularly in reference to *Enterobacteriaceae* has been expanding rapidly and become more problematic as it possess serious threat to both human and animals. The major contributors of AMR are Extended-spectrum β-lactamase (ESBL) and Carbapenemase producing *Enterobacteriaceae* (CPE) (Mustafai *et al.,* 2023). Nowadays Carbapenem resistance is one of the latest challenges faced by scientific community across the world because it is one of the latest groups of antibiotics which are used as last resort for treating the infections caused by multidrug-resistant gram negative bacilli. The most common mechanism of resistance is the production of carbapenem-hydrolysing enzymes that hydrolyse most β-lactam antibiotics (Tarashi *et al.,* 2016). Among CRE plasmid mediated horizontal transmission of carbapenem encoding resistance genes occur most commonly between inter and intra-species (Diene and Rolain, 2014). The major public health threat is because of transmissible carbapenemases, which not only increase the rate of mortality but also limit the choice of appropriate antibiotic therapy (Bush *et al.,* 2013). The transmissible enzymes are acquired randomly by important nosocomial pathogens such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and members of the family *Enterobacteriaceae* (Thomson, 2010). The most serious form of carbapenem

[1]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya-224 229, Uttar Pradesh, India.

[2]Department of Veterinary Public Health and Epidemiology, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya-224 229, Uttar Pradesh, India.

[3]Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya-224 229, Uttar Pradesh, India.

[4]Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya-224 229, Uttar Pradesh, India.

Corresponding Author: V. Yadav, Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya-224 229, Uttar Pradesh, India.
Email: vibhavet2005@gmail.com

How to cite this article: Yadav, V., Joshi, R.K., Joshi, N., Singh, S.V., Srivastva, D.P. and Yadav, D. (2025). Molecular Characterization and Antimicrobial Resistance Profiling of Carbapenemase Producing *E. Coli* and *Klebsiella* spp. Isolates of Bovine Origin in Awadh Region of Uttar Pradesh. Indian Journal of Animal Research. 1-8. doi: 10.18805/IJAR.B-5551.

Submitted: 14-01-2025 **Accepted:** 26-04-2025 **Online:** 09-06-2025

resistance is mediated by carbapenem-hydrolyzing β-lactamases, including metallo-β-lactamases (MBLs), such as imipenemase (IMP), Verona imipenemase (VIM), New Delhi metallo-β-lactamase (NDM), Ambler class A *Klebsiella pneumoniae* Carbapenemase (KPC) and class D oxacillinase-48 (OXA-48) (Iraz *et al.,* 2015). The CPE among livestock are of great social hygiene concern as these can be transferred to human and animal through adulterated food, water and environment. The research work on CPE in bovines including India and abroad is very limited and the incidence in animals is of most concern due to risk of transfer to human *via* food-chain, contact or environment.

Keeping in view the above facts, the present study was design to detect the Carbapenem resistant genes in *E. coli* and *Klebsiella* spp. Since, no in-depth study has been done on Carbapenemase producing enterobacteria in bovines in Awadh region of U.P. It will help the researchers, field veterinarians and policy makers to develop appropriate control strategy for the farmers of this region.

MATERIALS AND METHODS

Study area

The study was carried out in the Department of Veterinary Microbiology, C.V.Sc. and A.H. Kumarganj, Ayodhya. The samples were collected from Ayodhya and Sultanpur district of Eastern Plain Zone of Uttar Pradesh, India. The study was conducted between August 2020 and March 2021. The research work was duly permitted by Institutional Animal Ethical Committee (IAEC) as per letter no. IAEC/ C.V.Sc./2019/34 dated 10/12/2019.

Sample collection

In present study, total 240 faecal samples (160 normal and 80 diarrhoeic) of cattle and buffaloes were collected from 5 tehsils of Ayodhya and 3 tehsils of Sultanpur district.Sampling was done randomly and consisted of 10 apparently normal and 5 diarrhoeic faecal samples from each of the animal from total eight tehsils. The samples were collected aseptically and transported to laboratory under cold condition.

Isolation and identification

Samples were enriched with 2 ml nutrient broth and incubated at 37°C for 24 hrs. A loopful of inoculum was taken and directly streaked on MacConkey agar (MLA) plates added with 1mg/L imipenem and incubated at 37°C for 24 hours (Cruickshank *et al.,* 1975). Lactose fermenting colonies were picked up and streaked on Eosine Methylene Blue (EMB) agar plates. The colonies showing specific characteristics were identified by the method of Cruickshank *et al.* (1975). Further identification of the isolates was done by various biochemical and sugar fermentation reaction as per the method of Edward and Ewing (1972).

Extraction of genomic DNA

The DNA templates were prepared by using snap-chill method as described by Franco *et al.* (2008).

Confirmation of *E. coli* and *Klebsiella* spp. by PCR analysis

All presumptively positive *E. coli* and *Klebsiella* spp. isolates were confirmed by PCR amplification using spp. specific uidA and bacteria specific 16S rRNA gene as per method described by Anbazhagan *et al.* (2010) and Andersson *et al.* (2008) respectively (Table 1). PCR reaction was carried out in 25 µl volume consisted of 12.5 µl of 2X EmeraldAmp GT Master Mix, 8.5 µl nuclease free water, 1 µl mixture of the forward and reverse primers (0.5 µl each primer) and 3.0 µl of template DNA. Amplification was performed using thermal cycler (Bio-Rad, USA). The cycling conditions of PCR are mentioned in Table 1. Amplicons of PCR were stored at 4°C until electrophoresis.

Screening of carbapenemase producing isolates

All confirmed isolates of *E. coli* and *Klebsiella* spp. were subjected to screening test using imipenem and meropenem disc with 10 µg conc. (Hi-Media, India) disk diffusion method (Bauer *et al.,* 1966). The results were interpreted as per CLSI (2019) guidelines. The isolates showing reduced susceptibility to any one of these antibiotic disc were further confirmed by phenotypic tests.

Confirmation of carbapenemase positive isolates

Confirmation of carbapenemase producing isolates was done by using phenotypic methods.

Double disc synergy test (DDST)

The test was performed by placing the commercially available imipenem disc (10 µg) and combination of

Table 1: Detail of primer sequences used for molecular characterization of *E. coli* and *Klebsiella* spp.

Targeted gene	Primers' pair	Amplicon size (bp)	PCR conditions and cycles	References
	F 5CTGGTATCAGCGCGAAGTCT3 R 5'AGCGGGTAGATATCACACTC3	556	1 cycle of 5 minutes at 95°C,35 cycles of 45 seconds at 95°C, 55 seconds at 72°C,1 minute at 72°C, 1 cycle of 7 minutes at 72°C	Anbazhagan *et al.,* 2010
784F 1061R	F 5AGGATTAGATACCCTGGTA3 R 5CRRCACGAGCTGACGAC3	265	1 cycle of 5 minutes at 95°C, 35 cycles of 50 seconds at 95°C, 45 seconds 72°C, 1 minute at 72°C, 1 cycle of 7 minutes at 72°C	Andersson *et al.,* 2008

imipenem+EDTA (10/750 µg) discs (HiMedia, India) at 25 mm apart on Muller Hinton agar (MHA) (HiMedia) plate seeded with 1.5×10^8 organisms/ml and incubated at 37°C for 24 hrs (Fig 1). The results were interpreted as per CLSI guidelines (2019).

Minimum inhibitory concentration (MIC) MBL E-test

This test was performed by placing MBL E-strip on MHA plate seeded with 1.5×10^8 organisms/ml and incubated at 37°C for 24 hrs**.** Results were interpreted as per CLSI guidelines (2019) (Fig 2).

Detection of carbapenemase genes by PCR assay
Isolation of plasmid DNA

Single pure colony of each isolate was inoculated into 10 ml Luria-Bertani (LB) broth medium (HiMedia, India) and incubated at 37°C for 12-15 hrs in shaking incubator. The pellet was prepared by centrifugation of bacterial suspension at 10,000 rpm for 3 min. Plasmid DNA was isolated using the GeneJet plasmid Miniprep kit (Cat No. #K0503,Thermo Scientific) as per the instruction of the manufacturers.

Detection of *bla*-NDM, *bla*-KPC and *bla*-OXA-48 genes by PCR analysis

Genotypic analysis of phenotypically confirmed Carbapenemase producing isolates was performed by targeting *bla*-NDM, *bla*-KPC and *bla*-OXA-48 genes. PCR reaction was carried out in a total reaction volume of 25 µl as per method described by Mushi *et al*. (2014), Jones *et al*. (2009) and Dallenne *et al***.** (2010) for *bla*-NDM, *bla*-KPC and *bla*-OXA-48 genes respectively. The primer sequence of targeted genes and amplicon sizes are

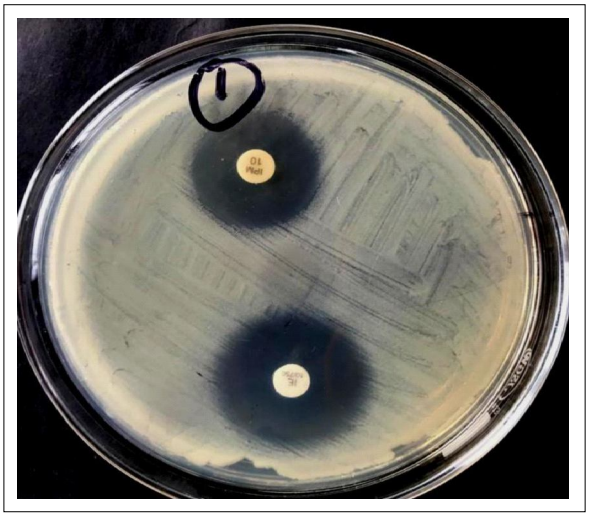

Fig 1: Double disc synergy test (DDST).

Table 2: Detail of primers used for Molecular characterization of carbapenemase genes.

Genes	Primers pair	Product size (bp)	References
bla-NDM	F-5GGTTTGGCGATCTGGTTTTC 3 R-5CGGAATGGCTCATCACGATC 3	521	Mushi *et al.,* 2014
bla-KPC	F-5ATGTCACTGTATCGCCGTCT3 R-5TTTTCAGAGCCTTACTGCCC3	892	Jones *et al.,* 2009
bla-OXA-48	F-5GCTTGATCGCCCTCGAT 3 R-R5GATTTGCTCCGTGGCCGAAA3	281	Dallenne *et al.,* 2010

Table 3: Isolation rate of *E. coli* and *Klebsiella* spp. in normal and diarrhoeic bovine faecal samples

Samples (Source/Origin)		Presumptive positive isolates (Biochemical tests)			Confirmed positive isolates (PCR analysis)		
		E. coli	*Klebsiella* spp.	Total	*E. coli*	*Klebsiella* spp.	Total
Cattle	Normal faecal (n=80)	77 (96.25%)	18 (22.5%)	84 (105.0%)	65 (81.25%)	11 (13.75%)	76 (95.0%)
	Diarrhoeic faecal (n=40)	35 (87.5%)	14 (35.0%)	38 (95.0%)	25 (62.5%)	8 (20.0%)	33 (82.5%)
Buffalo	Normal faecal (n=80)	70 (87.5%)	16 (20.0%)	75 (93.75%)	58 (72.5%)	15 (18.75%)	73 (91.25%)
	Diarrhoeic faecal (n=40)	37 (92.5%)	15 (37.5%)	40 (100.0%)	24 (60.0%)	12 (30.0%)	36 (90.0%)
Total	N=240	219 (91.25%)	63 (26.25%)	237 (98.75%)	172 (71.67%)	46 (19.16%)	218 (90.8%)

Table 4: Distribution of carbapenemase producing isolates according to various phenotypic confirmatory tests.

Tests	Carbapenemase positive isolates		
	E. coli (172)	*Klebsiella spp.*(46)	Total (218)
Screening test	27/172=15.69%	20/46=43.47%	47/218=21.55%
Double disc synergy test (DDST)	18/172=10.46%	18/46=39.13%	36/218=16.51%
MBLE test	13/172=7.55%	15/46=32.60%	28/218=12.84%
PCR	7/172=4.07 %	10/46=31.25%	17/218=7.80%

listed in table 2. Visualization of PCR product was done by mixing 5 µl of amplified products with 3 µl of bromophenol blue dye (6X) and electrophorased in 0.8% agarose gel in 1X TAE buffer mixed with ethidium bromide 1 µl (5µg/ml) in 60 ml and run slowly at 80-100V, 60-70 mA for 1 hrs and the gels were visualized using the UV illuminator (GeNei Bangalore, India). Ladder DNA of 1kb (Thermo Scientific # SM 0311) was used as a marker for band interpretation.

Study of antibiotic resistance profiling

All phenotypically confirmed Carbapenemase isolates of *E. coli* and *Klebsiella* spp. were subjected to antimicrobial resistance (AMR) profiling against 20 antibiotics of 12 different classes (Hi-Media) mentioned in table 7. The AMR profiling was performed by disk diffusion method on MHA (Hi-Media) plates seeded with 1.5×10^8organisms/ml and incubated at 37°C for 24 hrs and isolates were classified as susceptible and resistant as per interpretation criteria of CLSI (2019).

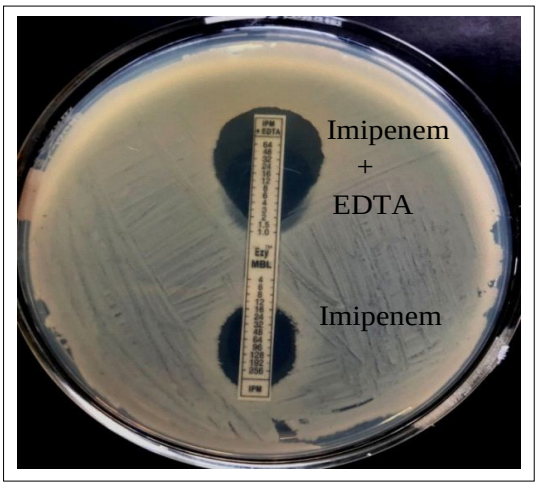

Fig 2: MIC MBL E-strip test .

RESULTS AND DISCUSSION

In present study, total 240 samples comprising 160 normal and 80 diarrhoeic faecal samples were processed for isolation and confirmation of *E. coli* and *Klebsiella* spp. The presumptive positive isolates of *E. coli* and *Klebsiella* spp. on the basis of their morphology, growth, cultural and biochemical characteristics were found 91.25% and 26.25% respectively (Table 3). Further PCR analysis revealed total 90.80% confirmed isolates comprising 71.67% *E. coli* and 19.16% *Klebsiella* spp. (Fig 3, 4 and Table 3). Similar findings have been also reported by Yadav *et al*. (2024) and Gupta *et al*. (2019). The isolation rate of *E. coli* was found much higher than *Klebsiella* spp. in both cattle and buffaloes which may be attributed to high prevalence of *E. coli* in GIT of ruminants as compared to other members of family *Enterobacteriaceae*.

The present study also aimed to detect the occurrence of carbapenemase producing isolates among the clinical and apparently healthy animal isolates. Total 218 isolates (172*E. coli* and 46 *Klebsiella* spp.) were subjected to screening and confirmatory phenotypic tests. Out of 218 confirmed isolates, 21.55% (47/218), 16.51% (36/218) and 12.84% (28/218) presumed as carbapenemase producer by screening test, DDST and MBL-E strip test respectively (Table 4). There was little difference in the sensitivity of both phenotypic confirmatory test used for detection of carbapenemase producers and this observation corroborated with the findings of Bora *et al*. (2014) and Yadav *et al*. (2021). Phenotypic confirmatory method revealed the occurrence of 8.75% (3.75% *E. coli* and 5.0% *Klebsiella* spp.) in normal faecal 12.5% (7.50% *E. coli* and 5.0% *Klebsiella* spp.) in diarrhoeic faecal, 11.25% (5.0% *E. coli* and 6.25% *Klebsiella* spp.) in normal faecal and 17.50% (7.50% *E. coli* and 10.0% *Klebsiella* spp) in diarrhoeic faecal samples of cattle and buffaloes respectively (Table 5). Similar to our finding, low percentage of carbapenemase positive *E. coli* has also been reported by Braun *et al*. (2016), Webb *et al*. (2016), Nirupama *et al*. (2018) from faecal samples of different spp. and Yadav *et al*. (2021) from milk samples of bovine. However some of the workers have reported higher

Table 5: Occurrence of carbapenemase producing of *E. coli* and *Klebsiella* spp. among various sources

Samples (Source/Origin)		*E. coli* isolates	Carbapenemase positive *E. coli*	*Klebsiella* spp. isolates	Carbapene-mase positive *Klebsiella* spp.	Total isolates	Total Carbapene-mase positive isolates
Cattle	Normal faecal (n=80)	65(90.0%)	3(3.75%)	11(13.75%)	4(5.0%)	76(95.0%)	7(8.75%)
	Diarrhoeic faecal(n=40)	25(62.5%)	3(7.50%)	8(20.0%)	2(5.0%)	33(82.5%)	5(12.5%)
Buffalo	Normal faecal (n=80)	58(72.5%)	4(5.0%)	15(18.75%)	5(6.25%)	73(91.25%)	9(11.25%)
	Diarrhoeic faecal (n=40)	24(60.0%)	3(7.50)	12(30.0%)	4(10.0%)	36(90.0%)	7(17.5%)
Total	N=240	172(71.67%)	13(5.41%)	46(19.16%)	15(6.25%)	218(90.8%)	28(11.7%)

Table 6: Distribution of carbapenemase genes according to various sources and organisms.

Samples (Source/Origin)		Carbapenemase producing *E. coli* (n=13)			Carbapenemase producing *Klebsiella* spp.(n=15)		
		bla-NDM	*bla*-OXA-48	*bla*-KPC	*bla*-NDM	*bla*-OXA-48	*bla*-KPC
Cattle	Normal faecal	01(7.69%)	Nil	Nil	Nil	Nil	02(13.33%)
	Diarrhoeic faecal	02 (15.38%)	Nil	01(7.69%)	Nil	2/15(13.33%)	01(6.67%)
Buffaloes	Normal faecal	Nil	Nil	Nil	Nil	Nil	03(20.0%)
	Diarrhoeic faecal	01 (7.69%)	Nil	02 (15.38%)	Nil	Nil	02(13.33%)
Total		4/13(30.77%)	Nil	3/13(23.07%)	Nil	2/15(13.33%)	8/15(53.33%)

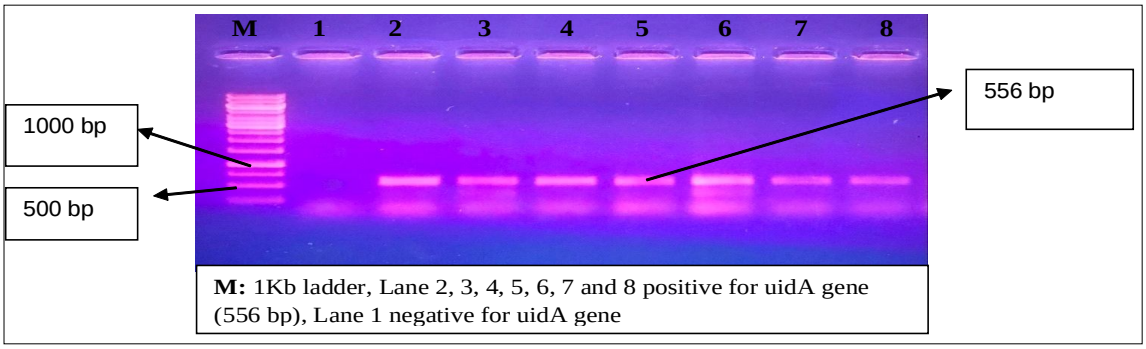

Fig 3: PCR amplification of uidA gene (556 bp)

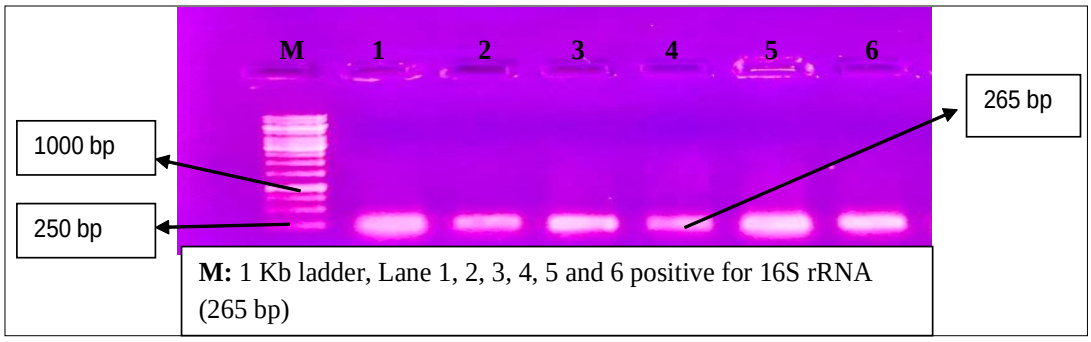

Fig 4: PCR amplification of 16S rRNA (265 bp)

occurrence of carbapenemase producers among faecal samples ranging from 21.64% to 29.03% from various parts of India (Gupta *et al.,* 2019; Murugan *et al.,* 2019; Pruthvishree *et al.,* 2017). These differences in the findings of various co-workers may be attributed to variations in source and animal husbandry practices in geographical locations.

Confirmation of carbapenemase producing isolates was done by PCR analysis by targeting *bla*-NDM, *bla*-$_{KPC}$ and *bla*-$_{OXA-48}$genes (Fig 5, 6, 7), which revealed 17/218 (7.80%) carbapenemase positive isolates comprising 7/172(4.07%) *E. coli* and 10/46(31.25%) *Klebsiella* spp. (Table 6). The overall gene distribution study showed 30.77% occurrence of *bla*-$_{NDM}$ and 23.07% *bla*-$_{KPC}$ in *E. coli* isolates while 53.33% of *bla*-$_{KPC}$ and 13.33% *bla*-OXA-$_{48}$ genes in *Klebsiella* spp. It was notable under this study that *bla*-$_{KPC}$ gene was reported both in *E. coli* and *Klebsiella* spp where as *bla*-$_{NDM}$ was only reported in *E. coli* and *bla*-$_{OXA-48}$ only in *Klebsiella* spp. (table-6). The occurrence of *bla*-$_{OXA-48}$ was found very low but it is very significant from public health point of view, as it is increasingly being reported from nosocomial infections in which *Klebsiella* spp. is most commonly implicated and in best of my knowledge, this is the first report of *bla*-$_{KPC}$ and *bla*-$_{OXA-48}$ gene detection in faecal samples of bovine in India. Similar to our finding

Table 7: AMR pattern of phenotypically positive carbapenemase positive isolates.

Group	Antibiotics (Hi-Media)	Conc. (µg/disc)	*E. coli* (n=13)	*Klebsiella* spp. (n=15)
			Resistance	Resistance
Aminogylcosides	Gentamicin (Gen)	10	0.0%	0.0%
	Amikacine (Ak)	30	0.0%	0.0%
Carbapenems	Imepenem (IMP)	10	100%	100%
	Meropenem (MRP)	10	84.61%	93.00%
3rd and 4th generation	Cefotaxime (CTX)	10	100%	100%
cephalosporins	Cefpodoxime(CPD)	10	100%	100%
	Ceftazidime (CAZ)	30	100%	100%
	Ceftriazone (CTR)	30	100%	100%
Monobactams	Aztreonam (AT)	30	100%	100%
2nd generation Cephalosporins	Cefoxitin (CX)	30	53.84%	46.66%
Penicillin	Ampicillin (AMP)	25	100%	100%
Polypeptides	Polymyxin-B (PB)	300 unit	0.0%	0.0%
Sulphonamides	Co-trimoxazole (COT)	25	53.84%	73.33%
	Trimethoprim (TR)	30	46.15%	60.0%
Quinolones	Enrofloxacin (EX)	10	46.15%	26.67%
	Ofloxacin (OF)	2	46.15%	26.67%
	Nalidixic acid (NA)	30	69.23%	80.0%
Tetracycline	Tetracycline (TE)	30	38.46%	40.0%
Amoxyclav	Amoxicillin/clavulanic (AMC)	(20/10)	38.46%	60.0%
Chloramphenicol	Chloramphenicol (C)	30	0.0%	0.0%

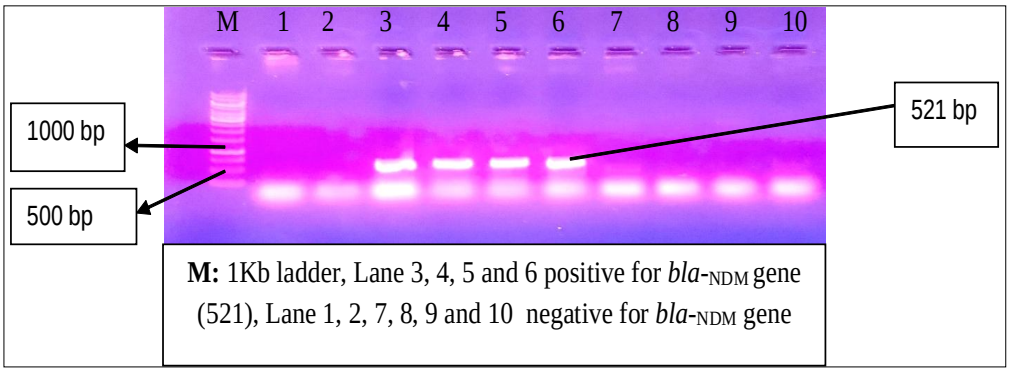

Fig 5: PCR amplification of *bla*-NDM gene (521 bp).

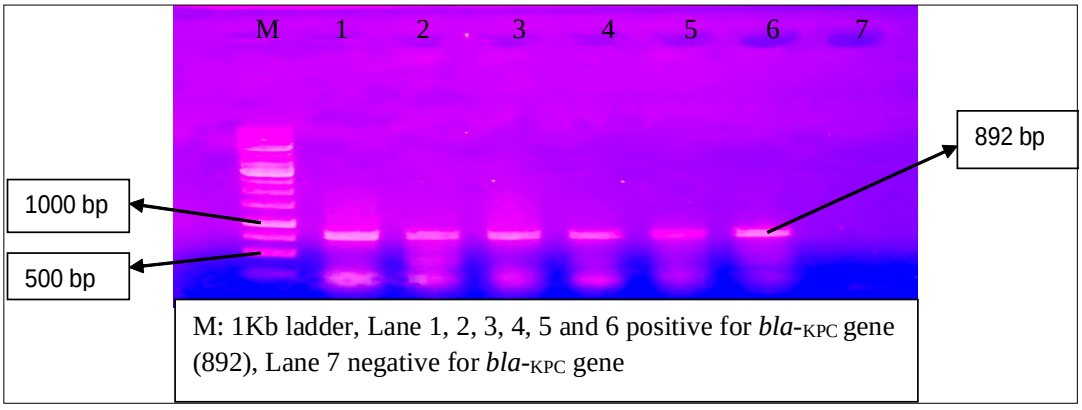

Fig 6: PCR amplification of *bla*-KPC gene (892 bp).

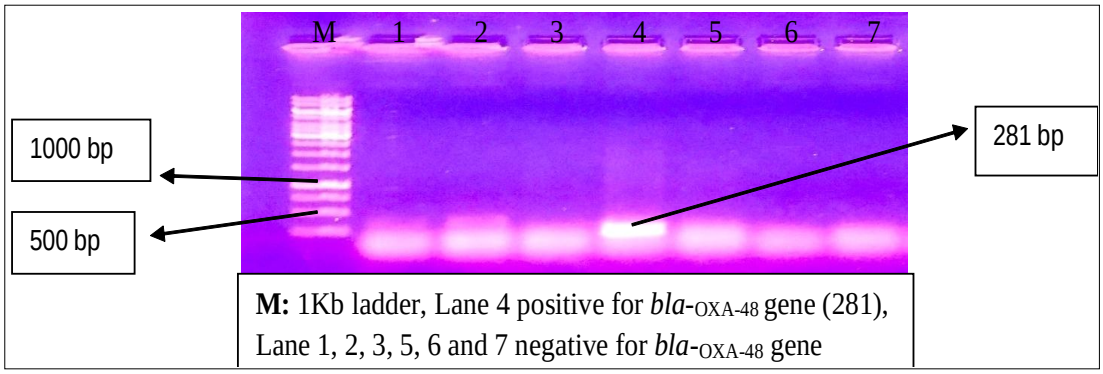

Fig 7: PCR amplification of *bla*-OXA-48 gene (281 bp).

these carbapenemase genes have been also reported from various parts of India and abroad in previous studies like*bla*-$_{NDM-1}$ and *bla*-$_{OXA-48}$ genes in piglets (Pruthvishree *et al.,* 2017; Nirupama *et al.,* 2018), *bla*-VIM in calves (Murugan *et al.,* 2019), *bla*-$_{OXA-48}$ in cattle faeces (Braun *et al.,* 2016) and in milk (Diab *et al.,* 2017; Yadav *et al.,* 2021). The occurrence of such genes in faecal samples of bovine is of great significant as can be transferred to human and animal horizontally through contaminated food, water and environment leading to various food-borne diseases.

Nowadays antimicrobial resistance is of great concern globally and a matter of serious threat to humanity which has received the attention of policy makers along with scientific community. In present study all *E. coli* and *Klebsiella* spp. isolates were 100% sensitive to aminogycosides, polypeptides and chloramphenicol whereas 84.61% to 100% resistant against carbapenems, 3rd and 4th generation cephalosporines, monobactams and penicillin group of antibiotics (Table 7). Both *E. coli* and *Klebsiella* spp. isolates showed resistance 26.67% to 80.0% against non-β-lactam group of antibiotics. There are several evidences that corroborate with these findings in India and abroad for both *E. coli* and *Klebsiella* spp. isolated from bovine (Gupta *et al.,* 2019; Ibrahim *et al.,* 2018; Yadav *et al.,* 2022). The plausible factors showing extreme level of resistance to several groups of antibiotic may be due to irrational and over use of these antibiotics without performing antibiotic sensitivity testing for treatment and in feed additives.

CONCLUSION

The findings of the present study are noteworthy with facts that carbapenems are not in use for treating the animals in this area; even though the isolates revealed complete resistance against these antibiotics, which is very threatening to public health point of view. It may be attributed to horizontal transfer of resistance genes between human and animal in community settings. This type of transfer of pathogens or genes from one species to another is likely to occur in ours populated country. This is important to consider in view of emergence of carbapenem resistance that the practice of routine carbapenem testing along with conventional antibiogram would be beneficial in all cases which will help appropriate selection of antibiotics along with prevention of further development of AMR. Therefore routine monitoring of resistance genes against carbapenem antibiotics in animal husbandry is warranted.

ACKNOWLEDGEMENT

The author is thankful to Dean, College of Veterinary Sciences and Animal Husbandry, Kumarganj and livestock owners of the Ayodhya and Sultanpur districts for their kind support during collection of samples.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

Anbazhagan D., Kathirvalu G.G., Mansor, M., Yan, G.O.S., Yusof, M.Y. and Sckaran S.D. (2010). Multiplex PCR assays for the detection of *Enterobacteriaeceae* in clinical samples. African Journal of Microbiology Research. 4(11): 1186-1191.

Andersson, A.F., Lindberg, M., Jakobsson, H., Backhed, F., Nyren, P. and Engstrand L. (2008). Comparative analysis of human gut microbiota by barcoded pyrosequencing. *PLoS One*:

Bauer, A.W., Kirby, W.M., Sherris, J.S. and Turck, M. (1966). Antibiotic susceptibility testing by standardized single disc method. American Journal of Clinical Pathology. 45:493.

Bora, A., Sanjana, R. Jha, B.K., Mahaseth. S.N. and Pokhrel, K. (2014). Incidence of metallo-β-lactamase producing clinical isolates of *E. coli* and *K. pneumoniae* in Central Nepal. BMC Research note. 7:557.

Braun, S.D., Ahmed, M.F.E., El-Adawy, H., Hotzel, H., Engelmann, I., Daniel W.D., Monecke, S. and Ehricht, R. (2016). Surveillance of ESBL producing *E. coli* in Dairy Cattle Farms in the Nile Delta, Egypt Frontiers in Microbiology. 7: 1020.

Bush K., Megan P., John, L. Lock, Anne MQueenan, James H Jorgensen, Ryan MLee, James SLewis, Deidre J. (2013). Detection systems for carbapenemase gene identification should include the SME serine carbapenemase. International. Journal of Antimicrobial Agents. 41(1): 1-4.

Clinical and Laboratory Standards Institute (2019). Performance standards for antimicrobial susceptibility testing. Twenty-Ninth Informational Supplement. CLSI document M100-S29. Wayne, PA: Clinical and Laboratory Standards Institute.

Cruickshank, R., Duguid, J.P., Marmion, B.P. and Ewing, W.H. (1975). Identification of *Enterobacteriaceae* 3rd ed. Burges publishing Co. *Atlanta, Georgia*, U.S.A. 152-154.

Dallenne, C., Costa, D.A., Decre, D., Favier, C. and Arlet, G. (2010). Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in *Enterobacteriaceae*. Journal of Antimicrobial Chemotherapy. 65(3): 490-495.

Diab, M., Hamze, M., Bonnet, R., Saras, E., Madec, J. and Haenni, M. (2017). OXA-48 and CTX-M-15 ESBL in raw milk in Lebanon: Epidemic spread of dominant *K. pneumoniae* clones. Journal of Medical Microbiology. 66: 1688-1691.

Diene, S.M., Rolain, J.M. (2014). Carbapenemase genes and genetic platforms in Gram-negative bacilli: *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* species. Clinical Micro biology and Infection. 20:831-838

Edward, P.R. and Ewing, W.H. (1972). Identification of *Enterobacteria mc eae* (3rd edn.). Burges publicity Co. Minneapolis, Minnesota. 55:415.

Franco S., Murphy M.M., Li, G., Borjeson, T., Boboila, C. and Alt, F.W. (2008). DNA-PKcs and joining phase of Immunoglobulin heavy chain class switch recombination. Journal of Experi mental Medicine. 205: 557-564.

Gupta, S., Abhishek, Shrivastav, S.and Verma, A.K. (2019). Isolation, Identification, Molecular characterization and antibiogram of *E. coli* isolates from neonatal calves. International Journal of Current microbiology and Applied Sciences. https://doi.org/10.20546/ijcmas.2019.806.2388 (6): 1996-2007.

Ibrahim, E.I., Sayed, F.H., Ashraf, M., Abd, E.I., Wahab, S.A.K. and Helmy A.T. (2018). Prevalence of ESBL producing *Entero bacteriaceae* isolated from bovine mastitis milk. Alexandria Journal of Veterinary Sciences. 58(1): 102-108.

Iraz, M., Azer O.D., Cemal, S., Mehmet, Z.D., Yasemin, A., Aysegul, S., Anton, Y.P., Osman B.O., Fatih, S.B., Hakan, K., Aysegul, C.C. (2015). Distribution of β-lactamase genes among carbapenem resistant *Klebsiella-pneumoniae* strains isolated from patients in Turkey. Annals of Laboratory Medicine. 35(6): 595-601.

Jones, C.H., Tuckman, M., Keeney, D., Ruzin, A. and Bradford, P.A. (2009). Characterization and sequence analysis of Extended-spectrum-β-lactamase-encoding genes from *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilus* iso lates collected during tigecycline phase 3 clinical trials. Antimicrobial Agents Chemotherapy. 53: 465-475.

Murugan, M.S., Sinha D.K., Vinodh, O.R., Yadav, A.K., Pruthvishree, B.S., Vadhana, P., Nirupama, K. R., Bhardwaj, M., Singh, B.R. (2019). Epidemiology of carbapenem resistant *E. coli* and first report of *bla*-VIM carbapenemase genes in calves from India. Epidemiology and Infection. 147(59):1-5.

Mushi, M.F., Mshana, S.E., Imirzalioglu, C., Bwanga, F. (2014). Carbapenemase genes among multidrug resistant gram negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. BioMed Research International: 303104.

Mustafai, M.M., Hafeez, M., Munawar, S., Basha, S., Rabaan, A.A., Halwani, M.A., Alawfi, A., Alshengeti, A., Najim, M.A., Alwarthan, S. (2023). Prevalence of carbapenemase and Extended-spectrum β-lactamase producing *Entero bacteriaceae*. A Cross-Sectional Study. Antibiotics. 12(1): 148. doi: 10.3390/antibiotics 12010148.

Nirupama *et al*. (2018). Molecular characterization of *bla*-OXA-48 carbapenemase, ESBL and Shiga-toxin producing *E. coli* isolated from farm piglets in India. Journal of Global Anti microbial Resistance. 13: 201-205.

Pruthvishree, B.S., Vinodh Kumar O.R., Sinha, D.K., Malik, Y.P.S., Dubal, Z.B., Desingu, P.A., Shivakumar, M., Krishnaswamy, N. and Singh B.R. (2017). Spatial molecular epidemiology of carbapenem-resistant and New Delhi metallo-β-lactamase (*bla*-NDM)-producing *E. coli* in the piglets of organized farms in India. Journal of Applied Microbiology. 122: 1537-1546.

Tarashi S., Hossein G., Soroor E., Ali P., Ali H. (2016). Phenotypic and molecular detection of Metallo-β-lactamase genes among imipenem resistant *Pseudomonasaeruginosa* and *Acinetobacter baumannii* strains isolated from patients with burn injuries. Archives of Clinical Infectious Diseases. 11(4): 1-6.

Thomson, K.S. (2010). Extended-spectrum-β-lactamase, AmpC and carbapenemase issues. Journal of Clinical Microbiology. 48 (4): 1019-1025.

Webb, H.E., Bugarel, M., Bakker, H.C., Nightingale, K.K., Granier, S.A., Scott, H.M. and Loneragan, G.H. (2016). Carbapenem resistant bacteria recovered from faeces of dairy cattle in the high plains region of the USA. PLoS ONE. 11(1): e0147363.

Yadav, V. Joshi, R.K., Joshi, N., Kumar, A. Singh, S.V. (2021). Detection of Carbapenemase genes *bla*-Ndm,*bla*-Kpc, *bla*-oxa-48 in *E. coli* and *Klebsiella* spp., isolated from milk samples of bovine in Eastern Zone of Uttar Pradesh. Indian Journal of Animal Research. 57(1): 66-73. doi: 10.18805/IJAR.B-4543.

Yadav, V. Joshi, R.K., Joshi, N., Singh, S.V. and Srivastva, D.P. (2024). Multidrug resistance pattern and plasmid profiling of ESBL and Carbapenemase producing *E. coli* isolates of bovine origin in Awadh region of Uttar Pradesh. Indian Journal of Animal Research. doi: 10.18805/IJAR.B-5322.

Yadav, V., Joshi, R.K., Joshi, N., Singh, S.V., Gupta, R.K., Niyogi, D., Yadav, D.K. (2022). Prevalence of extended-spectrum β-lactamase producing *E. coli* and *Klebsiella* spp. isolated from buffaloes in Eastern Plain Zone of Uttar Pradesh, Indian Journal of Animal Research. doi:10.18805/IJAR.B-5036.