A CROSS SECTIONAL STUDY ON ANTIMICROBIAL DRUG RESISTANCE PATTERN OF ESCHERICHIA COLI ISOLATED FROM HUMANS URINE AND STOOL SAMPLE IN KHULNA

[1]NASRIN AKHTER, [2]ZUBAIR AHMED RATAN, [3]NURUNNABI MOLLAH, [4]KANIZ FATEMA

Dept. of Biomedical Engineering, Khulna University of Engineering & Technology, Bangladesh
Department of Virology, BSMMU, Dhaka, Bangladesh
E-mail: [1]nasrin_dr@yahoo.com, [2]ratandmck62@gmail.com, [3]nurunnabim12@gmail.com, [4]fatemadr.kaniz@yahoo.com

Abstract - Escherichia coli (E.coli) is one of the most common causative organism for urinary tract infection (UTI) and diarrhoea in developing countries, specially in Bangladesh. We conducted a retrospective study of Escherichia coli isolates recovered from human urinary and stool samples during August, 2016–September, 2017 in Khulna city to generating primary data on the prevalence of antimicrobial drug resistance in E. coli in a sample population. In the present study a total of 524 E. coli isolates from human's urinary sample and 437 from human stool sample, were tested for susceptibility to 12 antimicrobial drugs. Our result reveals that E.coli is 97-100% sensitive to imipenem and meropenem isolated from both urine and stool sample. Most extensively used antibiotic Ciprofloxacin has become 33% and 25% resistant isolated from urine and stool sample respectively. 98.85% and 98.90% resistance to ampicillin, 97.98% and 88.17% resistance to amikacin, 95.34% and 87.13% resistant to tetracycline, 83.7% and 91.37% resistance to erythromycin, 93.6% and 91.72% resistance to co-trimoxazol, 63.57% and 68.56% resistance to ceftriaxon, 89.16%and 92% resistance to trimethoprim-sulfomethoxazole, 98.2% and 96.3% resistance to, nalidaxic acid and 49.03% and 51.34% resistant to gentamycin (urine and stool sample respectively). These data indicate that the multidrug resistance of E. coli is gradually increasing in Khulna city. Although, population density is lower in Khulna and the study were perform in a short period of time. Further details study including genomic and proteomic basis of resistance pattern is needed.

Index Terms - E. coli, Antimicrobial drug resistance, susceptibility, Multi drug resistance

I. INTRODUCTION

Escherichia coli (E. coli) is a gram-negative, facultative anaerobic, rod-shaped, coliform bacteria that normally live in the intestines of people and animals. However, some types of E. coli, particularly E. coli O157:H7, can cause intestinal infection like diarrhea. In developing countries, diarrhoeal diseases are one of the major causes of death in children under 5 years of age. On the other hand Urinary tract infections(UTI) are among the most common bacterial infections in humans and Escherichia coli is by far the most commonly recovered bacterial pathogen from both hospital and community patients with this infection [1]. Multiple antimicrobial drug-resistant bacteria have been recognized as a grave warning to the nation's public health. During the past few decades there are many studies about the antimicrobial drug resistance pattern and its prevalence have been scrutinized. Day by day the increasing resistances of microbes are now endangers the tremendous therapeutic advantage afforded by antibiotics. Two- thirds of the world's population lives in the developing nations of Asia, Africa and South America. Most of these populations exist under conditions of poverty, inadequate medical care, poor sanitation and nutrition [2]. There are different causes of increasing antimicrobial drug resistance among bacteria. The most common case are as follows; random use of antibiotics, over dose or inappropriate dosing, and incomplete treatment in both humans and also some animals , extensive agricultural use, leading to development of resistance in common bacteria [3]. Antibiotic resistance leads to higher medical costs, prolonged hospital stays, and increased mortality. Increased trend of drug resistance pattern, burden of treatment costs and higher frequency of infection to treatment failure insisted the scientists and physicians on doing research on UTI. It is surprisingly observed in the variation of the resistance pattern of UTI in the developing country compared to the developed one, most probably due to indiscriminate use of antibiotics [4]. It has been suggested that resistance in bacterial populations may spread from one ecosystem to another [5]. The wild dissemination of antimicrobial resistance among bacterial populations is an increasing problem worldwide. In the past various surveillance data show that resistance in E. coli is consistently highest for antimicrobial agents that have been in use the longest time in human and veterinary medicine [6]. The past 2 decades have witnessed major increases in emergence and spread of multidrug-resistant bacteria and increasing resistance to newer compounds, such as fluoroquinolones and certain cephalosporins. For example, a study of the susceptibility of E. coli isolates recovered from hospitals during a 12-year period (1971–1982) showed no major change in resistance to any of the antimicrobial drugs tested [7]. In contrast, a retrospective analysis of E. coli from urine specimens collected from patients during 1997–2007 showed an increasing resistance trend for ciprofloxacin, trimethoprim/sulfamethoxazole, and amoxicillin/clavulanic acid [8]. Similarly a 30-year

(1979–2009) follow-up study on E. coli in Sweden showed an increasing resistance trend for ampicillin, sulfonamide, trimethoprim, and gentamicin [9]. Also, the treatment of bacterial infections in individual patients is largely empirical, due to low access to diagnostic microbiological laboratories. The lack of surveillance data and feedback from clinical microbiology laboratories on etiology and susceptibility patterns of bacterial infections in individual patients hinders antibiotic stewardship and appropriate antibiotic use [10]. Although, lot of studies have been done for the resistance of antimicrobial drug in the world as well as in Bangladesh, Our aim of this study was to investigate the resistance pattern of E.coli in the Khulna region and generating primary data on the prevalence of antimicrobial drug resistance in E. coli in a sample population for the proper management of UTI and diarrhea caused by E.coli.

II. MATERIALS AND METHODS

A. Sample collection:

We conducted this prospective point-prevalence study in Khulna city during August, 2016–September, 2017 on different age group people from dissimilar socioeconomic status. The study design is abridged in Fig. 1 and Fig. 2. Urine and stool samples were collected from 960 patients at different hospital and diagnostic centers in Khulna city are included in this study. The participants were selected randomly from different hospitals. All participants were properly informed and signed an informed consent. The patients were categorized in five age groups. Table 1 shows the age group and specimen collected from UTI infected patient and patient with diarrhea.

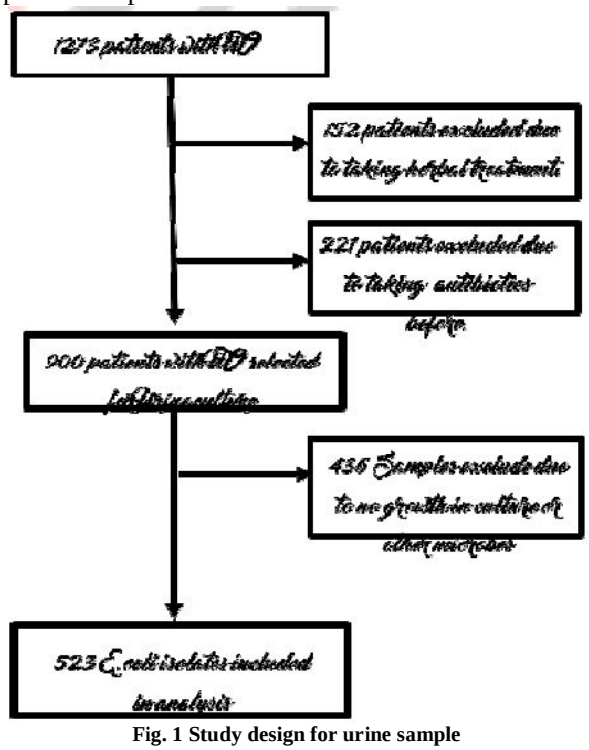


Fig. 1 Study design for urine sample

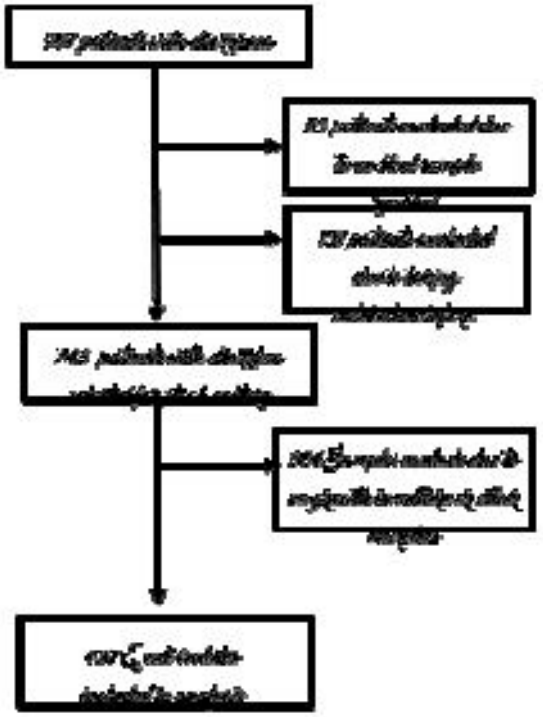


Fig. 2 Study design for stool sample

Age group	E.coli isolate from patient with UTI	E.coli isolate from Patient with diarrhoea
0-5	59	131
6-15	97	93
16-30	143	77
31-50	117	87
>50	107	49
Total	523	437

Table 1. Specimens collected for this study

B. Isolation of Colonies:

The aseptically collected samples were inoculated on nutrient agar media using a calibrated loop delivering 10 µL of the sample and incubated overnight at 37 °C. Processing of stool samples; Samples were allowed to reach room temperature and then 10gm of fresh fecal sample was mixed with 90ml of sterile normal saline and shook to form homogenous mixture. All samples were mixed by vigorous shaking. All bacterial specimens were cultured on blood agar and Mac Conkey agar, again incubated overnight at 37 °C and the isolates were identified by adopting standard microbiological procedure which includes colony morphology, Gram stain reaction, Microscopic examination of morphology bacteria include; The size, shape, arrangements and Gram reactions of the 24 hour bacterial cultures were observed in a microscopical field and biochemical reaction such as oxidase, catalase, sulfide indole motility test, citrate agar slant, methyl red test, Voges Proskauer test, triple sugar iron agar test and urease test. Biochemically confirmed isolates of E. coli were subjected to antimicrobial sensitivity testing [10, 11].

C. Antibiotic susceptibility testing

Susceptibility and resistance of all isolates were checked by performing the Kirby-Bauer disc diffusion method according to the guidelines of Clinical and Laboratory Standards Institute, formerly National Committee for Clinical Laboratory Standards. The antibiotic incorporated plates were incubated at 37°C and zones of inhibition around the antibiotic were measured after 24 hr and the standard disc diffusion method also known as Kirby Bauer method was used for the in vitro determination of the sensitivity to the antimicrobial agents [12, 13].

D. Antimicrobial discs

Commercially available antimicrobial discs (Mast Diagnostics, U.K) of Ampicillin (AM) (10 µg), Amikacin (AK) (30 µg) Erythromycin (ER) (15 µg), gentamycin (CN) (10 µg), Sulfamethoxazole-trimethoprim (SXT) (25 µg), Tetracycline (TE) (30 µg), Ciprofloxacin (CIP) (5 µg), Cotrimoxazol (CO) (25 µg), Meropenem (MEM) (10 µg), Imipenem (IMP) (10 µg), Ceftriaxone (CRO) (30 µg) and Nalidaxic acid (30 µg) were placed on Mueller–Hinton agar (Bio-Rad) seeded with bacteria cultured in their early log phase, as previously described CLSI [10-13].

E. Statistical analysis

Antimicrobial susceptibility data were expressed as percentages or the frequency of human isolates using excel. A one-way analysis of variance was used to estimate the overall difference between the percentages or frequencies of resistance of E. coli isolates. Student t test was done for p value. In all cases, $p < 0.05$ was regarded as statistically significant.

RESULTS

We conducted this study about antibiotic susceptibility test of E. coli isolates from urine sample of patients with UTI and stool sample of patient with diarrhea. A total of 523 E.coli isolates from urine sample of patients with UTI and 437 E.coli isolates from of patient with diarrhea in Khulna city, Bangladesh, were evaluated for their sensitivity and resistance to antibiotics. Twelve commonly prescribed and available antibiotics were used to assess. Antibiogram of these isolates showed that, E.coli was most susceptible towards imipenem and meropenem (for both Urine and stool sample) in Khulna ($P<0.05$). Whereas Ampicillin (98.85% and 98.90%), Amikacin (97.98% and 88.17%), Tetracyclin (95.34% and 87.13%), Erythromycin (83.7% and 91.37%), Trimethoprim-Sulfamethoxazol (89.16 and 92%) Co-trimoxazol (93.6% and 91.72%) and Nalidaxic acid (98.2% and 96.3%) was found most resistance in Khulna ($P<0.05$). On the other hand Gentamycin (49.03% and 51.34% resistance) and Ceftriaxone (63.57 % and 68.56 % resistance) (for Urine and stool sample respectively), were found less resistance and as well as less sensitive. The most commonly used antibiotics Ciprofloxacin found 33% and 25 % resistance for Urine and stool sample respectively Fig. 3 and Fig. 4.

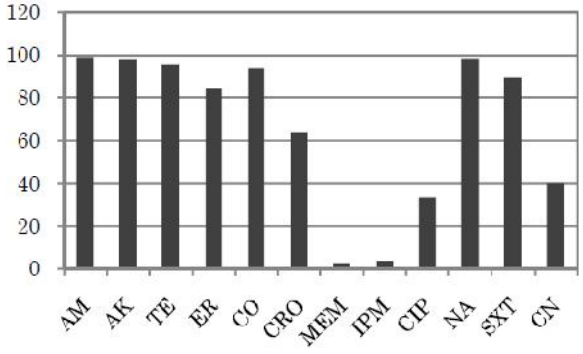


Fig. 3 Bar diagram showing % of resistance pattern for 12 most commonly used antibiotics to the E.coli isolated from urine sample

Ampicillin (AM), Amikacin (AK)), Tetracycline (TE), Erythromycin (ER), Cotrimoxazol(CO) Ceftriaxone (CRO), Meropenem (MEM), Imipenem (IMP), Ciprofloxacin (CIP), Nalidaxic acid, Sulfamethoxazole-trimethoprim (SXT), Gentamycin (CN)

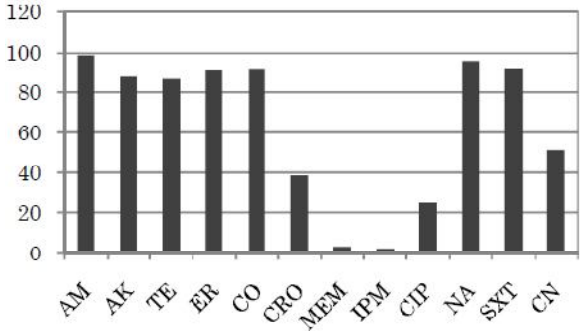


Fig. 4 Bar diagram showing % of resistance pattern for 12 most commonly used antibiotics to the E.coli isolated from stool sample

Ampicillin (AM), Amikacin (AK)), Tetracycline (TE), Erythromycin (ER), Cotrimoxazol(CO) Ceftriaxone (CRO), Meropenem (MEM), Imipenem (IMP), Ciprofloxacin (CIP), Nalidaxic acid, Sulfamethoxazole-trimethoprim (SXT), Gentamycin (CN)

DISCUSSION

Antibiotic resistance in E. coli is of particular concern because it is the most common Gram-negative pathogen in humans, the most common cause of urinary tract infections, a common cause of both community and hospital-acquired bacteraemia33 as well as a cause of diarrhea [14]. In this study, we examined antimicrobial resistance of E. coli isolates from patients due to UTI and diarrhea. Faecal E. coli is regarded as a useful indicator of the spread of acquired antibiotic resistance genes in the community [15, 16]. The highest levels of resistance were observed against

Ampicillin, Nalidaxic acid, Co-trimoxazole, and Trimethoprim-Sulfamethoxazol for pathogenic E. coli isolates from urine and stool, which may be caused by the frequent use of these antibiotics and the transfer of plasmids between bacteria. We found that higher resistance to the antibiotics most frequently prescribed for diarrhea was found in the isolates of not only the patients with diarrhea, but also patient with UTI [16]. The reason antibiotic resistance was different between the two sample remains unknown, and therefore requires further investigation. Different factors have been implicated in increasing antibiotic resistance of bacteria in Bangladesh. One of the most potent factors is the easy availability of antibiotics over the counter and the tendency of using antibiotics for most symptoms. Sometimes, studies showing antibiotic resistance can act as impetus to prescribe higher antibiotics as physicians may start to think that common drugs will be useless. But a multi-sector coordinated approach is needed to tackle the problem and preserve some medicines for future [17].

CONCLUSION

Our study adds to existing knowledge about resistance in E. coli in a Bangladeshi community setting. The large number of antibiotics included in the study provides a comprehensive overview of resistance pattern in E. coli. There is a need to enhance local and national research and surveillance efforts to monitor resistance trends of commensal E. coli in community.

REFERENCES

[1] F. Oluwasola, O. D. O. Kolawole and A. Lamikanra, "Antibiotic resistance and virulence properties in Escherichia coli strains from case of urinary tract infections", Afr. J. Infect. Dis., Vol. 7, No. 1, pp. 1 – 7, 2013.

[2] J. A. Walash, and K. S. Warren, "Selective primary health care; an interim strategy for disease control in developing countries", New Engl J Med, vol. 74, pp. 301-967, 1979.

[3] WHO Antimicrobial resistance, www.who.int/mediacentre/factsheets/fs194/en.

[4] S. Nasrin, F. T. Zohera, S. A. Mahbub, H. Akther, and M. Haque, "One year surveillance of antimicrobial sensitivity pattern of different antibiotics on clinical isolates of Escherichia coli with comparison of resistance against different antibiotics in four major cities in Bangladesh", ClinPharmacol, Vol. 5, pp. 607-612, 2003.

[5] J. R. Johnson, M. R. Sannes, C. Croy, B. Johnston, C. Clabots, M. A.Kuskowski, et al., "Antimicrobial drug-resistant Escherichia coli from humans and poultry products, Minnesota and Wisconsin, 2002–2004",Emerg Infect Dis., vol. 13, pp.838-46, 2007.

[6] US Food and Drug Administration. National antimicrobial resistancemonitoring system –enteric bacteria (NARMS): 2008 executivereport, Rockville (MD), 2010.

[7] B. A. Atkinson, and V. Lorian, "Antimicrobial agent susceptibility patternsof bacteria in hospitals from 1971 to 1982", J ClinMicrobiol, Vol. 20, pp. 791–6, 1984.

[8] L. Blaettler, D. Mertz, R. Frei, L. Elzi, A. F. Widmer, M. Battegay, et al., "Secular trend and risk factors for antimicrobial resistance inEscherichia coli isolates in Switzerland 1997–2007", Infection, vol. 37, pp. 534–9, 2009.

[9] D. A. Tadesse, S. Zhao, E. Tong, S. Ayers, A. Singh,M. J. Bartholomew, et al., "Antimicrobial Drug Resistancein Escherichia coli from Humansand Food Animals, United States, 1950–2002", Emerging Infectious Diseases, Vol. 18, pp.741-49, May 2012.

[10] M. M. B. Nagelkerke, K. Sikwewa, D. Makowa, I.D. Vries, S. Chisi and J. W. D. Zetsma, "Prevalence of antimicrobial drug resistant bacteria carried by in and outpatients attending a secondary care hospital in Zambia", BMC Res Notes, Vol. 10, pp. 378-89, 2017.

[11] S. Monira, S. A. Shabnam, S. I. Ali, A. Sadique, F. T. Johura, and K. Z. Rahman, "Multi‐drug resistant pathogenic bacteriain the gut of young children in Bangladesh", Gut Pathog, Vol. 9, 2017.

[12] M. S. Khan, N. Akhtar, M. E. Haque, A. Barua, T. Chowdhury, and R. Mullick, "Isolation and Identification of non-plasmid Multidrug Resistant E.coli from Poultry Wastes in Chittagong Region, Bangladesh", J Bacteriol Parasitol, vol. 5, 2014.

[13] A. Mannan, M. Shohel, S. Rajia, N. U. Mahmud, S. Kabir, and I. Hasan, "A cross sectional study on antibiotic resistance pattern of Salmonella typhiclinical isolates from Bangladesh", Asian Pac J Trop Biomed, vol. 4, pp. 306-311, 2014.

[14] P. Shakya, P. Barrett, V. Diwan, Y. Marothi, H. Shah, N. Chhari, et al., "Antibiotic resistance among Escherichia coli isolates from stool samples of children aged 3 to 14 years from Ujjain, India", BMC Infectious Diseases, vol. 13, pp. 477-485, 2013.

[15] S. C. Ester, P. M. D. Pilar, F. Wang, S. I. Perez, H. Jiang, and T. F. O'Brien, "The carriage of Escherichia coli resistant to antimicrobial agents by healthy children in Boston, in Caracas, Venezuela, and in Qin Pu China", N Engl J Med., vol. 5, pp. 285-289, 1990.

[16] S. Nys, I. N. Okeke, S. Kariuki, G. J. Dinant, C. Driessen, and E. E. Stobberingh, "Antibiotic resistance of faecal Escherichia coli from healthy volunteers from eight developing countries", J Antimicrob Chemother. Vol. 54, pp. 952-955, 2004.

[17] J. Ray, R. Paul, A. Haldar, S. Mondol "A study on antibiotic resistance pattern of Escherichia coli isolated from urine specimens in Eastern India", International Journal of Medical Science and Public Health, Vol .4, pp. 1670-1674, 2015.

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