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Approaches to characterize extended spectrum beta-lactamase/beta-lactamase producing *Escherichia coli* in healthy organized *vis-a-vis* backyard farmed pigs in India

Indranil Samanta [a,*], Siddhartha N. Joardar [a], Achintya Mahanti [a], Samiran Bandyopadhyay [b], Tapas K. Sar [c], Tapan K. Dutta [d]

[a] Department of Veterinary Microbiology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal & Fishery Sciences, 37, K.B. Sarani, Belgachia, Kolkata - 700037, WB, India
[b] Eastern Regional Station, Indian Veterinary Research Institute, Belgachia, Kolkata - 700037, WB, India
[c] Department of Veterinary Pharmacology & Toxicology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal & Fishery Sciences, 37, K.B. Sarani, Belgachia, Kolkata - 700037, WB, India
[d] Department of Veterinary Microbiology, Central Agricultural University, Aizawl, Mizoram, India.

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ABSTRACT

The study was undertaken to investigate the occurrence and to characterize the ESBL/beta-lactamase producing-*Escherichia coli* in healthy pigs of organized and backyard farms in West Bengal, India. Total 200 rectal swabs were collected randomly from healthy pigs maintained in four organized farms and 10 backyard farms (n = 100 each) and 76 isolates were identified as *E. coli* from organized (48/100, 48%) and backyard pigs (28/100, 28%). Twelve *E. coli* isolates (6%) in the present study were detected to possess any of the ESBL/beta-lactamase genes studied. ESBL/beta-lactamase producers were isolated with significantly more frequency from backyard pigs than the organized farm pigs ($p = 0.026$). Six of ESBL/beta-lactamase producing isolates were phenotypically confirmed as CTX-M producers and ten of them were confirmed as TEM/SHV producers. PCR and sequencing of the amplified product from representative isolates revealed the presence of $bla_{CTX\text{-}M\text{-}9}$, $bla_{SHV\text{-}12}$ and $bla_{TEM\text{-}1}$. No unique combination of the studied beta lactamase genes for organized and backyard farm pig isolates was noted. The ESBL isolates belonged to O13, O55, O133, O153, O157, O158, O166, rough and OUT serogroups. The association of heat labile toxin (*elt*) ($p < 0.0005$) with organized farm isolates and heat stable toxin (*estA*) ($p = 0.0143$) with backyard piggery sector was significantly higher. The ESBL/beta-lactamase producers from organized farm (Ak/Ex) and indigenous pigs (Ak/Ex/Te; Ak/CoT/G) showed a characteristic phenotypical antibiotic resistance pattern. Two pairs of isolates from organized and backyard farm pigs showed clonal relationship indicating a possible transmission between the farms which were situated adjacently. Thus the present study revealed backyard farm pigs as major source of ESBL/beta-lactamase producing-*E. coli* associated with STa and characteristic antibiotic resistance pattern in India.

1. Introduction

Food animals are quite often implicated as a major reservoir of human enteric pathogens and several human infections were traced following consumption of food products of animal origin. Close association of animals and human and interaction between commensal and pathogenic bacteria present in the animal gut possibly trigger such infection in human patients (Valentin et al., 2014). *Escherichia coli* are one of such commensal bacteria present in the gut of both animals and humans and the pathogenic strains are associated with intestinal and extra-intestinal infection both in human and animals (Samanta, 2013). The commensal *E. coli* present in food animals can also acquire the antibiotic resistance determinants via mobile genetic elements either from the animal itself which are maintained under different antibiotic selection pressures or from the environment (Gonzalez-Zorn and Escudero, 2012). *E. coli* are transmitted to human through the food chain during slaughter, improper cooking and unhygienic handling of food (Frye and Jackson, 2013). Transfer of antibiotic resistance determinants through *E. coli* may complicate the therapeutic regimen in the face of infection as the same families of antibiotics are used in human also (Samanta et al., 2014).

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; CTX-M, Cefotaxime; ECDC, European Centre for Disease Prevention and Control; EMB agar, Eosine Methylene Blue agar; ESBL, Extended spectrum beta-lactamase; ETEC, Enterotoxigenic *Escherichia coli*; LT, Heat labile toxin; ST, Heat stable toxin; SHV, Sulfhydryl variant; UT, Untypeable.

* Corresponding author at: Department of Veterinary Microbiology, West Bengal University of Animal & Fishery Sciences, 37, K.B. Sarani, Belgachia, Kolkata - 700037, WB, India.
E-mail address: isamanta76@gmail.com (I. Samanta).

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The commensal *E. coli* of *healthy* food animals possessing antibiotic resistance determinants is a global concern nowadays. Production of extended-spectrum β-lactamase (ESBL) enzymes is one of the major mechanisms instrumental in enterobacteriaceae family for their resistance which is detected against the penicillin, second, third and fourth generations of cephalosporins and monobactams (except cephamycins and carbapenems). There are three classical ESBLs i.e. TEM (except TEM-1), SHV (except SHV-1 and 2) and CTX-M (EFSA Panel on Biological Hazards, 2011). Among them, CTX-M is observed as the most prevalent type throughout the world (Carattoli, 2013).

Food animals are one of the important sources of ESBL producing *E. coli* in several countries in Europe (Cortés et al., 2010; Hille et al., 2014) and Asia (GuoBao et al., 2009; Hiroi et al., 2012). Healthy pigs among the food animals may play a major role in carriage of such ESBL-producing organisms which can increase the chance of infection in pig attendants, meat handlers as well as consumers of pork or pork products in manifold (Boonyasiri et al., 2014; Hammerum et al., 2014). A positive correlation was observed between ESBL-*E. coli* colonization and an Asian mother tongue with frequent consumption of meat products specially the pork (ECDC, 2011).

Enterotoxigenic *Escherichia coli* (ETEC) are intestinal pathotype of *E. coli* producing heat stable (STa and STb) and heat labile (LT) toxins and are associated with post weaning diarrhoea of piglets, traveller's diarrhoea, infant diarrhoea in human and neonatal watery diarrhoea in other mammals (Samanta, 2013). The O-serogroups of *E. coli* such as O8, O138, O139, O141, O147, O149 and O157 are considered as typical ETEC serogroups commonly detected in pigs (Heo et al., 2013). The presence of virulence genes (*elt*, *estA*/*estI*, *estB*/*estII*) encoding ETEC toxins (LT, STa, STb) is the key requirements for the pathogenicity (Wu et al., 2007). Commensal *E. coli* present in the gut of healthy pigs may also harbour the virulence genes but do not produce infection in their reservoirs due to lack of either correct virulence gene combinations or their expressions in suitable environment (Chapman et al., 2006). A positive linkage between antimicrobial resistance genes and virulence genes (of ETEC) in commensal *E. coli* of the porcine gut was detected (Travis et al., 2006). ETEC present in the gut may integrate plasmid or mobile elements bearing ESBL genes which are poorly investigated.

A few articles are available on detection of ESBL-*E. coli* from healthy or diseased swine population in Asian countries such as China (Duan et al., 2006; Hu et al., 2013), South Korea (Tamang et al., 2013) and Japan (Asai et al., 2011). In India, mostly exotic breeds of pigs are reared by organized farming and indigenous or non-descript breeds are reared by backyard farming and garbage belts (Banik et al., 2011). North-eastern and eastern states such as West Bengal are considered as hub of pig industry. In these states the food habits are favourable for pork consumption chiefly among the North-East inhabitants and Chinese people living in West Bengal (Tiwari and Arora, 2005). Earlier we have reported the occurrence of ESBL producing extraintestinal *E. coli* from bovine and poultry in West Bengal which indicates that food animals from eastern India are additional source of such infection (Kar et al., 2015; Bandyopadhyay et al., 2015).

So, the present study was undertaken to investigate the occurrence of ESBL/beta lactamase producing *E. coli* in healthy pigs with a comparative characterization and clonal relationship of the strains isolated from the organized and backyard farms in West Bengal, India.

2. Materials and methods

2.1. Samples

The rectal swabs (n = 200) were collected from apparently healthy pigs of either sex kept in organized or backyard farm (n = 100 each) in West Bengal (India) in 2012. The sample size was selected as per the earlier report (Hering et al., 2014). The samples were collected from four organized farms (Ao, Bo, Co, Do; n = 25 each) and 10 backyard farms (Ab, Bb, Cb, Db, Eb, Fb, Gb, Hb, Ib, Jb; n = 10 each). The samples were collected from less numbers of organized farms (n = 4) than backyard farms (n = 10) because numbers of organized piggery present in the studied area was comparatively lower than the backyard farms. The number of collected samples from each organized piggery was higher because the average number of pigs maintained in an organized piggery was comparatively higher than a backyard farm. The organized farms were selected on the basis of production level whereas the backyard farms were selected by the local veterinarian based on convenient location and willingness of the farmers to participate in the study. Some of the farms (Ao, Bo, Co, Do and Ab, Bb, Cb, Db) were located in the adjacent place in the same district whereas the other farms (Eb, Fb, Gb, Hb, Ib, Jb) were located in a different district. The rectal swabs were collected from the pigs selected randomly from both types of farms.

The pigs of organized farms were 1–2 years old with history of antibiotic intake such as enrofloxacin and cephalosporins during their sufferings. Incidentally the samples were collected after the antibiotic exposure. The indigenous pigs belonged to 5–6 months of age and all the studied backyard farms had no history of costly antibiotic intake (e.g. cephalosporins) throughout the period the pigs were kept. Occasionally the pigs were treated with tetracycline or gentamicin by local veterinarian or paraveterinarians. The similarity in age group of pigs between two types of farms could not be maintained as indigenous pigs were slaughtered or sold after attaining 6 months of age. The organized piggeries kept the pigs in brick made pens with concrete floors and asbestos shed. The backyard pigs were kept in houses during night only made of bamboo and jute-stick with earthen floors. The organized piggery and backyard farm offered the feed and water in cement-casted and earthen mangers, respectively. The pigs kept in backyard farms roamed the surroundings during daytime.

The rectal swabs were collected with sterile cotton swab sticks (HiMedia, India). The cotton swab sticks were directly put into sterile vials containing Stuart medium (HiMedia, India) for transport. All the samples collected were immediately brought into the laboratory maintaining the cold chain for further examination.

2.2. Isolation and identification of Escherichia coli

In the laboratory the samples were kept in the nutrient broth (HiMedia, India) & incubated at 37 °C for overnight. It was transferred to MacConkey's agar (HiMedia, India) and again incubated at 37 °C for overnight. Next day rose pink colonies (one colony per sample) were randomly picked and transferred to EMB agar (HiMedia, India) followed by an overnight incubation at 37 °C. Colonies were observed for metallic sheen and single colony was streaked into nutrient agar (HiMedia, India) slant for further biochemical confirmation. All the pure cultures obtained from nutrient agar slant were subjected to Gram's staining and standard biochemical tests as described earlier (Samanta et al., 2014).

2.3. Serogrouping

All the *Escherichia coli* isolates after confirmation by biochemical tests were sent for O-serogrouping to Central Research Institute, Kasuli, HP, India.

2.4. Antibiotic sensitivity of E. coli isolates

All the *E. coli* isolates were tested for their sensitivity and resistance to different antibiotics by the disc diffusion method (CLSI, 2008). The antibiotics used were amikacin (30 μg), chloramphenicol (10 μg), enrofloxacin (5 μg), tetracycline (30 μg), co-trimoxazole (25 μg), gentamicin (10 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefpodoxime (10 μg), cefuroxime (30 μg), cefotaxime (30 μg) (Hi Media, India).

2.5. Double disc test

The *E. coli* isolates found resistant (100%) to one or more of the extended spectrum cephalosporins (ceftazidime, ceftriaxone, cefpodoxime, cefuroxime, cefotaxime) were further subjected to double disc test for phenotypic confirmation of ESBL (CTX-M/TEM/SHV) production. Double disc test for detection of CTX-M production was carried out in an agar plate with a disc containing cefotaxime (30 μg, HiMedia) and a disc containing cefotaxime/clavulanate (30 μg/10 μg, HiMedia) placed 30 mm apart (center to center) as described earlier (Brenwald et al., 2003). Similarly for detection of TEM/SHV production double disc test with ceftazidime (30 μg, HiMedia) and ceftazidime/clavulanate (30 μg/10 μg, HiMedia) was carried out as described previously (Bedenic et al., 2007).

2.6. Detection of ESBL genes (bla_{CTX-M}, bla_{TEM}, bla_{SHV}) by PCR

The bacterial DNA was extracted from the *E. coli* isolates by the previously described method (Samanta et al., 2014). All the *E. coli* isolates including controls were subjected to PCR for detection of bla_{CTX-M}, bla_{TEM} and bla_{SHV} genes using the primers and the cycle conditions as described earlier (Table 1, Weill et al., 2004a, 2004b). The primers were procured from Genetix Biotechnology Asia Private Limited. The PCR was carried out in a 25 μl master mixture containing 2.5 μl DNA, 50 pmol of each primers, 200 μM dNTPs, 1.25 U Taq polymerase, 2 mM $MgCl_2$ (Genetix Biotechnology Asia Private Limited). The amplified product was visualized by gel documentation system (UVP, UK) after electrophoresis in 2% (W/V) agarose gel containing ethidium bromide (0.5 μg/ml) (SRL, India) (Sambrook and Russel, 2001). The selected PCR products (one product for each target gene) were sequenced from commercially available sources (Xcelris Genomics, India). The sequence homology searches were conducted using the BLAST algorithm (www. ncbi. nlm. nih. gov/BLAST).

2.7. Detection of ETEC toxin genes by PCR

The genes encoding major ETEC toxins (LT, STa, STb) were detected in all ESBL/beta-lactamase producing-*E. coli* isolates by PCR as per the cycle conditions and primers described earlier (Bandyopadhyay et al., 2011).

2.8. Characterization of ESBL/beta-lactamase producing-E. coli by RAPD-PCR and clonal relationship

All the ESBL/beta-lactamase producing-*E. coli* isolates were characterized by RAPD-PCR to detect the genetic diversity and clonal relationship among the strains. The RAPD-PCR was performed in a thermocycler (Eppendorf) using a single primer (OPB17: AGGGAACGAG, GCC Biotechnology, India) as per the conditions described earlier (Lim et al., 2009). The PCR products were then electrophoresed in 1.5% (w/v) agarose gel containing ethidium bromide (0.5 mg/ml) (Sambrook and Russel, 2001).

All the images taken by the gel documentation system (UVP, UK) were analyzed using the Doc-itLs image analysis software supplied with the system (UVP) as per manufacturer's instruction. An unrooted phylogenetic tree was constructed using neighbour-joining method, available in the software.

2.9. Statistical analysis

Occurrence of ESBL/beta-lactamase producing-producing *E. coli*, association of ETEC toxin genes and antibiotic resistance pattern in different types of pig farms was compared by chi-square test using SPSS software version 17.0 (SPSS Inc.).

3. Results and discussion

From the 200 rectal swabs of healthy pigs, 76 isolates were confirmed as *E. coli* of which 48 were from organized farms (48/100, 48%) and 28 were from backyard sector (28/100, 28%) (Table 2). All the *E. coli* isolates showed characteristic pink coloured colony in MacConkey agar, metallic sheen in EMB agar, gram negative small rod appearance in stained smear, and biochemically they were catalase (+ve), oxidase (−ve), indole-methyl red-voges proskauer-citrate (+ + − −) and urease (−ve). Higher occurrence (97%) of *E. coli* was detected in healthy pigs of organized farms in the Netherlands (Nijsten et al., 1996). In north-eastern India, 100% occurrence of *E. coli* in both healthy and diarrhoeic pigs was observed (Rajkhowa and Sarma, 2014). In the present study, antibiotic exposure of the pigs kept in organized farms before sample collection period and selection of single colony from each sample during isolation of *E. coli* are the probable causes of lower isolation rate. The *E. coli* status in healthy backyard or indigenous pigs of India is not available to compare the present finding. Former study has described the collection of rectal swabs from both organized and indigenous pigs but categorically did not illustrate the number of *E. coli* isolates obtained from both the sectors (Rajkhowa and Sarma, 2014).

From organized farm, *E. coli* isolates belonging to untypeable (UT) serogroup was isolated with maximum frequency (22/48, 45.8%) which was followed by rough (R) (6/48, 12.5%), O147 (4/48, 8.3%), O60 (4/48, 8.3%), O8 (2/48, 4.1%), O11 (2/48, 4.1%), O13 (2/48, 4.1%), O135 (2/48, 4.1%) and O166 (2/48, 4.1%) (Table 2). Whereas, from the indigenous pigs, O55 (5/28, 17.8%), O133 (5/28, 17.8%) and untypeable (UT) (5/28, 17.8%) were isolated most frequently which was followed by O153 (3/28, 10.7%), O157 (3/28, 10.7%) and O158 (3/28, 10.7%) (Table 2). The serogroups O5, O8, O147, UT and R were previously reported in healthy and diarrhoeic pigs from India (Rajkhowa and Sarma, 2014). Most of the enterotoxigenic *E. coli* of porcine origin were reported from the serogroups O8, O147 and O157 (Nagy et al., 1990; Harel et al., 1991). Higher occurrence (10.7%) of O157 in healthy indigenous pigs has public health importance as it is also considered as a major shiga toxin-producing *E. coli* (STEC) serogroup causing hemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) in human (Wani et al., 2004). In other countries also O157 was detected in faecal samples or intestinal contents of slaughtered pigs but with a lower occurrence rate viz. 0.7%–2.7% (Heuvelink et al., 1999; Bonardi et al., 2003). Earlier study in this part of India (West Bengal) detected higher occurrence of O157 in calves (7.5%, Manna et al., 2006). Further, two of the O157 isolates of the present study have both stx_1 and stx_2 (not *eaeA* and *ehxA*) genes indicating their pathogenic potentiality. While till date no human infection with O157 could be traced from pigs.

Table 1
Oligonucleotide primers used in PCR.

PCR target	Oligonucleotide sequence	Product size	Reference
bla_{TEM}	TEM-F:ATAAAATTCTTGAAGACGAAA TEM-R:GACAGTTACCAATGCTTAATC	1080 bp	Weill et al. (2004a)
bla_{SHV}	SHV-F:TTATCTCCCTGTTAGCCACC SHV-R: GATTTGCTGATTTCGCTCGG	795 bp	Weill et al. (2004a)
bla_{CTX-M} consensus	CTX-M-F: CRATGTGCAGYACCAGTAA CTX-M-R: CGCRATATCRTTGGTGGTG	540 bp	Weill et al. (2004b)

Table 2
Serogroups of *E. coli* isolated from organized and backyard farm pigs in West Bengal, India.

Source	Farm	Number of *E. coli* isolated	Serogroup
Organized farm pigs (n = 100)	Ao (n = 25)	15	O8, O13, O60,O147, O166, R (2), UT (8)
	Bo (n = 25)	11	O11, O13, O60, O147, R (2), UT (5)
	Co (n = 25)	11	O8, O60, O135, O147, O166, R, UT (5)
	Do (n = 25)	11	O5, O11, O60, O131, O135, O147, R, UT (4)
	Total	48 (48%)	
Backyard/indigenous pigs (n = 100)	Ab (n = 10)	3	O133, O153, UT
	Bb (n = 10)	4	O55, O133,O157, UT
	Cb (n = 10)	2	O133, O158
	Db (n = 10)	3	O55, O153, UT
	Eb (n = 10)	3	O55, O158, UT
	Fb (n = 10)	2	O8, O157
	Gb (n = 10)	4	O55, O133,O158, UT
	Hb (n = 10)	3	O11, O55, O133,
	Ib (n = 10)	2	O3, O153
	Jb (n = 10)	2	O7, O157
	Total	28 (28%)	

In the present study *E. coli*-isolates were screened for beta lactamase producing strains. Twelve *E. coli* isolates (12/200, 6%) consisting of four (4/100, 4%) from organized farm and eight from backyard farm (indigenous) pigs (8/100, 8%) were detected to possess any of the extended spectrum beta lactamase or beta lactamase genes studied (Table 3). Similar prevalence (5.7%) was detected in faecal samples from healthy pigs in Portugal (Machado et al., 2008). Higher prevalence was observed in faecal samples of healthy pigs kept in organized farms in Germany (37%, von Salviati et al., 2014) and Portugal (49%, Ramos et al., 2013). Lower occurrence (2%–3%) was observed in rectal swabs of healthy pigs in Hong Kong and Japan (Duan et al., 2006; Hiroi et al., 2012). No earlier report was apparently available regarding the prevalence in indigenous pigs to compare the finding. Analyzing the results linked to farms revealed at least one ESBL/beta lactamase-*E. coli* was isolated from two organized farms (50%, Table 3) and eight backyard farms (80%, Table 3), respectively.

Majority of ESBL/beta lactamase producing-*E. coli* (6/200, 3%) isolates were phenotypically confirmed as CTX-M producers by double disc test (cefotaxime and cefotaxime/clavulanate) and were detected to possess $bla_{CTX\text{-}M}$. The sequence of the PCR product was compared and found 98% cognate with $bla_{CTX\text{-}M\text{-}9}$ of *E. coli* from other reports. The primer for $bla_{CTX\text{-}M\text{-}9}$ was selected as the reports of CTX-M-9-*E. coli* is not uncommon in healthy pigs (Escudero et al., 2010; Hansen et al., 2013).

Similarly earlier studies in pigs detected CTX-M producing *E. coli* with maximum frequency than other beta lactamase producing strains (Corte´s et al., 2010; Ramos et al., 2013; Tamang et al., 2013). Previous reports from other parts of the world indicated comparatively higher prevalence of CTX-M-*E. coli* in healthy pigs such as in Switzerland (15%, Geser et al., 2012), China (2–10%, GuoBao et al., 2009) and South Korea (21%, Tamang et al., 2013) than the present finding (3%). Differences in geographical location, sampling pattern and other factors could have influenced the occurrence rate in the present study.

Ten *E. coli* isolates were phenotypically confirmed as TEM/SHV producers in double disc test with ceftazidime and ceftazidime/clavulanate. Among them only one isolate (1/48, 2%) from organized farm pigs was detected to possess bla_{SHV} and nine isolates (9/28, 32.1%) from the backyard pigs possessed both the bla_{SHV} (5/28, 17.8%) and bla_{TEM} (4/28, 14.2%) with or without $bl_{CTX\text{-}M}$ (Table 3). No predominant combination of bla_{SHV} and bla_{TEM} with $bla_{CTX\text{-}M}$ was noted but presence of only bla_{SHV} was detected in three isolates (Table 3). Similarly, lower prevalence of bla_{SHV} and bla_{TEM} was detected in organized pigs earlier (Ramos et al., 2013; Bardoň et al., 2013). Additionally bla_{SHV} alone was also detected earlier in *E. coli* isolated from healthy pigs (Bardoň et al., 2013). Higher association of bla_{TEM} with $bla_{CTX\text{-}M}$ in organized farm pigs was detected in former studies (Tamang et al., 2013; von Salviati et al., 2014) which was not observed in the isolates of organized farm pigs. Nevertheless the combination of bla_{TEM} with $bla_{CTX\text{-}M}$ was detected in a single isolate from the backyard pig in the present study. No unique combination of the studied beta lactamase genes for organized and backyard farm pig isolates was noted in the present study which is in line with the findings of Valentin et al. (2014). The nucleotide sequences of the selected PCR products (bla_{SHV} and bla_{TEM}) revealed 98% similarity with $bla_{SHV\text{-}12}$ and $bla_{TEM\text{-}1}$ in BLAST search. The primers were chosen due to association of $bla_{SHV\text{-}12}$ and $bla_{TEM\text{-}1}$ producing *E. coli* with healthy pigs (Blanc et al., 2006; Escudero et al., 2010; von Salviati et al., 2014). Our previous

Table 3
Serogroup, ESBL/beta lactamase and ETEC toxin gene profile and antibiotic resistance pattern of *E. coli* isolated from organized and backyard farm pigs in India.

	Isolate no	Farm	Serogroup	ESBL/beta lactamase gene profile			ETEC gene profile			Associated antibiotic resistance
				$bla_{CTX\text{-}M}$	bla_{SHV}	bla_{TEM}	*elt*	*estA*	*estB*	
Organized farm pigs	HP/C/9/A	Ao	UT	+	−	−	+	−	−	Ak, Ex
	HP/C/21/E	Ao	O166	−	+	−	+	−	−	−
	HP/C/5/A	Co	O13	+	−	−	+	−	−	Ak, Ex
	HP/C/5/C	Co	UT	+	−	−	+	−	−	Ak, Ex
		Total (2)	4	3	1	0	4	0	0	
Backyard farm pigs	PYT/5A	Ab	R	+	−	+	−	+	−	Ak, Ex, Te
	PYT/5B	Cb	O157	−	+	−	−	+	−	Ak, Ex, CoT
	PMM/3A	Bb	UT	−	+	+	−	+	−	Ak, CoT, G
	PMM/3B	Fb	O153	−	+	−	−	+	−	Ak, Ex, Te
	PMM/4B	Eb	O158	+	−	−	−	+	−	Ak, Ex, CoT
	PGG/4B	Db	UT	+	+	−	−	+	−	Ak, Ex, Te
	PGG/7A	Gb	O133	−	+	+	−	−	+	Ak, CoT, G
	PJP/1B	Hb	O55	−	−	+	−	−	+	Ak, CoT, G
		Total (8)	8	3	5	4	0	6	2	

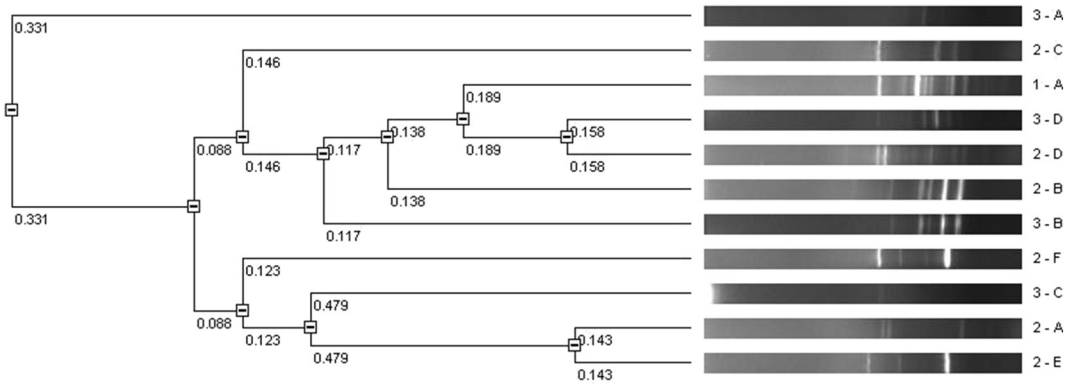

Fig. 1. Phylogenetic analysis of ESBL/beta lactamase-*E. coli* strains isolated from healthy pigs in West Bengal (India). The neighbour-joining method was used to summarize the similarity of RAPD-PCR profiles of ESBL/beta lactamase-*E. coli* strains in a dendogram.

studies have also reported presence of $bla_{SHV\text{-}12}$ and $bla_{TEM\text{-}1}$ producing *E. coli* in cattle and poultry in this region (Kar et al., 2015; Bandyopadhyay et al., 2015).

Comparative assessment revealed that the occurrence of ESBL/beta-lactamase producing-*E. coli* was significantly higher in indigenous pigs (8/28, 28.5%) than the organized farm pigs (4/48, 8.3%) ($p = 0.026$). This could not be related to the use of antibiotics as no history of costly antibiotic (cephalosporins) use was known in indigenous pigs. Overuse of antibiotic and emergence of resistant *E. coli* strains is not always positively correlated (Singer et al., 2008). No strong association could be detected between the prevalence of ceftriaxone resistant *E. coli* and use of ceftiofur (Tragesser et al., 2006). Probably the contaminated environment could be the source of ESBL/beta-lactamase-producing *E. coli* in the present study. The indigenous pigs roamed the surroundings during daytime and were more exposed to the contaminated environment. Previously, von Salviati et al. (2014) reported that the environment including the watering places might be the sources of ESBL/AmpC-*E. coli* infection in the pig farms. Another factor is the age group of indigenous pigs (5–6.5 months) which might enhance the shedding of ESBL-*E. coli* as detected in earlier study (Hansen et al., 2013).

The ESBL/beta-lactamase producing-*E coli* isolated from the studied pigs belonged to O13, O55, O133, O153, O157, O158, O166, rough and untypeable (UT) serogroups (Table 3). Among the serogroups isolated, untypeable was detected with maximum frequency (4/12, 33.3%) as ESBL/beta-lactamase producers. Similarly Mandakini et al. (2015) reported the highest frequency of UT (11/43, 25.5%) followed by O26 (8/43, 18.6%) in ESBL-*E. coli* isolated from diarrhoeic pigs in India.

All the ESBL/beta-lactamase-*E. coli* isolated from organized farm pigs (4) possessed *elt* (4/4, 100%) genes of ETEC and all the ESBL/beta-lactamase producing isolates from indigenous pigs possessed *estA* (6/8, 75%) and *estB* (2/8, 25%) (Table 3). The association of *elt* ($p < 0.0005$) with organized farm isolates and *estA* ($p = 0.0143$) with backyard piggery sector was significantly higher. Such positive correlation of antimicrobial resistance and toxin genes of *E. coli* was reported previously (Travis et al., 2006). Thus antimicrobial resistance generated in an *E. coli* strain can co-select virulence genes making the condition more severe. Furthermore, the heat stable toxin (ST) was more frequently associated with porcine and early childhood diarrhoea (Fairrbother et al., 1989; Shaheen et al., 2004). The indigenous pigs in the present study act as major public health threat due to more association of ESBL/beta-lactamase producing-*E. coli* along with STa.

The ESBL/beta-lactamase producing-*E. coli* isolates from organized farm (Ak/Ex) and indigenous pigs (Ak/Ex/Te and Ak/CoT/G) showed a typical antibiotic resistance pattern although the statistical correlation could not be ascertained due to lower numbers of isolates (Table 3). Similarly, Valentin et al. (2014) observed a common phenotypical antibiotic resistance pattern of ESBL-*E. coli* isolates [$bla_{CTX\text{-}M\text{-}15}$ RRR (resistant for gentamicin, chloramphenicol and sulfamethoxazole/trimethoprim)] in pigs and human.

Comparative analysis of the *E. coli* isolates other than ESBL/beta-lactamase producers (64) from the organized and backyard farm pigs revealed that isolates of backyard pigs were significantly more resistant against chloramphenicol ($p < 0.0001$), amikacin ($p < 0.0001$), co-trimoxazole ($p < 0.0001$), enrofloxacin ($p = 0.0006$), tetracycline ($p = 0.0018$) and gentamicin ($p = 0.0032$) than the isolates of organized farm pigs. *E. coli* isolates from pigs were found resistant to oxytetracycline (83.5%), fluroquinolone (61.9%), aminoglycosides (34%) and chloramphenicol in different studies (Nijsten et al., 1996; Sunde et al., 1998; Geser et al., 2012; Hu et al., 2013). Resistance to chloramphenicol is noteworthy due to its restricted use in pig industry. Earlier study has detected maintenance of chloramphenicol resistance in porcine *E. coli* isolates even in absence of selection pressure due to linkage of chloramphenicol resistance gene with other antibiotics used in pig industry (Bischoff et al., 2005). Such kind of linkage though was not evaluated in the present study. Furthermore, no study with organized farm and indigenous pigs was apparently available to compare the present finding.

All ESBL/beta-lactamase producing-*E. coli* strains (12) were characterized by RAPD-PCR to determine the genetic diversity among the strains. The majority of the strains (11) were typeable with primer OPB17 and they produced 11 different patterns. Two pairs of isolates each from organized and backyard pig farm (HP/C/9/A and PMM/3A, 2D and 3D in Fig. 1 and HP/C/5/C and PGG/4B, 2A and 2E in Fig. 1) were detected in the same cluster indicating their clonal relationship. All the four isolates belonged to untypeable (UT) serogroup, however, no other similarity in *bla* profile, virulence gene profile and phenotypical antibiotic resistance pattern was detected (Table 3). Further, all of them were isolated from the organized (Ao, Co, Table 3) and backyard farms (Bb, Db, Table 3) situated in the adjacent area. The RAPD based clonal relationship between them suggests the possibility of transmission between indigenous pigs and organized farms as observed earlier between the patients in human hospitals (Lim et al., 2009).

The present study detected comparative higher carriage of ESBL/beta-lactamase producing-*E. coli* in backyard farm (indigenous) pigs than the organized farm pigs. Many of the ESBL producers carried ETEC toxin genes. Isolation of O157 serogroup and association of STa with the *E. coli* isolates from backyard farm (indigenous) pigs make them a potential public health hazard. Clonal relationship between the ESBL/beta-lactamase producers of backyard and organized farm pigs and adjacent location of the farms suggests a cross transmission between them.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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