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Multidrug Resistance Pattern and Plasmid Profiling of ESBL and Carbapenemase Producing *E. coli* 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]

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ABSTRACT

Background: Emergence and dissemination of Extended-spectrum β-lactamase (ESBL) and Carbapenemase producing *Enterobacteriaceae* (CPE) constitutes a major public health concern. The extensive and inappropriate use of antibiotics may lead to development of multidrug resistant (MDR) organisms. The antibiotic resistant genes are mostly situated on conjugative plasmids, which are horizontally transmitted from one species to another in highly populous country like India.

Methods: Total 240 samples were collected from Ayodhya and Sultanpur districts of Awadh region of Uttar Pradesh (India). Confirmation of isolates was done by PCR analysis using species specific uidA gene. Then, ESBL and Carbapenemase producing isolates were confirmed using DDST, ESBL E-strip and MBL E-strip test respectively. Plasmid profiling of these isolates were performed using GeneJet Plasmid Miniprep Kit and gel-electrophoresis.

Result: In this study, a total 186 (77.5%) isolates of *E. coli* were confirmed by PCR analysis, out of which, 130(54.16%) isolates were confirmed as ESBL and 10(4.16%) as Carbapenemase producer using ESBL E-strip and MBL E-strip test. All ESBL and Carbapenemase positive isolates were found 99-100% sensitive against chloramphenicol, polypeptides and aminoglycosides group of antibiotics and 80-100% isolates were found resistance against 3rd, 4th group of cephalosporins, monobactam and penicillin group of antibiotics. Total 76.18% ESBL positive and 100% Carbapenemase positive isolates were found MDR. Plasmid profiling revealed 63.84% ESBL positive isolates, which carried plasmid of molecular weight in range of 1.5 to >10kb with 1-3 number of bands and 80.0% Carbapenemase positive isolates carried plasmid of molecular weight 3 to >10 kb with 1-3 number of bands.

Key words: Carbapenemase, *E. coli,* ESBL, MDR, Plasmid.

INTRODUCTION

Antimicrobial resistance (AMR) is a global public health concern and one of the latest challenges faced by scientific community across the world. The major contributors of AMR are Extended-spectrum β-lactamase (ESBL) and Carbapenemase producing *Enterobacteriaceae* (CPE) (Mustafai *et al*., 2023). Due to an increase in frequency of ESBL-producing *Enterobacteriaceae*, the use of Carbapenem antibiotics has increased to a very agitating level (Wang *et al*., 2015) hence MDR and CPE have been increasing to an alarming rate which restricts effective antimicrobial treatment (Singh *et al*., 2017). However in clinical conditions these bacteria act as opportunistic pathogens and develop resistance in treatment of life-threatening infections particularly in immune-compromised patients (Munoz-Price *et al*., 2013). The spread of the resistant bacteria into many species may occur through several means *viz*. food animals, sewage, waste water and environment. The resistant genes of the antibiotics are frequently located on mobile genetic elements which are horizontally transferred between the close families of bacteria and in the environment (Ansari *et al*., 2018). The plasmids also have ability to acquire AMR genes through mobile genetic elements and they also have tendency to replicate in wide host range which make them perfect vector for dissemination of AMR genes (Rozwandowicz *et al*., 2018).

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

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

[4]Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj-224 229, Ayodhya, 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-224 229, Ayodhya, Uttar Pradesh, India.
Email: vibhavet2005@gmail.com

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Molecular characterization of plasmids in ESBL and Carbapenemase producing isolates provides significant knowledge concerning to potential of transmission of AMR genes among the closely related bacteria and also in the environment, which will help the researchers, field vetenarians and policy makers in monitoring of disease outbreaks and ascertaining the evolution and spread of AMR genes among isolates in community setting. Keeping in view the above facts and due to confined information on AMR pattern and plasmid profiling of ESBL and Carbapenemase producing isolates of *Escherichia coli* (*E. coli*) of bovine origin in this region, present study was designed.

MATERIALS AND METHODS

Study area

The study was carried out in the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya. The samples were collected from Ayodhya and Sultanpur districts of Awadh region of Uttar Pradesh, India. The study was conducted between January 2020 to March 2021.

Collection of samples

In this study total 240 faecal samples (160 apparently normal and 80 diarrhoeic faeces) were collected from cattle and buffaloes from 5 tehsils of Ayodhya and 3 tehsils of Sultanpur district. Sampling was done randomly and comprising of 10 apparently normal and 5 diarrhoeic faecal samples from each of animal from above mentioned tehsils. The samples were collected into a sterilized test tube by swab technique. All collected samples were immediately transferred to bacteriological laboratory of Department of Veterinary Microbiology, under cold chain for further processing.

Isolation and Identification

All samples were inoculated into 2 mL nutrient broth and incubated at 37°C for 24 hrs. A loopful inoculums were taken and directly streaked on MacConkey agar plates and incubated at 37°C for 24 hrs. Individual lactose fermenting colony (rose pink) colour was picked up and directly streaked on Eosine Methylene Blue agar plates and colonies showing metallic sheen were tentatively suspected as *E. coli*. A pure individual colony was taken onto sterilized nutrient agar slant and further identification was done by Gram's staining and biochemical tests using KBM001 HiMotility^{TM}Biochemical kit for *E. coli* (Hi-media) having combination of 12 tests. The standard test procedure was followed as mentioned in the kit and results were interpreted as per provided interpretation chart by the manufactures of the kit.

Confirmation of the *E. coli* isolates by PCR analysis

Extraction of genomic DNA

The genomic DNA was extracted using the snap-chill method described by Franco *et al*. (2008).

Molecular confirmation of *E. coli* isolates

All presumptively positive *E. coli* isolates were confirmed by PCR analysis using species specific uidA gene as per method described by Anbazhagan *et al.* (2010) (Table 1). The PCR reaction was carried out in total volume of 25 µl volume comprising 12.5 µl of 2× EmeraldAmp GT Master Mixture, 8.5 µl nuclease free water, 1µl mixture of forward and reverse primers (0.5µl each primer, conc. 0.5µM each primer) and 3µl template DNA. Amplification was performed in thermo cycler (Bio-Rad, USA). The cycling conditions of the PCR are mentioned in Table 1.

Screening of ESBL and Carbapenemase producing isolates

All confirmed isolates of were subjected to 3rd, 4th generation cephalosprins and monobactam (Hi-Media, India) for screening of ESBLs and Carbapenem antibiotic discs (Hi-Media, India) for screening of Carbapenemase producing isolates. The result was interpreted as per CLSI (2019) guidelines.

Phenotypic confirmation of ESBL and Carbapenemase producing isolates

Double disc synergy test (DDST)

Screened ESBL producing isolates were further confirmed by using ESBL kit 1 and Kit 3 (Hi-media) and similarly Carbapenemase production was confirmed by commercially available imipenem disc (10 µg) alone and imipenem + EDTA (10/750 µg) discs (HiMedia, India). These commercially available discs were placed on Muller Hinton agar (MHA) (HiMedia) plates, infused with1.5×10^{8}organisms/ml and incubated at 37°C for 24 hrs. The results were interpreted as per CLSI guidelines (2019).

Minimum inhibitory concentration (MIC) tests

The MIC of ESBL and Carbapenemase producing isolates were determined by using ESBL E-strip (Fig 3) and MBL E-strip (Fig 4) placing on MHA plates infused with 1.5x10^{8} organisms/ml and incubated at 37°C for 24 hrs. The results were interpreted as per CLSI guidelines (2019).

Study of multi-drug resistance (MDR) pattern of ESBL and Carbapenemase producing isolates

All Phenotypically confirmed ESBL and Carbapenemase producing isolates were examined for their resistance against 20 antibiotics of 12 different classes (Hi-media) mentioned in Table 3. The antibiotic susceptibility test (ABST) was performed by disc diffusion method on MHA (Hi-media) plates seeded with 1.5×10^{8} organisms/ml and incubated at 37°C for 24 hrs and isolates were classified as susceptible and resistant as per interpretation criteria of CLSI (2019) guidelines and those organisms showing resistance to at least one antibiotic of three or more classes, were considered as MDR bacteria.

Plasmid profiling of ESBL and Carbapenemase producing isolates

Isolation of plasmid DNA from ESBL and Carbapenemase producing isolates

Plasmid DNA of each confirmed ESBL and Carbapenemase producing isolates were extracted using GeneJet plasmid Miniprep kit (Thermo Fisher Scientific, USA) as per manufacturer's protocol.

Analysis of plasmid profile of ESBL and Carbapenemase producing isolates

To analyze the plasmid profile, each extracted plasmid DNA samples (5 µl) mixed with 2 µl of loading dye (6X) and electrophoresis was done on 0.8% (w/v) agarose gel mixed 1µl ethidium bromide (conc. 5 µg/ml) at 80-100V for 1 to 1.5 hours using 1kb ladder as marker 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. Plasmid profile was analyzed for the number and size of plasmids.

RESULTS AND DISCUSSION

In present study total 240 samples (80 apparently normal and 40 diarrhoeic faeces) were collected aseptically from each cattle and buffaloes from 5 tehsils of Aoyodhya and 3 tehsils of Sultanpur district of Awadh region of Uttar Pradesh. Out of 240 samples, total 218 (90.83%) were presumptively identified as *E. coli* on the basis of morphological, cultural and biochemical characteristics (Table 2). Confirmation of these isolates was done by PCR analysis, which revealed total 186 (77.5%) *E. coli* isolates (Table 2, Fig 1). The findings were in concordance with the findings of Yadav *et al.* (2022a), Kotsoana *et al.* (2019) and Gupta *et al.* (2019). High isolation rate of *E. coli* in faecal samples may be attributed to high prevalence of *E. coli* in GIT of ruminants as compared to other members of family *Enterobacteriaceae.*

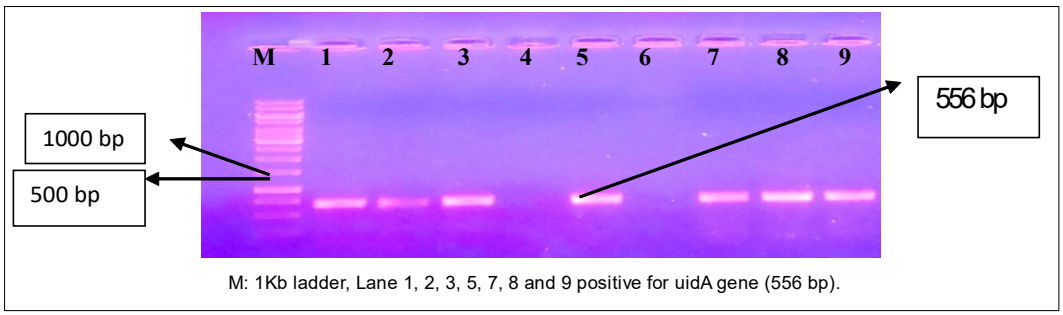
The present study was also focused to find out the occurrence of ESBL and Carbapenemase producing isolates. In this study total 130 (54.16%) isolates were found ESBL positive comprising 41 (51.25%), 22 (55.0%), 48 (60.0%) and 19 (47.5%) from apparently normal and

Table 1: Oligonucleotide primer sequences used for amplification of uidA genes and PCR cycling conditions used.

Targeted gene	Primer sequence (5′-3′)	Amplicon size (bp)	PCR conditions and cycles	References
uidA	F-5′CTGGTATCAGCGCGAAGTCT3′ R-5′AGCGGGTAGATATCACACTC3′	556	1 cycle of 5 minutes at 95°C, 35 cycles of 45 seconds at 95°C, 55 seconds at 56°C, 1 minutes at 72°C, 1 cycle of 7 minutes at 72°C	Anbazhagan *et al.*, 2010

Table 2: Isolation rate and distribution of ESBL and Carbapenemase producing isolates among various sources of bovine.

Samples (Source/Origin)		Presumptive positive isolates (Biochemical tests)	Confirmed isolates (PCR analysis)	ESBL positive isolates	Carbapenemase positive isolates
Cattle	Apparently normal faeces (n=80)	77 (96.25%)	65 (81.25%)	41 (51.25%)	1 (1.25%)
	Diarrhoeic faeces (n=40)	35 (87.5%)	28 (70.00%)	22 (55.0%)	3 (7.5%)
Buffaloes	Apparently normal faeces (n=80)	69 (86.25%)	63 (78.75%)	48 (60.0%)	4 (5.0%)
	Diarrhoeic faeces (n=40)	37 (92.5%)	30 (75.0%)	19 (47.5%)	2 (5.0%)
Total	N=240	218 (90.83%)	186 (77.5%)	130 (54.16%)	10 (4.16%)



M: 1Kb ladder, Lane 1, 2, 3, 5, 7, 8 and 9 positive for uidA gene (556 bp).

Fig 1: PCR amplification of uidA gene (556 bp).

diarrhoeic faecal samples of cattle and buffaloes respectively (Table 2, Fig 2). Likewise total 10 (4.16%) isolates were found Carbapenemase positive comprising 1 (1.25%), 3 (7.50%), 4 (5.0%) and 2 (5.0%) from apparently normal and diarrhoeic faecal samples of cattle and buffaloes respectively (Table 2, Fig 3). The Carbapenemase producing isolates were recovered in very low percentage in comparison to ESBL producing isolates. These results were in concordance with various workers (Yadav *et al*., 2022b; Kotsoana *et al*., 2019; Gupta *et al*., 2019). Similar to our findings in reference to Carbapenemase producing isolates, low percentage was also reported by various workers in India and abroad (Braun *et al*., 2016; Webb *et al*., 2016; Nirupama *et al*., 2018) from faecal samples of different species however, higher percentage was reported by (Murugan *et al*., 2019; Gupta *et al*., 2019).

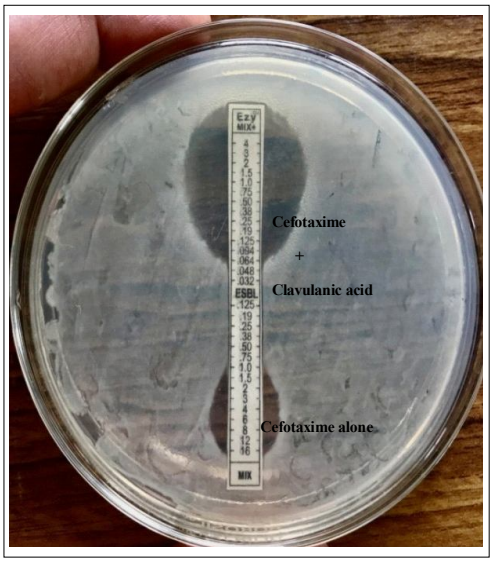


Fig 2: ESBL E-strip test for confirmation of ESBL producing isolates.

Antimicrobial resistance (AMR) is a serious problem around the globe and one of the burning issues faced by scientific community. AMR pattern in this study was found that ESBL positive isolates showed 90-100% resistance against 3rd and 4th generation cephalosporins and penicillin group antibiotics, 76.15% against monobactam, 10-15% against amoxyclav, cefoxitin and carbapenems antibiotics. The Carbapenemase positive isolates were found 100% resistant to carbapenems, monobactams, 80-100% resistant to 3rd and 4th generation cephalosporins and penicillin, 30-60% resistant to non-β-lactam group of antibiotics, while both ESBL and Carbapenem positive isolates showed 99-100% sensitivity to chloramphenicol, aminogylcosides and polymyxin-B group of antibiotics (Table 3). There are abundant evidences that corroborate with these findings (Gupta *et al*., 2019; Yadav *et al*., 2022b). The MDR is one of the latest challenges that are faced by scientific community across the world as they possess serious health

Table 3: AMR pattern of ESBL and Carbapenemase producing isolates of bovine origin.

Group	Antibiotics (Hi-Media)	Conc. (µg/disc)	ESBL positive isolates (n=130)	Carbapenemase positive isolates (n=10)
			Resistance	Resistance
Carbapenems	Imepenem (IMP)	10	13.84%	100%
	Meropenem (MRP)	30	11.54%	100%
3rd and 4th generation cephalosporins	Cefotaxime (CTX)	10	100 %	100%
	Cefpodoxime (CPD)	10	100%	100%
	Ceftazidime (CAZ)	30	90.76%	80.0%
	Ceftriazone (CTR)	30	100%	100%
Monobactams	Aztreonam (AT)	30	76.15%	100%
2nd generation cephalosporins	Cefoxitin (CX)	30	11.54%	60.0%
Penicillin	Ampicillin (AMP)	25	100%	80.0%
Polymixin B	Polymixin (PB)	300 unit	0.8%	0.0%
Aminogylcosides	Gentamicin (GEN)	10	0.0 %	0.0%
	Amikacin (AK)	30	0.0 %	0.0%
Quinolones	Enrofloxacin (EX)	10	21.53%	40.0%
	Ofloxacin (OF)	2	21.53%	30.0%
	Nalidixic acid (NA)	30	40.00%	60.0%
Sulphonamides	Co-trimoxazole (COT)	25	33.07%	50.0%
	Trimethoprim (TR)	30	29.23%	30.0%
Tetracycline	Tetracycline (TE)	30	22.30%	40.0%
Amoxyclav	Amoxicillin/Clavulanic (AMC)	(20/10)	13.84%	30.0%
Chloramphenicol	Chloramphenicol (C)	30	0.0 %	0.0%

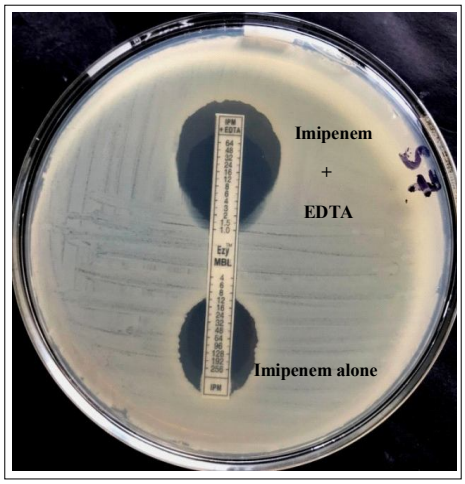


Fig 3: MBL E-strip test for confirmation of Carbapenemase producing isolates.

complications by limiting the therapeutic options. In this study total 76.18% ESBL positive and 100% Carbapenemase positive isolates were found MDR *i.e.* resistant to at least one antibiotic of three or more classes of antimicrobial groups, which is very worrisome situation for this area.

Plasmid profiling of ESBL and Carbapenemase positive isolates was performed, 63.84% ESBL positive isolates and 80.0% Carbapenemase positive isolates revealed the presence of plasmid band in the range of 1 to 3 with average plasmid size ranging 1 to >10 kb and 3 to >10 kb respectively (Table 4). Total 04 different plasmid patterns were observed based on their molecular weight and 03 plasmid patterns based on their number in ESBL positive isolates (Fig 4) and 02 different plasmid profiling patterns were observed based on their molecular weight and number in Carbapenemase positive isolates (Fig 5). One plasmid of >10 kb was found in 66.26% isolates with maximum number of isolates having 02 plasmid bands in

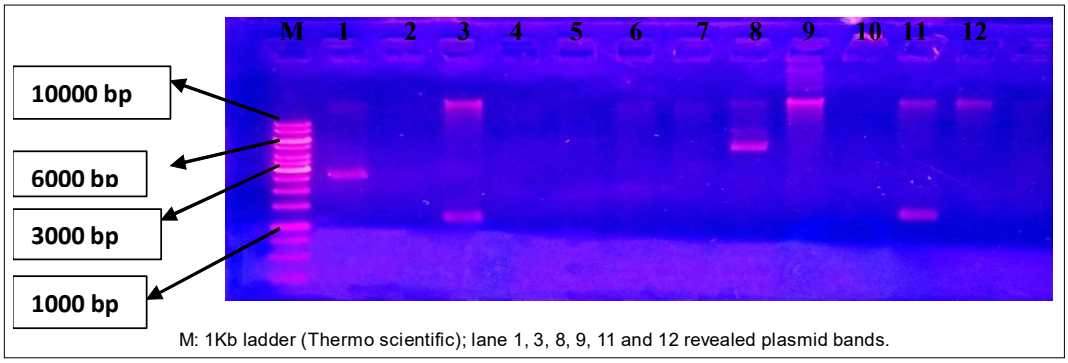


Fig 4: Plasmid profile of ESBL positive isolates.

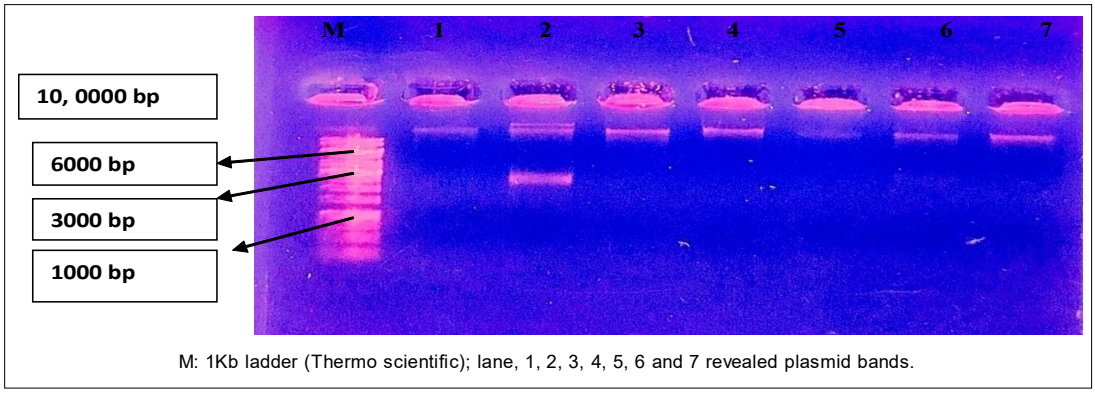


Fig 5: Plasmid profile of Carbapenemase positive isolates.

Table 4: Distribution of plasmids in ESBL and Carbapenemase positive isolates among various sources.

Samples (Source/Origin)		ESBL positive isolates	Pattern of plasmid profile			Carbapenemase positive isolates	Pattern of plasmid profile		
			Positive isolates	No. of bands	Average size (kb)		No. of bands	Average size (kb)	Positive isolates
Cattle	Normal faeces (n=80)	41	27	1-2	1.5 to >10	1	1	1	>10
	Diarrhoeic faeces (n=40)	22	14	1-2	6 to >10	3	2	1	>10
Buffaloes	Normal faeces (n=80)	48	30	1-2	1.5 to >10	4	3	1	>10
	Diarrhoeic faeces (n=40)	19	12	1-3	2.5 to >10	2	2	1-3	3 to >10
Total (N=180)		130	83	1-3	1.5 to >10	10	08	1-3	3 to >10

ESBL positive isolates while 100% Carbapenemase positive isolates revealed the presence of 01 plasmid >10 kb with maximum of isolates having 01 plasmids. The findings of this study were in conformity with the observations of many workers in India and abroad (Singh *et al*., 2020; Shrestha *et al*., 2020; Yadav *et al*., 2023).

CONCLUSION

It is noteworthy from present findings that Carbapenems are not used in animal husbandry practices any where across the world; even then resistance in animal isolates has been observed, which may be attributed to horizontal transmission of resistant genes between human and animal in community setting. ESBL positive isolates attributed 76.15% MDR whereas Carbapenemase positive isolates showed 100% MDR. Most interesting finding of this study was that both ESBL and Carbapenemase producing isolates revealed 100% sensitivity to aminoglycosides, polypeptides and chloramphenicol group of antibiotics which could be alternative therapeutic option for this area. Presence of plasmid and one plasmid with >10 kb molecular weight in most of the isolates clearly indicate the horizontal transmission of AMR genes in the environment and health care setting due to their extra-chromosomal and dynamic nature. This study serves as a base that would be helpful in taking necessary initiative to monitor and limiting the indiscriminate use of antibiotics and proper inspection following standard protocol throughout the nation.

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Conflict of interest

All authors declared 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.

Ansari, M., Munir, T., Sadd, N. (2018). Phenotypic identification, frequency distribution and antibiogram of carbapenemase producing *Enterobacteriaceae* in clinical isolates. Journal College of physicians Surgeons Pakistan. 28: 274-278.

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. 4(7): 1020. doi: 10.3389/fmicb.2016.01020.

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.

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 Experimental Medicine. 205: 557-564.

Gupta, S., Shrivastav, S.A. 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. 8(6): 1996-2007. https://doi.org/10.20546/ijcmas.2019.806.238.

Kotsoana, P., Montso, S.B.D., Ajay, K. and Collins, N.A. (2019). Antimicrobial resistance factors of ESBL producing *E. coli* and *K. pneumoniae* isolated from cattle farms and raw beef in North-west province, South Africa. BioMed Research Internationals. 6: 4318306. http://doi.org/10.1155/1019/4318306.

Munoz-Price, L.S., Arheart, K., Nordmann, P., Boulanger, A.E., Cleary, T., Alvarez, R., Pizano, I., Namias, N. *et al.* (2013). Eighteen years of experience with *Acinetobacter baumanni* in a tertiary care hospital. Critical Care Medicine. 41: 2733-2742.

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.

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 caebapenemase and extended-spectrum β-lactamase producing *Enterobacteriaceae*. A Cross-Sectional Study. Antibiotics. 12(1): 148. doi: 10.3390/antibiotics12010148.

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 Antimicrobial Resistance. 13: 201-205.

Rozwannowicz, M., Brouwer, MSM, Fischer, J. *et al*. (2018). Plasmids carrying antimicrobial resistance genes in *Enterobacteriaceae*. Journal of Antimicrobial Chemotherapy. 73(5): 1121-1137. doi:10.1093/jac/dkx488.

Shrestha, UT., Shrestha, S., Adhikari, N., Rijal, K.R., Shrestha, B., Adhikari, B., Banjara, M.R. and Ghimire, P. (2020). Plasmid profiling and occurrence of β-Lactamase Enzymes in multidrug-resistant uropathogenic *Escherichia coli* in Kathmandu, Nepal. Journal of Infection and Drug Resistance. 13: 1905-1917.

Singh, F., Hirpurkar, S.D., Rawat, N., Shakya S., Kumar, R., Rajput, P.K. and 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. and 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 Medicine. 47: 35-43.

Wang, J.T., Wu, U.I., Lauderdale, T.L., Chen, M.C., Li, S.Y., Hsu, L.Y. and Chang, S.C. (2015). Carbapenem non-susceptible *Enterobacteriaceae* in Taiwan. PLoS One. 10: e0121668. doi: 10.1371/journal.pone.0121668.

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. https://doi.org/10.1371/journal.pone.0147363.

Yadav, V., Joshi, R.K., Joshi, N., Kumar, A., Singh, S.V. and Niyogi, D. (2022b). Detection and molecular characterization of ESBL producing *E. coli* and *Klebsiella* spp. isolates of cattle origin in Eastern Plain Zone of Uttar Pradesh. Indian Journal of Animal Research. 56(11): 1369-1376. doi: 10.18805/IJAR, B-4762.

Yadav, V., Joshi, R.K., Joshi, N., Singh, S.V., Gupta, R.K., Niyogi, D. and Yadav, D.K. (2022a). 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.

Yadav, V., Joshi, R.K., Joshi, N., Singh, S.V., Maurya, S.K., Srivastva, D.P. and 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 and Biotechnology. 19(2): 10-16.