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Molecular and phylogenetic characterization of multidrug resistant extended spectrum beta-lactamase producing *Escherichia coli* isolated from poultry and cattle in Odisha, India

Debasish Kar, Samiran Bandyopadhyay *, Debaraj Bhattacharyya, Indranil Samanta [1], Achintya Mahanti [1], Pramod K. Nanda, Bimalendu Mondal, Premanshu Dandapat, Arun K. Das, Tapan K. Dutta [2], Subhasish Bandyopadhyay, Raj Kumar Singh [3]

Eastern Regional Station, Indian Veterinary Research Institute, 37 Belgachia Road, Kolkata 700 037, India

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ABSTRACT

The present study was undertaken to determine the occurrence and characterization of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* isolated from cattle and poultry in Odisha, India. Of 316 *E. coli* isolated from 305 samples (170 fecal samples from poultry and 135 milk samples from cattle), a total of 18 *E. coli* isolates were confirmed as ESBL producers by combination disc method and ESBL *E*-test. The isolates were resistant to oxyimino cephalosporins and monobactam as revealed by disc diffusion assay and determination of minimum inhibitory concentration. Resistance against other antibiotics was frequently noted as well. Further, beta-lactamase genes *viz*., *blaSHV*, *blaCTXM*, *blaTEM* and *blaampC* were detected in 17, 13, 9 and 2 isolates, respectively in PCR. Of the 18 ESBL strains, 16 were positive for class I integron (*int1*), nine of them carried sulphonamide resistance gene (*sul1*) and one harbored quinolone resistance gene (*qnrB*). Virulence markers for extraintestinal pathogenic *E. coli* like *astA*, *tsh* and *iucD* were also present in 4, 3 and 3 isolates, respectively. All the PCR amplified products were cloned and subjected to sequencing for homology analysis and data were submitted to gene bank. Sequence analysis of the amplified variable regions of class 1 integron of four representative isolates revealed the presence of *aadA*2 and *dfrA*12 gene cassettes conferring resistance to aminoglycosides and trimethoprim, respectively. Most of the ESBL producing strains emerged as single lineage through phylogenetic analysis by RAPD and ERIC PCR. This is the first ever systemic study on multidrug resistant ESBL producing *E. coli* in food producing animals from India.

1. Introduction

Emergence and dissemination of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* were mostly reported from nosocomial outbreaks, but in recent days, these are also being reported from community infections as well (Oteo et al., 2010). More importantly, detection of ESBL producing *E. coli* in food producing animals and edible animal products has become a serious cause of concern for the consumers (Geser et al., 2012; Machado et al., 2008). This poses a great challenge to medical and veterinary practitioners, as these ESBL producing *E. coli* can inactivate beta-lactam antibiotics containing oxyimino group, especially the 3rd and 4th generation cephalosporins and monobactam (Oteo et al., 2010). Added to this, ESBL producers are also resistant to other antibiotics such as fluoroquinones, aminoglycosides and trimethoprim–sulfamethoxazole (Machado et al., 2008; Pasom et al., 2013; Rath et al., 2014).

In India, ESBL producing *E. coli* have been reported in hospitalized patients, from various states over the last few years (Dutta et al., 2013; Mukherjee et al., 2013; Oberoi et al., 2013; Rath et al., 2014). In spite of this, no systematic study on ESBL producing *Enterobacteriaceae* has been conducted in food animals in India despite the fact that both animals and human share a close relationship. Besides, indiscriminate use of antibiotics in livestock often establishes selection pressure for emergence of several antibiotic resistant strains, including ESBL producers in animals. By this, these animals not only act as a mere reservoir but at times

* Corresponding author.
E-mail addresses: samiranicar@rediffmail.com, sbandyo@ivri.res.in, samiranvet@gmail.com (S. Bandyopadhyay).
[1] Department of Microbiology, WBUAFS, Kolkata 700 037, India.
[2] Department of Microbiology, C.V.Sc., CAU, Aizwal, Mizoram, India.
[3] Director, IVRI (Deemed University), Izatnagar 243 122, Bareilly, UP, India.

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may transmit these pathogens to human beings through direct and indirect contact. Furthermore, the presence of various cardinal virulence genes in ESBL producers may increase the pathogenicity and complicate the therapeutic strategy (Dutta et al., 2013).

Under these circumstances, the present study was undertaken to determine the occurrence of ESBL producing *E. coli* in poultry and cattle from different districts of Odisha, India and their further characterization through drug resistance pattern, identification of ESBL and other putative virulence genes, randomly amplified polymorphic DNA PCR (RAPD PCR) and enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC PCR).

2. Materials and methods

2.1. Sample collection and isolation of E. coli

A total of three hundred and five (n = 305) samples (170 poultry feces and 135 cattle milk) were collected from five different districts of Odisha (India) during July 2013 to March 2014 (Table 1). Out of 170 fecal samples, 33 were from healthy and 137 were from diarrheic birds. Of the 135 milk samples, 23 were from apparently healthy cow without any evidence of mastitis and 112 samples were from cattle with clinical and subclinical mastitis (determined by somatic cell count). All the samples were collected in sterile collection vials and transported to laboratory maintaining proper cold chain for isolation and biochemical characterization of *E. coli* following standard procedure (Quinn et al., 1994).

2.2. Phenotypic confirmation for extended spectrum beta-lactamase production by E. coli isolates

For phenotypic confirmation of ESBL production, all the confirmed *E. coli* isolates were screened for their susceptibility to 3rd and 4th generation cephalosporins and monobactam by disc diffusion method using the commercially available discs (BD BBL™, USA/HiMedia, India) of cefpodoxime (CPD 10 μg), ceftazidime (CAZ 30 μg), cefotaxime (CTX 30 μg), cefepime (CPM 30 μg) and aztreonam (ATZ 30 μg). The isolates exhibiting reduced susceptibility to any of these antibiotics were further subjected to combination disc method and ESBL *E*-test (Andrews, 2012).

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of all the phenotypically confirmed ESBL producing *E. coli* strains was performed by standard disc diffusion method against 19 different antibiotic discs [BD BBL™, USA/HiMedia, India] representing 10 diverse classes; amikacin (AK 30 μg), amoxicillin–clavulanic acid (AMC 20/10 μg), ceftriaxone (CTR 30 μg), cefepirome (CFP 30 μg), ceftizoxime (CZX 30 μg), cefepime–tazobactam (CPT 80/10 μg), chloramphenicol (C 30 μg), ciprofloxacin (CIP 5 μg), co-trimoxazole (COT 1.25/23.75 μg), gatifloxacin (GAT 5 μg), imipenem (IMP 10 μg), levofloxacin (LE 5 μg), lomefloxacin (LOM 10 μg), norfloxacin (NX 10 μg), piperacillin–tazobactam (PIT 100/10 μg), polymyxin B (PB 300 U), tetracycline (TE 30 μg) and tobramycin (TOB 10 μg). The isolates were categorized as sensitive, intermediately resistant or resistant based on the results interpreted following the recommendations of CLSI (2014).

2.4. Phenotypic assays for detection of AmpC beta-lactamase, carbapenemase and metallo-beta-lactamase production

All the ESBL producing isolates were subjected to cefoxitin–cloxacillin double disc synergy (CC-DDS) test and AmpC beta-lactamase (ACBL) detection *E*-test (HiMedia, India) for phenotypic

Table 1
Isolation of ESBL producing *Escherichia coli* from poultry and cattle in different districts of Odisha, India.

SL	Districts	Sampling areas	Poultry fecal samples (n)			Poultry *E. coli* isolates (n)			Cattle milk samples (n)				Cattle *E. coli* isolates (n)				ESBL producers (status of the animal)
			HB	DB	T	HB	DB	T	HC	CM	SCM	T	HC	CM	SCM	T	
1	Cuttack	Niali	3	18	21	4	28	32	4	2	9	15	1	1	0	2	PEC5 (DB), PEC7 (DB)
		Salipur	2	16	18	2	22	24	2	4	11	17	2	4	3	9	PEC6 (DB), PEC8 (DB)
2	Puri	Gop	3	12	15	5	12	17	2	1	9	12	1	2	2	5	PEC11 (DB), PEC12 (DB)
		Astaranga	2	10	12	3	16	19	3	3	8	14	2	5	2	9	PEC13 (DB)
		Sakhigopal	1	4	5	0	7	7	2	0	6	8	0	0	0	0	–
3	Khurda	Janla	6	16	22	8	22	30	1	4	9	14	0	3	4	7	PEC1 (DB), MEC1 (CM) PEC15 (DB)
		Khurda sadar	4	15	19	4	27	31	2	2	13	17	1	2	8	11	PEC3 (DB), PEC4 (HB) PEC2 (DB)
4	Balasore	Soro	2	14	16	1	21	22	1	1	5	7	1	1	4	6	PEC9 (DB)
		Jaleswar	1	13	14	0	23	23	1	3	7	11	0	1	2	3	MEC2 (SCM), PEC10 (DB)
5	Ganjam	Khalikot	2	5	7	3	9	12	1	2	2	5	0	1	2	3	–
		Bhanjanagar	4	8	12	3	14	17	2	1	2	5	0	2	2	4	PEC14 (DB)
		Chatrapur	3	6	9	6	12	18	2	3	5	10	1	1	3	5	PEC16 (DB)
Total			33	137	170	39	213	252	23	26	86	135	9	23	32	64	18

HB: healthy birds, DB: diarrheic birds, HC: healthy cattle, CM: clinical mastitis, SCM: subclinical mastitis, T: total.