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Prevalence and Antibiotic Sensitivity of Shiga Toxin Producing *Escherichia coli* in Gulbarga Region, India

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Abstract: The main objectives of this research was to isolate Shiga Toxin Producing *Escherichia coli* (STEC) from diarrheal samples, stool samples from cattle, beef, mutton samples, water and sewage samples collected from different places in Gulbarga region of Karnataka, India and determination of the antibiotic susceptibility patterns of STEC. The highest number of STEC was found in the Sewage sample (14.84%) where as urine sample did not contain any STEC. Among the 2109 sample 65 were confirmed as STEC. The highest (73%) incidence of resistance was recorded against Ampicillin, closely followed by that against streptomycin (70.77%) and cephalexin (69.23%).While only two antibiotics, chloramphenicol (21.54%) and gentamicin (12.3%) recorded comparatively lower incidence of resistance. The multiple antibiotic resistances were most common. More than 98% of the isolates were resistant to two or more antibiotics. Resistances to three (11 isolates), six (10 isolates) and five (9 isolates) antibiotics were most common. Alarming to note that, few isolates (4.61%) are resistant to all the 12 antibiotics tested and 21.5% of the isolates are resistant to ten or more antibiotics.

Key words: Shiga toxin producing *Escherichia coli*, antibiotic susceptibility, Kirby Bauer's method, Gulbarga region

INTRODUCTION

Most strains of the bacterium *Escherichia coli* are considered to be part of the normal microbial flora of the gastrointestinal tract of humans and other warm blooded animals. However, certain strains of the *E. coli* have been reported to possess a battery of virulence determinants which enable them to overcome the host's defense mechanisms and produce gastrointestinal diseases in both humans and animals. The cytotoxin produced by *E. coli* are either termed as Vero Toxins (VT), because of their activity on Vero cells or shiga toxins (Stx), because of their similarity with the toxin produced by *Shigella dysenteriae* (Melton-Celsa and O'Brien, 1998). Therefore these strains are either termed Shiga Toxin-Producing *E. coli* (STEC) or Vero Toxin-Producing *E. coli* (VTEC). Shiga toxin producing *E. coli* has in recent years emerged as a serious food borne pathogen, causing sporadic cases to severe outbreaks worldwide. The morbidity and mortality associated with several recent large outbreaks of gastrointestinal diseases due to shiga toxin-producing *E. coli* has highlighted the threat these organisms pose to the public health (Ahmed and Cowden, 1997). There are few reports about STEC infections in most of the Asian countries except Japan (Yoh *et al*., 1997). Few reports are available on the isolation of STEC from Hong Kong, Thailand, Japan, Malaysia, India and Sri Lanka.

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The Shiga toxins of STEC can be divided into two major groups- Stx 1 and Stx 2, in terms of their antigenic variations (Melton-Celsa and O'Brien, 2000). The Stx 1 is nearly identical to the toxin of *S. dysenteriae* serotype 1 and is neutralized by antisera against Stx (Melton-Celsa and O'Brien, 2000), while Stx 2 shares less than 60% amino acid sequence with Stx 1 and is not neutralized by anti-Stx1 antiserum (Melton-Celsa and O'Brien, 2000). Stx 1 is highly conserved and shows only little sequence variations (Zhang *et al*., 2002). Several variants of Stx 2 with altered antigenic and biological characteristics have been identified. Such toxins have been termed Stx 2c, Stx 2d, Stx 2dac, Stx 2e, Stx 2f or Stx 2g (Leung *et al*., 2003). Several reports on other Stx 2 variants produced by single strains (Scheutz *et al*., 2001). The variants of Stx 2 share 84-99% similarity with the Stx2 (Cherla *et al*., 2003). The clinical manifestations of STEC infection range from asymptomatic carriage to a wide variety of clinical symptoms like non-bloody diarrhea, Hemorrhagic Colitis (HC), Hemolytic Ureamic Syndrome (HUS), Thrombotic Thrombocytopenic Purpura (TTP) and death (Mead and Griffin, 1998). The use of antimicrobial drugs is controversial and not recommended since they may increase the risk for developing HUS and the bloody diarrheal phase may last longer (Zimmerhackl, 2000) although a recent, meta-analysis did not show a higher risk of HUS and antimicrobial therapy (Safdar *et al*., 2002).

The main objectives of this research was to isolate Shiga Toxin Producing *Escherichia coli* (STEC) from diarrheal samples, stool samples from cattle, beef, mutton samples, water and sewage samples collected from different places in Gulbarga region and determination of the antibiotic susceptibility patterns of STEC.

MATERIALS AND METHODS

The research was carried out during 2003-2006. The samples were mainly collected from the Gulbarga city, Jewargi, Chitapur, Aland, Chincholi and Sedam of Karnataka, India.

Sampling of Specimens

Stool Samples

Eight hundred and eighty five Stool samples were collected from diarrheal patients attending Government Hospital, Gulbarga, Basaveshwara Teaching and General Hospital (BTGH), Khaja Banda Nawaz Teaching and General Hospital (KBNTGH), Pooja diagnostics, Primary Health Centers (PHCs) of Jewargi, Aland, Chitapur, Chincholi, Sedam, during outbreaks of gastroenteritis. Stool samples were collected in wide mouthed sterile bottles; sterile cotton tipped swabs were used to take rectal swabs from patients from whom stool could not be obtained. Rectal swabs were placed in Cary-Blair medium and stool samples were transported to the Laboratory for further investigation.

Urine Samples

Two hundred and seventy eight urine samples of patients were collected in sterile screw cap tubes and immediately transported to the Laboratory for further process from the above mentioned centers.

Food Samples

Ten grams of ground beef (205 samples) and meat samples (157 samples) were collected in sterile flasks containing lactose broth and kept in ice box for transportation to the Laboratory for processing.

Water Samples

Two hundred and forty seven water samples were collected in sterile containers from different part of Gulbarga Region.

Table 1: Antimicrobial discs used and the concentration of each antibiotic

Antibiotics	Concentration (µg)
Tetracycline (T)	30
Chloramphenicol (C)	30
Ciprofloxacin (Cf)	05
Gentamicin (G)	10
Ampicillin (A)	10
Co-Trimoxazole (Co)	25
Neomycin (N)	30
Nalidixic acid (Na)	30
Norfloxacin (Nx)	10
Streptomycin (S)	10
Cephalexin (Cp)	30
Amoxicillin (Am)	10

Sewage Samples

One hundred and eighty two sewage samples were collected in sterile screw cap containers from different part of Gulbarga Region.

Animal Fecal Samples

One hundred and fifty eight fecal samples were collected directly from the rectum of diarrheic animals into polythene bags and carried to the Laboratory on ice.

Isolation and Screening of *E. coli*

All stool specimens, food and environmental samples were screened simultaneously for *E. coli* and STEC as follows: Stool specimens were plated on MacConkey's agar plates. After overnight incubation at 37°C, pink colonies were picked up and subcultured on Eosin Methylene Blue (EMB) agar plates to observe the characteristic metallic sheen. The well separated colonies were picked up on nutrient agar slants as pure cultures and subjected to standard 70 biochemical tests.

Isolation and Screening of STEC

The samples were simultaneously streaked on to Sorbitol MacConkey's agar plates with cefixime-tellurite supplements (CT-SMAC agar) and were incubated overnight at 37°C for the isolation of STEC. Sorbitol negative colonies were tested for the production of β-Glucuronidase, fermentation of cellobiose and growth in the presence of potassium cyanide by standard techniques to differentiate STEC strains from other strains of *E. coli* as they are unable to ferment sorbitol and MUG. Fermentation of different sugars and decarboxylation of amino acids were tested. The sorbitol and MUG negative colonies were further tested for the presence of *stx* genes by the PCR.

Antibiotic Susceptibility Testing of STEC

Antibiotic susceptibility testing was performed as per the reliability and reproducibility guidelines of National Committee for Clinical Laboratory Standards (NCCLS). The method adopted is the Kirby Bauer's Agar Disk Diffusion Assay (Baur *et al*., 1966).

The antibiotic susceptibility discs and the Mueller Hinton agar were obtained from Hi-Media Laboratories Mumbai, India. The disc diameter is 6 mm. Table 1 shows the types of antibiotics and its concentrations.

RESULTS

The highest number of STEC was found in the Sewage sample (14.84%) where as urine sample did not contain any STEC. Among the 2109 sample 65 were confirmed as STEC (Table 2). Table 3 shows the antibiotic resistance of STEC whereas Table 4 indicates the multiple antibiotic resistance pattern of STEC.

Table 2: Incidence of STEC in different samples

Sample	No. of isolates	Percentage of isolates
Stool		
Samples	885	1.36
STEC	12	
Urine		
Samples	278	0.00
STEC	Nil	
Beef		
Samples	205	1.95
STEC	4	
Meat		
Samples	157	0.64
STEC	1	
Water		
Samples	247	2.43
STEC	6	
Sewage		
Samples	182	14.84
STEC	27	
Animal fecal		
Samples	158	9.67
STEC	15	
Total		
Samples	2109	3.08
STEC	65	

Table 3: Antibiotic resistance of STEC

	Incidence of resistance	
Antibiotics	No. of isolates	Percentage
Tetracycline (T)	44	67.69
Chloramphenicol (C)	14	21.54
Ciprofloxacin (Cf)	33	47.69
Gentamicin (G)	8	12.30
Ampicillin (A)	48	7.85
Co-Trimoxazole (Co)	25	38.46
Neomycin (N)	43	66.16
Nalidixic acid (Na)	34	52.30
Norfloxacin (Nx)	23	35.38
Streptomycin (S)	46	70.77
Cephalexin (Cp)	45	69.23
Amoxicillin (Am)	41	63.07

Table 4: Multiple antibiotic resistance pattern of STEC

	Incidence of resistance	
No. of antibiotics	No. of isolates	Percentage
One	1	1.54
Two	7	10.76
Three	11	16.92
Four	2	3.08
Five	9	13.85
Six	10	15.38
Seven	3	4.61
Eight	4	6.15
Nine	4	6.15
Ten	5	7.69
Eleven	6	9.23
Twelve	3	4.61

Comparison of the distribution of antibiotic resistance among STEC isolates from different sources indicated a random and diversified pattern. The highest (73%) incidence of resistance was recorded against Ampicillin, closely followed by that against Streptomycin (70.77%) and Cephalexin

(69.23%). While only two antibiotics, chloramphenicol (21.54%) and gentamicin (12.3%) recorded comparatively lower incidence of resistance. Half the strains isolated from human diarrheal samples are resistant for nine or more antibiotics and constituted almost 50% of the all the STEC isolates showing resistance to 9 or more antibiotics. Out of the 12 isolates from diarrheal samples 11 were resistant to tetracycline, amoxicillin and cephalexin. Ten isolates were resistant streptomycin. All the isolates from diarrheal samples were resistant to Ampicillin. All the four isolates of STEC from beef samples were resistant to tetracycline, ampicillin, streptomycin, cephlexin and amoxicillin. Similarly all the STEC isolates from water samples are resistant to ciprofloxacin and Nalidixic acid.

DISCUSSION

Antimicrobial drugs have undoubtedly saved the lives of millions of people. However, the widespread use of such drugs in hospitals, health centers, the community and agriculture has led to the emergence of resistance among bacteria. Antimicrobials are commonly used in food producing animals for treatment, prophylaxis and growth promotion. However, such use can also lead to the development of drug-resistant bacteria, which may be transmitted to humans through the food supply. Over the past years, the emergence and spread of antimicrobial resistance has become a major public health concern. The incidence of *Escherichia coli* in the stool samples of the patients suffering from gastroenteritis was found to be 77.49%. The location-wise incidence of *E. coli* among different areas of Gulbarga has been assessed and highest (81.20%) incidence was reported in Jewargi and the lowest (68.62%) incidence in Sedam. The multiple antibiotic resistances were most common. More than 98% of the isolates were resistant to two or more antibiotics. Resistances to three (11 isolates), six (10 isolates) and five (9 isolates) antibiotics were most common. Alarming to note that, few isolates (4.61%) are resistant to all the 12 antibiotics tested and 21.5% of the isolates are resistant to ten or more antibiotics.

In India there seems to be scanty literature on antimicrobial resistance pattern on STEC. Chattopadhyay *et al*. (2001) studied the antibiotic sensitivity pattern of STEC strains from animal, human and food products and reported that STEC strains were uniformly sensitive to common antibiotics except tetracycline, cephalexin, dicloxacillin, erythromycin and lincomycin. STEC represents the only pathogenic group of *E. coli* that has a definite zoonotic origin, with domestic animals, especially cattle and sheep being recognized as the major reservoirs and sources of infections. Contaminated food items such as meat and meat products, dairy products, green produce like sprouts and salads, acid products like dry sausage, apple juice and mayonnaise, drinking water and swimming pools have been recognized as main vehicles of spread of infection to humans (Tilden *et al*., 1996). Several monitoring programs have been initiated to generate baseline data about the prevalence of resistance in different bacterial species, including *Escherichia coli* (Aarestrup, 2004). The genetic mechanisms that lead to bacterial resistance are manifold and their spread in different bacterial populations is enabled by highly efficient transfer systems of mobile genetic elements (Schwarz and Chaslus-Dancla, 2001). During the recent years, the importance of integrons (mobile gene expression systems) for the dissemination of resistance in *E. coli* has been established (Guerra *et al*., 2003; Singh *et al*., 2005). The characterization of resistance mechanisms provides additional information about the epidemiology of resistant clones (Aarestrup *et al*., 2004). Because of its epidemiological importance, the prevalence and nature of antimicrobial resistance in zoonotic Shiga Toxin Producing *E. coli* (STEC) has been the subject of many studies (Singh *et al*., 2005; Mora *et al*., 2005). The further studies needed to found molecular epidemiology of STEC to know the antibiotic resistance mechanism in the genetic level.

REFERENCES

Aarestrup, F.M., 2004. Monitoring of antimicrobial resistance among food animals: Principles and limitations. J. Vet. Med. B Infect Dis. Vet. Public. Health, 51: 380-388.

Ahmed, S. and J. Cowden, 1997. An outbreak of *E. coli* O157 in central Scotland. 3rd International Conference and Workshop on Shiga Toxin (verotoxin) Producing *Escherichia coli* Infections, Lois Joy Galler Foundation for Haemolytic Uraemic Syndrome Inc., Melville, NY. USA. Abstr. 191/I, pp: 22.

Baur, A.W., W.M.M. Kirby, J.C. Sherries and M. Truck, 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol., 45: 493-496.

Chattopadhyay, U.K., S. Dutta, A. Deb and D. Pal, 2001. Verotoxin producing *Escherichia coli* an environment-induced emerging zoonosis in and around Calcutta. Int. J. Environ. Health Res., 1: 107-112.

Cherla, R.P., S.Y. Lee and V.L. Tesh, 2003. Shiga toxins and apoptosis. FEMS Microbiol. Lett., 228: 159-166.

Guerra, B., E. Junker and A. Schroeter *et al*., 2003. Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine and poultry. J. Antimicrob. Chemother., 52: 489-492.

Leung, P.H.M., J.S.M. Peiris, W.W.S. Ng, R.M. Robins-Browne, K.A. Bettelheim and W.C. Yam, 2003. A newly discovered verotoxin variant, VT2g, produced by bovine verocytotoxigenic *Escherichia coli*. Applied Environ. Microbiol., 69: 7549-7553.

Mead, P.S. and P.M. Griffin, 1998. *Escherichia coli* O157: H7. Seminar. Lancet, 352: 1207-1212.

Melton-Celsa, A.R. and A.D. O'Brien, 1998. Structure, Biology and Relative Toxicity of Shiga Toxin Family Members for Cells and Animals. In: *Escherichia coli* O157: H7 and Other Shiga Toxin-producing *E. coli* Strains. Kaper, J.B. and A.D. O'Brien (Eds.), ASM Press, Washington, DC., pp: 121-128.

Melton-Celsa, A.R. and A.D. O' Brien, 2000. Shiga toxins of *Shigella dysenteriae* and *Escherichia coli*. Bact. Prot. Tox., 145: 385-406.

Mora, A., J.E. Blanco and M. Blanco *et al*., 2005. Antimicrobial resistance of Shiga toxin (Verotoxin)-producing *Escherichia coli* O157: H7 and non- O157 strains isolated from humans, cattle, sheep and food in Spain. Res. Microbiol., 156: 793-806.

Safdar, N., A. Said, R.E. Gangnon and D.G. Maki, 2002. Risk of hemolytic uremic syndrome after antibiotic treatment of *Escherichia coli* O157: H7 enteritis a meta-analysis. JAMA., 288: 996-1001.

Scheutz, F., L. Beutin, D. Piérard and H.R. Smith, 2001. Appendix Nomenclature of Erocytotoxins. In: Verocytotoxigenic *E. coli*. Duffy, G., P. Garvey and D.A. McDowell (Eds.), Food and Nutrition Press, Trumbull, CT, USA., pp: 447-452.

Schwarz, S. and E. Chaslus-Dancla, 2001. Use of antimicrobials in veterinary medicine and mechanisms of resistance. Vet. Res., 32: 201-225.

Singh, R., C.M. Schroeder and J. Meng *et al*., 2005. Identification of antimicrobial resistance and class 1 integrons in Shiga toxin-producing *Escherichia coli* recovered from humans and food animals. J. Antimicrob. Chemother., 56: 216-219.

Tilden, J., W. Young, A.M. McNamara, C. Custer, B. Boesel, M.A. Lambert-Fair, J. Majkowski, D. Vugia, S.B. Werner, J. Hollingsworth and J.G. Morris, 1996. A new route of transmission for *Escherichia coli*: Infection from dry fermented salami. Am. J. Public Health, 86: 1142-1145.

Yoh, M., T. Aoki, M. Akao, Y. Sakaue, E. Tsubura and T. Honda, 1997. Report of questionnaire about enterohemorrhagic *Escherichia coli* cases caused in the area including Sakai City in 1996. Kansenshogaku Zasshi, 71: 1144-1154.

Zhang, W.L., M. Bielaszewska, A. Liesegang, H. Tschäpe, H. Schmidt, M. Bitzan and H. Karch, 2000. Molecular characteristics and epidemiological significance of Shiga toxin-producing *Escherichia coli* O26 strains. J. Clin. Microbiol., 38: 2134-2140.

Zimmerhackl, L.B., 2000. *E. coli* antibiotics and the hemolytic-uremic syndrome. New Eng. J. Med., 342: 1990-1991.