Indian Journal of Animal Sciences **95** (4): 279–285, April 2025/Article
https://doi.org/10.56093/ijans.v95i4.157437

Study on prevalence of ESBL producing multi drug resistant *E. coli* in livestock and poultry in Patna district of Bihar state, India

ARCHANA[1], PURUSHOTTAM KAUSHIK[1]✉, ANJAY[1], BHOOMIKA[1], SEULI SAHA ROY[1], BABLU KUMAR[2], ANIL KUMAR[3], MANOJ KUMAR[4], SAVITA KUMARI[4] and SAURABH SWAMI[1]

Bihar Veterinary College, Bihar Animal Sciences University, Patna 800014, India

Received: 03 October 2024; Accepted: 30 June 2025

ABSTRACT

Antimicrobial resistance is considered one of the greatest public health threat undermining the effectiveness of antibiotics. Occurrence of Extended spectrum beta-lactamase (ESBL) producing multidrug resistant (MDR) *E. coli* in human and animal poses a clinical and epidemiological challenge. The aim of the present study was to determine the occurrence of ESBL-producing MDR *E. coli* among healthy livestock and chicken and to investigate the types of ESBL genes circulating among livestock in Patna district of Bihar. A total of 254 samples from apparently healthy animals and chickens were collected from sixteen villages of Patna district which comprised of raw milk, rectal swabs and chicken cloacal swab. Out of 254 samples processed a total 148 isolates were confirmed as *E. coli.* After isolation and confirmation of *E. Coli* resistance profile were generated by disc diffusion method. The antimicrobial resistance profile of isolates revealed that 85.13% isolates were resistant to amoxicillin/ clavulanate; 72.97% to cefpodoxime; 37.83% resistant to ampicillin; 33.10% to ceftazidime; 27.02% to nalidixic acid; 26.35% to cefoxitin; and 23.64% to trimethoprim. However, 97.98% isolates were found susceptible to amikacin and 100% were sensitive to imipenem. The MDR analysis showed that 54.05% of isolates have MAR index ≥0.25 and 8.1% of isolates have MAR index ≥0.5. Further, out of 148 *E. coli* isolates tested for ESBL production, 30 isolates were found ESBL producer. The study provides data on prevalence of MDR ESBL producing *E. coli* among livestock in Bihar, India, indicating the risk of transmission of pathogens to humans through horizontal transmission.

Keywords: AMR, Animal, *E. coli*, ESBL, MDR

Antimicrobial resistance (AMR) stands as a global threat and is recognized as among the top 10 challenges to public health by the World Health Organization (WHO). It poses catastrophic outcomes, limiting arsenal against severe and life-threatening infections (Nobrega *et al*. 2021). The rampant use of antibiotics, coupled with improper dosing and termination of premature treatment in both humans and animals, are some of the major factors of developing bacterial resistance to antibiotics (Rahman *et al*. 2020). This renders the antibiotics ineffective, complicating the management of infections, and in the process elevate both morbidity and mortality rates. The development of resistance among the bacterial population also undermines the advancements made in modern medicine, raising concerns about the potential onset of a post-antimicrobial era in the 21st century (Suay-García and Pérez-Gracia 2019). In a report on AMR by O'Neill (2016), 10 million deaths associated to AMR has been projected, out of which up to 90% deaths has been projected to take place in low- and middle-income countries (LMICs). Among these LMICs, India bears a significant burden of AMR-related infection (CDDEP 2020).

Extended spectrum beta lactamases (ESBLs) are enzymes commonly produced by *Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella pneumoniae* which hydrolyzes β-lactam ring of broad-spectrum β-lactam antibiotics such as oxyimino-cephalosporins, the third-generation cephalosporins, (cefotaxime, ceftazidime, ceftriaxone, cefuroxime, cefepime) and monobactams (aztreonam), however these enzymes could not inactivates cephamycins (cefoxitin, cefotetan) or carbapenems (imipenem, meropenem, ertapenem, doripenem) (Blair *et al*. 2015). These enzymes can be inhibited by β-lactamase inhibitors such as clavulanate, sulbactam & tazobactam, which are considered as older β-lactamase inhibitors, while, avibactam, relebactam and vaborbactam are the latest Food and Drug Administration (FDA) approved β-lactamase inhibitors (Bush and Bradford 2020). Production of ESBLs by the bacterium depends on the presence of genes encoding ESBL commonly harboured on bacterial transposons or insertion sequences of plasmids. These genes can be horizontally transferred to other bacterial species (pathogenic or commensals) making them resistant to antibiotics. These genes are diverse in nature, leading

Present address: [1]Bihar Veterinary College, Bihar Animal Sciences University, Patna, India, [2]Indian Veterinary Research Institute, Izatnagar, India. ✉Corresponding author email: drkaushikvet@gmail.com

to a continuous increase in the number of reported ESBLs (Rahman *et al*. 2018). Based on the amino acid sequences of enzymes, ESBL have been subdivided into ten families—CTX-M, TEM, SHV, SFO, PER, VEB, GES, TLA, BES and OXA. Among these, CTX-M, TEM, and SHV ESBLs are the most common among *Enterobacteriaceae*.

Livestock, particularly chicken has been reported to carry ESBL producing Enterobacteriaceae. Such bacteria producing ESBL has also been reported from raw milk (Odenthal *et al*. 2016) and has been correlated with spread of resistant bacterium among human population (Carattoli 2008). The gut microbiota of livestock serves as an ideal reservoir for antibiotic resistance genes accumulated in due course of time due to overuse of antibiotics (Bennett 2008) in animal husbandry.

The current work was aimed to determine the occurrence of ESBL-producing MDR *E. coli* among healthy livestock and chicken and to investigate the types of ESBL gene circulating among healthy livestock of Bihar, India.

MATERIAL AND METHODS

Sample collection: A total of 254 samples were collected from farm animals and chickens during the period December 2021 to July 2022. The sample were collected aseptically from healthy animals, from different blocks of Patna, Bihar that comprised of raw milk from cattle (n=72) and buffalo (n=64), rectal swabs from goat (n=67) and chicken cloacal swab (n=51). The milk samples were collected in sterile vial (HiMedia, Mumbai, India) after washing the teats with clean water followed by swabbing with antiseptic. Before collecting the milk, first few strips of milk were discarded. The rectal and cloacal swabs were collected with the help of sterile cotton swab sticks (HiMedia, Mumbai, India) kept in sterile vial with transport media as per the standard guideline. All the samples were transported to the laboratory under cold temperature for further processing.

Isolation and PCR based confirmation of E. coli: Isolation of *E. coli* was made by enrichment of samples in Mac-Conkey broth with overnight incubation at 44°C. The suspected tubes with bacterial growth were streaked on Eosin Methylene Blue agar (EMB agar, HiMedia, Mumbai, India) for selective growth of *E. coli*. The colony showing metallic sheen were selected and sub cultured in order to get pure *E. coli* isolates. The isolates were subjected to biochemical characterization as per standard microbiological procedures (Quinn *et al*. 1994). The biochemically confirmed isolates were further confirmed by PCR targeting 16S rRNA (Table 1) as described by Sabat *et al*. (2000).

Antimicrobial resistance (AMR) profile of E. coli: The AMR profile of all confirmed *E. coli* isolates were generated against antibiotics: ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), ceftriaxone (30 µg), cefpodoxime (10 µg), aztreonam (30 µg), cefotaxime (30 µg), imipenem (10 µg), amikacin (30 µg), tetracycline (30 µg), enrofloxacin (5 µg), nalidixic acid (30 µg), trimethoprim (5 µg) and chloramphenicol (30 µg) using Mueller–Hinton agar (HiMedia, Mumbai, India), by disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI VET08) guideline. Based on zone of inhibition, isolates were classified as resistant or susceptible to a particular antibiotic using standard reference values as per CLSI VET08. *E. coli*, ATCC 25922 was used as a reference strain for the test. The multiple antibiotic resistance (MAR) index was determined using the formula: MAR index = a/b, where 'a' represents the number of isolates resistant to antibiotics and 'b' denotes the total number of antibiotics used. The isolates found resistant to three or more than three classes of antibiotics were defined as Multidrug-resistant (MDR) isolate.

Detection of ESBL production by E. coli: Initially all the confirmed *E. coli* isolates were screened for ESBL production by standard disc diffusion using antibiotic disc cefpodoxime (10 µg), ceftazidime (30 µg), aztreonam (30 µg), cefotaxime (30 µg) and ceftriaxone (30 µg) on Mueller Hinton agar pre-inoculated with 0.5 McFarland inoculum. *E. coli*, ATCC 25922 was used as a reference strain for the test. The isolates exhibiting resistance to any of these five antibiotics were suspected for ESBL production. These suspected isolates were further confirmed as ESBL producer by combined disc method (CLSI VET08) on Mueller-Hinton agar pre-inoculated with 0.5 McFarland

Table 1. Primer for amplification of *16S rRNA* and ESBL genes of *E. coli*

Primer sequence	Target gene	Amplicon size (bp)	Reference
F- 5'GAAGAAGCTTGCTTCTTTGCTGAC3' R- 5'GCCCGGGGATTTCACATCTGACTTA3'	*16S rRNA* *E. coli*	544	Sabat *et al*. 2000
F-5'CATTTCCGTGTCGCCCTTATTC3' R-5'CGTTCATCCATAGTTGCCTGAC3'	*TEM*-1& *TEM*-2	800	
F-5'AGCCGCTTGAGCAAATTAAAC3' R-5'ATCCCGCAGATAAATCACCAC3'	*SHV*-1	713	
F-5'GGCACCAGATTCAACTTTCAAG3' R-5'GACCCCAAGTTTCCTGTAAGTG3'	*OXA*-1, *OXA-4* & *OXA*-30	564	Dallenne *et al*. 2010
F-5'AACRCRCAGACGCTCTAC3' R-5'TCGAGCCGGAASGTGTYAT3'	*CTX-M* 8/25	326	
F-5'CCC CGC TTA TAG AGC AAC AA3' R-5'TCA ATG GTC GAC TTC ACA CC3'	*AmpC*	634	

inoculum of suspected isolates. The combined disc diffusion test was performed with antibiotic disc containing cefotaxime (30 µg) or ceftazidime (30 µg) alone and in combination with clavulanic acid (10 µg) placed diagonally with a distance of 25 mm (center to center) incubated at 35°C for 16-18 hours. An increase of 5 mm or more in the zone of inhibition around the combined disc containing clavulanic acid than the corresponding disc with cefotaxime or ceftazidime alone was considered positive for ESBL production. Furthermore, cefoxitin–cloxacillin double disc synergy (CCDDS) test was performed with all the isolates for phenotypic confirmation of AmpC beta lactamase (ACBL) production by *E. coli* isolates (Tan *et al*. 2009).

Molecular detection of ESBL genes in E. coli: The isolates phenotypically confirmed for ESBL production were investigated for the presence of ESBL gene(s) like blaCTX-M, blaSHV, blaTEM, blaOXA and AmpC by PCR (Table 1) as described by Dallenne *et al*. (2010). The template DNA from ESBL producer isolates were extracted by snap and chill method as described earlier by Kaushik *et al*. (2018). The PCR reaction mixture was prepared in 25 µL reaction volume each containing 2.5 µL 10X PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH-8.3; 15 mM $MgCl_2$), 0.5 µL of dNTP mixture (10 mM), 2 µL (10 pmol/ µL) of forward and reverse primers (Table-1), 0.2 µL (5 U/ µL) Taq DNA polymerase, 3 µL of DNA template and 14.8 µL nuclease free water. The amplification process consisted of an initial denaturation at 94ºC for 10 min, followed by 30 cycles of denaturation at 94ºC for 40 sec, annealing at 60ºC for 40 sec and elongation at 72ºC for 1 min with a final elongation phase at 72ºC for 7 min. The PCR amplicon was analysed in 1.5% agarose gel electrophoresis and visualized under gel documentation system (VILBER).

RESULTS AND DISCUSSION

AMR has become a global challenge for both human and animal health. A survey report on mapping of AMR in animals among developing countries envisages China and India (north eastern) as hotspots of AMR (Van Boeckel *et al*. 2019). WHO also focussed on the ESBL-producing *E. coli* as an indicator pathogen for integrated surveillance programme on AMR (WHO 2021). The current study was undertaken to know the occurrence of ESBL producing *E. coli* in milk from apparently healthy cattle/ buffalo, faeces of goats and chickens in Patna. A total of 148 *E. coli* (25 cattle milk, 31 buffalo milk, 52 goat rectal swab and 40 chicken cloacal swab) were isolated and confirmed by PCR amplification of 16S rRNA gene (Table 2) which were further studied for antimicrobial resistance (AMR) and ESBL production. The resistance profile was found to be 85.13%, 72.97%, 37.83%, 33.10%, 27.02%, 26.35%, 23.64%, 18.24%, 12.83%, 8.78%, 7.4%, and 5.4%, against amoxicillin/ clavulanate, cefpodoxime, ampicillin, ceftazidime, nalidixic acid, cefoxitin, trimethoprim, tetracycline, ceftriaxone, enrofloxacin, aztreonam and chloramphenicol, respectively, however, 97.98% and 100%

Table 2. Sample wise confirmation by PCR and resistance pattern of *E. coli* based on disc diffusion

Animal Species	Sample type	No. of samples	*Confirmation of E. coli* by PCR targeting *16S rRNA*	Percent resistance (number of isolate)											
				AMP	AMC	CTR	CPD	CAZ	AT	CTX	CX	IPM	AK	TE	EX	NA	TR	C
Cow	Milk	72	25 (34.72%)	20 (5)	80 (20)	8 (2)	76 (19)	40 (10)	8 (2)	20 (5)	36 (9)	0(0)	0(0)	8 (2)	4(1)	20 (5)	8 (2)	0(0)
Buffalo	Milk	64	31 (48.43%)	29 (9)	90.3 (28)	6.4(2)	77.4 (24)	32.2 (10)	0 (0)	16.1 (5)	38.7 (12)	0(0)	0(0)	3.2(1)	0(0)	16(5)	9.6 (3)	0(0)
Goat	Rectal swab	67	52 (77.61%)	25(13)	76.9 (40)	13.4 (7)	61.5 (32)	32.6 (17)	0 (0)	19 (10)	17.3 (9)	0(0)	0(0)	7.6 (4)	1.9 (1)	17.3 (9)	17.3 (9)	1.9 (1)
Poultry	Cloacal swab	51	40 (78.43%)	72.5(29)	95 (38)	20 (8)	82.5 (33)	30 (12)	7.5 (3)	32.5 (13)	22.5 (9)	0(0)	7.5 (3)	50 (20)	25(10)	52.5(21)	52.5(21)	17.5 (7)
	Total	254	148 (58.26%)	37.83 (56)	85.13 (126)	12.83 (19)	72.97 (108)	33.10 (49)	7.4 (11)	22.29 (33)	26.35 (39)	0 (0)	2.02 (3)	18.24 (27)	8.78 (13)	27.02 (40)	23.64 (35)	5.4 (8)

AMP: Ampicillin, AME: Amoxicillin-clavulanic acid, CTR: Ceftriaxone, CPD: Cefpodoxime, CAZ: Ceftazidime, AT: Aztreonam, CTX: Cefotaxime, CX: Cefoxitin, IPM: Imipenem, AK: Amikacin, TE: Tetracycline, EX: Enrofloxacin, NA: Nalidixic acid, TR: Trimethoprim, C: Chloramphenicol.

isolates were found sensitive to amikacin and imipenem, respectively (Table 2). Besides amoxicillin/ clavulanic acid, cefpodoxime, ceftazidime and cefoxitin, in the present study it was observed that *E. coli* isolates are also resistant to ampicillin (37.83%); ceftazidime (33.10%); nalidixic acid (27.02%); trimethoprim (23.64%); tetracycline (18.24 %); ceftriaxone (12.83%); enrofloxacin (8.78%); aztreonam (7.4%); chloramphenicol 5.4% which is not surprising seeing other studies (Kar *et al*. 2015, Brower *et al*. 2017, Jindal *et al*. 2021, Lalhruaipuii *et al*. 2021, Jain *et al*. 2021, Athanasakopoulou *et al*. 2021, Banerjee *et al*. 2022). The current finding reveals that 97.98% isolates are susceptible to amikacin and 100% were sensitive to imipenem which is in line with the previous findings in *E. coli*. (Jana and Mondal 2013, Suay-García *et al*. 2019, Jain *et al*. 2021). The isolation rate of *E. coli* in the study was higher in the faecal samples of goat and chickens (Table 2) compared to milk which may be because the organism naturally inhabits in the GI tract of animals. The resistance of *E. coli* to amoxicillin/ clavulanic acid was shown by 85.13% of isolates which was highest among all antibiotics tested. The high resistance against amoxicillin/ clavulanic acid observed among *E coli* isolates from animal sources may be correlated with the indiscriminate use of these drug in livestock for therapeutic purposes or as growth promotor in poultry feed (Kar *et al*. 2015, Haulisah *et al*. 2021). This can also be correlated with exposure of animals to the environmental resistance gene pool. It was also observed in the study that 72.97% and 33.10% of isolates were resistant to cefpodoxime and ceftazidime (3rd generation cephalosporin), respectively, whereas 26.35% were resistant to cefoxitin (2nd generation cephalosporin). Resistance shown by *E. coli* isolates from apparently healthy animals, in the study, against molecules such as ceftazidime raises major concern over the use of these antibiotics as these are considered strategic molecules for bacterial infections used under hospital setting (Suay-García *et al*. 2019). These variations in AMR pattern among the isolates could be attributed to the prevailing usage and drug abuse in the study area, which warrants stringent and continuous surveillance on antibiotic resistance pattern in the area. This finding also supports the fact that the drug like carbapenem are not being used in the treatment of livestock in India (Murugan *et al*. 2019), however resistance to amikacin was seen among few isolates (2.02%) from chickens only though, this is encouraging, seeing as this is widely used in clinical practice, in both veterinary and human medicine.

With regard to MDR among *E. coli* in the present study, 54.05% of isolates showed MAR index ≥0.25 and 8.1% of isolates showed MAR index ≥0.5 which indicates a very high prevalence of MDR among non-clinical isolates of *E. coli* in the region. Such MDR bacterial isolates are considered as reservoirs for resistance and virulence genes which can be transferred to other strains of the same or the other species, thereby increasing the source of antibiotic resistance (Martínez-Vázquez *et al*. 2022). In accordance to the findings of present study, earlier reports are also available, on high prevalence of MDR *E. coli* from livestock and poultry in India (Lalhruaipuii *et al*. 2021, Jaiswal *et al*. 2024).

The predominance of antimicrobial resistance genes significantly drives the emergence of MDR bacteria. While data on the frequency of antibiotic resistance in *E. coli* from clinical animal sources is available, very limited information is available from Bihar, India, regarding prevalence of *E. coli,* from nonclinical sources, harbouring antibiotic resistance genes (WHO 2021). The current findings indicate that 20.27% (30 out of 148) of *E. coli* isolates exhibit ESBL production. Among these, 56% (17 isolates) were identified from chicken cloacal swabs, 26.6% (8 isolates) from goat faeces, and 16.6% (5 isolates) from buffalo milk (Table 3). There are several reports regarding ESBL producer *E. coli* from livestock sector is available in India which corroborate with the present findings (Samanta *et al*. 2014, Kar *et al*. 2015, Brower *et al*. 2017, Murugan *et al*. 2019, Tewari *et al*. 2019), however, we could not find any report from Bihar, India. Brower *et al*. (2017) in his comparative study on occurrence of MDR and ESBL producing *E. coli,* among layer and broiler birds, reported 94% and 87% as MDR and ESBL producing *E. coli,* respectively in broiler chicken in Punjab, India. In contrast, the occurrence of ESBL producing *E. coli* has been reported to be more frequent from human clinical samples (Dallenne *et al*. 2010, Mukherjee *et al*. 2013, Harwalkar *et al*. 2013, Bhoomika *et al*. 2016, Rohit *et al*. 2019). Tewari *et al*. (2022) in a recent study reported the occurrence of ESBL genes among 50% (101 out of 203) of *E. coli* isolated from human clinical samples.

The blaTEM and blaSHV type ESBL enzymes in *E. coli* isolates are frequently reported in India (Bhoomika *et al*. 2016, Tewari *et al*. 2019, Agrawal *et al*. 2021, Das *et al*. 2017), in which point mutations are suggested to give rise to ESBLs (Ramadan *et al*. 2019). The present study revealed blaTEM (blaTEM1/2) as predominant ESBL gene in 13 (43.33%) isolates followed by blaSHV-1 in 4 (13.33%) isolates (Table 3). It is interesting to note that all the *E. coli* isolates harbouring blaTEM and/or blaSHV in the current study was resistant to antibiotic amoxicillin/ clavulanic acids, a β-lactam-β-lactamase inhibitor combination, suggesting the presence of inhibitor-resistant TEMs and SHV variants of *E. coli* in animals which might have evolved due to antibiotic selective pressure due to indiscriminate use of antibiotics (Prinarakis *et al*. 1997, Jacquier *et al*. 2013, Robin *et al*. 2011).

Several studies have suggested that animals could potentially transmit ESBL-producing bacteria and/or bacteria with ESBL-encoding genes to humans, either through direct contact or via food chain (Ewers *et al*. 2012). The widespread use of antibiotics in the food and farming industries is considered one of the most significant risk factors contributing to the high prevalence of ESBL-producing *E. coli* among healthy animals (Bailar and Travers 2002, Larson 2007). The presence of such *E. coli* variants in livestock poses a serious threat to public health

Table 3. Antibiotic Resistance (Signatures), Beta-Lactamase Production and multiple antibiotic resistant (MAR) index among thirty beta lactamase producing *E. coli* Strains isolated from different livestock sources

Isolate	Animal spp	Signature profile (No. of Antibiotics)	β-Lactamase	MAR index
B15BI	Buffalo	AMC, CPD, CX, NA	blaSHV-1, AmpC	0.25
B6PS	Buffalo	AMP, AMC, CPD, CAZ, CTX, NA, CL	blaSHV-1, AmpC	0.44
B9PS	Buffalo	AMP, AMC, CTR, CPD, CAZ, CTX, NA,TR	blaTEM-1/2, AmpC	0.5
B5PH	Buffalo	AMC, CPD, CAZ, CL	AmpC	0.25
B14PH	Buffalo	AMP, AMC, CPD, CAZ, CX, CL	AmpC	0.37
G9BI	Goat	AMC, CPD, TE, EX, NA, TR	AmpC	0.37
G4D	Goat	AMP, AMC, CPD, CAZ, NA	blaTEM-1/2, AmpC	0.31
G10D	Goat	AMC, CPD, TE, NA, C	AmpC	0.31
G12D	Goat	AMP, AMC, CTR, CPD, CAZ, CTX, NA	AmpC	0.43
G13PS	Goat	AMP, AMC, CPD, TE, NA, TR	blaTEM-1/2	0.37
G1PH	Goat	AMP, AMC, CTR, CPD, CAZ, CTX, TR	AmpC	0.43
G6PH	Goat	AMP, AMC, CTR, CPD, CAZ, CTX, CX, TR	AmpC	0.5
G12PH	Goat	AMP, AMC, CTR, CPD, CTX, TR	AmpC	0.37
P2BI	Chicken	AMP, AMC, CPD, AK, TE, EX, NA	blaTEM-1/2, AmpC	0.43
P3BI	Chicken	AMP, AMC, CTR, CPD, CAZ, AT, CTX, TE, TR, C	blaSHV-1, blaTEM-1/2, AmpC	0.62
P8BI	Chicken	AMC, CPD, CTX, C	AmpC	0.25
P9BI	Chicken	AMP, AMC, CTR, CPD, CTX, TE	AmpC	0.37
P12BI	Chicken	AMP, AMC, CPD, TE, TR	blaSHV-1, AmpC	0.31
P1D	Chicken	AMP, AMC, CTR, CPD, CAZ, AT, CTX, CX, TE, NA, TR, C	blaTEM-1/2, AmpC	0.75
P5D	Chicken	AMP, AMC, CTR, CPD, AT, CTX, CX, TE, NA, TR, C, CL	AmpC	0.75
P4PS	Chicken	AMP, AMC, CTR, CPD, CAZ, CTX, TE, EX, NA, TR, C	blaTEM-1/2, AmpC	0.68
P6PS	Chicken	AMP, AMC, CPD, TE, NA	blaTEM-1/2, AmpC	0.31
P7PS	Chicken	AMP, AMC, CAZ, TE, NA	blaTEM-1/2, AmpC	0.31
P8PS	Chicken	AMP, AMC, CTR, CPD, CAZ, CTX, TE, EX,NA, TR,C	AmpC	0.68
P9PS	Chicken	AMP, AMC, CPD, CX, TR	AmpC	0.31
P13PS	Chicken	AMP, AMC, CPD, TE, NA, TR	blaTEM-1/2, AmpC	0.37
P3PH	Chicken	AMP, AMC, CTR, CPD, CAZ, CTX, AK, EX, NA	blaTEM-1/2, AmpC	0.56
P6PH	Chicken	AMP, AMC, CPD, CX, TE, NA	blaTEM-1/2, AmpC	0.37
P9PH	Chicken	AMP, AMC, CPD, TE, NA	blaTEM-1/2, AmpC	0.31
P11PH	Chicken	AMP, AMC, CTR, CPD, CAZ, CTX, AK, EX, NA, CL	AmpC	0.62

AMP: Ampicillin, AME: Amoxicillin-clavulanic acid, CTR: Ceftriaxone, CPD: Cefpodoxime, CAZ: Ceftazidime, AT: Aztreonam, CTX: Cefotaxime, CX: Cefoxitin, IPM: Imipenem, AK: Amikacin, TE: Tetracycline, EX: Enrofloxacin, NA: Nalidixic acid, TR: Trimethoprim, C: Chloramphenicol.

due to their potential for transmission to humans through environmental contamination or food chain (Ewers *et al*. 2012). The co-occurrence of AmpC with blaTEM and blaSHV in *E. coli* isolates, resistant to the amoxicillin/ clavulanic acid and other cephalosporins, from healthy livestock species indicates multi-drug-resistant bacteria circulating in animal that pose risks to environmental transmission.

REFERENCES

Agrawal S, Singh A P, Singh R, Saikia R, Choudhury S, Shukla A, Prabhu S N and Agrawal J. 2021. Molecular characterization of extended-spectrum β-lactamase-producing *Escherichia coli* isolated from postpartum uterine infection in dairy cattle in India. *Veterinary World* **14**: 200-09.

Athanasakopoulou Z, Reinicke M, Diezel C, Sofia M, Chatzopoulos D C, Braun S D, Reissig A, Spyrou V, Monecke S, Ehricht R and Tsilipounidaki K. 2021. Antimicrobial resistance genes in ESBL- producing *Escherichia coli* isolates from animals in Greece. *Antibiotics* **10**(4): 389.

Bailar III J C and Travers K. 2002. Review of assessments of the human health risk associated with the use of antimicrobial agents in agriculture. *Clinical Infectious Diseases* **34**(Suppl3): S135-S143.

Banerjee J, Bhattacharyya D, Habib M, Chaudhary S, Biswas S, Maji C, Nanda P K, Das A K, Dandapat P, Samanta I and Lorenzo J M. 2022. Antimicrobial resistance pattern, clustering mechanisms and correlation matrix of drug-resistant *Escherichia coli* in black Bengal Goats in West Bengal, India. *Antibiotics* **11**(10): 1344.

Bennett P M. 2008. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology* **153**: S347-S357.

Bhoomika, Shakya S, Patyal A and Gade N E. 2016. Occurrence and characteristics of extended-spectrum β-lactamases producing *Escherichia coli* in foods of animal origin and human clinical samples in Chhattisgarh, India. *Veterinary World* **9**(9): 996-1000.

Blair J M, Webber M A, Baylay A J, Ogbolu D O and Piddock L J. 2015. Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology* **13**(1): 42-51.

Brower C H, Mandal S, Hayer S, Sran M, Zehra A, Patel S J, Kaur R, Chatterjee L, Mishra S, Das B R and Singh P. 2017. The prevalence of Extended-Spectrum Beta-Lactamase-producing Multidrug-Resistant *Escherichia coli* in poultry chickens and variation according to farming practices in Punjab, India. *Environmental Health Perspectives* **125**(7): 077015.

Bush K and Bradford P A. 2020. Epidemiology of β-Lactamase-Producing Pathogens. *Clinical Microbiology Reviews* **33**(2): e00047-19.

Carattoli A. 2008. Animal reservoirs for extended spectrum beta-lactamase producers. *Clinical Microbiology and Infection* Suppl **1**: 117-23. doi: 10.1111/j.1469-0691.2007.01851.x.

CDDEP. 2020. The Center for Disease Dynamics, Economics & Policy. Resistance Map: Antibiotic resistance.

Dallenne C, Da Costa A, Decré D, Favier C and Arlet G. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. *Journal of Antimicrobial Chemotherapy* **65**(3): 490-95.

Das A, Guha C, Biswas U, Jana P S, Chatterjee A and Samanta I. 2017. Detection of emerging antibiotic resistance in bacteria isolated from subclinical mastitis in cattle in West Bengal. *Veterinary World* **10**(5): 517-20.

Ewers C, Bethe A, Semmler T, Guenther S and Wieler L H. 2012. Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. *Clinical Microbiology and Infection* **18**(7): 646-55.

Harwalkar A, Sataraddi J, Gupta S, Yoganand R, Rao A and Srinivasa H. 2013. The detection of ESBL-producing *Escherichia coli* in patients with symptomatic urinary tract infections using different diffusion methods in a rural setting. *Journal of Infection and Public Health* **6**(2): 108-14.

Haulisah N A, Hassan L, Bejo S K, Jajere S M and Ahmad N I. 2021. High levels of antibiotic resistance in isolates from diseased livestock. *Frontiers In Veterinary Science* **8**: 652351.

Jacquier H, Birgy A, Le Nagard H, Mechulam Y, Schmitt E, Glodt J, Bercot B, Petit E, Poulain J, Barnaud G and Gros P A. 2013. Capturing the mutational landscape of the beta-lactamase TEM-1. *The Proceedings of the National Academy of Sciences USA* **110**(32): 13067-72.

Jain P, Bepari A K, Sen P K, Rafe T, Imtiaz R, Hossain M and Reza H M. 2021. High prevalence of multiple antibiotic resistance in clinical *E. coli* isolates from Bangladesh and prediction of molecular resistance determinants using WGS of an XDR isolate. *Scientific Reports* **11**(1): 22859.

Jaiswal A, Khan A, Yogi A, Singh S, Pal A K, Soni R, Tripathi P, Lal J A and Tripathi V. 2024. Isolation and molecular characterization of multidrugresistant *Escherichia coli* from chicken meat. *3 Biotech* **14**(4): 107.

Jana A and Mondal A. 2013. Serotyping, pathogenicity and antibiogram of *Escherichia coli* isolated from raw poultry meat in West Bengal, India. *Veterinaria Italiana* **49**(4): 361-65.

Jindal P, Bedi J, Singh R, Aulakh R and Gill J. 2021. Phenotypic and genotypic antimicrobial resistance patterns of *Escherichia coli* and *Klebsiella* isolated from dairy farm milk, farm slurry and water in Punjab, India. *Environmental Science and Pollution Research* **28**(22): 28556-70.

Kar D, Bandyopadhyay S, Bhattacharyya D, Samanta I, Mahanti A, Nanda P K, Mondal B, Dandapat P, Das A K, Dutta T K and Bandyopadhyay S. 2015. Molecular and phylogenetic characterization of multidrug resistant extended spectrum beta-lactamase producing *Escherichia coli* isolated from poultry and cattle in Odisha, India. *Infection, Genetics and Evolution* **29**: 82-90.

Kaushik P, Anjay A, Kumari S, Dayal S and Kumar S. 2018. Antimicrobial resistance and molecular characterisation of *E. coli* from poultry in Eastern India. *Veterinaria Italiana* **54**(3): 197-204.

Lalhruaipuii K, Dutta T K, Roychoudhury P, Chakraborty S, Subudhi P K, Samanta I, Bandyopadhayay S and Singh S B. 2021. Multidrug-resistant extended-spectrum β-lactamase-producing *Escherichia coli* pathotypes in north eastern region of India: Backyard small ruminants-human-water interface. *Microbial Drug Resistance* **27**(12): 1664-71.

Larson E. 2007. Community factors in the development of antibiotic resistance. *Annual Review of Public Health* **28**: 435-47.

Martínez-Vázquez A V, Mandujano A, Cruz-Gonzalez E, Guerrero A, Vazquez J, Cruz-Pulido W L, Rivera G and Bocanegra-García V. 2022. Evaluation of retail meat as a source of ESBL *Escherichia coli* in Tamaulipas, Mexico. *Antibiotics* **11**(12): 1795.

Mukherjee M, Basu S, Mukherjee S K and Majumder M. 2013. Multidrug-resistance and Extended Spectrum Beta-Lactamase production in Uropathogenic *E. coli* which were isolated from hospitalized patients in Kolkata. *Journal of Clinical and Diagnostic Research* **7**(3): 449-53.

Murugan M S, Sinha D K, Kumar O V, Yadav A K, Pruthvishree B S, Vadhana P, Nirupama K R, Bhardwaj M and Singh B R. 2019. Epidemiology of carbapenem-resistant *Escherichia coli* and first report of blaVIM carbapenemases gene in calves from India. *Epidemiology and Infection* **147**: e159.

Nobrega D B, Tang K L, Caffrey N P, De Buck J, Cork S C, Ronksley P E and Barkema H W. 2021. Prevalence of antimicrobial resistance genes and its association with restricted antimicrobial use in food producing animals: a systematic review and meta-analysis. *Journal of Antimicrobial Chemotherapy* **76**(3): 561-75.

O'Neill J. 2016. Tackling drug-resistant infections globally: final report and recommendations. 1-84. 160518_Final paper_with cover.pdf (amr-review.org)

Odenthal S, Akineden Ö and Usleber E. 2016. Extended-spectrum β-lactamase producing Enterobacteriaceae in bulk tank milk from German dairy farms. *International Journal of Food Microbiology* **238**: 72-78.

Prinarakis E E, Miriagou V, Tzelepi E, Gazouli M and Tzouvelekis L S. 1997. Emergence of an inhibitor-resistant beta-lactamase (SHV-10) derived from an SHV-5 variant. *Antimicrobial Agents and Chemotherapy* **41**(4): 838-40.

Quinn P J, Carter M, Markey B and Carter G R. 1994. *Clinical Veterinary Microbiology*. Mosby Wolfe, Wolfe, UK.

Rahman M M, Husna A, Elshabrawy H A, Alam J, Runa N Y, Badruzzaman A T, Banu N A, Al Mamun M, Paul B, Das S and Rahman M M. 2020. Isolation and molecular characterization of multidrug-resistant *Escherichia coli* from chicken meat. *Scientific Reports* **10**(1): 21999.

Ramadan A A, Abdelaziz N A, Amin M A and Aziz R K. 2019. Novel blaCTX-M variants and genotype-phenotype correlations among clinical isolates of extended spectrum beta lactamase-producing *Escherichia coli*. *Scientific Reports* **9**(1): 4224.

Robin F, Krebs M, Delmas J, Gibold L, Mirande C and Bonnet R.

2011. In vitro efficiency of the piperacillin/tazobactam combination against inhibitor-resistant TEM- and complex mutant TEM-producing clinical strains of *Escherichia coli*. *Journal of Antimicrobial Chemotherapy* **66**(5): 1052-56.

Rohit A, Deekshit V K, Balaraj M, Alandur V S, Abraham G, Karunasagar I and Karunasagar I. 2019. CTX-M type extended-spectrum β-lactamase in *Escherichia coli* isolated from extra-intestinal infections in a tertiary care hospital in South India. *Indian Journal of Medical Research* **149**(2): 281-84.

Sabat G, Rose P, Hickey W J and Harkin J M. 2000. Selective and sensitive method for PCR amplification of *Escherichia coli* 16S rRNA genes in soil. *Applied and Environmental Microbiology* **66**(2): 844-49.

Samanta I, Joardar S N, Das P K, Das P, Sar T K, Dutta T K, Bandyopadhyay S, Batabyal S and Isore D P. 2014. Virulence repertoire, characterization, and antibiotic resistance pattern analysis of *Escherichia coli* isolated from backyard layers and their environment in India. *Avian Diseases* **58**(1): 39-45.

Suay-García B and Pérez-Gracia M T. 2019. Present and future of carbapenem-resistant *Enterobacteriaceae* infections. *Antibiotics* **8**(122): 1-16.

Suay-García B, Galán F, Rodríguez-Iglesias M A and Pérez-Gracia M T. 2019. Detection and characterization of Extended-Spectrum Beta-Lactamases-producing *Escherichia coli* in animals. *Vector-Borne and Zoonotic Diseases* **19**(2): 115-20.

Tan T Y, Ng L S, He J, Koh T H and Hsu L Y. 2009. Evaluation of screening methods to detect plasmid-mediated AmpC in *Escherichia coli, Klebsiella pneumoniae*, and *Proteus mirabilis*. *Antimicrobial Agents and Chemotherapy* **53**(1): 146-49.

Tewari R, Ganaie F, Venugopal N, Mitra S, Shome R and Shome B R. 2022. Occurrence and characterization of genetic determinants of β-lactam-resistance in *Escherichia coli* clinical isolates. *Infection, Genetics and Evolution* **100**: 105257.

Tewari R, Mitra S, Ganaie F, Das S, Chakraborty A, Venugopal N, Shome R, Rahman H and Shome B R. 2019. Dissemination and characterisation of *Escherichia coli* producing extended-spectrum β-lactamases, AmpC β-lactamases and metallo-β-lactamases from livestock and poultry in Northeast India: A molecular surveillance approach. *Journal of Global Antimicrobial Resistance* **17**: 209-15.

Ur Rahman S, Ali T, Ali I, Khan N A, Han B and Gao J. 2018. The growing genetic and functional diversity of Extended Spectrum Beta-Lactamases. *BioMed Research International* 9519718.

Van Boeckel T P, Pires J, Silvester R, Zhao C, Song J, Criscuolo N G, Gilbert M, Bonhoeffer S and Laxminarayan R. 2019. Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science* **365**(6459): eaaw1944.

WHO. 2021. WHO integrated global surveillance on ESBL-producing *E. coli* using a "One Health" approach: Implementation and opportunities. *ISBN* 978-92-4-002140-2 (electronic version).