P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Short Communication

Prevalence and Antimicrobial Susceptibility Pattern of Shiga Toxin Producing *Escherichia coli* (STEC) from Pigs and Cattle

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Article History: Received: February 19, 2015 Revised: June 01, 2015 Accepted: June 06, 2015

ABSTRACT

Shiga toxin-producing *E. coli* (STEC) is one of the emerging zoonotic foodborne pathogens with great public health importance. It can be found in the faecal flora of a wide variety of animals. The present study was under taken to investigate the prevalence and antibiotic sensitivity pattern of STEC from cattle and pig faecal samples. The prevelance of STEC from cattle fecal samples was recorded as 12(16.21%) out of 74 *Escherichia coli* isolates and in pig it was recorded to be 6(6.74%) out of 89 *Escherichia coli* isolates. In- vitro susceptibility testing of STEC isolates from cattle against 15 different antimicrobial agents showed highest sensitivity towards ciprofloxacin (100%) followed by norfloxacin (91.67%), chloramphenicol (83.33%), nalidixic acid (83.33%), co- trimoxazol (75%) and cephotaxim (66.67%), gentamicin (58.33%), streptomycin (58.33%), enrofloxacin (41.67%), tetracycline (33.33%) and amoxicillin (16.67%). Resistance pattern was seen towards amikacin, ampicillin, furazolidone and cloxacillin. While STEC isolates from pigs showed highest sensitivity to ciprofloxacin (100%) followed by norfloxacin (100%), chloramphenicol (83.33%), cephotaxime (83.33%), nalidixic acid (66.67%), co-trimoxazole (66.67%), gentamicin (50%), streptomycin (33.33%), enrofloxacin (33.33%), amoxicillin (16.67%) and tetracycline (16.67%). The resistance pattern was seen towards amikacin, furazolidone, ampicillin and cloxacillin. The resistance pattern of the bacterial strain against number of antibiotics has become one of the major public health concerns.

Key words: Shiga toxin producing *Escherichia coli*, Sensitivity, Emergence, Susceptibility, Resistance

INTRODUCTION

Escherichia coli (E. coli) a member of family *Enterobacteriaceae* is a short gram negative, non-spore forming and usually peritrichous and fimbriate bacillus. Verotoxin-producing *E. coli* (VTEC) is also termed as shiga-like toxin producing *E. coli* (SLTEC) or shiga toxin producing *E. coli* or STEC (Paton and Paton, 1998). There are at least 200 serotypes of *E. coli* that are capable of producing shiga toxins (Johnson *et al.*, 1996) however of these serotypes *E. coli* 0157:H7 is the most well known to both microbiologists and the general public. This organism was first recognized in 1982 following an outbreak of haemorrhagic colitis (HC) in the USA (Riley *et al.*, 1983). Since then, STEC O157 has been implicated in sporadic cases and outbreaks of diarrhoea, haemorrhagic colitis and haemolytic syndrome world-wide.

Shiga toxin-producing *E. coli* can be found in the faecal flora of a wide variety of animals including cattle, sheep, goats, pigs, cats, dogs, chickens (Johnson *et al.*, 1996; Wallace *et al.*, 1997). High rates of colonization of shiga toxin positive *E. coli* have been found in bovine herds in many countries (Burnens *et al.*, 1995). Though STEC strains from diarrhoeic calves, lambs, infants and beef have been isolated from India (Khan *et al.*, 2002; Chattopadhyay *et al.*, 2003; Wani *et al*., 2004).

There has been considerable increase in the incidence of drug resistance in bacteria due to extensive and indiscriminate use of antimicrobial agents for therapy, prophylaxis or growth promotion in recent years. The emergence of drug resistant strains following continuous feeding of antibiotics to livestock has led to loss of their efficacy (Singh *et al.*, 1992).

This combination of virulence coupled with multiple drug resistance has posed an increasing threat to successful treatment of *E. coli* related veterinary diseases. This suggests that the use of antibiotics in animals could lead to a reservoir of antibiotic-resistant bacteria that could potentially infect humans.

Cite This Article as: Begum S, GC Hazarika and S Rajkhowa, 2015. Prevalence and antimicrobial susceptibility pattern of Shiga toxin producing *Escherichia coli* (STEC) from pigs and cattle. Inter J Vet Sci, 4(4): 221-223. www.ijvets.com

Hence the present study was carried out with the objective to isolate shiga toxin producing *E.coli* from cattle and pig and further characterization of their susceptibility patterns to different antibiotics.

MATERIALS AND METHODS

The samples were collected aseptically in sterile vials. A total of 150 and 110 rectal swabs were collected from swine and cattle farm (Table1). The samples were collected aseptically in sterile vials and transported in an icebox to laboratory for further processing and microbiological analysis. Mac Conkey's broth (Hi-Media) was used as enrichment medium. All the samples collected were inoculated into 5-10 ml of the broth and incubated at 37^0 C for 24 hours. Mac Conkey's lactose agar media (MLA) (Hi-Media) was used for primary isolation of *Escherichia coli***.** Eosin Methylene Blue agar media was used for purification of colonies. The morphological characteristics of the isolates were studied after staining the fresh culture smears by gram's staining method.

The bacterial isolates were subjected to *in vitro* antibiotic sensitivity test as per the method of Bauer *et al* (1966). Positive isolates were grown in Brain Heart infusion (BHI) broth overnight and plates of Mueller Hinton (MH) agar were seeded with about one ml of inoculum. The incoulum was allowed to dry. Antibiotic discs were placed on inoculated agar surface at about two cm apart. The plates were incubated at 37^0 C overnight and diameter of the zones of inhibition was measured and values obtained from the National Committee on Clinical Laboratory Standards (NCCLS, 1999) were used to interpret the results obtained.

RESULTS

In this study it was found that out of 74 *Escherichia coli* isolates 12(16.21%) were positive for STEC and in case of pig out of 89 *E.coli* isolates 6(6.74%) were positive for STEC (Table 1).

Antimicrobial sensitivity and resistance pattern of STEC isolates obtained from cattle

In- vitro susceptibility testing of STEC isolates from cattle showed highest sensitivity towards ciprofloxacin (100%) followed by norfloxacin (91.67%), chloramphenicol (83.33%), nalidixic acid (83.33%), co-trimoxazole (75.00%) and cephotaxime (66.67%) and all the isolates were resistance towards amikacin, ampicillin, furazolidone and cloxacillin, respectively (Table 2).

Antimicrobial sensitivity and resistance pattern of STEC isolates from Pigs

In- vitro susceptibility testing of STEC isolates obtained from pigs showed highest sensitivity to ciprofloxacin (100%) and norfloxacin (100%) followed by chloramphenicol (83.33%), cephotaxime (83.33%), co-trimoxazole (66.67%) and nalidixic acid (66.67%) and all the isolates were resistant towards amikacin, furazolidone, ampicillin and cloxacillin, respectively (Table 2).

DISCUSSION

Shiga toxin producing *E.coli* (STEC) are zoonotic agents which cause a potentially fatal human illness. It is recognized as an important food borne pathogen, responsible for sporadic cases to serious outbreak worldwide (Wani *et al*., 2004). Currently there is a little information available regarding the epidemiology of STEC in domestic animals in India.

In-vitro susceptibility testing of STEC isolate against fifteen different antimicrobial agents showed highest sensitivity towards ciprofloxacin followed by norfloxacin, co-trimoxazole, chloramphenicol and cephotaxime. Resistance pattern was seen towards amikacin, ampicillin, furazolidone and cloxacillin. Sensitivity of STEC isolates to ciprofloxacin, norfloxacin, chloramphenicol, nalidixic

Table 1: Prevalence of STEC in faecal samples of cattle and pigs

Animals	Total no. of fecal samples collected	No. of Positive *Escherichia coli* samples	No. of STEC positive samples	Percent positive (%) of STEC isolates
Cattle	110	74	12	16.21
Pig	150	89	6	6.74

Table 2: In-vitro antimicrobial drug sensitivity and resistance pattern of STEC isolates from cattle and pigs.

Antibiotics Discs	Number of STEC isolates from cattle (N=12)				Number of STEC isolates from pig (N=6)			
	Sensitivity		Resistance		Sensitivity		Resistance	
	No	% positive	No	% positive	No	% positive	No	% positive
Amikacin	0	0.00	12	100	0	0.00	6	100
Gentamicin	7	58.33	5	41.67	3	50	3	50
Ciprofloxacin	12	100	0	0.00	6	100	0	0.00
Furazolidone	0	0.00	12	100	0	0.00	6	100
Tetracycline	4	33.33	8	66.67	1	16.67	5	83.33
Norfloxacin	11	91.67	1	8.33	6	100	0	0.00
Chloramphenicol	10	83.33	2	16.67	5	83.33	1	16.67
Ampicillin	0	0.00	12	100	0	0.00	6	100
Co-Trimoxazole	9	75.00	3	25	4	66.67	2	33.33
Cloxacillin	0	0.00	12	100	0	0.00	6	100
Streptomycin	7	58.33	5	41.67	2	33.33	4	66.67
Cephotaxime	8	66.67	4	33.33	5	83.33	1	16.67
Amoxicillin	2	16.67	10	83.33	1	16.67	5	83.33
Nalidixic acid	10	83.33	2	16.67	4	66.67	2	33.33
Enrofloxacin	5	41.67	7	58.33	2	33.33	4	66.67

acid and co-trimoxazole was also reported by various authors (Puii, 2005 and Baruah, 2005). Resistance of STEC isolates to ampicillin and cloxacillin as observed in the present study was also reported by many workers (Puii, 2005 and Baruah, 2005).

In-vitro susceptibility testing of STEC isolates from pigs against 15 different antimicrobial agents showed highest sensitivity to ciprofloxacin followed by norfloxacin and chloramphenicol and the resistance pattern was seen towards amikacin, furazolidone, ampicillin and cloxacillin. Baruah (2005) also recorded highest sensitivity of the isolates to norfloxacin, ciprofloxacin, chloramphenicol and nalidixic acid. He also observed that the isolates were resistance to ampicillin and furazolidone.

High resistance of STEC isolates against common antibiotics observed in this study may be due to indiscriminate usuage of antimicrobial agents for therapy, prophylaxis or growth promotion.

Conclusion

This study revealed the prevalence of STEC in cattle and pig as well as 100 percent antibiotic resistance towards amikacin, furazolidone, ampicillin and cloxacillin, while highest sensitivity was observed towards ciprofloxacin, norfloxacin, chloramphenicol, nalidixic acid, co-trimoxazole and cephotaxime.

Acknowledgments

The authors is very thankful to Department of Veterinary Public Health, College of Veterinary Science, Khanapara, Guwahati (Assam), India were these study was carried out.

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