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Characterization of MDR and ESBL-producing *E. coli* strains from healthy swine herds of north-eastern India

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ABSTRACT

Fecal samples (225) from apparently healthy pigs were analysed for *E. coli* isolation. Multidrug-resistant (MDR) isolates (42) were identified from 225 *E. coli* isolates. Forty isolates exhibited extended-spectrum beta-lactamases (ESBL) phenotype harbouring single to multiple-sized plasmids (1 kb to ≥ 25 kb). Overall bla_{TEM} (85.7%) was the commonest genotype, followed by $bla_{\text{CTX-M}}$ (61.9%), bla_{OXA} (17%) and bla_{SHV} was not recovered. Other antimicrobial resistant genes (ARGs) identified were *sul*2 (100%), *str*A (95.2%), *tet*A (92.9%), *sul1* (76%), *aad*A (71.4%), *tet*B (50%), *cat* (42.9%) and *aac(3)IV* (26.2%). Plasmid-mediated quinolone resistance (PMQR) positive strains totalled to 59.5% for *qnrB,* 19.04% for *qnrS* but *qnrA* was not detected. Integron genes were detected either singly or combined: *intI1* (76.2%), *intI2* (26.2%). Conjugation experiment was successful for 4 ESBL producers. The present study detected higher carriage of ESBL producing *E. coli* in pigs, and highlights the dominance of resistance and integron genes circulating in MDR *E. coli* from swine of northeast India, posing a threat of horizontal gene transfer.

Key words: Antimicrobial resistant genes, *E. coli,* ESBLs, MDR, Northeastern India, Pig

Antimicrobial resistance (AMR) is a burning and serious challenge in human and veterinary medicine. The increasing exploitation of antibiotics in livestock due to rising demand for animal-source protein worldwide is of great concern. Use of antibiotics in animals has been related to drug-resistant infections in animals and humans. Intensive production systems have largely exploited antimicrobials for nontherapeutic use, such as antimicrobial growth promoter, chemoprophylactic etc. Multidrug-resistant (MDR) *Escherichia coli* have been a grave concern, looming worldwide with the potential to circulate in food animals. The dissemination of broad-spectrum beta-lactams resistant enterobacterial strains along with co-resistance to other antibiotic families is an emerging threat to animal and public health (EFSA 2013).

India is one of the largest consumers of antibiotics in the world with 13 billion standard units in 2010 (CDDEP 2015). India is burdened with highest bacterial diseases, explosive population, maximum antibiotic consumption; poor health care and vast livestock sector with rampant use of growth promoter. On the contrary, stewardship policy intended to curtail this rise is in its infancy. All these factors lead to the development of MDR, as one of the foremost problems in the country beyond the umbrella of defined norms or accountability. Despite the growing trend, available data are primarily restricted to hospital-based human cases. Information or research pertaining to livestock sector is scarce (Mandakini *et al*. 2014). In India, previous studies undertaken to study antimicrobial resistance in *E. coli* isolated from pigs were only focused either in ESBL or carbapenemase-producing *E. coli* (Mandakini *et al.* 2014, Samanta *et al.* 2015, Pruthvishree *et al.* 2017, Nirupama *et al*. 2018). The co-resistance of ESBL producing *E. coli* to other antibiotics is hardly addressed.

It may be noted that pig farming in north-eastern India is an important livelihood option, often in the close interface with human habitation. Thus the role of pigs in the swift circulation of MDR, with accompanying threats to one health cannot be undermined. With this backdrop, this study was undertaken to understand the phenotypic and genotypic determinants of ESBL, other antibiotic resistance, plasmids, and integrons of fecal *E. coli* from pigs raised on intensive farms of Northeast India.

MATERIALS AND METHODS

Samples were collected randomly from scattered swine farms of north-eastern region of India (Asom, Meghalaya, Mizoram, and Nagaland), which differs geographically and

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demographically from other regions of India. Fecal samples (225) were collected from apparently healthy pigs irrespective of sex, age, and breed. The samples were transported in phosphate buffer saline and stored at 4°C until further analysis. None of the pigs were having diarrhoea and there was no record of antibiotic therapy for at least 3 months preceding the sample collections.

The isolation and identification were carried out as per standard microbiological and biochemical techniques (Quinn *et al*. 1994) by streaking on MacConkey agar and Eosin Methylene blue agar (HiMedia). The isolated *E. coli* were subjected to *in vitro* antimicrobial susceptibility profiling by disc diffusion using impregnated antibiotic discs against 4 commonly used antibiotics (tetracycline, sulphonamides, ampicillin, and colistin). Isolates which showed resistance to all the four antibiotics were further subjected to a panel of 24 antibiotics (HiMedia) classified into 8 groups of antibiotics using the following commercially available antibiotic discs (HiMedia, India) namely amikacin (10 µg), gentamicin (30 µg), streptomycin (25 µg), kanamycin (30 µg), neomycin (30 µg), cephotaxime (30 µg), ceftazidime (30 µg), chloramphenicol (10 µg), ampicillin (25 µg), amoxycillin (10 µg), carbenicillin (100 µg), cloxacillin (30 µg), colistin (10 µg), polymyxin B (30 µg), sulphadiazine (100 µg), sulphafurazole (300 µg), Co-trimaxazole (25 µg), trimethoprim (30 µg), enrofloxacin (10 µg), ofloxacin (5 µg), norfloxacin (10 µg), nalidixic acid (30 µg), chlortetracycline (30 µg) and oxytetracycline (30 µg). Antibiogram was performed on Mueller-Hinton agar plate as per Clinical Laboratory Standard Institute (CSLI) guidelines and interpreted as per CLSI (2008). Resistance to ≥3 classes of antibiotics was considered MDR.

Multidrug resistant *E. coli* (MDRE) were subjected to double disc synergy test (DDST) for phenotypic confirmation of ESBL producing isolates using cefotaxime (30 µg), ceftazidime (30 µg), cefotaxime/Clavulanate (30/10 µg), and ceftazidime/clavulanate (30/10 µg), in combination as per CLSI (2008). DNA was extracted from MDR *E. coli* isolates following conventional phenol chloroform method. The amplification was carried out for 4 ESBL genes, 11 antibiotic resistant genes (ARGs) and 2 mobile genetic elements (MGEs) genes. The targeted genes were, *bla*TEM, *bla*CTX-M, *bla*SHV, *bla*OXA, *aac(3)IV, aadA, cat, sul1, sul2, strA, tetA, tetB, qnrA, qnrB, qnrS, intI1* and *intI2*. Polymerase chain reactions (PCRs) were carried out in thermal cycler (BioRad) according to previously described protocols with minor variations (Boerlin *et al.* 2005, Jianyu *et al.* 2006, Cattoir et *al.* 2007, Dallenne *et al.* 2010, Literak *et al.* 2010). The primer details and cycling conditions are mentioned in Table 1.

Plasmids were extracted from MDR *E. coli* using HiPurA plasmid kit (HiMedia, Mumbai, India) and visualised by gel electrophoresis (0.8%) using lambda *EcoRI-HindIII* digested marker. Broth mating was employed to study *in vitro* transfer of ESBLs carrying plasmids (Cattoir *et al*. 2007). Seven ESBLs carrying isolates was randomly selected for this experiment. Transfer was done between MDR *E. coli* as donors to plasmid free DH5α *E. coli* (lac negative) susceptible to all antibiotics as recipient, with antibiotic selection pressure. Donor and recipient strains were separately grown in antibiotic-free medium and antibiotic medium (control). Transconjugants were selected on MacConkey's agar supplemented with cefotaxime (3 µg/ml) and ceftriaxone (3 µg/ml). Selected transconjugants were characterized for their antibiotic susceptibility, ESBL production, plasmid presence and the detection of the ESBL genes (*bla*CTX-M-15 and/or *bla*TEM) by PCR.

Table 1. Primers sequences and PCR conditions

Primer	Amplicon size (bp)	PCR condition	Reference
*bla*TEM	800	1 cycle of 3 min at 94°C;	Dallenne
*bla*OXA	564	35 cycles of 45 sec at 94°C,	*et al.* (2010)
*bla*SHV	713	45 sec at 60°C, 45 sec at 72°C; 1 cycle of 4 min at 72°C	
*bla*CTX-M	688	1 cycle of 7 min at 94°C; 35 cycles of 50 sec at 94°C, 40 sec at 50°C, 60 sec at 72°C; 1 cycle of 5 min at 72°C	Dallenne *et al.* (2010)
tetA	210	1 cycle of 3 min at 94°C;	Literak *et al.*
tetB	659	35 cycles of 45 sec at 94°C,	(2010)
cat	623	45 sec at 55°C, 45 sec at	
aadA	284	72°C; 1 cycle of 4 min at 72°C	
aac (3)IV	*653*	1 cycle of 3 min at 94°C;	Dallenne
sul1	248	35 cycles of 45 sec at 94°C,	*et al.* (2010)
sul2	417	45 sec at 60°C, 45 sec at	Boerlin
strA	250	72°C; 1 cycle of 4 min at 72°C	*et al* (2005)
intI1	565	1 cycle of 5 min at 94°C;	Jianyu *et al.*
intI2	403	30 cycles of 50 sec at 94°C, 50 sec at 52°C, 2 min at 72°C; 1 cycle of 7 min at 72°C	(2006)
qnrA	*580*	1 cycle of 5 min at 95°C;	Cattoir *et al.*
qnrB	264	35 cycles of 1 min at 94°C,	(2007)
qnrS	428	1 min at 56°C, 1 min at 72°C; 1 cycle of 10 min at 72°C	

RESULTS AND DISCUSSION

Out of 225 fecal samples screened, 225 *E. coli* isolates were recovered on the basis of characteristic pink-coloured colonies on MacConkey's agar, metallic sheen on EMB agar. All the 225 confirmed *E. coli* isolates were subjected to preliminary antibiotic susceptibility testing employing discs of 4 commonly used antibiotics (tetracycline, sulphonamides, ampicillin, and colistin). Around 90.2% isolates (203) were found resistant to at least two antibiotics. Interestingly, 18.66% of the isolates (42/225) were found resistant to all the four antibiotics. This result was concordant with an earlier study conducted in pigs, where 85% *E. coli* isolates showed resistance to more than 2 antimicrobials (Rajkhowa and Sarma 2014). The predominance of resistance phenotypes of *E. coli* against several antibiotics in food, companion, and wild animals

were reported by various workers worldwide and India (Poirel *et al.* 2012, Schmiedel *et al*. 2014, Bandyopadhyay *et al.* 2015). These 42 isolates were designated as multi-drug resistant *E. coli* and selected for further study.

MDR *E. coli* (42) were subjected to a panel of 24 antibiotics belonging to 8 groups of antibiotics. Antibiogram of 42 MDRE revealed that all isolates showed resistance to a minimum of 6 antibiotics and maximum of 23 antibiotics but were sensitive to amikacin. Majority of the isolates showed a high frequency of resistance against various antibiotics ranging from 45.2 to 100%. Intriguingly, 100% isolates were resistant to amoxicillin, ceftazidime, and polymyxin B (Table 2). The most frequently observed resistance phenotypes was against 5 antibiotics, viz. penicillin (ampicillin, cloxacillin), colistin, sulphonamides (sulphadiazine, sulphafurazole), and other 7 antibiotics, i.e. streptomycin, neomycin, cotrimaxazole, trimethoprim, nalidixic acid, chlortetracycline, and oxytetracycline showed resistance in 95.2% isolates (Table 2). Earlier studies conducted in *E. coli* isolated from pigs in India showed a varied level of resistance to different antibiotics, which was partially concordant with the current study. A study conducted in *E. coli* isolated from pigs of Mizoram showed phenotypic resistance for chlortetracycline (87.5%), streptomycin (50%), ampicillin (75%), and enrofloxacin (75%) (Lalzampuia *et al*. 2013). Another study conducted in shiga toxin producing *E. coli* (STEC) recovered from pigs showed phenotypic resistance for tetracycline (99%), streptomycin (98%), ampicillin (100%), gentamicin (88%), kanamycin (85%), sulfadiazine (94%) and nalidixic acid (95%) (Rajkhowa and Sarma 2014). Another study carried out with the samples collected from diarrhoeic piglets and infants in the NE India revealed 58.3%, 8.3%, 16.7%, 14.6%, 6.2%, 85.4%, 60.4%, 33.3%, 27% resistance in *E. coli* for amoxicillin, gentamicin, ofloxacin, enrofloxacin, chloramphenicol, nalidixic acid, polymyxin B, kanamycin, and cefotaxime, respectively (Begum *et al*. 2015). Plausible factors for this high resistance in our study could be previous antibiotic pressure remaining persistently latent or acquired from the environmental sources, other livestock or farm workers.

Table 2. Groups of antibiotics, isolates with resistance phenotypes and associated genes

Group (8)	Antibiotic (24)	Isolates with resistance phenotypes (%) (42)	Associated genes	Isolates with resistance genotypes (%)
Amino-glycosides	Amikacin (AK)	0	*aac(3)IV*	26.2
	Gentamicin (G)	69.04		
	Streptomycin (S)	95.2	*strA*	95.2
	Kanamycin (K)	45.2	*aadA*	71.4
	Neomycin (N)	95.2		
Cephalo-sporins	Cefotaxime (CE)	47.6	$bla_{\text{CTX-M}}$	61.9
	Ceftazidime (CA)	100		
Chloram-phenicol	Chloram-phenicol (C)	52.3	*cat*	42.9
Penicillins	Ampicillin (A)	97.6	bla_{TEM}	85.7
	Amoxicillin (AM)	100	bla_{OXA}	17.0
	Carbenicillin (CB)	64.3	bla_{SHV}	0
	Cloxacillin (CX)	97.6		
Polypeptides	Colistin (CL)	97.6	–	–
	Polymyxin B (PB)	100		
Sulphona-mides	Sulphadiazine	97.6	*sul1*	76.0
	Sulphafurazole (SZ)	(SF)97.6	*sul2*	100.0
	Co-trimaxazole (CO)	95.2	–	–
	Trimethoprim (TR)	97.6		
Quinolones	Enrofloxacin (EX)	64.2	*qnrB*	59.5
	Ofloxacin (OF)	69.04	*qnrS*	19.0
	Norfloxacin (NX)	66.6	*qnrA*	0
	Nalidixic acid (NA)	97.6		
Tetracyclines	Chlortetracycline (CT)	95.2	*tetA*	92.9
	Oxytetracycline (OT)	95.2	*tetB*	50.0

Out of 42 MDR *E. coli*, 40 were phenotypically confirmed to be ESBL producing by DDST. Earlier studies conducted in pigs in India detected 25.3%, 6% and 32.8% ESBL producers among *E. coli* (Mandakini *et al*. 2014, Samanta *et al*. 2015, Nirupama *et al*. 2018). This suggests the importance of food animals, which may play an important role in the transmission of ESBL resistance to human beings. Overall 32 resistant patterns were observed ranging from 6 to 23 antibiotics. Notably, 4 isolates showed resistance to 23 antibiotics, with maximum multiple antibiotic resistance (MAR) index of 0.96. Seven isolates were resistant to 22 antibiotics (MAR index – 0.92) with four different profiles. Thirteen isolates exhibited resistance to 19–21 antibiotics with 11 different patterns (MAR index 0.88–0.80). Remaining 18 isolates showed 16 resistant patterns showing resistance to 6–18 antibiotics and MAR index of 0.28 to 0.76 (Table 3).

Sul2 was the most common ARG with 100% of the isolates harbouring it. Above 85% of the isolates possessed *strA, tetA,* and *bla*TEM while more than 50% isolates carried *sul1, aadA*, *bla*CTX-M and *tetB*. The frequency of *bla*OXA, *aac(3)IV,* and *cat* were 42.9, 26.2, and 17% respectively. *qnrB* and *qnrS* were detected in 59.5 and 19.04% of isolates, respectively. None of the isolates harboured *bla*SHV and *qnrA* genes. All the 40 phenotypically characterized ESBL producing *E. coli* carried at least one *bla* genes. Two isolates possessed 3 targeted *bla* genes (*bla*TEM, *bla*CTX-M, *bla*OXA); 24 isolates carried a different combination of two *bla* genes and 13 strains possessed either *bla*CTX-M or *bla*TEM. In the present study, 85.7, 61.9 and 17% MDR

Table 3. Pattern of antibiotic resistance and MAR index

Sample IDs	Antibiotic resistance pattern	MAR index (24)
P2, P4, P5, P14	EX, CL, OF, CT, CA, NX, COT, N, G, PB, SZ, SF, S, TR, NA, O, CX, CE, K, A, AM, C, CB	0.96
P6, P7	EX, CL, OF, CT, CA, NX, COT, N, G, PB, SZ, SF, S, TR, NA, O, CX, CE, A, AM, C, CB	0.92
P1	EX, CL, OF, CT, CA, NX, COT, G, PB, SZ, SF, S, TR, NA, O, CX, CE, K, A, AM, C, CB	0.92
P10, P23, P15	EX, CL, OF, CT, CA, NX, COT, N, G, PB, SZ, SF, TR, NA, O, CX, CE, K, A, AM, C, CB	0.92
P8	EX, CL, OF, CT, CA, NX, COT, G, PB, SZ, SF, S, TR, NA, O, CX, CE, K, A, AM, C, CB	0.92
P3	EX, CL, OF, CT, CA, NX, COT, G, PB, SZ, SF, TR, NA, O, CX, CE, K, A, AM, C, CB	0.88
P25	EX, OF, CT, CA, NX, COT, N, G, PB, SZ, SF, TR, NA, O, CX, CE, K, A, AM, C, CB	0.88
P16	EX, CL, OF, CT, CA, NX, COT, N, G, PB, SZ, SF, TR, NA, O, CX, K, A, AM, C, CB	0.88
P17, P22, P24	EX, CL, OF, CT, CA, NX, COT, G, PB, SZ, SF, TR, NA, O, CX, CE, K, A, AM, CB	0.84
P41	EX, CL, OF, CT, CA, NX, COT, N, G, PB, SZ, SF, TR, NA, O, CX, A, AM, C, CB	0.84
P42	EX, CL, OF, CT, CA, NX, COT, N, G, PB, SZ, SF, S, TR, NA, O, CX, A, AM, CB	0.84
P12	EX, CL, OF, CT, CA, NX, COT, PB, SZ, SF, S, TR, NA, O, CX, K, A, AM, C, CB	0.84
P20	EX, CL, OF, CT, CA, NX, COT, PB, SZ, SF, TR, NA, O, CX, CE, A, AM, C, CB	0.80
P37	EX, CL, OF, CT, CA, NX, COT, G, PB, SZ, SF, TR, NA, O, CX, A, AM, C, CB	0.80
P33	EX, CL, OF, CT, CA, NX, COT, G, PB, SZ, SF, G, TR, NA, O, CX, K, AM, C	0.80
P13	CL, CT, CA, NX, COT, N, G, PB, SZ, SF, TR, NA, O, CX, K, A, AM, C, CB	0.80
P9	EX, CL, OF, CT, CA, NX, COT, G, PB, SZ, SF, TR, NA, O, CX, CE, A, AM	0.76
P29	EX, CL, OF, CT, CA, NX, COT, G, PB, SZ, SF, TR, NA, O, CX, A, AM, C	0.76
P19	EX, CL, OF, CT, CA, NX, COT, N, G, PB, SZ, SF, TR, NA, O, CX, A, AM	0.76
P30	EX, CL, OF, CT, CA, NX, COT, PB, SZ, SF, TR, NA, O, CX, CE, A, AM	0.72
P11	CL, CT, CA, COT, PB, SZ, SF, S, TR, NA, O, CX, K, A, AM, C, CB	0.72
P40	CL, CT, CA, COT, G, PB, SZ, SF, TR, NA, O, CX, A, AM, CB	0.64
P27	CL, CT, CA, CO, PB, SZ, SF, TR, NA, O, CX, A, AM, CB	0.60
P18	CL, CT, CA, COT, G, PB, SZ, SF, TR, NA, O, CX, A, AM	0.60
P28	CL, CT, CA, COT, PB, SZ, SF, TR, NA, O, CX, A, AM	0.60
P39	CL, CA, PB, SZ, SF, TR, NA, O, CX, CE, A, AM	0.56
P32	CL, CT, CA, COT, G, PB, SZ, SF, TR, NA, A, AM	0.52
P21, P38	CL, CT, COT, PB, SZ, SF, TR, NA,O, CX, A, AM	0.52
P31	CA, PB, SZ, SF, TR, NA, O, CX, A, AM, CB	0.48
P34	CL, CT, CA, CO, G, PB, SZ, SF, CX, AM	0.44
P35, P36	CL, OF, CA, PB, SZ, SF, CX, A, AM	0.40
P26	CL, CA, PB, CX, A, AM	0.28

EX, enrofloxacin; CL, colistin; OF, ofloxacin; CT, chlortetracycline; CA, ceftazidim; NX, norfloxacin; CO, co-trimaxazole; N, neomycin; G, gentamicin; PB, polymyxin B; Z, sulphadiazine; SF, sulphafurazole; S, streptomycin; TR, trimethoprim; NA, nalidixic acid; O, oxytetracycline; CX, cloxacillin; CE, cefotaxime; K, kanamycin; A, ampicillin; AK, amikacin; AM, amoxicillin; C, chloramphenicol; CB, carbenicillin.

E. coli isolates were detected to possess *bla*TEM, *bla*CTX-M and *bla*OXA, respectively, which was in harmony with a former study (Talukdar *et al.* 2013). None of the isolates harboured *bla*SHV gene. A higher prevalence of CTX-M and TEM-producing *E. coli* was also reported earlier in pigs in India due to extensive use of third-generation cephalosporins or may be related with the high mobilization of the encoding genes (Lalzampuia *et al*. 2013). Among the aminoglycosides resistance genes, the occurrence of *strA, aac(3)IV* and *aadA* genes were 95.2, 26.2 and 71.4%, respectively. The predominance of *strA* was also observed earlier in *E. coli* isolated from pigs (Rosengren *et al*. 2009). With respect to sulphonamides, *sul2* (100%) was more dominant compared to *sul1* (76%) gene, which is concordant with previous reports (Machado *et al*. 2013, Schmiedel *et al*. 2014). However, both the genes in the majority of 32 isolates (76%) were found. Sulphonamides are the recurrently used antibiotic in the treatment of pigs and high prevalence of resistance has been noted in human and food of animal origin. The predominance of *sul2* as compared to *sul1* or *sul3* and its rapid and extensive dissemination has been correlated with widespread plasmid replicon such as IncF. Moreover, *sul* genes carrying plasmid has been established to be the most important source of the non-human pool of reserved genes with the capability of transfer to human (Machado *et al*. 2013, Schmiedel *et al*. 2014). The chloramphenicol resistance gene (*catA*) was detected in 42.9% isolates and phenotypic resistance was observed in 52.3% isolates. It is noteworthy since chloramphenicol is hardly used in pig husbandry. A previous study has noticed the maintenance of chloramphenicol resistance in swine *E. coli* isolates inspite of the absence of chloramphenicol selection pressure due to the connection of chloramphenicol resistance gene with other antibiotics used in pig industry (Bischoff *et al*. 2005). Regarding tetracycline group, *tetA* (92.9%) was more common than *tetB* (50%), which is in line with findings of other researchers (Horton *et al*. 2011). Interestingly, we have noted 50% (21) MDR *E. coli* isolates carrying both *tetA* and *tetB* genes, nevertheless some researcher have deduced the existence of negative

association between these genes, attributing to plasmid incompatibilities. The incidence of tetracycline resistance is often described to be pervasive in *Enterobacteriaceae*, which has been linked to plasmid carrying class 1 integron enabling horizontal transfer to other inter or intra-species bacterial population irrespective of the hosts (Jurado *et al*. 2014). Additionally, we found the association of plasmid-mediated *qnr* genes (59.5% *qnrB;* 19% *qnrS*) with ESBL genes and this concurrence is a nascent trepidation. The degree of resistance conferred by *qnr* gene increases manifold with ESBL, which can be a latent hazard. Such phenomenon has been increasingly described from various countries in arrays of bacterial species and hosts including human, livestock, swine, poultry, companion animals, equine, wild birds, zoo animals from non-clinical or clinical cases (Cattoir *et al*. 2007, Schmiedel *et al.* 2014, Poirel *et al.* 2012).

Mobile genetic elements (Integrons) were detected either singly or in combination of both, i.e. *int1* (32) and *int2* (11). The distribution of targeted genes in MDR *E. coli* isolates is presented in Table 4. The frequency of integron class 1 was much more than class 2 which is in congruence with previous reports (Machado *et al*. 2013, Rajkhowa and Sarma 2014). Transmissibility of integrons is linked to its ability to get mobilized by wide and diverse host range conjugative plasmids or transposons. The propensity of integrons to impound, integrate and articulate gene cassettes corresponding to antibiotic resistance in clinical *E. coli*

Table 4. Presence of AR genes, ESBL genes, integrons and plasmids in 42 MDR *E. coli*

Sample IDs	Antibiotic resistance genes	*bla* genes	Integrons	Plasmids
P1	*tetA, tetB, cat, aad, strA, sul1, sul2, qnrB, qnrS*	TEM, CTX-M	*int1*	+
P2	*tetA, cat, aad, strA, sul1, sul2, qnrB*	TEM, OXA	*int1*	+
P3	*tetA, aad, strA, sul1, sul2, aac*	TEM	–	+
P4	*tetA, cat, aad, strA, sul1, sul2, aac, qnrB*	TEM, CTX–M	*int1 int2*	+
P5	*tetA, tetB, cat, aad, strA, sul1, sul2, aac, qnrB, qnrS*	TEM, CTXM, OXA	*int1 int2*	+
P6	*tetA, tetB, cat, aad, strA, sul1, sul2, qnrS*	TEM, CTX-M	*int1*	+
P7	*tetA, tetB, aad, strA, sul1, sul2, qnrB, qnrS*	TEM, CTX-M, OXA,	*int1 int2*	+
P8	*tetA, aad, strA, sul2, qnrS*	TEM	*int1*	+
P9	*tetA, tetB, aad, strA, sul2, qnrS*	TEM, OXA	*int1*	+
P10	*tetA, tetB, cat, aad, strA, sul1, sul2, aac, qnrB*	TEM OXA, CTX-M	*int1 int2*	+
P11	*tetA, aad, strA, sul2, qnrB*	TEM, CTX-M	*int1*	+
P12	*tetA, tetB, aad, strA, sul1, sul2, qnrB*	TEM, OXA	*int1*	+
P13	*tetA, aad, strA, sul2, qnrB*	TEM, CTX-M	*int1*	+
P14	*tetA, tetB, aad, strA, sul2, aac, qnrB*	TEM	*int1*	+
P15	*tetA, aad, strA, sul1, sul2, qnrB*	TEM, CTX-M	*int2*	+
P16	*tetA, tetB, aad, strA, sul1, sul2*	TEM	*int2*	+
P17	*tetA, tetB, cat, aad, strA, sul1, sul2*	TEM	–	+
P18	*tetA, cat, aad, strA, sul1, sul2, qnrB*	TEM, CTX-M	*int1*	+
P19	*tetA, tetB, cat, aad, strA, sul1, sul2, qnrB*	CTX-M	*int1 int2*	+
P20	*strA, sul2, aac, qnrB*	TEM, CTX-M	*int1*	+
P21	*tetA, cat, strA, sul2*	–	–	–
P22	*tetA, aad, strA, sul2, qnrB*	TEM, CTX-M	*int1 int2*	+
P23	*tetA, strA, sul1, sul2, aac, qnrB*	TEM, CTX-M	*int1*	+
P24	*tetA, cat, strA, sul2, aac, qnrB*	TEM, OXA	*int1*	+
P25	*tetA, cat, aad, strA, sul1, sul2, aac, qnrB, qnrS*	TEM, CTX-M	*int1 int2*	+
P26	*tetA, strA, sul1, sul2, qnrS*	TEM	-	+
P27	*tetA, strA, sul2, qnrB*	TEM, CTX-M	*int1*	+
P28	*sul1, sul2*	–	–	–
P29	*tetA, aad, strA, sul1, sul2*	TEM	–	+
P30	*tetA, tetB, cat, strA, sul1, sul2, qnrB*	TEM, CTX-M	*int1 int2*	+
P31	*tetA, tetB, strA, sul1, sul2, qnrB*	TEM, CTX-M	*int1 int2*	+
P32	*tetA, cat, aad, strA, sul1, sul2*	CTX-M	*int1*	+
P33	*tetA, tetB, cat, aad, strA, sul1, sul2*	TEM	–	+
P34	*tetA, tetB, aad, strA, sul1, sul2, qnrB*	TEM, CTX-M	*int1*	+
P35	*tetA, tetB, cat, aad, sul1, sul2, aac*	CTXM	*int1*	+
P36	*tetA, tetB, cat, aad, strA, sul1, sul2, aac*	TEM, CTX-M	*int1*	+
P37	*tetA, strA, sul1, sul2, qnrB*	TEM, CTX-M	*int1*	+
P38	*tetA, tetB, aad, strA, sul1, sul2, qnrB*	TEM, CTX-M	*int1*	+
P39	*tetA, tetB, cat, aad, strA, sul1, sul2, qnrB*	TEM, CTX-M	*int1*	+
P40	*tetB, aad, strA, sul1, sul2*	TEM, CTX-M	*int1*	+
P41	*tetA, strA, sul1, sul2*	CTX-M	*int1*	+
P42	*tetA, tetB, strA, sul1, sul2*	TEM	–	+

isolates asserts its role as the reservoir (Machado *et al*. 2013).

Out of 42 MDRE isolates, 40 isolates (Table 4) carried one to seven plasmids with molecular sizes ranging from 1 kb to ≥ 25 kb and harboured singly or in combination which is also in concurrence with the reports of Akortha and Filgona (2009), who reported that plasmids of *E. coli* isolated from human patients spread extensively with vast diversity. Majority of the isolates had a distinct pattern of plasmid excepting those harbouring single plasmid. Conjugation experiment was successful for 4 ESBL producers and remaining 3 isolates failed to transfer this phenotype/genotypic trait. It was confirmed by antibiotic susceptibility and *bla* genes (*bla*CTX-M and/or *bla*TEM) detection by PCR. On antibiogram, the transconjugant also exhibited resistance to tetracycline, sulphadiazine, sulphafurazole, and trimethoprim, besides ampicillin, cloxacillin and cefotaxime. Similar observations were made by other workers (Nuesch-Inderbinen *et al*. 1996, Schmitt *et al*. 2007). This may have a putative role in the horizontal spread of resistance genes. As reported earlier, conjugation failure in three isolates may be due to the presence of small-sized plasmids in the donors (Yuan *et al*. 2000).

The results of this study show alarming resistance frequencies in *E. coli* from swine in Northeastern India where both pigs and humans reside in close proximity. The present study also detected higher carriage of ESBL producing *E. coli* in pigs. Many of the ESBL producers carried other antimicrobial resistant genes. ESBL genes could be transferred from the isolates to recipient strains. These findings indicate that resistance to a broad range of antimicrobials is alarmingly circulating among fecal *E. coli* of apparently healthy pigs, and this may constitute an impending reservoir for resistance genes pertinent to public health. Alliance of these resistance genes with mobile genetic elements could be assigned as the basis for dissemination. This definitely warrants the need for an incessant scrutiny in livestock population in diverse agro climatic and socio-economic population representing the region and India en masse for evolving tangible measures to contain the menace. Research priority may be given to AMR surveillance and usage of antibiotic in livestock and poultry farming in India for rational and judicious use of these precious drugs in both human and animal health under a defined regulation.

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