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Antimicrobial sensitivity and resistance of *Shiga toxin producing Escherichia coli* from animal faecal samples

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Abstract
Shiga toxin-producing *Escherichia coli* (*STEC*) strains and is considered to be most common food-borne zoonotic pathogen causing various disease conditions in both animals and humans. At present antimicrobial resistance among these isolates is a major problem. Hence a small study was planned to identify antimicrobial resistance or sensitivity against STEC isolates obtained from animal faecal samples of different livestock species against selected antibiotics like Ampicillin (Amp) 10µg/ disc, Cephalothin (Cef) 100 µg/disc, Chloromphenicol (Chl) 30µg/disc, Colistin (Cst) 25µg/disc, Gentamycin (Gen) 10µg/ disc, Kanamycin (K) 30µg/disc, Sulphonamides (sul) 10µg/ disc, Streptomycin (S) 10µg/disc, Tetracycline (Tet) 30µg/disc and Trimethoprim (Tmp) 10µg/ disc. Antimicrobial susceptibility of the isolates was established by the disk diffusion assay with Muller Hinton agar in accordance with French National Antibiogram Committee Guidelines. The resistance was highest to Ampicillin, Cepahlothin (100%) followed by Tetracycline (95.5%), Streptomycin (93.3%), Sulphonamides (89.9%), Trimethoprim (84.4%), Colistin (32.9%), Kanamycin (21.2), Chloromphenicol (17.3%) and least resistant to Gentamycin (8.3%). The STEC isolates were more sensitive to Gentamycin, Chloromphenicol, Colistin, Kanamycin, Trimethoprim and sulphonamides.

Keywords: Antibiotics, antimicrobial resistance/sensitivity, public health, *Shiga toxin producing Escherichia coli*

Introduction
Escherichia coli is a Gram-negative, nonsporulating, rod shaped, flagellated, and facultative anaerobic bacteria belonging to the Enterobacteriaceae Family. *E. coli* is one of the main inhabitants of the intestinal tract of most mammalian species, including humans. Hence recovery of *E. coli* from livestock products and environmental samples like water is used as reliable indicator of fecal contamination and indicates a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard. Most *E. coli* are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man (Kaper *et al.*, 2004) [1].

EHEC refers to a subset of Shiga toxin-producing *Escherichia coli* (*STEC*) strains and is considered to be most common food-borne zoonotic pathogen causing various disease conditions in both animals and humans (Kumar *et al.*, 2014) [2]. Ruminants are considered as important source of *STEC* with cattle being regarded as the primary reservoir (Sang *et al.*, 2012; Perera *et al.*, 2015) [3, 4]. Shiga toxin is the key factor in *STEC* pathogenesis (Acheson, 2000) [5] which is toxic to human colonic, ileal epithelial (Schmidt *et al.*, 1999) [6] and endothelial cells (Obrig, 1998) [7].

Several antimicrobial agents are used therapeutically in human and veterinary medicine. In addition, some antibiotics are routinely used for disease prevention and growth promotion in animal production. This practice leads to development of specific antibiotic resistance, which then enter the human population and may create a public health problem (Witte, 1998; Tollefson *et al.*, 1999; Van den Bogaard and Sobberingh, 1999) [8-10]. The emergence of antimicrobial-resistant bacterial pathogens is a public health problem. Hence the present study was designed to identify the resistance levels of STEC towards ten commercially available antibiotic discs and their concentrations (g) used in this study were Ampicillin (Amp) 10µg/ disc, Cephalothin (Cef) 100 µg/disc, Chloromphenicol (Chl) 30µg/disc, Colistin (Cst) 25µg/disc, Gentamycin (Gen) 10µg/ disc, Kanamycin (K) 30µg/disc, Sulphonamides (sul) 10µg/ disc, Streptomycin (S) 10µg/disc, Tetracycline (Tet) 30µg/disc and Trimethoprim (Tmp) 10µg/ discs in animal faecal samples.

Materials and Methods

Muller–Hinton agar, the recommended medium for disc diffusion test was employed in this study. The prepared medium was autoclaved. When the temperature of medium reached between 45 – 50 ^{0}C it was mixed well and approximately 15-20ml was added to the sterile petridishes and incubated overnight at 37 ^{0}C for sterility testing and the uncontaminated plates were wrapped with aluminium foil and they were stored at 4 ^{0}C till use.

Preparation of inoculum

The sterile cotton swab was dipped in the standardized inoculum (turbidity so adjusted) and rotated several times. Then the cotton swab was gently pressed on the upper inside wall of the test tube to remove excess inoculums and streaked over the entire surface of the muller-hinton agar plate for three times. To ensure even distribution of the inoculums, the plate was turned at 60^0 angle between each streak. A final sweep of the swab was made around the agar rim and allowed the inoculums to dry for 5 to 15 minutes. Selected antimicrobial discs were placed at least 24mm apart by using a disc dispenser and gently pressed down on to the agar surface to provide uniform contact. The inoculated plates were inverted and incubated for 24-48 hours at 37 ^{0}C. After incubation each plate was examined and measured the diameter of zones of inhibition up to the nearest whole millimetre with ruler in non reflecting background. The zone margin was the area where no obvious growth was visible and the readings were compared with that specified readings in the interpretive chart supplied by the manufacturer of the antibiotic discs and the results were documented as sensitive (S), intermediate (I) and resistant (R).

Results

The positive STEC isolates by PCR from animal faecal samples were found to be highly resistant to Ampicillin, Cepahlothin (100%) followed by Tetracycline (95.5%), Streptomycin (93.3%), Sulphonamides (89.9%), Trimethoprim (84.4%), Colistin (32.9%), Kanamycin (21.2), Chloromphenicol (17.3%) and least resistant to Gentamycin (8.3%). The results were given in the following table.

Table 1: Antibiotic Resistance of STEC from animal faecal samples (n=179) by phenotypic methods

S. No.	Antibiotic	Sensitive	Intermediate	Resistance
1.	Ampicillin (10mcg)	0	0	179(100%)
2.	Cephalothin	0	0	179(100%)
3.	Chloramphenicol (30mcg)	135(75.4)	13(7.2)	31(17.3)
4.	Colistin	102(56.9)	18(10)	59(32.9)
5.	Gentamycin (10mcg)	152(85)	12(6.7)	15(8.3)
6.	Kanamycin (K) 30µg	66(36.8)	18(10)	38(21.2)
7.	Sulphonamides	06(3.3)	12(6.7)	161(89.9)
8.	Streptomycin (S) 10µg	03(1.6)	09(5)	167(93.3)
9.	Tetracycline (T) 30µg.	04(2.2)	04(2.2)	171(95.5)
10.	Trimethoprim	12(6.7)	16(8.9)	151(84.4)

The positive STEC isolates by PCR from animal faecal samples were found to be highly sensitive to Gentamycin (85%), followed by Chloromphenicol (75.4%), Colistin (56.9%), Kanamycin (36.8%), Trimethoprim (6.7%), Sulphonamides (3.3%), Tetracyclin (2.2%), Streptomycin (1.6%) and no sensitivity to Ampicillin and Cephalothin.

The positive STEC isolates by PCR from animal faecal samples were found to be intermediately resistant to Colistin, Kanamycin (10%), followed by Trimethoprim (8.9%), Chloromphenicol (7.2%), Gentamycin and Sulphonamides (6.7%), Streptomycin (5%) and least 2.2% to tetracycline.

Discussion

The *STEC* isolates from animal faecal samples had 100% resistance against ampicillin in the present study, slightly less resistance of 88.4% was reported by Niranjan and Malini (2014) [11]. Low resistance of 58%, 31.5% and 21.1% was reported by Akond *et al.* (2009) [12], Mishra *et al.*, (2018) [13] and Bok *et al.* (2015) [14] respectively, whereas very low resistance of 8% was reported by Sharma *et al.* (2007) [15] than the present study. On contrary to the present findings higher sensitivity of 86%, 66%, 61.6% and 26% was reported by Sharma *et al.* (2007) [15], Mishra *et al.* (2018) [13] Hanson *et al.* (2002) [16] and Akond *et al.* (2009) [12] respectively than the present study in pooled faecal samples.

The *STEC* isolates from animal faecal samples had high sensitivity (75.4%) for chloromphenicol in the present study, which was slightly lower sensitivity of 80% and 78.9% reported by Akond *et al.* (2009) [12] and Mishra *et al.* (2018) [13] respectively, whereas lower sensitivity of 68% was reported by Sharma *et al.* (2007) [15]. The resistance of isolates against chloromphenicol was less (17.3%) in the present study compared to the resistance of 100%, 86%, 73%, 61.4%, 55% and 20% observed by Joshi *et al.* (2012) [17], Rehman *et al.* (2013) [18], Nontongana *et al.* (2014) [19], Vinita *et al.* (2010) [20], Van Den Bogaard *et al.* (2000) [21] and Akond *et al.* (2009) [12], whereas very low resistance of 10.52%, 10%, 5.4% and 3.1% was reported by Mishra *et al.* (2018) [13], Sharma *et al.* (2007) [15], Bok *et al.* (2015) [14] and Nontongana *et al.* (2014) [19] respectively. Chloromphenicol intermediate resistance/ sensitivity was 7.2% in the present study which was higher than 22% and 10.5% observed by Sharma *et al.* (2007) [15] and Mishra *et al.* (2018) [13].

The resistance of *STEC* isolates against colistin from animal faecal samples was 32.9% in the present study, which was almost similar resistance (34%) reported by Sharma *et al.* (2007) [15], whereas lower resistance of 6.3% was noticed by Morales *et al.* (2012) [22]. The sensitivity (56.9%) of the isolates for colistin was higher than the sensitivity (24%) reported by Sharma *et al.* (2007) [15], whereas the intermediate resistance of 10% was observed in the present study was less than 42% repo [13] rted by Sharma *et al.* (2007) [15].

Sensitivity of 88% and 84.2% against Gentamycin for the *STEC* isolates from animal faecal samples was observed by Sharma *et al.* (2007) [15] and Mishra *et al.* (2018) [13] respectively and almost similar sensitivity (85%) was observed in the present study. The resistance against gentamycin observed in the present study was low (8.3%) which was almost similar resistance(8%) reported by Sharma *et al.* (2003) [15], whereas higher resistance of 82.5%, 70.86%,

32%, 17.3% and 11.6% reported by Arabi & Vinazavihi, (2013) [23], Vinita *et al.* (2010) [20], Zinnah *et al.* (2008) [24], Alshara (2010) [25] and Bok *et al.* (2015) [14] respectively. Lower resistance of 5.26% and 5% than the present study was reported by Mishra *et al.* (2018) [13] and Ali *et al.* (2014) [26] respectively.

The sensitivity of the *STEC* isolates from pooled samples against kanamycin in the present study was 36.8%, which was less than the sensitivity (74%) reported by Sharma *et al.* (2003) [15]. The resistance against kanamycin in the present study observed was 21.2%, which was less than 88% reported by Akond *et al.* (2009) [12].

The resistance of *STEC* isolates from animal faecal samples against *sul*phonamides in the present study was 89.9% which was higher than the resistance 6% observed by Katakweba *et al.* (2014) [27] and 12.9% by Bok *et al.* (2015) [14].

High resistance of 94.3% against streptomycin for the *STEC* isolates from animal faecal samples was observed in the present study than the resistance of 77%, 72.4%, 70%,48.6%, 47.3%, 37.8%, 30.1% and 20.4% observed by Nontongana *et al.* (2014) [19], Saeed *et al.* (2009) [28], Akond *et al.* (2009) [12], Vladimirpyatov *et al.* (2014) [29], Mishra *et al.* (2018) [13], Osek *et al.* (2004) [30], Sabir *et al.* (2004) [31] and Bok *et al.* (2015)[14] respectively. The sensitivity against streptomycin in the present study was very less (1.1%), whereas higher sensitivity of 36.8% and 30% was reported by Mishra *et al.* (2018) [13] and Akond *et al.* (2009) [12].

Very high resistance (95.5%) by the *STEC* isolates from animal faecal samples against tetracyclin was observed in the present study, which was less than the resistance (98%) reported by Van Den Bogaard *et al.* (2000) [21]. Less resistance of 91.5% by Hanson *et al.* (2002) [16], 77% by Nontongana *et al.* (2014) [19], 66% by Saleem *et al.* (2003) [32] and Sharma *et al.* (2003) [15], 60% by Zinnah *et al.* (2008) [24] and Koga *et al.* (2015) [33], 52.6% by Mishra *et al.* (2018) [13], 52% by Akond *et al.* (2009) [12], 40.4% by Aminu & David (2015) [34], 37.8% by Osek *et al.* (2004) [30] and 23.8% by Bok *et al.* (2015) [14], were reported from different animals and different conditions of rearing conditions. Very less sensitivity (2.2%) against tetracycline from animal faecal samples was observed in the present study, than the moderately high sensitivity of 36%, 26.3% and 22% reported by Akond *et al.* (2009) [12], Mishra *et al.* (2018) [13] and Sharma *et al.* (2003) [15] respectively.

Van Den Bogaard *et al.* (2000) [21] reported a resistance of 62% for the *STEC* isolates from animal faecal samples against trimethoprim, which was less than the resistance (84.5%) observed in the present study, whereas a very low resistance of 3.4% was reported by Bok *et al.* (2015) [14].

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