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Short Communication

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

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ABSTRACT

Objectives: The aim of this study was to identify and characterise probable extended-spectrum β-lactamase (ESBL)-, AmpC lactamase- and/or metallo-β-lactamase (MBL)-producing *Escherichia coli* variants circulating in the livestock and poultry environment to establish their epidemiological significance, genetic diversity, antimicrobial resistance (AMR) trends and virulence.
Methods: The culture method and *E. coli*-specific multiplex PCR identified 78 *E. coli* strains from faecal samples of healthy livestock and poultry. The antibiogram was determined by the disk diffusion and minimum inhibitory concentration (MIC) methods. Antimicrobial-resistant *E. coli* isolates were screened for the presence of ESBL, AmpC and MBL genes. Isolates were further characterised by plasmid replicon typing, integron assay and virulence gene analysis. Genetic diversity was assessed by random amplification of polymorphic DNA (RAPD) analysis and multilocus sequence typing (MLST).
Results: ESBL (CTX-M group 1, CTX-M group 4, TEM), AmpC (EBC, FOX, CMY, DHA) and MBL (IMP, SIM) resistance determinants were identified in 75%, 19% and 6% of isolates, respectively. Nine plasmid replicon types were distributed among resistant *E. coli* strains, with the most common plasmid replicon types being L/M and Y. Integrons were detected in 19% of *E. coli* isolates. RAPD analysis categorised the *E. coli* isolates into three clusters. MLST revealed seven different sequence types (STs), with ST10 being the most common.
Conclusions: This study demonstrated a high prevalence of animals carrying potential ESBL- and AmpC-producing *E. coli* and emphasises the need for rigorous surveillance in the animal sector to identify critical control points conducive to prevent the rapid dissemination of AMR.

1. Introduction

Antibiotics are used in veterinary and human medicine for the treatment, control and prevention of infectious diseases. However, their repeated off-label overuse can have unpredicted adverse effects, including the development of bacterial antimicrobial resistance (AMR) to modern β-lactam antibiotics [1].

The occurrence of extended-spectrum β-lactamase (ESBL)-, AmpC β-lactamase- and metallo-β-lactamase (MBL)-producing Enterobacteriaceae, in particular *Escherichia coli*, in animals may constitute a public-health issue [2]. Initially, ESBL/AmpC/MBL-producing *E. coli* were only observed in human medical practice, but the recent observation of these bacteria, first in companion animals and increasingly in livestock, has prompted monitoring studies concentrating on livestock [3]. An increase in the number of

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ESBL/AmpC/MBL-producing *E. coli* isolates is now being observed in food-producing animals, favouring the postulation that animals might become the natural persistent source of infection or even reservoirs [4].

Multifactorial and complex interrelationships contribute to the spread of these bacteria among animals and humans. ESBL-, AmpC β-lactamase- and MBL-encoding genes are often located on mobile genetic elements such as integrons, insertion sequences, transposons and plasmids, favouring their dissemination [4,5]. Although limited information is available on the direct transfer of ESBL/AmpC/MBL-producing bacteria between animals and humans through close contact, the commensal ESBL/AmpC/MBL reservoir in non-human sources is strikingly increasing [2,5]. This may represent an indirect risk to public health by increasing the gene pool from which pathogenic bacteria can acquire ESBL/AmpC/MBL genes [3,6].

The emergence and dissemination of prospective ESBL/AmpC/MBL-producing *E. coli* should be assessed through molecular surveillance studies since local, regional, national and international information related to this phenomenon is largely incomplete in various geographical locations, including India [5,7,8]. The present study investigated β-lactam-resistant *E. coli* variants circulating in the livestock and poultry environment in India to understand their prevalence, genetic diversity, AMR trends and virulence using a molecular surveillance approach.

2. Materials and methods

2.1. Study area

This observational, molecular surveillance study was carried out in two states (Meghalaya and Assam) in Northeast India. In Meghalaya, samples were collected from two districts comprising three cattle farms (Farms 1, 2 and 3), three pig farms (Farms 4, 5 and 6) and two poultry farms (Farms 8 and 9). In Assam, samples were collected from one goat farm (Farm 7). Farms 1–5, 7 and 8 were organised maintained in an intensive system, whereas Farms 6 and 9 were unorganised maintained in a semi-intensive or extensive system.

2.2. Sample collection and laboratory processing

A total of 78 samples were collected by stratified random sampling strategy with 20% of samples representing each farm/stratum (Table 1). Rectal specimens were collected from livestock (cattle, pigs and goats) and cloacal specimens from poultry using sterile, polyester-tipped swabs with a plastic shaft. Faecal-stained swabs were placed in a sterile tube filled with 500 μL of viral transport medium (HiMedia, Mumbai, India) and were stored frozen at −80 °C in a local laboratory. Specimens were transferred within 72 h to ICAR–National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI, Bengaluru, India) for laboratory processing. Faecal specimens were inoculated on MacConkey agar plates (HiMedia) and were incubated at 37 °C for 18–24 h. Identification of *E. coli* was done based on colony morphology, lactose fermentation, Gram staining, IMViC tests (indole +ve; methyl red +ve; Voges–Proskauer, −ve; citrate utilisation: −ve) and oxidase test.

2.3. DNA extraction and E. coli-specific multiplex PCR

DNA was extracted from overnight-grown pure cultures of Gram-negative bacteria using a QIAmp® DNA Mini Kit (QIAGEN, Duesseldorf, Germany) according to the manufacturer's instructions. The concentration and integrity of the extracted genomic DNA was determined spectrophotometrically using Nanodrop 2000c (Thermo Fisher Scientific Inc., Wilmington, DE) and 1% agarose gel electrophoresis, respectively.

Extracted DNA from the bacterial isolates was subjected to *E. coli*-specific multiplex PCR as described previously [9]. Confirmed *E. coli* isolates and their corresponding DNA samples were stored at −80 °C for further characterisation.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the *E. coli* isolates was performed by the Kirby–Bauer disk diffusion method for third-generation cephalosporins (cefotaxime, ceftiofur and ceftazidime) and other β-lactams (ampicillin, cefoxitin, imipenem and meropenem) in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. Isolates resistant to at least three β-lactam antibiotics were tested for minimum inhibitory concentration (MIC) by microbroth dilution using a VITEK®2 system (bioMérieux, Marcy-l'Étoile, France) for 18 antimicrobial agents (Table 1).

2.5. Screening of antimicrobial resistance genes

β-Lactam-resistant *E. coli* isolates were screened for 17 different antimicrobial resistance genes (Table 2) as follows: (i) six uniplex PCR assays targeting ESBL genes (namely bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}Mgroups1,2,3and4}$) [2,11]; (ii) multiplex PCR-1 targeting bla_{AmpC} family genes (namely $bla_{FOX(FOX\text{-}1to\text{-}5b)}$, $bla_{MOX(MOX\text{-}1and\text{-}2,CMY\text{-}1,\text{-}8to\text{-}11)}$, bla_{ACC}, $bla_{DHA(DHA\text{-}1and\text{-}2)}$, $bla_{EBC(MIR\text{-}1,ACT\text{-}1)}$ and $bla_{CIT(LAT\text{-}1to\text{-}4,CMY\text{-}2to\text{-}7,BIL\text{-}1)}$) [12]; and (iii) multiplex PCR-2 targeting MBL genes (namely bla_{IMP}, bla_{VIM}, bla_{GIM}, bla_{SIM} and bla_{SPM}) [13].

2.6. PCR-based replicon typing (PBRT)

Plasmid incompatibility (Inc) groups were determined by PBRT using genomic DNA of β-lactam-resistant *E. coli* strains as template. Amplification by PCR was performed with 18 pairs of primers recognising FIA, FIB, FIC, HI1, HI2, I1-Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FIIA in three multiplex PCR assays according to Johnson et al. [14].

2.7. Detection of integrons

The presence of class 1, 2 and 3 integrons in all β-lactam-resistant *E. coli* strains were tested by multiplex PCR using primers specific for the *intI1*, *intI2* and *intI3* integrase genes [15].

2.8. Virulence gene analysis

A group of nine virulence genes reported to be associated with different *E. coli* pathotypes was selected for analysis, namely *stx1A*, *stx2*, *eae*, *cnf1*, *cnf2*, *sfa*, *papG*, *hlyA* and *traT*. PCR analysis of these virulence genes was performed as described by Modak et al. [9] at an annealing temperature of 60 °C.

2.9. Random amplification of polymorphic DNA (RAPD) analysis

RAPD profiles of the probable ESBL/AmpC/MBL-producing *E. coli* strains were generated using a single decamer primer (5′-GTTTCGCTCC-3′) as previously described [16]. an RAPD dendrogram was constructed according to the unweighted pair-group method with arithmetic mean (UPGMA) based on the Pearson correlation coefficient using BioNumerics v.7.6 (Applied Maths, Kortrijk, Belgium).

2.10. Multilocus sequence typing (MLST)

MLST sequence types (STs) were assigned to a subset of 10 representative *E. coli* strains from the RAPD clusters. PCR amplification and sequencing of seven housekeeping genes (*adk*,

Table 1
Antimicrobial resistance (AMR) profile and distribution of AMR genes, plasmid replicons, integrons and virulence genes in β-lactam-resistant *Escherichia coli* isolates.

Host/ stratum	Farm ID	No. of samples collected/no. of animals in farm (20% per farm[a])	Farm location	Total *E. coli*/ total GNB	Sample ID of β-lactam resistant *E. coli*	AMR profile (MIC method)	β-Lactamase genes			Plasmid replicons ($n=25$)	Integrons ($n=6$)	Virulence genes ($n=26$)	MLST
							ESBLs ($n=24$)	MBLs ($n=2$)	AmpC ($n=6$)				
Cattle	Farm 1	8/40	Meghalaya	6/12	NE-C-2	CIP, CRO, CXM, IPM, NAL, SXT, TZP	CTX-M group 4	–	–	Y	–	*traT*	ST1727
					NE-C-4	AMC, AMP, CRO, CXM	CTX-M group 1	–	–	–	–	–	ND
					NE-C-5	CXM, NIT, SXT	CTX-M group 4	–	–	Y	–	*fliC*, *traT*	ND
					NE-C-8	CRO, CXM, FEP, GEN, IPM, MEM, NAL, NIT	CTX-M group 4	–	–	L/M	–	*stx2*, *fliC*	ST10
	Farm 2	12/20	Meghalaya	13/15	NE-C-28	AMP, CIP, CRO, CXM	CTX-M group 4	–	–	L/M	–	*traT*	ND
					NE-C-29	AMC, AMP, CRO, CXM	CTX-M group 4	–	–	–	–	–	ND
					NE-C-33	CRO, CXM, NIT, SXT	–	–	EBC	L/M	–	*cnf1*, *traT*	ND
					NE-C-34	CIP, CRO, CXM, NAL, NIT	CTX-M group 4	–	–	L/M	–	*stx2*, *traT*	ND
					NE-C-35	AMC, AMK, CRO, CXM, GEN, IPM	CTX-M group 4	–	–	–	–	*stx2*, *traT*	ST58
					NE-C-37	CXM, SXT, TZP	CTX-M group 1	–	–	HI1	–	*stx2*, *traT*	ND
					NE-C-38	AMP, CXM, NAL	CTX-M group 1	–	–	–	–	*stx2*, *traT*	ND
					NE-C-39	CIP, CRO, CXM, ETP, FEP, GEN, NAL	CTX-M group 1, TEM	–	–	L/M	Int-2	*stx2*, *traT*	ST10
	Farm 3	8/40	Meghalaya	7/11	NE-C-53	AMP, CRO, CXM, IPM, NAL, SXT	CTX-M group 1, TEM	–	–	N	Int-2	–	ST10
Pigs	Farm 4	4/60	Meghalaya	5/6	NE-PG-20	AMP, CRO, CXM, IPM, MEM, NAL, NIT	CTX-M group 4	–	–	Y, L/M	–	–	ST1079
					NE-PG-24	CXM, NAL, SXT	CTX-M group 4	–	–	L/M	–	*traT*	ND
	Farm 5	12/60	Meghalaya	14/18	NE-PG-59	CIP, CRO, CXM, ETP	–	SIM	–	A/C	–	*fliC*, *traT*	ST206
					NE-PG-60	CIP, CXM, NAL	CTX-M group 1	–	–	–	–	*traT*	ND
					NE-PG-69	AMC, AMP, CXM	CTX-M group 1	–	–	Y	–	*traT*	ND
					NE-PG-70	CRO, CXM, GEN, NAL	CTX-M group 4	–	–	Y	–	*traT*	ND
					NE-PG-71	AMP, CRO, CXM	CTX-M group 1	–	–	L/M	–	*traT*	ND
					NE-PG-72	AMP, CXM, NIT	CTX-M group 4	–	–	M	–	*traT*	ST617
					NE-PG-74	AMC, CRO, CXM, ETP, FEP, IPM, NAL, SXT	CTX-M group 1	–	CMY	A/C, L/M	Int-2, Int-3		
	Farm 6	7/35	Meghalaya	8/11	NE-PG-80	AMP, CRO, CXM, ETP, IPM, MEM, SXT, TZP	–	IMP	–	FIC, A/C, HI1, HI2	–	*traT*	ST10
Goat	Farm 7	7/35	Assam	5/9	NE-G-89	AMP, CIP, CRO, CXM, ETP, FEP, NAL, NIT	–	–	DHA, FOX	I1, HI2	–	*traT*	ST1079
Poultry	Farm 8	12/60	Meghalaya	12/16	NE-P-42	AMP, CRO, CXM, SXT	CTX-M group 4	–	–	Y, L/M	–	*traT*	ND
					NE-P-43	AMP, IPM, NAL	CTX-M group 4	–	–	–	–	–	ND
					NE-P-44	AMC, AMP, CIP, CRO, CXM, ETP, FEP, IPM	CTX-M group 1, TEM	–	–	–	–	*traT*	ST746
					NE-P-45	CRO, CXM, MEM, NAL, SXT	CTX-M group 4	–	–	B/O	–	*traT*	ND
					NE-P-46		–	–		Y, B/O	Int-3	*traT*	ND

Table 1 (*Continued*)

Host/ stratum	Farm ID	No. of samples collected/no. of animals in farm (20% per farm[a])	Farm location	Total *E. coli*/ total GNB	Sample ID of β-lactam resistant *E. coli*	AMR profile (MIC method)	β-Lactamase genes			Plasmid replicons (*n* = 25)	Integrons (*n* = 6)	Virulence genes (*n* = 26)	MLST
							ESBLs (*n* = 24)	MBLs (*n* = 2)	AmpC (*n* = 6)				
						AMP, CRO, CXM, ETP, SXT			FOX, CMY				
					NE-P-48	AMP, CRO, CXM, FEP, NAL	–	–	FOX, CMY	Y, L/M, B/O	Int-3	*traT*	ND
	Farm 9	8/40	Meghalaya	8/11	NE-P-49	CIP, ETP, NAL, NIT	–	–	FOX, CMY	B/O, HI2	Int-3	*traT*	ND
					NE-P-50	AMC, CIP, CRO, NIT, TZP	–	–	–	N, Y	–	*traT*	ND

GNB, Gram-negative bacteria; MIC, minimum inhibitory concentration; ESBL, extended-spectrum β-lactamase; MBL, metallo β-lactamase; MLST, multilocus sequence typing; CIP, ciprofloxacin; CRO, ceftriaxone; CXM, cefuroxime; IPM, imipenem; NAL, nalidixic acid; SXT, trimethoprim/sulfamethoxazole; TZP, piperacillin/tazobactam; AMC, amoxicillin/clavulanic acid; AMP, ampicillin; NIT, nitrofurantoin; FEP, cefepime; GEN, gentamicin; MEM, meropenem; AMK, amikacin; ETP, ertapenem; ND, not done.

[a] The sampling criterion (i.e. 20% per farm) was met for all farms except Farm 2 and Farm 4.

Table 2
Summary of six uniplex (sets 1–6) and two multiplex (sets 7 and 8) primer sets for the amplification of 17 antimicrobial resistance genes.

Primer set	Gene name/target	Antimicrobial resistance	Primer	Oligonucleotide sequence (5′ → 3′)	Amplified product (bp)	Annealing temperature (°C)	Reference
1	TEM	ESBL	TEM-F	ATGAGTATTCAACATTTTCG	861	60	[2]
			TEM-R	TTACCAATGCTTAATCAGTG			
2	SHV	ESBL	SHV-F	ATGCGTTATATTCGCCTGTG	860	60	[2]
			SHV-R	TTAGCGTTGCCAGTGCTCGA			
3[a]	CTX-M group 1	ESBL	CTXM-I-F	GACGATGTCACTGGCTGAGC	499	55	[11]
			CTXM-I-R	AGCCGCCGACGCTAATACA			
4[a]	CTX-M group 2	ESBL	CTXM-II-F	GCGACCTGGTTAACTACAATCC	351	55	[11]
			CTXM-II-R	CGGTAGTATTGCCCTTAAGCC			
5[a]	CTX-M group 3	ESBL	CTXM-III-F	CGCTTTGCCATGTGCAGCACC	307	62	[11]
			CTXM-III-R	GCTCAGTACGATCGAGCC			
6[a]	CTX-M group 4	ESBL	CTXM-IV-F	GCTGGAGAAAAGCAGCGGAG	474	62	[11]
			CTXM-IV-R	GTAAGCTGACGCAACGTCTG			
7a[b]	MOX-1, MOX-2, CMY-1, CMY-8 to -11	AmpC	MOXM-F	GCTGCTCAAGGAGCACAGGAT	520	64	[12]
			MOXM-R	CACATTGACATAGGTGTGGTGC			
7b[b]	LAT-1 to -4, CMY-2 to -7, BIL-1	AmpC	CITM-F	TGGCCAGAACTGACAGGCAAA	462	64	[12]
			CITM-R	TTTCTCCTGAACGTGGCTGGC			
7c[b]	DHA-1, DHA-2	AmpC	DHAM-F	AACTTTCACAGGTGTGCTGGGT	405	64	[12]
			DHAM-R	CCGTACGCATACTGGCTTTGC			
7d[b]	ACC	AmpC	ACCM-F	AACAGCCTCAGCAGCCGGTTA	346	64	[12]
			ACCM-R	TTCGCCGCAATCATCCCTAGC			
7e[b]	MIR-1, ACT-1	AmpC	EBCM-F	TCGGTAAAGCCGATGTTGCGG	302	64	[12]
			EBCM-R	CTTCCACTGCGGCTGCCAGTT			
7f[b]	FOX-1 to -5b	AmpC	FOXM-F	AACATGGGGTATCAGGGAGATG	190	64	[12]
			FOXM-R	CAAAGCGCGTAACCGGATTGG			
8	IMP	MBL	IMP-F	GAATAG(A/G)(A/G)TGGCTTAA (C/T)TCTC	188	53	[13]
			IMP-R	CCAAAC(C/T)ACTA(G/C)GTTATC			
	VIM	MBL	VIM-F	GTTTGGTCGCATATCGCAAC	382	53	[13]
			VIM-R	AATGCGCAGCACCAGGATAG			
	GIM	MBL	GIM-F	TCAATTAGCTCTTGGGCTGAC	72	53	[13]
			GIM-R	CGGAACGACCATTTGAATGG			
	SIM	MBL	SIM-F	GTACAAGGGATTCGGCATCG	569	53	[13]
			SIM-R	TGGCCTGTTCCCATGTGAG			
	SPM	MBL	SPM-F	CTAAATCGAGAGCCCTGCTTG	798	53	[13]
			SPM-R	CCTTTTCCGCGACCTTGATC			

ESBL, extended-spectrum β-lactamase; MBL, metallo β-lactamase.

[$] Detection of metallo-β-lactamase enzymes (MβL) genes.

[a] Group 1 includes CTX-M-1, -3, -10 to -12, -15 (UOE-1), -22, -23, -28, -29 and -30; group 2 includes CTX-M-2, -4 to -7 and -20 and Toho-1; group 3 includes CTX-M-8; and group 4 includes CTX-M-9, -13, -14, -16 to -19, -21 and -27 and Toho-2.

[b] Detection of family-specific plasmid-mediated AmpC β-lactamase identifies the family-specific bla_{AmpC} gene responsible for AmpC β-lactamase expression.

fumC, *gyrB*, *icd*, *mdh*, *purA* and *recA*) was performed following the protocols specified at the *E. coli* MLST website (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli).

3. Results

3.1. Isolation and identification of E. coli isolates

A total of 109 Gram-negative bacterial isolates were recovered from 78 faecal samples. The culture method and *E. coli*-specific multiplex PCR identified 78 isolates as *E. coli* and 31 isolates as other Gram-negative bacteria. Details of the *E. coli* isolates recovered from different farms are presented in Table 1.

3.2. Antimicrobial susceptibility testing

Among the 78 *E. coli* isolates, 32 isolates (41%) resistant to at least three β-lactam antibiotics were detected by the disk diffusion method. MIC testing of these 32 resistant *E. coli* strains revealed resistance to cefuroxime, ceftriaxone and ampicillin in 91% (29/32), 72% (23/32) and 50% (16/32) of isolates, respectively. The antibiogram for other common antibiotics determined by the VITEK®2 system is given in Table 1.

3.3. Molecular detection of antimicrobial resistance genes

Molecular analysis of the 32 β-lactam-resistant *E. coli* strains identified ESBL ($bla_{\text{CTX-Mgroup1}}$, $bla_{\text{CTX-Mgroup4}}$ and bla_{TEM}), AmpC (bla_{EBC}, bla_{FOX}, bla_{CMY} and bla_{DHA}) and MBL (bla_{IMP} and bla_{SIM}) resistance determinants in 75% (24/32), 19% (6/32) and 6% (2/32) of isolates, respectively (Table 1). $bla_{\text{CTX-Mgroup4}}$ (58%; 14/24) was the most common ESBL gene detected. Of these probable ESBL/AmpC/MBL-producing *E. coli*, 13 were from cattle, 10 from pigs, 8 from chickens and 1 from a goat.

3.4. Distribution of plasmid replicon types and integrons among potential ESBL/AmpC/MBL-producing E. coli

Nine plasmid replicon types (B/O, FIC, A/C, Y, I1, HI1, N, L/M and HI2) were distributed among 25 (78%) of the 32 β-lactam-resistant *E. coli* strains. Multiple plasmids were observed in 28% (9/32) of isolates. The most common plasmid replicon types identified were L/M and Y. Integrons were detected in 19% (6/32) of the *E. coli* isolates. Class 2 and 3 integrons were observed in 9% (3/32) and 13% (4/32) of isolates, respectively; 1 isolate contained both classes of integron (Table 1).

3.5. Distribution of virulence genes among potential ESBL/AmpC/MBL-producing E. coli

Virulence genes were detected in 81% (26/32) of the *E. coli* isolates. The most common virulence genes detected were *traT* (78%; 25/32) and *stx2* (19%; 6/32). Other genes detected were *fliC* (9%; 3/32) and *cnf1* (3%; 1/32) (Table 1).

3.6. RAPD analysis

RAPD cluster analysis categorised the 32 *E. coli* isolates into three clusters (I–III) with possible ESBL-producing *E. coli* distributed widely in all of the clusters (Fig. 1). Notably, the likely Shiga toxin-producing *E. coli* isolates were grouped exclusively in cluster II.

3.7. Multilocus sequence typing and phylogenetic analysis

Seven different sequence types (ST10, ST1079, ST1727, ST206, ST58, ST746 and ST617) were distributed across the β-lactam-resistant *E. coli* strains. Of these, ST10 was the common sequence type isolated from cattle and pig hosts from different, yet close, geographical areas (Farms 2, 3 and 6) (Table 1).

4. Discussion

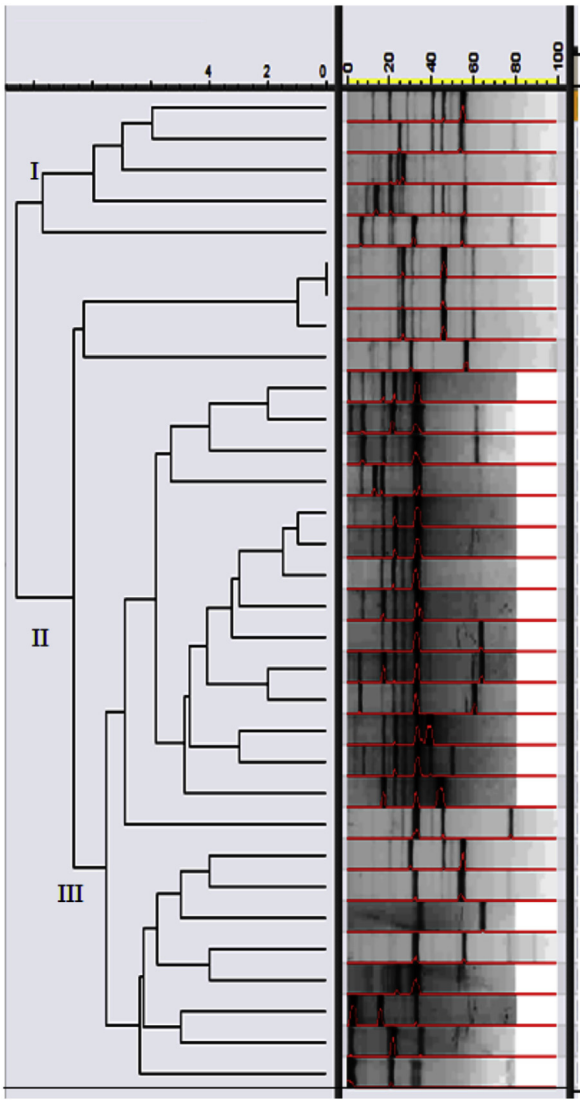
Due to the growing problem of AMR worldwide, focus on the epidemiology of β-lactam-resistant *E. coli* has increased as this organism is generally considered a useful indicator of AMR owing to its medical importance and its presence in a wide range of hosts [3].

Molecular characterisation of 32 β-lactam-resistant *E. coli* isolates in this study showed 75% (24/32) as potential ESBL-producers with major occurrence of the $bla_{\text{CTX-Mgroup4}}$ gene (58%; 14/24), followed by $bla_{\text{CTX-Mgroup1}}$ (42%; 10/24). AmpC and MBL determinants were observed in 19% and 6% of isolates, respectively. The high concentration of prospective ESBL-producing *E. coli* isolates in faecal samples and the high level of diversity of ESBL genes illustrates the abundance of these bacteria in animals and the ease with which they are acquired and presumably lost [5,6]. The MBL resistance determinant panel did not included bla_{NDM}, bla_{FIM} and bla_{AIM} genes as these resistance determinants are rarely found in food-producing animals [7]. In India, the most common MBL resistance determinants reported are bla_{VIM} and bla_{IMP} [17]; however, with the increase in reports of bla_{NDM} in human health care and considering the dissemination potential of these drug-resistant pathogens, future studies under the One Health concept warrant the inclusion of bla_{NDM} and other important resistance genes.

In accordance with our observations, a study from Italy noted the significant emergence of isolates producing the CTX-M-1 enzyme from healthy and diseased pets [4]. Similarly, in a study from France, $bla_{\text{CTX-Mgroup1}}$ genes associated with IncI1 in *E. coli* suggested a potential link for dissemination of this gene between humans and animals [18]. In the poultry population in the current study, a predominance of potential AmpC-producing *E. coli* was observed in addition to ESBL-producers. Similar findings have been reported by other studies, affirming poultry as a reservoir of ESBL/AmpC-producing *E. coli* [5].

Nine different plasmid replicons were observed in the present study. IncL/M was identified in eight $bla_{\text{CTX-M}}$-positive, two bla_{AmpC}-positive and one $bla_{\text{CTX-M}} + bla_{\text{AmpC}}$-positive *E. coli* isolates. In the study by Marcadé et al., $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-3}}$ genes were carried on IncL/M plasmids along with IncI1 and IncN [18]. Another important replicon type, IncA/C, was detected in $bla_{\text{CTX-Mgroup1}}$-, AmpC- and MBL-positive *E. coli* strains isolated from the pig population. These findings are in accordance with the study by Mulvey et al. wherein replicon type IncA/C was detected in *E. coli* strains isolated from beef, chicken, turkey and pork in different regions of the USA, highlighting the dissemination of this common plasmid backbone among resistant zoonotic pathogens [6].

A high frequency of multiple plasmid replicon types were observed in *E. coli* strains isolated from chicken (5/8), pig (3/10) and goat (1/1) hosts. One probable AmpC-producing *E. coli* carrying plasmid replicons IncI1 and HI2 was identified. Johnson et al. [14] and Carratoli [4] in their independent studies reported that IncI1 plasmids are associated with the spread of several other ESBL genes and have been reported to be widespread in *E. coli* animal strains. Subsequently, we identified integron classes 3 and 2 predominantly in *E. coli* isolates of poultry and cattle origin, respectively. One *E. coli* isolate of pig origin was found to be positive for both integron classes 2 and 3, unlike a recent study from Eastern India where 88% of *E. coli* isolates were positive for a class 1 integron [8]. Studies emphasise the importance of identification of plasmid types and integrons prevalent in the resistant bacterial population. The information may be useful to identify drugs targeting these mobile genetic element families for the treatment of drug-resistant bacteria [4,14].



Sl.N.	*E. coli* Strain ID	MLST	*ESBL/MBL/AmpC* genes
1	Pig, NE PG-24, Farm 4		ESBL (ctxmIV)
2	Chicken, NE P- 44, Farm 8	ST746	ESBL (ctxmIV, tem)
3	Pig, NE PG-69, Farm 5		ESBL(ctxmI)
4	Chicken, NE P-42, Farm 8		ESBL(ctxmIV)
5	Pig, NE PG-20 Farm 4	ST 1079	ESBL (ctxmIV)
6	Pig, NE PG-72 Farm 5	ST 617	ESBL (ctxmIV)
7	Pig, NE PG-74, Farm 5		ESBL (ctxmI,cmy)
8	Pig NE PG-71, Farm 5		ESBL (ctxmI)
9	Pig NE PG-60, Farm 5		ESBL (ctxmI)
10	Cattle, NE C-2, Farm 1	ST 1727	ESBL (ctxmIV)
11	Chicken, NE-P46, Farm 8		AmpC (Fox,cmy)
12	Chicken, NE-P48, Farm 8		AmpC (Fox,cmy)
13	Cattle, NE C-39, Farm 2	ST10	ESBL (ctxmI,tem) ; *stx2*
14	Cattle, NE-C5, Farm 1		ESBL (ctxmIV)
15	Cattle, NE-C29, Farm 2		ESBL (ctxmIV)
16	Cattle, NE-C53, Farm 3	ST10	ESBL (ctxmI,tem)
17	Cattle, NE-C33, Farm 2		AmpC (ebc)
18	Cattle, NE-C37, Farm 2		ESBL(ctxmI); *stx2*
19	Cattle, NE-C 34, Farm 2		ESBL (ctxmIV); stx2
20	Cattle, NE-C38, Farm2		ESBL (ctxmI); *stx2*
21	Cattle, NE-C8, Farm 1	ST10	ESBL (ctxmIV); *stx2*
22	Cattle, NE-C28, Farm2		ESBL (ctxmIV)
23	Cattle, NE-C4, Farm 1		ESBL (ctxmI)
24	Pig NE-PG-70, Farm 5		ESBL (ctxmIV)
25	Pig, NE-PG-59, Farm 5	ST-206	MBL (sim)
26	Chicken, NE-P-43 Farm 8		ESBL (ctxmIV)
27	Chicken, NE-P50 Farm 9		
28	Pig, NE-PG-80, Farm 6	ST10	MBL (imp)
29	Goat, NE-G-89, Farm 7	ST1079	AmpC (Dha,fox)
30	Cattle, NEC-35, Farm 2	ST58	ESBL (ctxmIV) ; *stx2*
31	Chicken, NE-P49, Farm 9		AmpC (Fox,cmy)
32	Chicken, NE- P45, Farm 8		ESBL (ctxmIV)

Fig. 1. Random amplification of polymorphic DNA (RAPD) cluster analysis of the 32 *Escherichia coli* isolates. MLST, multilocus sequence typing; ESBL, extended-spectrum β-lactamase; MBL, metallo β-lactamase.

The current study showed that 81% of β-lactam-resistant *E. coli* strains carried virulence genes. The genes *traT* (53%; 17/32) and *stx*2 (19%; 6/32) showed a strong association with $bla_{\text{CTX-Mgroup1}}$ and $bla_{\text{CTX-Mgroup4}}$ resistance determinants. Jensen et al. reported ESBL-positive, Shiga toxin-producing *E. coli* (STEC) as a rare phenomenon [19]. In contrast, in the current study six probable ESBL (three $bla_{\text{CTX-Mgroup1}}$ and three $bla_{\text{CTX-Mgroup4}}$)-producing *E. coli* harbouring *stx2* were identified. The presence of drug-resistant pathogenic *E. coli* strains in dairy and/or companion animals is of grave concern because of the high possibility of zoonotic transmission [6,8].

RAPD clustering identified three major clusters, namely clusters I, II and III primarily in pig, cattle and poultry isolates, respectively. However, ESBL-producing isolates were distributed commonly in all of the clusters. RAPD clustering of multidrug-resistant *E. coli* suggests that there might be host jump/movement of isolates across animals species [6,16]. MLST analysis of representative *E. coli* strains from each cluster showed different sequence types. Notably, ST10 was found as a common clonal strain of *E. coli* detected in cattle and pigs. Previous studies have also reported the occurrence of ST10 isolates in dogs, cats, horses, cattle, pigs and birds [3,20]. Interestingly, MBL-producing *E. coli* of pig origin harbouring FIC, A/C, HI1 and HI2 plasmids were recognised as ST10, and an *E. coli* variant harbouring AmpC and CTX-M-1 along with A/C and L/M as well as integron classes 2 and 3 were documented as ST676. Overall, isolates in this study showed a distinct allelic profile compared with the STs of isolates reported from different parts of the world [20], thus increasing the threat associated with the emergence of high-risk antimicrobial-resistant *E. coli* clones.

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Competing interests

None declared.

Ethical approval

Not required.

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