



ANTIBIOTIC RESISTANCE AMONG SHIGA TOXIN PRODUCING- *ESCHERICHIA COLI* (STEC) ISOLATED FROM HUMANS

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ABSTRACT

Shiga toxin – producing *Escherichia coli* (STEC) can cause food borne infections, and fatal diseases in children, such as haemorrhagic colitis and hemolytic uremic syndrome, the progressive increase in antibiotic resistance among enteric pathogens in developing countries is becoming a critical area of concern. The aim of our study was to determine shiga toxin producing *Escherichia coli* resistance to the most used antibiotics. Isolates of STEC were assessed for susceptibility based on disk diffusion test following the recommendations of CLSI. Of the 28 STEC isolates, 100, 85.71, 85.71, 82.14, 64.29, 64.29, 60.71, 57.14, 57.14, 53.57, 50, 50, 46.43, 10.71, 0 percentage were resistant to: Amoxicillin, cefaclor, cefotaxime, trimethoprim-sulfamethoxazol, ceftazidime, gentamicin, ciprofloxacin, tobramycin, doxycyclin, chloramphenicol, cefepime, Amikacin, amoxicillin-clavulanic acid, Nitrofurantoin, Imipenem respectively. Findings from this study suggest antimicrobial resistance is widespread among *E. coli* O157, O26, O103, O111, and O145. Our findings from this study suggest antimicrobial resistance is widespread among STEC in our area. Therefore, regional information regarding antibiotic resistance should be used in clinical management, and treatment guidelines should be updated.

Keywords: *E. Coli*, Shiga toxin, Antibiotic resistance.

INTRODUCTION

Diarrheal diseases caused by microorganisms and their toxins are a major cause of mortality and morbidity throughout the world¹. There are over 2 million deaths occurring each year, particularly among infants younger than 5 years². It is now recognized that there is a very broad spectrum of human disease associated with Shiga toxin (Stx)-producing organisms. Stx-producing *Escherichia coli* (STEC) are an important cause of haemorrhagic colitis and the diarrhea-associated form of the Haemolytic Uraemic Syndrome (HUS)³. The STEC-related disease may involve either sporadic cases or large outbreaks involving a common contaminated food source⁴. These *E. coli* species produce either one or both phage encoded potent cytotoxic termed shiga toxins (stx)⁵.

Antibiotics have revolutionized the treatment of common bacterial infections and play a crucial in reducing mortality, antimicrobial therapy should be used in severe cases of diarrheal disease to reduce the duration of illness and may also used to prevent traveler's diarrhea⁶. However, the progressive increase in antibiotic resistance among enteric pathogens in developing countries is becoming a critical area of concern. In addition, the overuse and misuse of antibiotics in the treatment of diarrhea could lead to an increase in antibiotic resistance.⁷ Diarrhea caused by multidrug-resistant bacteria is an important public health dilemma among children and is set as a research priority of the control of diarrheal disease program for developing countries by the World Health Organization.⁸

In this study, the susceptibility of 28 STEC strains to different antibiotics are evaluated.

MATERIALS AND METHODS

Bacterial strains: in our study STEC were detected in 28 diarrheal case, determined by the presence of shiga toxin in stool samples by using ELISA kit (DRG, Germany), and STEC isolates were serologically typed, using commercially available O Antisera (O157, O26, O103, O111, O145) (Denka Seiken, Japan).

Antimicrobial agents

The following antibiotics were used for susceptibility testing: Amoxicillin (ABTEK, UK), Cefaclor (ABTEK, UK), Cefotaxime (ABTEK, UK), Trimethoprim-Sulfamethoxazol (BBL, USA), Ceftazidime (ABTEK, UK), Gentamicin (ABTEK, UK), Ciprofloxacin (BBL, USA), Tobramycin (ABTEK, UK), Doxycyclin (ABTEK, UK), Chloramphenicol (ABTEK, UK), Cefepime (ABTEK, UK), Amikacin (BBL, USA), Amoxicillin-Clavulanic acid (ABTEK, UK), Nitrofurantoin (ABTEK, UK), and Imipenem (BBL, USA).

Susceptibility testing

Susceptibility testing was performed by disk diffusion method by the following the recommendations of the clinical and laboratory standards institute (CLSI, formerly the national committee for clinical laboratory standards (NCCLS)), According to next protocol:

Preparation of Muller-Hinton (MH) plate

1) MH agar plates were got out from refrigerator to come to room temperature.



2) If the surface of the agar has visible liquid present, the plates were set inverted ajar on its lid to allow the excess liquid to drain from the agar surface and evaporate plates were set in 35°C incubator (usually to 30 minutes).

Preparation of inoculums

Four or five isolated colonies of each isolate of STEC were selected from MacConkey agar by using a sterile inoculating loop, then it was transferred or suspended to a tube containing 2 ml of sterile saline, to have bacterial suspension, saline tube was vortexed then the turbidity of this suspension was adjusted to 0.5 McFarland, by comparing the bacterial suspension and 0.5 McFarland standards against a white background to match the turbidity.

The inoculums suspension should be used within 15 minutes of preparation.

1) Inoculation of the MH plate

A sterile cotton swab was dipped into the bacterial suspension, pressed and rotated firmly against the side of the tube to express excess liquid, Or A sterile cotton swab was dipped into the inoculums tube, then the swab was rotated against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. Then the dried surface of a MH agar plate was inoculated by streaking the swab three times over the entire agar surface, the plate was rimmed with the swab to pick up any excess liquid. Then the lid was left slightly ajar, and the plate was placed at room temperature at least 3 to 5 minutes but not more than 15 minutes, for the surface of the agar plate to dry.

2) Placement of the antibiotic disks

The antibiotic disks were placed evenly (no closer than 24 mm from center to center) on the surface of the agar plate by using a sterile FORCEPS. No more than 5 disks should be placed on a plate. The disks were pressed firmly to ensure contact with agar. Within 15 minutes of disk placement, plates were inverted and placed in a 35 ° c air incubator for 16-18 hours.

3) Reading plates and test interpretation

After incubation, each plate was examined, and the diameters of the zones of complete inhibition were measured, using a ruler. Plates were placed above a black surface, and zones were examined from the back side (agar side) of the plate. Large colonies growing within a clear zone of inhibition were subcultured, reidentified and retested it. Once zone measurements had been made, the millimeter reading for each antimicrobial agent was compared with that specified in the interpretive tables of the CLSI documents, and results are interpreted as either susceptible, resistant, or intermediate.

» Preparation of 0.5 McFarland standard

0.5 McFarland standard can be prepared by adding 99.5 ml of 1% sulfuric acid and 0.5 ml of 1.175% barium chloride. This solution is dispensed into tubes comparable

to those used for inoculums preparation. The 0.5 McFarland standard provides a turbidity comparable to that of a bacterial suspension containing approximately 1.5×10^8 cfu/ml.

RESULTS AND DISCUSSION

Results

Detection of shiga toxin and Serotyping of STEC isolates

We detected stx in 28 stool samples by ELISA and these 28 STEC isolates belong to the following serogroups: O26 (n=18 isolates), O111 (n=3 isolates), O157 (n=2 isolates), O103 (n=1 isolate), O145 (n=1 isolate), Other serogroups (n=3 isolates).

Antimicrobial resistance

Of the 28 STEC isolates, 100, 85.71, 85.71, 82.14, 64.29, 64.29, 60.71, 57.14, 57.14, 53.57, 50, 50, 46.43, 10.71, 0 percentage were resistant to: Amoxicillin, cefaclor, cefotaxime, trimethoprim-sulfamethoxazol, ceftazidime, gentamicin, ciprofloxacin, tobramycin, doxycyclin, chloramphenicol, cefepime, Amikacin, amoxicillin-clavulanic acid, Nitrofurantoin, Imipenem respectively. The antimicrobial resistance of STEC isolates of serogroups O157, O111, and O26 are shown in (figure 1) and (table 1).

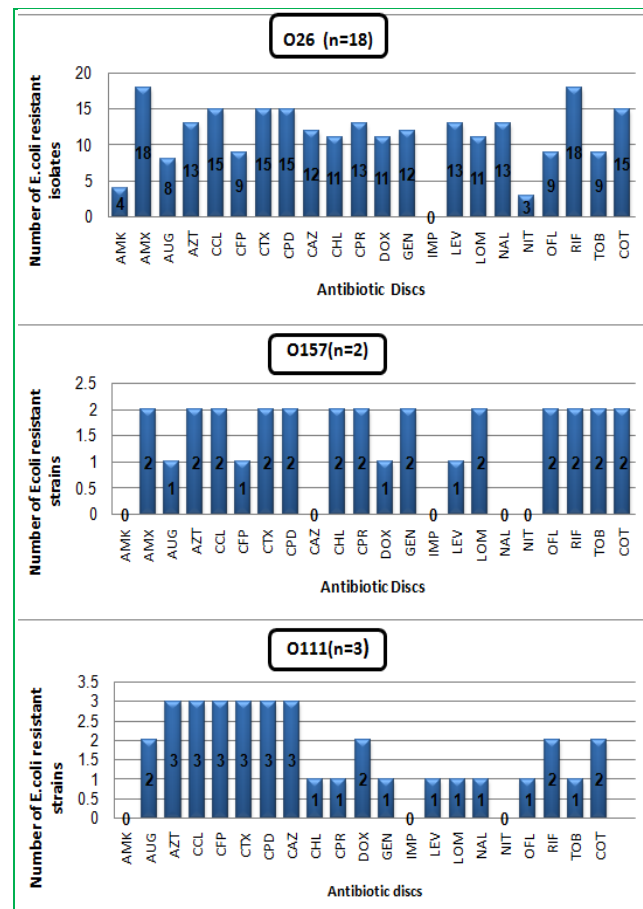


Figure 1: Comparison of antimicrobial resistance frequencies between Shiga toxin-producing *Escherichia coli* most isolated Serotypes.



Table 1: Antibiotic resistance of 28 shiga toxin-producing E.Coli isolated from diarrheal patients

Antimicrobial Agent	No. of Resistance	% Resistance
Amoxycillin	28	100
Cefaclor	24	85.71
Cefotaxime	24	85.71
Trimethoprim-sulfamethoxazol,	23	82.14
Ceftazidime	18	64.29
Gentamicin	18	64.29
Ciprofloxacin	17	60.71
Nalidixic Acid	17	60.71
Tobramycin	16	57.14
Doxycyclin	16	57.14
Chloramphenicol	15	53.57
Lomefloxacin	14	50
Cefepime	14	50
Amikacin	14	50
Ofloxacin	13	46.43
Amoxycillin-clavulanic acid,	13	46.43
Nitrofurantoin	3	10.71
Imipenem	0	0

Resistance to more than four antibiotics was observed in 89.3 % (n=25) of STEC isolates, and 71.4 % were resistant to more than six antibiotics.

According to the definition of multidrug resistance by lockhart et al [multidrug resistance defined as resistance to at least one extended spectrum cephalosporins (ceftazidime, ceftriaxone or cefotaxime), one aminoglycoside (Amikacin, or tobramycin) and ciprofloxacin], Five STEC (17.85%) isolates were multidrug resistance. These isolates were resistance to at least eight antimicrobials from these classes.

Discussion

Diarrheal diseases due to the Shiga toxin producing E.coli are a health problem in many parts of the world. It has been estimated that infectious diseases cause 9.2 million deaths in the developing countries, and diarrheal diseases are the fourth most common cause of mortality. Most mild diarrhea cases are effectively treated by oral rehydration slats (ORS) and only patients with severe or persistent diarrhea should receive antibiotics⁹.

Resistance to antibiotics is very common in bacterial isolates all around the world. Regular Surveillance of antibiotic resistance provides data for antibiotic therapy and resistance control. Information of antimicrobial resistance patterns is important in choosing the appropriate antibiotic therapy. Diarrhea caused by multidrug-resistant bacteria is an important public health dilemma among children and is set as a research priority of the control of diarrheal disease program for developing countries by the World Health Organazation.⁸

In the present study, most STEC strains were resistant to at least 3 commonly used antibiotics amoxicillin, cephalosporins, and trimethoprim-sulfamethoxazole. Some previous studies have shown high prevalence of

resistance to these antibiotics^{9,10}. Fazeli⁷ reported lower percentage of resistance to trimethoprim-Sulfamethoxazole and Amoxicillin in E.coli compared to what we have determined. However the resistance against tetracycline was higher than what our data shows. Our findings are comparable with the study of Borjian¹¹ which the resistance against these antibiotics may be due to the higher rate of prescription in treatment of diarrhea because of their low cost and easy application.

According to our findings, amoxicillin, and trimethoprim-sulfamethoxazole are not recommended for the treatment of diarrhea in this population. Therefore, local information about antibiotic resistance should be used in clinical management, and treatment guidelines should be updated. Although the cephalosporins are not indicated for the treatment of diarrhea, we have tested the susceptibilities of STEC strains to these antibiotics. In Orrett study in India¹², the greatest efficacy were observed for Imipenem, gentamycin, ciprofloxacin, and the cephalosporins ceftazidime and ceftriaxone. In our study, the results showed that cefepime and ceftazidime indicated moderate activity against STEC strains, and ceftriaxone indicated low activity while the greatest efficacy were observed for Impenem.

In this study, we find that Fluoroquinolone (Lomefloxacin, Ofloxacin) and quinolone (nalidexic acid) have moderate activity against resistant STEC strains, while in Fazeli sudy⁷ all STEC strains were susceptible to ofloxacin. These antibiotics are now commonly used to treat infections, including diarrhea. They have also been recommended for treatment of travelers diarrhea.

The multi resistance to amoxicillin, cephotaxime and trimethoprim-sulfamethoxazole were most prevalent. Multi resistance has been also reported in previous studies¹³. We found that 50% of STEC strains were resistant to amoxicillin, Doxycyclin, and trimethoprim-sulfamethoxazole, and 71% were resistant to either nalidixic acid or ciprofloxacin. That means the patients infected with these E.Coli strains may risk a treatment failure. Its also indicated that multi resistance of different categories of STEC strains is emerging in our country where these antibiotics (both early and new) have been widely used.

CONCLUSION

The results of Our study suggest that antimicrobial resistance is wide spread among shiga Toxin-producing E.coli strains, and it should be a major concern, and more extensive studies are required to establish the levels of antibiotic resistance among populations of bacteria isolated from diarrheal patients.

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