RESEARCH ARTICLE

Antimicrobial Resistance and Plasmid Profiling of ESBL Producing *Klebsiella* spp. of Bovine Origin of Eastern Plain Zone of Uttar Pradesh

Vibha Yadav[1]*, Rajesh Kumar Joshi[1], Satyavrat Singh[2], Satyendra Kumar Maurya[3], Debasish Niyogi[4], Dharam Prakash Shrivastva[4] , Deepak Yadav[2]

Abstract

Emergence of antimicrobial resistance in animal husbandry is of utmost concern as it has resulted in therapeutic failure and nowadays it is one of the latest challenges faced by scientific community across the world. Extensive use of antibiotics without performing antibiotic susceptibility testing may lead to development of multidrug resistant (MDR) organisms. This study was undertaken to assess the AMR pattern and their correlation with plasmid profiling in ESBL producing *Klebsiella* spp**.** from bovine origin. Total 160 samples (80 mastitic milk and 80 diarrhoeic faeces) were collected from 2 district of Eastern Plain Zone of Uttar Pradesh (India) and 63 (39.37%) isolates were confirmed as *Klebsiella* spp. by conventional methods (cultural characteristics on selective media & biochemical tests). Antimicrobial susceptibility testing of confirmed isolates revealed 82.0%-94.0% resistance to 3rd & 4th generation cephalosporin, 100% to ampicillin and all isolates were found 100% sensitive to aminoglycosides, chloramphenicol and polypeptides class of antibiotics. Total 49 (77.8%) isolates were confirmed as ESBL producers using DDST and ESBL E-strip test, out of which 45 (71.43%) isolates revealed the presence of *bla*-$_{CTX-M}$ gp genes by PCR analysis. On plasmid profiling, total 82.82% isolates revealed the presence of plasmid with molecular weight between 2.5 kb to >10 kb and number 1-2. These high molecular weight plasmids are mostly situated on conjugative plasmid, which are horizontally transferred from one species to another in highly populous country like India.

Key words: Bovine, ESBLs, *Klebsiella* spp., MDR, Plasmid.

Ind J Vet Sci and Biotech (2023): 10.48165/ijvsbt.19.5.11

Introduction

Nowadays resistance to beta-lactam group of antibiotics is expanding rapidly throughout the world and is a point of discussion as it is one of the latest challenges faced by scientific community since it has resulted in therapeutic failure. Several disease in animals are caused by variety of bacteria; among them *Klebsiella* spp. is an important Gram negative pathogen and noticed as an emerging pathogen (Saini *et al*., 2012). It has also been reported as opportunistic pathogen that can cause primary bacteraemia as well as urinary tract infection (UTI) in human and animals (Mansour *et al*., 2014). Among *Enterobacteriaceae E. coli* and *Klebsiella* spp. are major ESBL producers and their increasing prevalence in livestock during last few years has been identified as global threat (Reuland *et al*., 2013). Most of the antibiotic resistant genes in Gram negative bacteria are located on plasmids which are horizontally transmitted between close families of bacteria and are considered as the important vector in retaining and dissemination of antibiotic resistant genes as their tendency to replicate in wide host range (Rozwannowicz *et al*., 2018).

There is limited information on antimicrobial resistance pattern and plasmid profiling of ESBL producing isolates of *Klebsiella* spp. of bovine origin. The present study was

[1]Department of Veterinary Microbiology, College of Veterinary Science & Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology (ANDUAT), Kumarganj- 224229, Ayodhya (UP), India

[2]Department of Veterinary Medicine, College of Veterinary Science & Animal Husbandry, ANDUAT, Kumarganj-224229, Ayodhya (UP), India

[3]Department of Veterinary Biochemistry & Physiology, College of Veterinary Science & Animal Husbandry, ANDUAT, Kumarganj-224229,Ayodhya (UP), India

[4]Department of Veterinary Pathology, College of Veterinary Science & Animal Husbandry, ANDUAT, Kumarganj-224229,Ayodhya (UP), India

Corresponding Author: Vibha Yadav, Department of Veterinary Microbiology, College of Veterinary Science & Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology (ANDUAT), Kumarganj- 224229, Ayodhya (UP), India. e-mail: vibhavet2005@gmail.com

How to cite this article: Yadav, V., Joshi, R. K., Singh, S., Maurya, S. K., Niyogi, D., Shrivastva, D. P., & Yadav, D. (2023). Antimicrobial Resistance and Plasmid Profiling of ESBL Producing *Klebsiella* spp. of Bovine Origin of Eastern Plain Zone of Uttar Pradesh. Ind J Vet Sci and Biotech. 19(5), 55-59.

Source of support: Nil

Conflict of interest: None

Submitted:20/07/2023 **Accepted:** 05/08/2023 **Published:** 10/09/2023

© The Author(s). 2023 Open Access This work is licensed under a Creative Commons Attribution-Non Commercial-No Derivatives 4.0 International License.

designed to understand the characteristics of plasmids and their possible role in the transmission of AMR genes among bacteria of closely related families and also in environment.

Materials and Methods

Collection of Samples: In the present study, total 160 random samples (80 mastitic milk and 80 diarrhoeic faeces) were collected aseptically from 5 tehsils of Ambedkar Nagar and 3 tehsils of Barabanki district of Eastern plain zone of Uttar Pradesh (India) during the period from January, 2022 to January 2023. Sampling was done randomly and consisted of 5 diarrhoeic faecal samples and 5 mastitic milk samples from both cattle and buffaloes of above mentioned tehsils. Approximately 5 mL of milk was collected in sterilized test tube and Calofornia mastitis test was used for screening of mastitic milk samples and faecal samples were collected by swab techniques. After collection, the samples were immediately transported to Veterinary Microbiology Laboratory in ice box for further processing.

Isolation and Identification: All samples were enriched with 2 mL tetrathionate broth and incubated at 37°C for 24 h. A loopful inoculum was taken and streaked directly on MacConkey agar plates and incubated at 37°C for 24 h. Pink colonies with mucoid appearance were picked up and streaked on Eosine Methylene Blue agar plates. Colonies showing light purple mucoid appearance were tentatively suspected as *Klebsiella* spp. After this, pure individual colonies were taken onto sterilized nutrient agar slant and further identification was done by various biochemical tests, *viz.*, IMViC pattern, nitrate reduction, urease test, triple sugar iron agar sugar fermentation reaction and catalase test as per standard method in practice.

Antibiotic Resistance Profiling: All the confirmed isolates (63) were subjected to *in vitro* antibiotic sensitivity test against 17 antibiotics of 11 different classes (Table 3) using standard disc diffusion method and results were interpreted as per guideline of CLSI (2019). The isolates showing reduced susceptibility towards 3rd, 4th generation cephalosporines and monobactam classes of antibiotics were screened as ESBL producers.

Confirmation of ESBL Producing Isolates by Phenotypic Methods

Double disc synergy test (DDST): The screened isolates were further confirmed by using ESBL Kit 1 and Kit 3 (Hi-media). These commercially available discs were placed on MHA plates inoculated with $1.5x10^8$ organism/mL at 25 mm apart and incubated at 37°C for 24 h. The results were interpreted as per CLSI guidelines (2019).

Minimum inhibitory concentration (MIC) ESBL E-test: This test was done by using E-strip (Hi-media) on MHA plates inoculated with $1.5x10^8$ organism/mL and incubated at 37°C for 24 h. The result was interpreted as per CLSI guidelines (2019).

Confirmation of ESBL Producing Isolates by Polymerase Chain Reaction

Extraction of plasmid DNA: Single pure colony of phenotypically confirmed ESBL positive isolates was inoculated into 10 mL of Luria-Bertani (LB) broth medium (HiMedia, India) and incubated at 37°C for 18 h in shaking incubator. Plasmid DNA was extracted using GeneJet plasmid Miniprep kit (Thermo Scientific) as per the instruction of the manufacturers.

Detection of bla-$_{CTX-M-1}$ and bla-$_{CTX-M-9}$ genes: Genotypic confirmation of ESBL genes (*bla*-$_{CTX-M-1}$ and *bla*-$_{CTX-M-9}$) was done in a total reaction volume of 25 µL for CTX-M gene as per method described by Dallenne *et al*. (2010). Amplicon size, primer sequence of targeted genes and cyclic conditions of PCR are mentioned in Table 1. Multiplex PCR was performed for CTX-M genes and amplified products (5 µL) were mixed with 3 µL of bromophenol blue dye (6X) and electrophoresis was done in 0.8% agarose gel for CTX-M group genes using 1 kb ladder at 60-70 mA for 40 min and gel was visualized using the UV illuminator (GeNei Bangalore, India).

Profiling of Plasmid DNA: Plasmid DNA from genotypically confirmed ESBL producing isolates was extracted, using GeneJet plasmid Miniprep kit (Thermo Fisher Scientific, USA) following the manufacturer's protocol and was stored at -20°C till further processing. Each extracted plasmid DNA samples (5 µL) was mixed with 2 µL of loading dye (6X) and electrophoresis was done on 0.8% (w/v) agarose gel mixed with 1 µL ethidium bromide (conc. 5 µg/mL) at 80-100V for 1 to 1.5 h using 1 kb ladder DNA. At the end of electrophoresis, the gel was visualized under UV trans-illuminator (EZ Gel Documentation system, Bio-Rad) fitted with high resolution digital camera for the presence, number and size of plasmid.

Results and Discussion

In this study, out of total 160 samples (80 mastitic milk and 80 diarrhoeic faeces) 63 (39.37%) isolates were confirmed

Table 1: Detail of primers and PCR conditions used for detection of ESBLs gene in *Klebsiella* spp. isolates (Dallenne *et al*., 2010)

Targeted genes	Primer sequence (5'-3')	Amplicon size (bp)	PCR conditions and cycles
bla-$_{CTX-M-1}$gp	F-5'TTAGGAARTGTGCCGCTGYA3' R-5'CGATATCGTTGGTGGTRCCAT3'	688	1 cycle of 10 min at 94 °C, 30 cycles of 40 sec. at 94 °C, 40 sec. at 60°C, 1 min at 72°C, 1 cycle of 7 min. at 72 °C
bla-$_{CTX-M-9}$gp	F-5'TCAAGCCTGCCGATCTGGT3' R-5'TGATTCTCGCCGCTGAAG3'	561	

Table 2: Occurrence of *Klebsiella* spp. isolates from clinical samples of bovine origin

Samples (Source/Origin)		Confirmed *Klebsiella* spp. isolates	ESBL positive *Klebsiella* spp. isolates
Cattle	Mastitic milk (n=40)	16 (40.0%)	12 (30.0%)
	Diarrhoeic faeces (n=40)	10 (25.0%)	7 (17.5%)
Buffalo	Mastitic milk (n=40)	22 (55.0%)	15 (37.5%)
	Diarrhoeic faeces (n=40)	15 (37.5%)	11 (27.5%)
Total	**(N=160)**	**63 (39.37%)**	**45 (28.12%)**

Table 3: Antimicrobial resistance pattern of *Klebsiella* spp. isolates (n=63)

Group	Antibiotics (Hi-Media)	Conc. (µg/disc)	*Klebsiella* spp. (AMR%)
Carbapenems	Imepenem (IMP)	10	17.46%
	Meropenem (MRP)	10	14.28%
3rd and 4th generation Cephalosporins	Cefotaxime (CTX)	10	93.65%
	Cefpodoxime(CPD)	30	88.91%
	Ceftazidime (CAZ)	30	85.71%
	Ceftriazone (CTR)	30	82.53%
Monobactams	Aztreonam (AT)	30	76.19%
2nd generation Cephalosporins	Cefoxitin (CX)	30	28.57%
Penicillin	Ampicillin (AMP)	25	100%
Polypeptides	Polymyxin B (PB)	300 unit	0. 00%
Aminoglycosides	Gentamicin (GEN)	30	0. 00%
	Amikacin (AK)	30	0. 00%
Tetracycline	Tetracycline (TE)	30	34.92%
Sulphonamides	Trimethoprim (TR)	30	36.50%
Quinolons	Ofloxacin (OF)	2	31.74%
	Nalidixic acid (NA)	30	34.92%
Chloramphenicol	Chloramphenicol (C)	30	0. 00%

as *Klebsiella* spp. on the basis of morphological, cultural and biochemical characteristics. The isolation rate was highest in mastitic milk of buffaloes (55.0%) followed by mastitic milk of cattle (40.0%) and diarrhoeic faecal samples of buffaloes (37.5%) and cattle (25.0%) (Table 2). In this study, isolation rate of *Klebsiella* spp. predominated in milk samples as compared to diarrhoeic faecal samples of bovine. These findings are in agreement with the observations of Badri *et al*. (2017), Koovapra *et al*. (2016) and Rawat *et al.* (2018), who have reported 63.0% *Klebsiella* spp. from raw cow milk samples, 68.5%, 59.6% and 10.8% *Klebsiella* spp. from subclinical, clinical and healthy cow milk, respectively, and 16.6% *Klebsiella* spp. from faecal samples of dairy cattle.

Antimicrobial resistance (AMR) is currently one of the major problems faced by scientific community across the world. In present study, antimicrobial susceptibility test (AST) of all confirmed *Klebsiella* spp. isolates was performed against 17 antibiotics of 11 different classes. All isolates of *Klebsiella* spp. showed 100% resistance to ampicillin followed by 82-94% to 3rd, 4th generation cephalosporins and 76.19% to aztreonam. The reasonable factors for this high degree of resistance against these antibiotics might be due to continuous antibiotic pressure or acquired by horizontal transmission through plasmids, transposons or insertion sequences. Susceptibility pattern of these isolates varied to different classes of non-β-lactam antibiotics. These isolates showed 100% susceptibility to chloramphenicol, polypeptides and aminogycosides classes of antibiotics (Table 3). There are abundant evidences that corroborate with these findings of *Klebsiella* spp. isolated from milk samples of bovine (Olowe *et al*., 2015; Badri *et al*., 2017; Rawat *et al*., 1018; Salauddin *et al*., 2019).

In this study, carbapenem antibiotics like imipenem and meropenem were also found resistant against these isolates, which were 17.46% and 14.28%, respectively. Badri *et al*. (2017) reported 49.4% resistance of *Klebsiella* spp. to imipenem isolated from raw milk of bovine in Sudan, while Singh *et al*. (2017) found 53.50% resistance to meropenem and 22.5% resistance to imipenem in enterobacteria isolated from human and environment in India. Although these antibiotics are not used for treatment of animal anywhere in India, but across the world, it might be due to use of these antibiotics for treatment of human patients and *Klebsiella* spp. being most commonly implicated in outbreaks of nosocomial infections.

Table 4: Distribution of ESBL genes and plasmid in ESBL positive *Klebsiella* spp. isolates among various sources

Samples (Source/Origin)		ESBL positive isolates	*bla*-$_{CTX-M}$ gp genes		Plasmid profile in *Klebsiella* spp.		
			bla-$_{CTX-M-1}$	*bla*-$_{CTX-M-9}$	Positive isolates	No. of bands	Average size (kb)
Cattle	Mastitic milk (n=40)	12 (30.0%)	10 (25.0%)	6 (15.0%)	11	1-2	2.5 to 6.0
	Diarrhoeic sample (n=40)	7 (17.5%)	5(12.5%)	2 (5.0%)	6	1	2.5- >10
Buffaloes	Mastitic milk (n=40)	15 (37.5%)	12 (30.0%)	4 (10.0%)	15	1-2	3.5-> 10
	Diarrhoeic sample (n=40)	11(27.5%)	10 (25.0%)	4 (10.0%)	5	1	4.0-8.0
Total (n=160)		**45 (28.12%)**	**37 (23.12%)**	**16 (10.0%)**	37	**1-2**	**2.5->10**

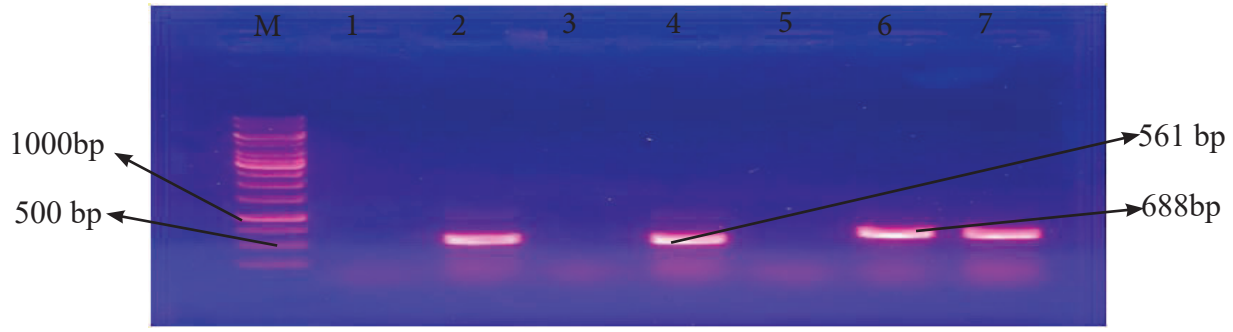

Fig.1: Multiplex PCR, Amplification of *bla*-$_{CTX-M-1}$ and *bla*-$_{CTX-M-9}$

M: 1 kb ladder (Thermo Scientific) Lane 2 & 4 positive for *bla*-$_{CTX-M-9}$ gene (561bp), Lane 6 and 7 positive for *bla*-$_{CTX-M-1}$ gene (688 bp)

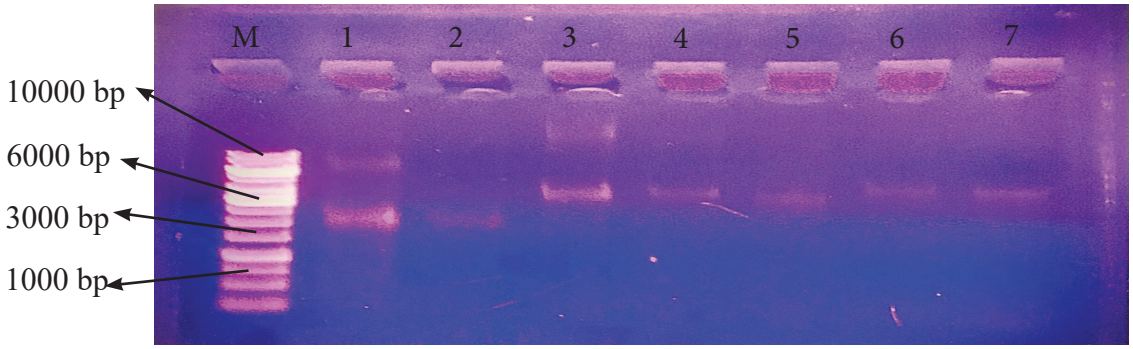

Fig. 2: Plasmid profile of ESBL positive *Klebsiella* spp. isolates

M: 1 Kb ladder (Thermo Scientific); lane, 1, 2, 3, 4, 5, 6 and 7 revealed plasmid bands

Nowadays, Multi-drug resistance (MDR) is the major cause of concern as they posses serious health problem by limiting the therapeutic options. In present study, 76.12% (48/63) isolates were found to be MDR, which highlighted the potential risk by limiting the treatment options. These results corroborated with the findings of Salauddin *et al*. (2019) and Yadav *et al*. (2022[a]).

This study also had aim to confirm ESBL producing isolates using phenotypic confirmatory methods and PCR analysis. Total 63 confirmed isolates of *Klebsiella* spp. were subjected to screening and phenotypic confirmatory tests. Out of total 63 confirmed isolates, 52 (82.53%) were screened as ESBL producers using disc diffusion method, 50 (79.36%) were confirmed as ESBL producer by DDST and 49 (77.8%) by ESBL-E strip test, and final confirmation was done by PCR analysis, which revealed 45 (71.43%) ESBL producing isolates. There was very little difference in the sensitivity of both phenotypic and genotypic confirmatory method and this was very similar to findings of Olowe *et al*. (2015) and Badri *et al*. (2017).

Phenotypically confirmed ESBL positive isolates were subjected to PCR analysis for detection of *bla*-$_{CTX-M-1}$ and *bla*-$_{CTX-M-9}$ genes. The molecular characterization revealed total 45 (28.12%) isolates to be ESBL positive comprising 12 (30.0%) and 07 (17.5%) from mastitic milk and diarrhoeic faecal samples of cattle and 15 (37.5%) and 11 (25.7%) from mastitic milk and diarrhoeic faecal samples of buffalo (Table 2, Fig. 1). This study revealed 37 (23.12%) existence of *bla*-$_{CTX-M-1}$ gene and 16 (10.0%) *bla*-$_{CTX-M-9}$ genes (Table 4). Similarly various co-workers across the world have also reported high prevalence of *bla*-$_{CTX-M}$ gene in different sample sources (Olowe *et al*., 2015; Badri *et al*., 2017; Yadav *et al*., 2019; Yadav *et al*., 2022[b]). This study also revealed the co-existence of *bla*-$_{CTX-M-1}$ and *bla*-$_{CTX-M-9}$ genes in 8 (5.0%) isolates.

Plasmid profiling of ESBL positive isolates was performed, total 37/45 (82.22%) isolates of *Klebsiella* spp. revealed plasmid

band in range of 1 to 2 with average plasmid size ranging from 2.5 to > 10 kb. Total 5 different plasmid profiling patterns were observed based on molecular weight and 2 on their numbers. One plasmid of > 10 kb was found in 67.56% (25/37) of isolates and maximum number of isolates (72.97%, 27/37) having 01 plasmid band (Table 4, Fig. 2). The results of this study were found in accordance with the findings of many workers of India (Rawat *et al*., 2018; Singh *et al*., 2020; Yadav *et al*., 2023).

Conclusions

Present study revealed that 76.0% isolates were MDR and most of the isolates showed resistance to 3rd, 4th generation cephalosporins, ampicillin and aztreonam, which is not a good sign. Most interesting finding of this study was that all isolates revealed 100% sensitivity to aminoglycosides, chloramphenicol and polypeptide group of antibiotics, which may be alternative therapeutic option for this area. Most of the ESBL positive isolates revealed the presence of at least one plasmid with molecular weight ranging from 2.5 kb to >10 kb. Due to extra-chromosomal and dynamic nature of plasmids their number and size may differ and a single bacterium can acquire several plasmids from multiple donors. The presence of plasmids clearly indicates the horizontal transmission of antibiotic resistant genes in the environment and healthcare settings. This study serves as a base that would be helpful in taking necessary initiative to monitor and limit the indiscriminate use of antibiotics, as well as proper inspection following standard protocol throughout the nation.

Acknowledgements

The authors are thankful to the Dean, College of Veterinary Sciences and Animal Husbandry, Kumarganj and livestock owners for their kind support for this work.

References

Badri, A.M., Ibrahim, T.I., Mohamed, S. ., Garbi, M. ., Kabbashi, A., & Arbab, M.H. (2017). Prevalence of ESBL producing *E. coli* and *K. pneumoniae* isolated from raw milk samples in Al Jazirah State, Sudan. *Molecular Biology*: *Open Access, 7*(1)**,** 1-4.

CLSI (2019). Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 29th Informational Supplement. CLSI document M100-S29. Wayne, PA, USA.

Dallenne, C., Costa, D.A., Decre, D., Favier, C., & 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.

Koovapra, S., Bandyopadhyay, S. Das, G., Bhattacharya, D., Banerjee, J., Mahanti, A., Samanta, P.K., Nanda, P.K., Kumar, A., Mukherjee, R., Dimri, U., & Singh, R.K. (2016). Molecular signature of ESBL-producing *Klebsiella pneumoniae* isolated from bovine milk in eastern and north-eastern India. *Infection, Genetics and Evolution*, *44,* 395-402.

Mansour, M.A., Zaki, H.M., Hassan, N.A., & Al-Humiany, A.A. (2014). Molecular characterization and immune-protective activity of capsular polysaccharides of *Klebsiella pneumoniae* isolated from farm animals at Taif Governorate. *American Journal of Infectious Diseases*, *10*(1), 1-14.

Olowe, O.A., Adewumi, O., Odewale, G., Ojurongbe, O., & Adefioye, O.J. (2015). Phenotypic and molecular characterization of Extended-spectrum β-lactamase producing *E. coli* obtained from animal faecal samples in Ado Ekiti, Nigeria. *Journal of Environmental and Public health*, *2015, ID 497980,* http://dx.doi.org/10.1155/2015/ 497980.

Rawat, N., Singh, F., Hirpurkar, S.D., Sannat, C., & Gade, N.E. (2018). Detection and characterization of extended spectrum β-lactamase genes (bla_{TEM} and bla_{SHV}) among β-lactam-resistant faecal coliforms of dairy cattle from Chhattisgarh, India. *Turkish Journal of Veterinary and Animal Sci*ences, *42,* 503-511.

Reuland, E.A., Overdevest, I.T., Al Naiemi, N., Kalpoe, J.S., Rijnsburger, M.C., Raadsen, S.A., Ligtenberg-Burgman, I., vanderZwaluw, KW., Heck, M., & Savelkoul, P.H., *et al*. (2013). High prevalence of ESBL-producing *Enterobacteriaceae* carriage in Dutch community patients with gastrointestinal complaints. *Clinical Microbiology and Infection, 19*(6)**,** 542-549.

Rozwannowicz, M., Brouwer, M.S.M., & Fischer, J., *et al*. (2018). Plasmids carrying antimicrobial resistance genes in *Enterobacteriaceae*. *Journal of Antimicrobial Chemotherapy*, *73*(5), 1121-1137.

Saini, V, McClure, J.T., Leger, D., & Morck, D.W. (2012). Antimicrobial resistance profiles of common mastitis pathogens on Canadian dairy farms. *Journal of Dairy Science, 95,* 4319-4332.

Salauddin, M., Akter, M.R., Hossain, M.K., & Rahman, M.M. (2019). Isolation of multi-drug resistant *Klebsiella* spp. from bovine mastitis samples in Rangpur, Bangladesh, *Journal of Advanced Veterinary and Animal Research*, 6(3), 362-365.

Singh, F., Hirpurkar, S.D., Rawat, N., Shakya S., Kumar, R., Rajput, P.K., & Kumar, S. (2020). Occurrence of the genes encoding carbapenemases, ESBLs and class 1 integron-integrase among fermenting and non-fermenting bacteria from retail goat meat. *Applied Microbiology, 71*(6), 611-619.

Singh, F., Hirpurkar, S.D., Shakya, S., Rawat, N., Devangan, P., Khan, F.F., & Bhandekar, S.K. (2017). Presence of enterobacteria producing Extended-spectrum β-lactamases and/or carbapenemases in animals, humans and environment in India. *Thai Journal of Veterinary Med*icine, *47,* 35-43.

Yadav, A., Joshi, N., & Joshi, R.K. (2019). Occurrence of ESBLs producing Enterobacteria in animal products and their environments. *International Journal of Current Microbiology and Applied Sciences*, *8*(5), 2255-2264.

Yadav, V., Joshi, R.K., Joshi, N., Kumar, A., Singh, S.V., & Niyogi, D. (2022[a]). Detection and molecular characterization of Extended-spectrum β-lactamase producing *E. coli* & *Klebsiella* spp. isolates of cattle origin in Eastern Plain Zone of Uttar Pradesh. *Indian Journal of Animal Research*, *56*(11), 1369-1376.

Yadav, V., Joshi, R.K., Joshi, N., Singh, S.V., Gupta, R.K., Niyogi, D., & Yadav, D.K. (2022[b]). Prevalence of Extended-spectrum β-lactamase genes (bla-$_{CTX-M}$, bla-$_{TEM}$ and bla-$_{SHV}$) in *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.

Yadav, V., Joshi, R.K., Joshi, N., Singh, S.V., Maurya, S.K., Srivastva, D.P., & Gupta, R.K. (2023). Status of MDR and plasmid profiling of ESBL producing *E. coli* and *Klebsiella* spp. isolated from milk of bovine in Gangetic Plain Zone of Uttar Pradesh. *Indian Journal of Veterinary Science & Biotechnology*, *19*(2), 16-22.