Bangladesh Journal of Veterinary and Animal Sciences, Vol. 2, No. 1 & 2, December 2013

ISSN 2227-6416

Bangladesh Journal of Veterinary and Animal Sciences

Journal home page: www.cvasu.ac.bd/journal/userlist

Research article

Antimicrobial susceptibility pattern of sorbitol non-fermenting including shiga toxin producing *Escherichia coli* isolated from Black Bengal goat

Gupta, M. D.[1*], Das, A.[2], Ahad A.[1], Biswas, P. K.[1]

[1]*Department of Microbiology, Chittagong Veterinary and Animal Sciences University, Chittagong–4225, Bangladesh*
[2]*Department of Genetics and Animal Breeding, Chittagong Veterinary and Animal Sciences University, Chittagong–4225, Bangladesh*

ARTICLE INFO

Article history:
Received 24 November 2013
Revised 10 December 2013
Accepted 19 December 2013

Keywords:
Goat
Escherichia coli
Shiga toxin
Antimicrobial sensitivity

*Corresponding author:
Email: mukta_as@yahoo.com
Tel.: +88 01675438402

ABSTRACT

Sorbitol non-fermenting (SN-F) shiga toxin producing *Escherichia coli* (STEC) is an important group of emerging zoonotic pathogen. Cattle are recognized as main natural reservoir of these organisms; however, small ruminants like goat, may represent an equally serious risk to harbor the pathogen for human infections as evidenced by recent reports. In this study, we performed antimicrobial sensitivity test on 32 SN-F isolates including 12 STEC collected from Black Bengal goats to determine their susceptibility patterns against 10 antimicrobials. Bauer-Kirby disk-diffusion method was used for the antimicrobial sensitivity test. Seventy-five percent (75%) isolates were sensitive to chloramphenicol and gentamicin, whereas 69% were sensitive to ciprofloxacin and doxycyline. Fifty-six percent were sensitive to ampicillin and amoxicillin. On the other hand, a high resistance profile was found against trimethoprim-sulfomethoxazole (53%) and oxytetracycline (47%). Fifty percent of the isolates containing shiga toxin-producing genes - *stx1* and/or *stx2* showed resistance to trimethoprim-sulfomethoxazole. Forty-three percent of the isolates having the *hlyA* gene were resistant to tetracycline, whereas 50% isolates harboring the *eae* gene to oxytetracycline.

To cite this paper: *Gupta, M. D., Das, A., Ahad A., Biswas, P. K. 2013. Antimicrobial susceptibility pattern of sorbitol non-fermenting including shiga toxin-producing Escherichia coli isolated from Black Bengal goat. Bangladesh Journal of Veterinary and Animal Sciences, 2: 8-13.*

INTRODUCTION

Shiga toxin producing *Escherichia coli* (STEC) can be found in the gut of numerous animal species, but ruminants particularly, cattle are considered to be major reservoir of STEC that are highly virulent to humans (Caprioli *et al.*, 2005). Other small ruminants like sheep, goats, and deer are known to harbor these bacteria (Bhat *et al.*, 2008; Orden *et al.*, 2005; Sanchez *et al.*, 2009), and occasionally other animals (Garcia and Fox, 2003; Schouten *et al.*, 2005). Although, a number of transmission routes exist (La Ragione *et al.*, 2009), contaminated foods, or water by fecal material from ruminants are the major source of transmission (Caprioli *et al.*, 2005). More recently, cattle in Bangladesh are reported to harboring STEC with a prevalence of

9.1% (Islam *et al.*, 2008). In Bangladesh, the total goat population is about 34.5 million (FAO, 2011) and approximately 90% of them are of Black Bengal breed. More than 95% goats are being reared under the smallholding farming system in the rural areas. In rural areas, farmers especially women and children have more contact with goat than others. Sharing the same homesteads for living and rearing of goats in such close proximity of human contact favors a higher chance for STEC being transmitted to humans. Antibiotics are used for the treatment of most bacterial diseases of goat in Bangladesh. Uses of antibiotics with suboptimal dose and incomplete course may lead to the development of resistance in bacterial populations of both pathogenic and non-pathogenic ones. *E. coli* isolates from biological and environmental samples in Bangladesh were found to be at an intermediate state for conversion from sensitive to resistant against antibiotics (Zinnah *et al.*, 2008), and about 60% of SN-F STEC isolates from cattle in smallholdings in Bangladesh were reported to be resistant against ≥2 of commonly used antibiotics: ceftriaxone, nalidixic acid, ciprofloxacin, tetracycline, doxycycline and trimethoprim-sulfomethoxazole (Islam *et al.*, 2013). Acquired resistance in STEC to multiple drugs could be sources of antimicrobial resistant genes (Srinivasan *et al.*, 2007) that might be transferred to both commensals and pathogenic bacteria, resulting in treatment failures in humans. In this study, we tested antibiotic sensitivity of SN-F including STEC isolates from Black Bengal goats to assess the spectrum of antimicrobial resistance they have acquired.

MATERIALS AND METHODS

Source of the isolates tested

In this study, we used 32 SN-F *E. coli* isolates collected from a previously prevalence study on Black Bengal goats in Bangladesh (Gupta, 2013). Of these isolates 12 were STEC. The organism was isolated from recto-anal junction (RAJ) of Black Bengal goats selected randomly from three districts in Bangladesh namely Chittagong, Feni and Noakhali. Sampled animals were diverse in distributions of age, sex and health status.

Antimicrobial sensitivity testing

The isolates were tested against ten selected antimicrobials: ceftriaxone, ciprofloxacin, tetracycline, ampicillin, doxycycline, sulfomethoxazole-trimethoprim, gentamicin, chloramphenicol, amoxicillin and oxytetracycline. Bauer-Kirby disk-diffusion procedure (Bauer *et al.*, 1966) was used to perform the antimicrobial sensitivity testing. For that purpose Mueller-Hinton (MH) agar, prepared according to the manufacturer's instructions (Oxoid), was used. The antimicrobial micro-disks from Oxoid Ltd. Basingstoke, Hampshire, England were used and the result was interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2007) (Table 1). The isolates were considered as "resistant (R)", "intermediately resistant (I)" and "sensitive (S)" according to their zone of inhibition and group of antibiotics used for the assays, based on the performance standards from the CLSI (CLSI, 2007).

RESULTS

The numbers of isolates that were sensitive, intermediately resistant and resistant to the antimicrobials tested are shown in Figure 1. Seventy-five per cent SN-F *E. coli* isolates were sensitive to chloramphenicol and gentamicin. The percentage of isolates sensitive to ciprofloxacin and doxycycline was 69% and 56% were sensitive to ampicillin and amoxicillin. On the other hand, 53% were resistant against trimethoprim-sulfomethoxazole followed by oxytetracycline (47%). Thirty-seven per cent isolates were resistant against tetracycline. The frequencies of the isolates showing different zones of inhibition to the 10 different antimicrobials tested are illustrated in Figure 2. The antimicrobial susceptibility profiles of the isolates harboring the *stx1, stx2, hly* and *eae* genes are displayed in Table 2.

Three (50%) and 2 (33%) of the *stx1* gene-containing isolates were resistant to trimethoprim-sulfomethoxazole and ampicillin, respectively; 50% and 33% of the *stx2* gene-containing isolates were resistant to trimethoprim-sulfomethoxazole and oxytetracycline, respectively.

On the other hand, 43% *hly* gene-containing isolates were resistant to tetracycline, whereas 50% *eae* gene-containing isolates to oxytetracycline. Only 4 (12%) isolates showed susceptibility to all the 10 antimicrobials tested and 4 (12%) isolates were found to be susceptible against 9 antimicrobials. In total, 24 (75%) isolates were resistant against ≥2 antimicrobials.

Table 1. The concentrations and interpretative standard zone diameters of different antimicrobials (CLSI, 2007) used for this study to interpret the results

Group of antimicrobial agent	Antimicrobial agent	Disk content (µg)	Zone diameter, nearest whole mm			Origin
			R	I	S	
Penicillin	Ampicillin	10	≤ 13	14-16	≥ 17	Oxoid Ltd.
B-lactamase inhibitor	Amoxicillin-clavulanic acid	20/10	≤ 13	14-17	≥ 18	Basingstoke,
Cephems	Ceftriaxone	30	≤ 13	14-20	≥ 21	Hampshire,
Amino glycosides	Gentamicin	10	≤ 12	13-14	≥ 15	England
Tetracycline	Tetracycline	30	≤ 11	12-14	≥ 15	
	Doxycycline	30	≤ 10	11-13	≥ 14	
	Oxytetracycline	30	≤ 14	15-18	≥ 19	
Fluoroquinolones	Ciprofloxacin	5	≤ 15	16-20	≥ 21	
Folate pathway inhibitor	Trimethoprim-sulfomethoxazole	1.25/23.7	≤ 10	11-15	≥ 16	
Phenicoles	Chloramphenicol	30	≤ 12	13-17	≥ 18	

Table 2. Frequencies of antimicrobial resistance in sorbitol non-fermenting shiga toxin producing *Escherichia coli* isolates of Black Bengal goat origin

Antimicrobial	No. resistant isolates (%) containing virulent genes				
	stx1 (*n*=6)	*stx2* (*n*=6)	*hly* (*n*=7)	*eae* (*n*=2)	Without virulent genes *(n=11)*
CRO	1 (17%)	0 (0%)	0 (0%)	0 (0%)	6 (54%)
CIP	0 (0%)	0 (0%)	1 (14%)	0 (0%)	5 (45%)
TET	0 (0%)	1 (17%)	3 (43%)	0 (0%)	8 (73%)
AMP	2 (33%)	2 (33%)	2 (28%)	0 (0%)	6 (54%)
DOC	1 (17%)	0 (0%)	1 (14%)	0 (0%)	4 (36%)
SXT	3 (50%)	3 (50%)	1 (14%)	1 (50%)	9 (82%)
GNT	1 (17%)	1 (17%)	0 (0%)	0 (0%)	4 (36%)
CHL	0 (0%)	0 (0%)	1 (14%)	0 (0%)	4 (36%)
AML	2 (33%)	1 (17%)	2 (28%)	0 (0%)	6 (54%)
OXT	1 (17%)	2 (33%)	2 (28%)	1 (50%)	9 (82%)

Here, CRO, Ceftriaxone; CIP, Ciprofloxacin; TET, Tetracycline; AMP, Ampicillin; DOC, Doxycycline; SXT, Sulfomethoxazole-Trimethoprim; GNT, Gentamicin; CHL, Chloramphenicol; AML, Amoxicillin; and OXT, Oxytetracycline.

DISCUSSION

Although a previous study (Islam *et al.*, 2013) reported the antibiotic resistance profiles of STEC in cattle in smallholdings, there is no inland report on antimicrobial susceptibility patterns of SN-F including STEC of goat origin. The antimicrobial susceptibility patterns observed in the SN-F including STEC isolates from Black Bengal goats towards 10 antimicrobials summarized that the isolates were diverse in their antimicrobial resistance spectrum. Most isolates were sensitive to chloramphenicol and gentamicin, an agreement with the susceptibly pattern observed for SN-F STEC from cattle on smallholdings in Bangladesh (Islam *et al.*, 2013). A similar pattern of antimicrobial susceptibility in both studies might be related to the common geographic origin and similar nature of use of antibiotics for goats and cattle in the study area. A moderate resistance of the SN-F STEC isolates to ampicillin and amoxicillin is similar to that of a previous study (Nizza *et al.*, 2010). This finding justifies the treatment effectiveness of the amino-penicillin, such as ampicillin and amoxicillin against diseases attributable to several aerobic Gram-negative enteric bacilli, such as *E. coli* (Hackley *et al.*,

2007). A high rate of resistance was observed in the isolates to sulfamethoxazole and oxytetracycline, an agreement with some previous reports (Islam *et al.*, 2013; Galland *et al.*, 2001; Zhao *et al.*, 2001). This resistance pattern might be linked to wide use of sulfamethoxazole and oxytetracycline in treating enteric bacterial and parasitic infection of goats (Islam *et al.*, 2003). Furthermore, the resistance against tetracycline is corroborated with the observation of Nizza *et al.* (2010). A high resistance against tetracycline is not surprising because tetracycline has been commonly used in food animal production since its approval in 1948 as growth promoter in animal feed and antibiotic therapy (Walsh, 2003; McEwen and Fedorka-Cray, 2002).

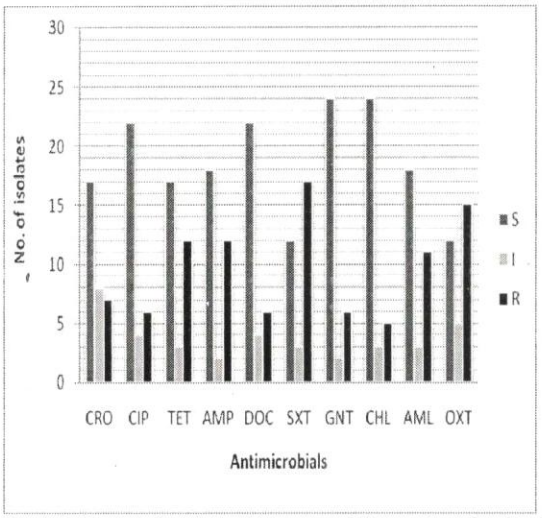

Figure 1. Results of antimicrobial susceptibility testing of sorbitol non-fermenting *Escherichia coli* from Black Bengal goats; S, I, R = proportional representations of sensitive, intermediately-resistant and resistant isolates, respectively, against the antimicrobials tested; CRO, Ceftriaxone; CIP, Ciprofloxacin; TET, Tetracycline; AMP, Ampicillin; DOC, Doxycycline; SXT, Sulfomethoxazole-Trimethoprim; GNT, Gentamicin; CHL, Chloramphenicol; AML, Amoxicillin; and OXT, Oxytetracycline.

In this study, the observed antimicrobial susceptibly pattern of the isolates harboring virulent genes-*stx1, stx2, hly* and *eae* is almost similar to the susceptibility pattern reported by Islam *et al.* (2013). The anticipation for this finding is that the isolates isolated from Black Bengal goats harboring the shiga toxin-producing genes might have been come from the isolates circulating in cattle population because cattle in Bangladesh have been identified with such isolates and they are the major source of STEC worldwide (Islam, 2012; Caprioli *et al.*, 2005). A good number of SN-F including STEC isolates showed resistance against ≥2 drugs which is higher than the resistance pattern in bovine isolates (Islam *et al.*, 2013) from the same geographical area. This kind of multidrug resistance could be an outcome of more frequent and inappropriate use of antibiotics.

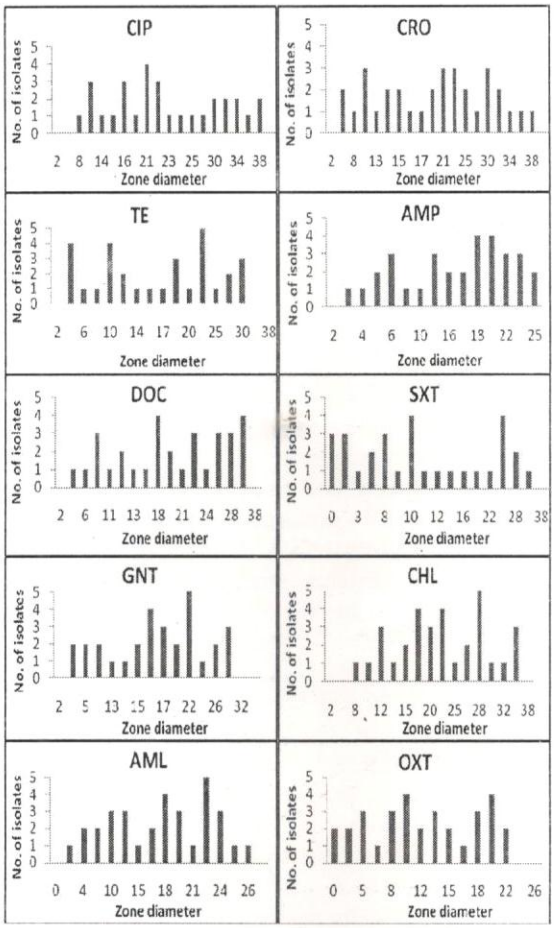

Figure 2. Frequency distributions based on zones of inhibition to 10 antimicrobials tested

Antimicrobial resistant SN-F *E. coli* including STEC can gain entrance to human food chain and drinking water to infect humans, and the antimicrobial resistant genes might be transferred to the other closely related human bacterial pathogens. Therefore, careful selection of appropriate antibiotics with optimal doses might have an impact on reducing the rate of emerging multidrug resistance in SN-F STEC.

CONCLUSION

The antimicrobial resistance profiles of the SN-F *E. coli* being harbored in Black Bengal goats varied

substantially. Therefore, Black Bengal goat could be an important source of infection of it particularly STEC to humans in rural environment of Bangladesh.

ACKNOWLEDGEMENTS

This study was financially supported from Regional Fisheries & Livestock Development Component (RFLDC), Noakhali through an applied research project implemented from the Department of Microbiology, CVASU. The cooperation of Dr. Himel Barua and Dr. MD. Zohorul Islam is thankfully acknowledged.

REFERENCES

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 36: 493–496.

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.

Caprioli, A., Morabito, S. ,Brugere, H. and Oswald, E. 2005. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. Veterinary Research, 36: 289–311.

Carter, G. R. and Cole, J. R. J. 1990. Diagnostic Procedure in Veterinary Bacteriology and Mycology, Fifth edition, Academic Press, Inc., Harcourt Brace Jovanovich, Publishers, New York, 484 p.

Clinical Laboratory Standards Institute (CLSI). 2007. Performance standards for antimicrobial susceptibility testing; Seventeenth information Supplement. CLSI document M100-S17. 27: 3.

Gupta, M. D. 2013. Prevalence and diversity of *Escherichia coli* in Black Bengal goat while using selective medium for enterohemorragic group. MS thesis, Department of Microbiology, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh.

Food and Agricultural Organization (FAO). 2011. The State of the world's Animal Genetic Resources for Food and Agriculture. Rome, Italy.

Galland, J. C., Hyatt, D. R., Crupper, S. S. and Acheson, D. W. 2001. Prevalence, antibiotic susceptibility, and diversity of *Escherichia coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. Applied and Environmental Microbiology, 67: 1619–1627.

Garcia, A. and Fox, J. G. 2003. The rabbit as a new reservoir host of enterohemorrhagic *Escherichia coli*. Emerging Infectious Diseases, 9: 1592–1597.

Hackley, B., Kriebs, J. M. and Rousseau, M. E. 2007. Primary care of women: a guide for midwives and women's health providers, Jones and Bartlett Publishers, Inc., 40 Tall Pine Drive, Sudbury MA 01776, 123 p.

Islam, M. Z., Ahad, A. and Biwas, P. K. 2013. Antimicrobial resistance profile of sorbitol non–fermenting shiga toxin producing *Escherichia coli* isolated from small holdings cattle. Journal of Infection and Molecular Biology (In press).

Islam, M. Z. 2012. An investigation on clonal diversity of *Escherichia coli* from cattle in smallholdings while using selective media for O157 strains. MS thesis, Department of Microbiology, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh.

Islam, M. A., Mondol, A. S. Boer, E.-de, Beumer, R. R., Zwietering, M. H. Talukder, K. A. and Heuvelink, A. E. 2008. Prevalence and genetic characterization of shiga toxin-producing *Escherichia coli* isolates from slaughtered animals in Bangladesh. Applied and Environvironmental Microbiology, 74: 5414–5421.

Islam, M. R., Giasuddin, M., Rahman, M. M. and Kafi, M. A. 2003. Antibiotic combined hyperimmune serum therapy in peste des petits ruminants infected goats. Bangladesh Journal of Veterinary Medicine, 1: 49–51.

La Ragione, R. M., Best,A., Woodward, M. J. and Wales, A. D. 2009. *Escherichia coli* O157:H7 colonization in small domestic ruminants. FEMS Microbiology Reviews, 33: 394– 410.

McEwen, S. A. and Fedorka-Cray, P. 2002. Antimicrobial use and resistance in animals. Clinical Infectious Diseases, 34: S93–106.

Nizza, S., Mallardo, K., Marullo, A., Iovane, V., Martino, L. D. and Pagnini, U. 2010. Antibiotic susceptibility of haemolytic *E. coli* strains isolated from diarrhoeic faeces of buffalo calves. Italian Journal of Animal Science, 9: e26

Orden, J. A., Cortes, C., Horcajo, P., De la Fuente, R., Blanco, J. E., Mora, A., Lopez, C., Blanco, J.,

Contreras, A., Sanchez, A., Corrales, J. C. and Domınguez-Bernal, G. 2008. A longitudinal study of verotoxin-producing *Escherichia coli* in two dairy goat herds. Veterinary Microbiology, 132: 428–434.

Sanchez, S., Garcıa-Sanchez, A., Martınez, R., Blanco, J., Blanco, J. E., Blanco, M., Dahbi, G., Mora, A., Hermoso de Mendozaa, J., Alonsoa, J. M. and Rey. J. 2009. Detection and characterisation of Shiga toxin-producing *Escherichia coli* other than *Escherichia coli* O157:H7 in wild ruminants. The Veterinary Journal, 180: 384–388.

Schouten, J. M., van de Giessen, A. W., Frankena, K., De Jong, M. C. M. and Graat, E. A. M. 2005. *Escherichia coli* O157 prevalence in Dutch poultry, pig finishing and veal herds and risk factors in Dutch veal herds. Preventive Veterinary Medicine, 70: 1–15.

Srinivasan, V., Nguyen, L. T., Headrick, S. I., Murinda, S. E. and Oliver, S. P. 2007. Antimicrobial resistance patterns of Shiga toxin-producing *Escherichia coli* O157:H7 and O157:H7- from different origins. Microbial Drug Resistance, 13: 44–51.

Walsh, C. T. 2003 Antibiotics: actions, origins, resistance. Washington (DC): American Society for Microbiology Press.

Zhao, S., White, D. G., Ge, D., Ayers, S., Friedman, S., English, L., Wagner, D., Gaines, S. and Meng, J. 2001. Identification and characterization of integron- mediated antibiotic resistance among Shiga toxin-producing *Escherichia coli* isolates. Applied and Environvironmental Microbiology, 67: 1558–1564.

Zinnah, M., Haque, M. H., Islam, M. T., Hossain, M. T., Bari, M. R., Babu, S. A. M., Rahman, M. T. and Islam, M. A. 2008. Drug sensitivity pattern of *Escherichia coli* isolated from samples of different biological and environmental sources. Bangladesh Journal of Veterinary Medicine, 6: 13–18.