



Detection and characterization of shiga toxigenic *Escherichia coli* from piglets with or without diarrhoea in Mizoram

T K DUTTA¹, P ROYCHOUDHURY², S BANDYOPADHYAY³ and RAJESH CHANDRA⁴

Central Agricultural University, Selesih, Aizawl, Mizoram 796014 India
and

National Research Centre on Yak, Dirang, Arunachal Pradesh 790 101 India

Received: 2 January 2010; Accepted: 20 April 2011

ABSTRACT

Escherichia coli (774) were isolated from faecal samples of piglets with or without diarrhoea from different districts of Mizoram, India. Virulence genes (stx1, stx2, eaeA and hlyA) were detected by multiplex PCR assay. Majority of the isolates were multi-drug resistant. Altogether, 48 serogroups were recorded and O60 was most prevalent. Commonest porcine pathogens under serogroup O8, O103, O108, O138, O141 and O147 were isolated, of which O103 causes haemolytic uremic syndrome in human. Out of 774 isolates, 153 (32.35%) carried at least one virulence gene studied. The virulence gene profiles of *E. coli* from both diarrhoeic and non diarrhoeic piglets were almost similar. Presence of high rate of coliform bacteria in the healthy and diarrhoeic piglets indicates that these animal species may represent as an important reservoir of these organisms for human infection.

Key words: EPEC, Mizoram, Piglets, STEC, Virulence genes

Shiga-toxin-producing *Escherichia coli* (STEC), the cause of several outbreaks of gastrointestinal diseases (haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS), leading to diarrhoea of man and animals, pose a serious threat to public health (Paton and Paton 1998).

In India, there is a paucity of information on STEC and it has not been identified as a significant etiological agent of diarrhoea for humans (Wani *et al.* 2004). Till date, only few reports are available on isolation, identification and characterization of STEC in human and animals (Wani *et al.* 2004, Barman *et al.* 2008). Reports are not available on association of STEC in pigs in this region of country. Present study was undertaken on isolation and characterization of STEC from diarrhoeic and healthy piglets in Mizoram.

MATERIALS AND METHODS

Animals and sampling: Piglets between the age group of 1–8 weeks were studied for presence of STEC/EPEC in their faeces. Rectal swabs were collected from clinically healthy (231) and diarrhoeic (379) piglets during the period from April 2007 to August 2008 from organized and unorganized small farms located at different districts of Mizoram, India. Samples were collected by sterilized cotton swabs and

transported to the laboratory under cold chain.

Isolation and identification of *E. coli*: Samples were inoculated on MacConkey's agar plate and incubated at 37°C for 18–24 hours. Five red-pink coloured lactose fermenting colonies were randomly picked up and subcultured on eosin methylene blue (EMB) agar plates to observe the characteristic metallic sheen of *E. coli*. The well separated pure colonies were inoculated on nutrient agar slants as pure culture and subjected for standard morphological and biochemical tests (Edwards and Ewing 1972).

Serotyping of *E. coli*: Entire *E. coli* isolates were serotyped based on their somatic (O) antigens at National Salmonella and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh, India.

Antibiogram of *E. coli*: All the isolates were subjected to *in vitro* antibiotic sensitivity assay as per Kirby Bauer disc diffusion method (Bauer *et al.* 1966) employing 18 commonly used antibacterial agents.

Preparation of *E. coli* DNA for PCR assay: Isolates were inoculated to 2 ml Luria Bertani (LB) broth and incubated at 37°C under constant shaking for 18 h. After incubation, 1 ml broth was taken in a 1.5 ml microcentrifuge tube and centrifuged at 8000 rpm for 10 min. The pellet was washed twice in sterilized NSS and re-suspended in 400 µl of nuclease free sterilized distilled water and boiled for 10 min followed by immediate chilling. Cell debris was removed by

Present address: ^{1,2,4}(e mail: tapandutta@rediffmail.com)
Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry

Table 1. Oligonucleotide primers used in multiplex PCR assay

Primers	Primer sequences	References
<i>stx</i> ₁ F	5'-ATA AAT CGC CAT - TCG TTG ACT AC-3'	Paton and Paton (1998)
<i>stx</i> ₁ R	5'- AGA ACG CCC ACT - GAG ATC ATC -3'	
<i>stx</i> ₂ F	5'- GGC ACT GTC TGA AAC TGC TCC -3'	Paton and paton (1998)
<i>stx</i> ₂ R	5'- TCG CCA GTT ATC - TGA CAT TCT G -3'	
<i>eaeA</i> F	5'- GAC CCG GCA CAA GCA TAA GC -3'	Paton and paton (1998)
<i>eaeA</i> R	5'- CCA CCT GCA GCA - ACA AGA GG -3'	
<i>ehxA</i> F	5'- GCA TCA TCA AGC - GTA CGT TCC -3'	Paton and paton (1998)
<i>ehxA</i> R	5'-AAT GAG CCA AGC- TGG TTA AGC T-3'	

centrifugation at 2000 rpm for 5 min. The supernatant was used as template DNA for the PCR reaction.

Detection of virulence genes by multiplex PCR: A multiplex PCR was carried out using 4 sets of oligonucleotide primers for *stx*₁, *stx*₂, *eaeA* and *hlyA* genes. The detail of the primers used in this study is depicted in Table 1. The multiplex PCR mixture of 25 µl contained 1X PCR buffer, 1.5 mM of MgCl₂, each primer within the four primer sets at a concentration of 40 nM, 200 µM each of dNTPs, 1.0 U of Taq DNA polymerase and 2.0 µl of template DNA. The PCR reaction was performed in a thermal cycler using standard

cycling condition: an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 59°C for 1 min and extension at 72°C for 1 min and a final extension at 72°C for 6 min. Amplified products were separated by agarose gel (2% in 1X Tris-borate-EDTA buffer) electrophoresis at 5v/cm for 2 h and stained with ethidium bromide (0.5 µg/ml). Standard molecular size marker (100 bp DNA ladder) was included in each gel. DNA fragments were observed by ultra violet transilluminator and photographed in a gel documentation system. The PCR reaction was performed 3 times to ensure the repeatability of the techniques and to make sure that strains were correctly assigned to respective patterns.

RESULTS AND DISCUSSION

Bacterial isolation and characterization: A total of 774 *E. coli* were isolated from 610 faecal samples. Of 473 *E. coli* isolates from 379 piglets with diarrhoea, 318 belonged to 48 serogroups, 147 were untypable and rests 8 were rough strains. Similarly, of 301 *E. coli* strains from 231 healthy piglets, 199 belonged to 42 serogroups, 98 were untypable and 4 were rough strains.

Antimicrobial sensitivity test: The result of antimicrobial sensitivity patterns of *E. coli* isolates against 18 commonly used antimicrobial agents are presented in Table 2. Majority of the STEC strains exhibited high frequency of resistance to ampicillin (84.56%), cefixime (81.62%), erythromycin (83.82%), lincomycin (89.71%), nalidixic acid (87.50%), oxytetracyclin (82.36%), penicillin (83.09%), roxythromycin (83.82%), streptomycin (74.26%) and sulphadiazine

Table 2. Antimicrobial sensitivity/resistance pattern of STEC and EPEC against 20 commonly used antibacterial agents

Antimicrobial agents	Concentration	Strains (%) sensitive		Strains (%) intermediate		Strains (%) resistance	
		STEC (n= 136)	EPEC (n= 85)	STEC (n= 136)	EPEC (n= 85)	STEC (n= 136)	EPEC (n= 85)
Amoxycillin (Am)	10 µg	98(72.06)	69(81.18)	32(23.53)	13(15.29)	6(4.41)	3(3.53)
Ampicillin (A)	10 µg	10(7.35)	5(5.88)	11(8.09)	2(2.36)	115(84.56)	78(91.76)
Cefixime (Cfx)	5 µg	8(5.88)	3(3.53)	13(9.56)	11(12.94)	111(81.62)	71(83.53)
Cephataxime (Ce)	10 µg	88(64.71)	57(67.06)	14(10.29)	8(9.41)	34(25.00)	20(23.53)
Chloramphenicol (C)	10 µg	76(55.88)	49(57.65)	17(12.50)	13(15.29)	43(31.62)	23(27.06)
Chlortetracycline (Ct)	30 µg	91(66.91)	68(80.00)	8(5.88)	5(5.88)	37(27.21)	12(14.12)
Ciprofloxacin (Cf)	10 µg	79(58.09)	58(68.24)	17(12.50)	6(7.06)	40(29.41)	21(24.71)
Erythromycin (E)	10 µg	11(8.09)	7(8.24)	11(8.09)	5(5.88)	114(83.82)	73(85.88)
Enrofloxacin (Ex)	10 µg	95(69.85)	69(81.18)	17(12.50)	12(14.12)	24(17.65)	4(4.71)
Gentamycin (G)	10 µg	32(23.53)	19(22.35)	5(3.67)	38(44.71)	49(36.03)	18(21.18)
Lincomycin (L)	10 µg	6(4.41)	3(3.53)	8(5.88)	7(8.24)	122(89.71)	75(88.24)
Nalidixic acid (Na)	30 µg	3(2.21)	3(3.53)	14(10.29)	11(12.94)	119(87.50)	61(71.76)
Oxytetracyclin (O)	30 µg	13(9.56)	9(10.59)	11(8.09)	9(10.59)	112(82.36)	67(78.82)
Penicillin (P)	10 µg	15(11.03)	12(14.12)	8(5.88)	9(10.59)	113(83.09)	64(75.29)
Roxythromycin (Ro)	30 µg	5(3.67)	5(5.88)	17(12.50)	12(14.12)	114(83.82)	68(80.00)
Streptomycin (S)	10 µg	22(16.18)	11(12.94)	13(9.56)	9(10.59)	101(74.26)	65(76.47)
Sulfadiazine (Sz)	100 µg	15(11.03)	11(12.94)	3(2.21)	7(8.24)	118(86.76)	67(78.82)
Tetracycline (T)	10 µg	19(13.97)	11 (12.94)	60 (44.12)	36 (42.35)	57(41.91)	38(44.71)

Figures in parenthesis indicate the percentage

(86.76%). similarly, most of the EPEC strains also showed high resistance against ampicillin (91.76%), cefixime (83.53%), erythromycin (85.88%), lincomycin (88.24%), nalidixic acid (71.76%), oxytetracyclin (78.82%), penicillin (75.29%), roxythromycin (80.00%), streptomycin (76.47%) and sulphadiazine (78.82%). The most effective antimicrobial agents in terms of sensitivity were amoxycillin (72.06%) followed by enrofloxacin (69.85%), chlortetracycline (66.91%), cephotaxime (64.71%), ciprofloxacin (58.09%) and chloramphenicol (55.88%) for STEC strains. Similarly, for EPEC strains, the best antimicrobial agents were amoxycillin and enrofloxacin (81.18%) followed by chlortetracycline (80.00%), ciprofloxacin (68.24%), cephotaxime (67.06%), and chloramphenicol (57.65%).

Serogrouping of *E. coli* strains: Amongst the 774 *E. coli* isolates 48 serogroups were recorded; O60 was the most prevalent serogroup, and 136 STEC and 85 EPEC isolates belonged to 39 and 31 serogroups, respectively. Result of serotyping is given in Table 3.

Multiplex PCR for virulence genes: Multiplex PCR assay gave amplified products of ~180 bp, ~255bp, ~384bp and ~534 bp specific for stx1, stx2 and eaeA and hlyA genes, respectively. Out of 473 *E. coli* isolates from piglets with diarrhoea, 153 (32.35%) carried at least one virulence gene studied, of which 91 (19.24%) and 62 (13.11%) isolates were detected as STEC and EPEC, respectively. Amongst the 301 *E. coli* isolates from piglets without diarrhoea, 68 (22.59%) carried at least one virulence gene of which, 45 (19.48%) and 23 (7.64%) were STEC and EPEC, respectively. Of 136 (17.57%) STEC isolates from piglets with or without diarrhoea, 39 (5.04%) carried only stx1, 11 (1.42%) had only

stx2, 45 (5.81%) were with both stx1 and stx2, 26 (3.36%) carried stx1, stx2 and hlyA and 19 (2.45%) carried stx1, stx2, eaeA and hlyA genes. Similarly, of 85 (10.98%) EPEC isolates, 66 (8.53%) carried only hlyA gene and 19 (2.45%) had both eaeA and hlyA genes. Details of the virulence genes profile amongst STEC and EPEC is given in Table 4.

STEC in animals including cattle (Pal *et al.* 1999), sheep (Wani *et al.* 2004, Bhat *et al.* 2008), fish (Kumar *et al.* 2001) as well as from beef (Khan *et al.* 2002) and human faeces (Chattopadhyaya *et al.* 2001, Khan *et al.* 2002) is limited. Information on prevalence of STEC and EPEC in pigs is rare in India. In Mizoram, pork is the major source of animal protein and people used to rare 5–10 pigs near to their house and share the common source of water. Therefore, chances of getting infection of STEC/EPEC/EHEC through contaminated water are very high.

In this study, majority of the STEC and EPEC strains expressed the resistance to more than one antimicrobial agent (Table 2). Schroeder *et al.* (2002) reported the highest prevalence of antimicrobial resistance among swine O157 isolates, where greater than 50% of all isolates were resistance to sulfamethoxazole, cephalothin, tetracycline and more than 20% isolates were resistance to ampicillin and gentamicin. Similar types of observations were also reported by other workers from Japan (Uemura *et al.* 2003) and Korea (Choi *et al.* 2002). Multiple antimicrobial resistances in STEC and non STEC may partly result from the spread of genetic elements including plasmids, transposons and integrons. Resistant bacteria may be readily transferred from food animals to humans (Schroeder *et al.* 2002).

Of 48 different serogroups recorded from 774 *E. coli*

Table 3. Serogroups of *E. coli* isolated from piglets with or without diarrhoea in Mizoram

Piglets screened	<i>E. coli</i> isolated	Serogroups
Piglets with diarrhoea (379)	473	UT(147), O60(37), O159(20), O20(19), O103(12), O138(12), O69(11), O89(11), O21(9), O63(9), O79(9), R(8), O14(7), O54(7), O100(7), O4(5), O17(5), O98(5), O108(5), O109(5), O146(5), O149(5), O162(5), O45(4), O142(4), O41(3), O51(3), O85(3), O132(3), O141(3), O166(3), O168(3), O9(2), O10(2), O34(2), O48(2), O75(2), O80(2), O84(2), O90(2), O101(2), O105(2), O112(2), O116(2), O147(2), O155(2), O169(2), O8(1), O24(1), O97(1)
Piglets with out diarrhoea (231)	301	UT(98), O60(25), O159(16), O20(14), O103(11), O162(9), O14(8), O89(8), O138(8), O69(7), O79(7), O21(6), O54(6), O17(5), O51(5), O100(5), O108(5), O63(4), O146(4), R(4), O4(3), O85(3), O116(3), O149(3), O34(2), O41(2), O45(2), O80(2), O90(2), O98(2), O101(2), O109(2), O132(2), O141(2), O142(2), O166(2), O168(2), O169(2), O10(1), O48(1), O84(1), O105(1), O112(1), O155(1),
610	774	UT(245), O60(62), O159(36), O20(33), O103(23), O138(20), O89(19), O69(18), O79(16), O14(15), O21(15), O162(14), O54(13), O63(13), O100(12), R(12), O17(10), O108(10), O146(9), O4(8), O51(8), O149(8), O98(7), O109(7), O45(6), O85(6), O142(6), O41(5), O116(5), O132(5), O141(5), O166(5), O168(5), O34(4), O80(4), O90(4), O101(4), O169(4), O10(3), O48(3), O84(3), O105(3), O112(3), O155(3), O9(2), O75(2), O147(2), O8(1), O24(1), O97(1)

UT, untypable; R, rough.

isolates, O60 was the predominant. Schierack *et al.* (2006) reported that porcine pathogenic *E. coli* strains belong to the limited number of serogroups, with O8, O108, O138, O139, O141, O149 and O157. Barman *et al.* (2008) also reported that most of the oedema causing STEC in pigs belongs to the serogroup O138, O139, O141 and O147 as well as few untypable strains. In our study, O8, O103, O108, O138, O141 and O147 have been isolated from diarrhoeic pigs but O8 and O147 could not be detected from healthy pigs. Pigs in this region are potential reservoir of pathogenic *E. coli*. Except serogroup O103 none of the other serogroups (O26, O111, O113, O145 and O157) responsible for HUS in human being were detected in this study. But isolation of high level of serogroup O103 STEC from both diarrhoeic (12) and non diarrhoeic (11) pigs warrants the chance of transmission of this pathogen from pig to human being in this region and is a potential public health concern.

Of 774 *E. coli* isolates tested in this study, 159 (20.54%) carried at least one virulence gene studied, of which 140 (18.09%) and 19 (2.45%) strains were detected as STEC and EPEC, respectively. Prevalence of *stx1* gene in STEC isolates is higher than *stx2* genes, which are in agreement with the findings of other investigators in India and abroad, who also mentioned the similar trend in STEC from animals in India (Bhat *et al.* 2008), Australia (Djordjevic *et al.* 2001) and Spain (Orden *et al.* 2003), respectively.

Of 473 *E. coli* isolates from 379 piglets with diarrhoea 107 carried at least one virulence gene studied. Ninety one (19.24%) and 16 (3.38%) isolates were detected as STEC and EPEC, respectively. Similarly, of 301 *E. coli* isolates from 231 healthy piglets, 52 carried at least one virulence gene studied. 49 (16.28%) and 3 (0.1%) isolates were detected as STEC and EPEC, respectively (Table 4). Because of non availability of any previous data, our result could not be compared in respect of prevalence of STEC and EPEC in pigs in India. There is only one published report on detection of STEC from pigs (Barman *et al.* 2008). They have reported an outbreak of oedema disease in an organized pig farm in Asom and association of STEC. Overall incidence of STEC and EPEC is variable in different domestic animals as

Table 4. Distribution of virulence genes in *E. coli* isolated from piglets with or without diarrhoea in Mizoram

Virulence genes	No of isolates		
	Diarrhoeic	Non-diarrhoeic	Total
<i>stx1</i> only	20 (4.23)	19 (8.23)	39 (5.04)
<i>stx2</i> only	11 (2.33)	—	11 (1.42)
<i>stx1</i> , <i>stx2</i>	32 (6.77)	13 (5.63)	45 (5.81)
<i>eaeA</i> , <i>ehxA</i>	16 (3.38)	3 (1.30)	19 (2.45)
<i>stx1</i> , <i>stx2</i> , <i>ehxA</i>	10 (2.11)	16 (6.93)	26 (3.36)
<i>stx1</i> , <i>stx2</i> , <i>eaeA</i> , <i>ehxA</i>	18 (3.81)	1 (0.04)	19 (2.45)
Total	107(22.62)	52(17.28)	159 (20.54)

Figures in parenthesis indicate the percentage.

published by other workers (Bhat *et al.* 2008).

Only 5.81% of STEC isolates were contained both *stx1* and *stx2* genes which were lower than the earlier report by other workers (Bhat *et al.* 2008) in which they have reported 45.7% of STEC with both *stx1* and *stx2* genes in lambs. But rare information is available in pigs in India to compare our findings. A total of 19 (2.45%) EPEC isolates carried both *eaeA* and *hlyA* genes, rare information but none of the isolates were carried *eaeA* gene alone. The major pathogenicity of EPEC is mainly mediated by the products of the locus of enterocyte effacement (LEE), pathogenicity island, with the *eae* gene that encodes for the intimin protein involved in the intimate adhesion of bacteria to enterocytes and production of AE lesion on the intestinal mucosa (Paton and Paton 1998). Besides, a specific plasmid encoded haemolysin called EHEC haemolysin, which is encoded by *hlyA* gene, might contribute to the virulence of STEC in humans. Therefore, presence of such kind of *E. coli* in pig's environment might increase the possibility of infection to the human beings. Presence of even low level of *eae* gene in STEC isolates should not be overlooked because some *eae* negative STEC have been isolated from human beings with clinical symptoms (Kumar *et al.* 2004).

The virulence factor gene profiles of *E. coli* from both diarrhoeic and non diarrhoeic piglets were almost similar, making it difficult to identify a particular clone as the causative agent of the diarrhoea. In addition, in case of healthy piglets, the virulence genes of the *E. coli* isolates were either not or only very poorly expressed in the intestine, as a result the ability of those *E. coli* isolates to cause disease might be suppressed or these *E. coli* isolates represented a very small fraction of the total intestinal flora (Schierack *et al.* 2006).

ACKNOWLEDGEMENTS

The authors are highly thankful to the Director National Salmonella and Escherichia Center, CRI, Kasauli, HP, India for serotyping the isolates, Dr S A Wani, FVSc&AH, SKUAST-K, Alusteng, Srinagar, Jammu & Kashmir for providing standard cultures and to the Dean, CVSc&AH, CAU, Selesih, Aizawl, Mizoram for providing necessary funds and facilities to carry out the investigations.

REFERENCES

- Barman N N, Reema D, Ramamurthy T, Sharma R K, Borah P, Wani S A and Kalita D. 2008. Molecular characterization of shiga like toxin - producing *Escherichia coli* (STEC) isolates from pigs oedema. *Indian Journal of Medical Research* **127**: 602–06.
- Bauer A W, Kirby W M, Sherriz J C and Tuck N. 1966. Antibiotic susceptibility testing by standardized single disc method. *American Journal of Clinical Pathology* **45**: 493–96.
- Bhat M A, Nishikawa Y and Wani S A. 2008. Prevalence and virulence gene profiles of Shiga toxin - producing *Escherichia coli* and enteropathogenic *Escherichia coli* from diarrhoeic and

- healthy lambs in India. *Small Ruminant Research* **75**: 65–70.
- Chattopadhyaya U K, Dutta S, Deb A and Pal D. 2001. Verotoxin producing *Escherichia coli* - an environment induced emerging zoonosis in and around Calcutta. *Internal Journal of Environmental Health Research* **1**: 107–12.
- Choi C, Ham H J, Kwon D, Kim J, Cheon D S and Min K. 2002. Antimicrobial susceptibility of pathogenic *Escherichia coli* isolated from pigs in Korea. *Journal of Veterinary Medical Science* **64**: 71–73.
- Djordjevic S P, Hornitzky M A, Bailey G, Gill P, Vanselow B, Walker K and Battelheim K A. 2001. Virulence properties and serotypes of Shiga toxin - producing *Escherichia coli* from healthy Australian slaughter age sheep. *Journal of Clinical Microbiology* **39**: 2017–21.
- Edwards P R and Ewing W H. 1972. *Identification of Enterobacteriaceae*. 3rd edn. Burgess Publishing Company, Minneapolis, M N.
- Khan A, Das S C, Ramamurthy T, Sikdar A, Khanan J, Yamasaki S. 2002. Prevalence and genetic profiling of virulence determinants of non-O157 Shiga-toxin producing *Escherichia coli* isolated from cattle, beef and humans, Calcutta, India. *Emerging Infectious Diseases* **8**: 54–62.
- Kumar S H, Otta S K, Karunasagar I and Karunasagar I. 2001. Detection of Shiga-toxigenic *Escherichia coli* (STEC) in fresh seafood and meat marketed in Mangalore, India by PCR. *Letters in Applied Microbiology* **33**: 334–38.
- Kumar S H, Karunasagar I, Karunasagar I, Teizou T, Shima K and Yamasaki S. 2004. Characterization of Shiga toxin - producing *Escherichia coli* (STEC) isolated from seafood and beef. *FEMS Microbiology Letter* **233**: 173–78.
- Orden J A, Ruiz-Santa-Quiteria J A, Blanco M, Blanco J E, Mora A, Cid D, Gonzalea G A. 2003. Prevalence and characterization of verocytotoxin - producing *Escherichia coli* isolated from diarrhoeic and healthy sheep and goats. *Epidemiology and Infections* **130**: 313–21.
- Paton J C and Paton A W. 1998. Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohaemorrhagic *E. coli* hlyA, rfbO111 and rfbO157. *Journal of Clinical Microbiology* **36**: 598–602.
- Schierack P, Steinruck H, Kleta S and Vahjen W. 2006. Virulence factors gene profiles of *Escherichia coli* isolates from clinically healthy pigs. *Applied Environmental Microbiology* **72**: 6680–86.
- Schroeder C M, Zhao C, DebRoy C, Torcolini J, Zhao S. 2002. Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine and food. *Applied Environmental Microbiology* **68**: 576–81.
- Uemura R, Sueyoshi M, Nagayoshi M and Nagatomo H. 2003. Antimicrobial susceptibilities of Shiga toxin- producing *Escherichia coli* isolates from pigs with edema disease in Japan. *Microbiology and Immunology* **47**: 57–61.
- Wani S A, Pandit F, Samanta I, Bhat M A. and Buchh A S. 2004. Molecular epidemiology of Shiga toxin- producing *Escherichia coli* in India. *Current Science* **87**: 1345–53.