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Antibiotic Resistance of Sorbitol Non-Fermenting Shiga Toxin Producing *Escherichia coli* Isolated from Buffaloes

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ABSTRACT

Sorbitol non-fermenting Shiga toxin producing *Escherichia coli* (SNF-STEC) is considered as a significant emerging pathogen. Though, cattle and buffaloes are the chief reservoir, species like goat, sheep, deer and other ruminants can also harbor this pathogen. Therefore, this pathogen can easily be transmitted to human and other animals through food chain and their environment. The present study, aimed to ascertain the antibiotic resistance profile of SNF-STEC isolates from buffaloes as well as to detect the resistance genes. A total of 33 sorbitol non-fermenting (SNF) *E. coli* isolates were tested against ten commonly used antibiotics both in human and veterinary medicine. Results revealed that 78.8% isolates were resistant to sulfamethoxazole-trimethoprim and nalidixic acid whereas 60.6% to tetracycline and 48.5% to doxycycline. The majority of the isolates were found sensitive to both gentamycin and ciprofloxacin (90%) followed by erythromycin (66.7%) and ceftriaxone (51.5%). Of 33 SNF *E. coli*, 12 were STEC harboring both *stx*1 and *stx*2 gene that dictated 66.7% isolates were found resistant to sulfamethoxazole-trimethoprim and nalidixic acid followed by ampicillin (58.3%) and tetracycline (58.3%). bla_{TEM} was detected in 66.7% ampicillin resistant isolates and *sul*2 was exposed in 34.6% sulfamethoxazole-trimethoprim resistant isolates. *sul*1 gene was negative for the sulfamethoxazole-trimethoprim resistant isolates.

Key words: Antibiotic resistance, Buffalo, *Escherichia coli* and SNF-STEC.

INTRODUCTION

Escherichia coli, enteric pathogens, shares multi host system including human, livestock and primates (Mercat et al. 2014). Shiga toxin producing *E. coli* (STEC) is considered as foremost food borne pathogens having public health significance and causes diarrhea (Asakura et al. 2014). SNF-STEC is unable to ferment sorbitol which causes acute and serious diseases like hemolytic uremic syndrome (HUS), hemorrhagic colitis and thrombotic thrombocytopenia in humans (Sun et al. 2016; Kwan et al. 2019). Domestic animal particularly bovine and ovine is mostly affected through this pathogen worldwide (Oliveira et al. 2007). Cattle, major reservoir for STEC (Bono et al. 2012) and other species like goat, sheep or deer also act as a reservoir (Bhat et al. 2008; Sanchez et al. 2009). Human get infection of STEC through contaminated food (Kwan et al. 2019) but in the farm land it can be transmitted through contaminated feces, water, insects, wild birds and even throughout animal (Vogeleer et al. 2004). However, buffalo, another ruminant, can also be acted as reservoir for pathogenic STEC (Beraldo et al. 2014).

The total buffalo population in Bangladesh is about 1.47 million and most of them reared in saline coastal area, mainly used for draught animal as well as for meat and milk production (Hamid et al. 2016). Treatment of different bacterial diseases by antimicrobials in a suboptimal or incomplete dose may develop antimicrobial resistance in animals. Recently in Bangladesh, a report has been published on STEC in buffalo calves with a prevalence of 11.01% (Gupta et al. 2018) and another study in India with a prevalence of 35.01% (Srivani et al. 2017). Limited number

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of works has been done so far in Bangladesh on STEC in different farm animals especially in cattle (Islam et al. 2014) and buffalo (Gupta et al. 2018). However, antibiotic resistant STEC might be source of resistance genes that could be transmitted horizontally to human leads to treatment failure. Therefore, the current study was conducted to detect the antibiotic resistance along with the resistant genes in SNF-STEC isolates of buffalo.

MATERIALS AND METHODS

Source of Isolates

A total of 33 SNF *E. coli* isolates, of which 12 were STEC used from a preceding study (Mannan, 2015). In brief, total 308 fecal swab samples from recto-anal junction of apparently healthy buffalos were collected aseptically from coastal area in Chattogram Division, Bangladesh during the period January to June 2014. After bacteriological and biochemical confirmation, 33 isolates were detected as SNF *E. coli* that we have been used in our present study.

Antibiotic Susceptibility Testing

Mueller-Hinton agar (Oxoid, UK) plate was used to perform the antibiotic susceptibility of SNF *E. coli* isolates based on the guidelines and recommendations of CLSI (2012). The following ten commonly used antibiotics both in human and veterinary practices were tested namely ampicillin (10µg), ceftriaxone (30µg), chloramphenicol (30µg), tetracycline (30µg), sulfamethoxazole-trimethoprim (25 µg), nalidixic acid (30µg), erythromycin (15 µg), doxycycline (5µg), gentamicin (10µg) and ciprofloxacin (5µg) (Oxoid, UK). The antibiotic susceptibility results were interpreted by CLSI (2012).

Chromosomal DNA Extraction

Boiling method was used to extract the total DNA (Sánchez et al. 2009). Briefly, freshly cultured two to three colonies were taken into 1.5ml sterile Eppendorf tube confined 200µl of nuclease free water and vortexed thoroughly. At 99°C, the tube was heated for 10 minutes and centrifuged at 15000rpm for 2 minutes. The collected supernatant was used as a DNA template and for further use stored at −20°C.

Amplification of Antibiotic Resistant Genes

To amplify the antibiotic resistant genes, PCR was performed in a Thermocycler (Applied Biosystems, 2720 Thermal cycler, USA), using 25µl final volume containing of 12.5µl Go Taq master mixes (Promega, USA), 0.5µl of each primer, 2µl DNA template and deionized water, 9.5µl. Conditions of PCR were described in the earlier published paper (Belaaouaj et al. 1994; Sunde, 2005; Change et al. 2007). The primers used in this study are enlisted in Table 1. 1.5% agarose gels stained with ethidium bromide (10mg/ml) (Thermo Scientific, USA) was used to electrophorese the PCR products and finally inspected under an ultraviolet transilluminator (BDA digital, Germany).

RESULTS

All the 33 SNF *E. coli* isolates were tested against 10 different antibiotic agents for susceptibility testing that represented in Fig. 1. Results revealed that 78.8% isolates were resistant to sulfamethoxazole-trimethoprim and nalidixic acid. Tetracycline and doxycycline were resistant to 60.6% and 48.5% isolates, respectively. On the contrary, 90.9% isolates were found sensitive to gentamycin and ciprofloxacin followed by erythromycin (66.7%), doxycycline (51.5%) and ceftriaxone (51.5%).

The susceptibility of *stx*1 and *stx*2 genotypic isolates are exhibited in Table 2. Among 12 STEC (carrying both *stx*1 and *stx*2 gene) isolates, highest resistance (66.7%) was observed to sulfamethoxazole-trimethoprim and nalidixic acid. Of the tested *stx*1 genotypic isolates, 75% were found resistance against sulfamethoxazole-trimethoprim whereas ampicillin (75%) and nalidixic acid (75%) was the most prevalent resistance in *stx*2 isolates.

Three of the targeting antibiotics resistant geneshave amplified, that is bla_{TEM} for ampicillin resistant and *sul*1 and *sul*2 for sulfamathoxazole-trimethoprim resistant. Out of 15 ampicillin resistant isolates, 10 (66.7%) isolates exposed bla_{TEM} genes. In case of 26 sulfamathoxazole-trimethoprim resistant isolates, *sul*2 was the prevalent (34.6%, n=9/26) gene whereas none of the isolates revealed *sul*1 gene.

Table 1: Primers used to identify antibiotic resistant genes, bla_{TEM}, *sul*1 and *sul*2

Target genes	Primer sequence (5´-3´)	Amplicon size	References
bla_{TEM}	F: TACGATACGGGAGGGCTTAC R: TTCCTGTTTTTGCTCACCCA	716-bp	Belaaouaj et al. (1994)
sul 1	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCG	433-bp	Sunde (2005)
sul 2	F: GAAGCGCAGCCGCAATTCAT R: TGTGCGGATGAAGTCAGCTC	435-bp	Change et al. (2007)

Table 2: Frequency of antibiotic resistance in relation to Shiga toxin producing genes

Antibiotic agents	*stx1* n=8 (%)	*stx*2 n=4 (%)	*stx*1/*stx*2 n=12 (%)
AMP	4 (50.0)	3 (75)	7 (58.3)
CRO	3 (37.5)	1 (25)	4 (33.3)
C	3 (37.5)	2 (50)	5 (41.7)
TE	5 (62.5)	2 (50)	7 (58.3)
SXT	6 (75.0)	2 (50)	8 (66.7)
NA	5 (62.5)	3 (75)	8 (66.7)
E	3 (37.5)	1 (25)	4 (33.3)
DOX	3 (37.5)	2 (50)	5 41.7)
CN	0 (0)	0 (0)	0 (0)
CIP	1 (12.5)	0 (0)	1 (8.3)

AMP; ampicillin, CRO; ceftriaxone, C; chloramphenicol, TE; tetracycline, SXT; sulfamethoxazole-trimethoprim, NA; nalidixic acid, E; erythromycin, DOX; doxycycline, CN; gentamicin, CIP; ciprofloxacin.

DISCUSSION

Based on vigorous literature searches, this is the first report of antibiotic resistant SNF *E. coli* isolates in buffaloes in Bangladesh. However, at the same study area, antibiotic resistant of SNF *E. coli* was determined in cattle (Islam et al. 2014). In this study, high resistance rate was perceived to sulfamethoxazole-trimethoprim, nalidixic acid and tetracycline, an agreement with the previous reports on buffalo in Egypt and Iran (Shahrani et al. 2014; Abdulgayeid et al. 2015). Higher resistance to these antibiotics was also found in other species in several preceding studies (Galland et al. 2001; Islam et al. 2014; Dorgham et al. 2019; El Jalil et al. 2020).

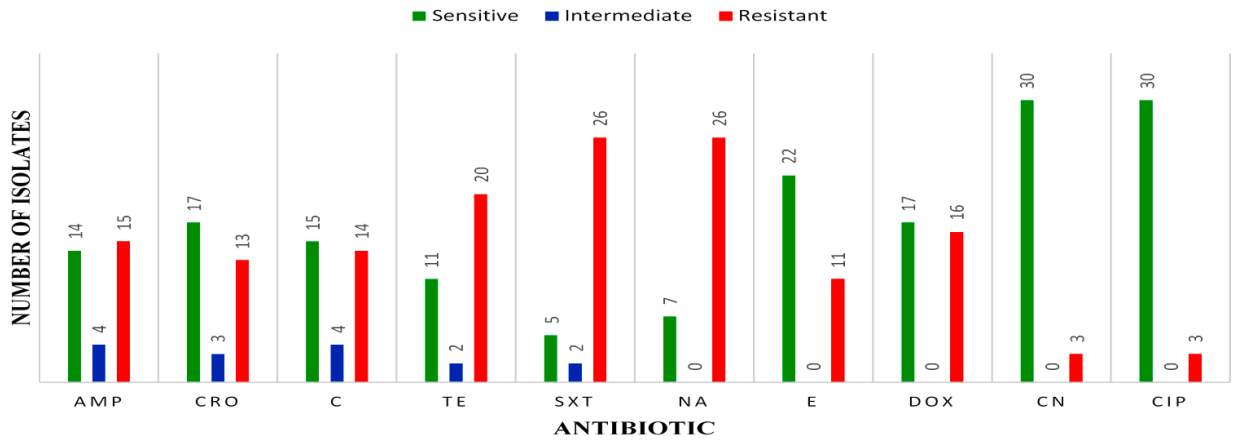

Fig. 1: Antibiotic susceptibility results of SNF *E. coli* isolates (N=33).

Resistances of these drugs are not surprising as they are very commonly used to treat bacterial diseases in buffalo. Tetracycline is commonly used in food animal production as growth promoter in animal feed and antibiotic therapy. However, isolates were found sensitive to gentamicin, ciprofloxacin, erythromycin and ceftriaxone, agreement with the resistant pattern of SNF-STEC from cattle in Bangladesh (Islam et al. 2014; Mehmood et al. 2020).

In current study, STEC isolates harboring *stx*1 and *stx*2 both genes were found resistance (66.7%) to sulfamethoxazole-trimethoprim and nalidixic acid followed by tetracycline (58.3%) and ampicillin (58.3%) which is parallel with the findings of Gupta et al. (2018), informed that STEC genes were found resistant to sulfamethoxazole-trimethoprim (73%) and ampicillin (55%). On the contrary, Srivani et al. (2017) noted lower resistance against sulfamethoxazole (28.30%) and amoxicillin (20.75%). This variation could be attributed to the variation of sample size, geographical location, choice of antibiotics etc. Fascinatingly, isolates also found sensitive to gentamicin, ciprofloxacin, ceftriaxone, erythromycin, and chloramphenicol. This finding was in the line of Gupta et al. (2018), who claimed that chloramphenicol, ciprofloxacin and gentamycin were sensitive to STEC isolates of buffalo calves. These antibiotics could be a good alternative for STEC infection in human and animals, though further clinical trials are suggested.

Among three antibiotic resistance genes, bla_{TEM} was the most prevalent one, 66.7%. However, bla_{TEM} was found in lesser extent in Iran (Dehdashti et al. 2019). 34.6% resistant isolates in this study exposed *sul*2 that comparatively higher than another study, discovered 11% (Dehdashti et al. 2019). *E. coli* isolates from buffalo were positive for *sul*2 genes, 31% in Pakistan closer to our current findings (Idrees et al. 2011). None of the sulfamethoxazole-trimethoprim resistant isolates exposed *sul*1 gene. Resembling to our study, antibiotic resistance and these genes have been determined in *E. coli* isolates from sheep, goat, cattle, buffaloes, broiler and even in the wildlife in Bangladesh (Zinnah et al. 2008; Gupta et al. 2017; Sarket et al. 2019a; Sarker et al. 2019b). These resistance genes can easily transmit to human and animals through food chain, drinking water or environment which might pose serious health risk. So, careful selection of antimicrobial drug and maintaining proper dose and course might reduce the emergence of such SN-F-STEC organisms.

Conclusions

The study revealed the high frequency of antibiotic resistant with the existence of resistant genes in SNF STEC isolates in healthy buffaloes for the first time in Bangladesh. In this study, high resistance was found to sulfamethoxazole-trimethoprim (78.8%), nalidixic acid (78.8%) and tetracycline (60.6%). Of 33 SNF *E. coli*, 12 were STEC harboring both *stx*1 and *stx*2 gene. Of the 3 tested genes, bla_{TEM} was detected in 66.7% and *sul*2 was exposed in 34.6% resistant isolates. None of the sulfamethoxazole-trimethoprim resistant isolates was positive for *sul*1 gene.

Author's contribution

MSM and FR designed the research. MSM and ZBB performed the laboratory work. AAMS and MSS wrote the manuscript. MKR and MSS critically revised the manuscript.

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