Antibiotic Resistance and Plasmid Profiling of *Escherichia coli* Isolated from Human Sewage Samples

Microbiology Insights
Volume 14: 1–6
© The Author(s) 2021
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/11786361211016808

Sanjida Akter*, A. M. Masudul Azad Chowdhury* and Sohana Akter Mina

Department of Genetic Engineering and Biotechnology, Faculty of Biological Sciences, University of Chittagong, Chittagong, Bangladesh.

ABSTRACT: In developing countries, the occurrence of antibiotic resistance is increasing day by day and antibiotic resistant microorganisms are being found in almost every environmental setting. Plasmids are considered as the main vector in the procurement and propagation of antibiotic resistance in many microorganisms such as *Escherichia coli* (*E. coli*). The goal of this study was to examine the antibiotic resistance and screening of plasmid in *E. coli* strains which were previously identified from human sewage samples. During this study antibiotic susceptibility of *E. coli* isolates were determined by Kirby-Bauer disk diffusion method against 5 antibiotics (ampicilin, ceftriaxone, amoxicillin, ciprofloxacin, azithromycin). Furthermore, plasmid extraction of each isolate was done according to the protocol of FavorPrepTMPlasmid Mini Kit and plasmid profiling was done by agarose gel electrophoresis. In antibiotic sensitivity test, all *E. coli* strains showed resistance to ampicilin, amoxicillin, and ceftriaxone. In the plasmid profiling, it was revealed that all the isolates of *E. coli* harbored plasmids. The plasmid sizes ranged from approximately 1.5 to 15 kb. The findings of this study prove the consequences of antibiotic resistance as well as relationship of plasmid with antibiotic resistance which necessitates proper surveillance on antibiotic usage in the developing countries.

KEYWORDS: *E. coli*, antibiotic resistance, plasmid

RECEIVED: February 12, 2021. **ACCEPTED:** April 3, 2021.

TYPE: Original Research

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research is partially funded by Research and Publication Offices, University of Chittagong, Bangladesh for experimental works. The authors received no financial support for the authorship and publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: A. M. Masudul Azad Chowdhury, Department of Genetic Engineering and Biotechnology, University of Chittagong, Chittagong 4331, Bangladesh. Email: masud.geb@cu.ac.bd

Introduction

Antibiotics include a range of powerful medications that are used to treat diseases caused by bacteria as they destroy or slow-down the growth of bacteria. For decades, the marked increase in antibiotic usage has accelerated the natural phenomena 'antibiotic resistance'. Antibiotic resistance happens when bacteria develop the ability to defeat the drug that is designed to destroy them. In fact, antibiotic resistance has been named as 1 of the 3 most important health risks of 21st century by the World Health Organization.[1] Health complications caused by resistant microbes includes increased mortality rate, treatment difficulties and prolonged time of infection. Moreover, antibiotic resistant organism causes deaths which are of around 23 000 people annually in the United States.[2]

The impendence of antibiotic resistance is greater in developing countries because of the comprehensive misuse of antibiotics, lacking of inspection, poor quality of drugs etc.[3] Bangladesh being a developing country of Southeast Asia poses a global and regional threat having high risk of antibiotic resistance problem.[4] The healthcare system of Bangladesh is very low that leads to chronic and repeated infections. Irrational prescribing by doctors, a habit of self-medication among patients[5] and the indiscriminate use of antibiotics in agriculture and farming in many areas of the country have also been found recently.[6]

Antibiotic resistance emerges not only in pathogenic and disease causing organisms but also commensal strains like *Escherichia coli* (*E. coli*) that is a member of the normal flora in the gastrointestinal tract of human and warm blood animals.[7] *E. coli* belongs to the family Enterobacteriaceae, is a Gram-negative, rod shaped, non-sporulating, a non-fastidious, motile, and facultative anaerobic bacterium. *E. coli* is widely used as an indicator organism for the microbiological quality of water and food.[8] *E. coli* is widely dispersed in the natural environment (water, soil, sometimes plants used as food) through human or animal excretion. It is transmitted via fecal–oral route.[9] The existence of *E. coli* in nature is diverse, that range from exhibiting commensalism to those causing diseases on human or animal hosts.[10] The commensal *E. coli* when are exposed to antibiotics, are forced to develop different strategies to survive and grow in the toxic environment. Antibiotic resistant *E. coli* was found in healthy human stool, street food and drinks and surface.[11] If *E. coli*, especially the pathogenic ones are present in open environment and factors that influence their survival rate are very troublesome issues in case of disease occurrence.[10]

E. coli can develop resistance mechanism mainly by both the efflux pumps interruption and the resistance genes located on plasmids.[12] Plasmids are considered as the main vector in the procurement and propagation of multi-resistant either phenotypically or genotypically.[13] Horizontal gene transfer of plasmid encoded resistant genes is the prevalent mechanism at the origin of acquisition of antibiotic

* These authors contributed equally to this work.

resistance and plasmid-encoded antibiotic resistance encompasses most currently used clinically relevant classes of antibiotics.[14]

The present study was designed to understand the antibiotic resistance pattern and plasmid profiling of previously identified *E. coli* isolates[15] from human sewage samples of poor hygienic regions of Chattogram city, Bangladesh to evaluate the misuses of commonly used antibiotics in those areas.

Materials and Methods

Bacterial strain

Eight *E. coli* strains[15] were studied in this research. All the isolates were previously isolated and identified from human sewage samples of 4 poor hygienic areas (Chittagong Railway station, Jhautola station, Akbarshah, Karnaphuli market) of Chattogram city, Bangladesh[15] All the *E. coli* stains were isolated with proper microbiological, biochemical and molecular methods and coded as Ec-CRS1 (*E. coli* Chittagong Railway Station 1), Ec-RS2 (*E. coli* Chittagong Railway Station 2), Ec-JHT3 (*E. coli* Jhautola station 3), Ec-AKS4 (*E. coli* Akbarshah 4), Ec-AKS5(*E. coli* Akbarshah 5). Ec-AKS6 (*E. coli* Akbarshah 6), Ec-KPM7(*E. coli* Karnaphuli market 7), Ec-KPM8 (*E. coli* Karnaphuli market 8).[15]

Selection of antibiotic

In this study commercially available and most commonly used antimicrobial discs (Himedia Laboratory limited, Mumbai, India) were used for antibiotic resistance test. The following antimicrobial agents were tested against the identified *E. coli* isolates:[15] Ampicillin (AMP)(25 µg), Azithromycin (AZM) (30 µg), Ciprofloxacin(CIP) (5 µg), Amoxicillin (AMX) (30 µg),Ceftriaxone (CTR)(30 µg).

Antimicrobial susceptibility testing

Kirby-Bauer Disk diffusion method[16] was performed to assess the antibiotic susceptibility of all the *E. coli* isolates using Mueller-Hinton agar plate according to the guidelines and recommendations of CLSI.[17] The procedure involved measuring the diameter of the zone of inhibition that results from pervasion of the agent into the medium surrounding the disc.

Extraction of plasmid DNA and Plasmid profiling

To determine, whether the antibiotic resistance was plasmid-mediated or not, these isolates were subjected to plasmid-DNA extraction procedure and agarose gel electrophoresis. For plasmid DNA extraction high copy number protocol was followed according to FavorPrepTMPlasmid Mini Kit (Cat. No. -FAPDE 100). The isolated DNA samples were then electrophoresed in 1% agarose gels stained with ethidium bromide (0.5 µg/ml) and visualized under an ultraviolet transilluminator.

Results

Antimicrobial susceptibility test

Antibiotic pollution in various environmental setting is quite common and has been reported in several recent articles.[18,19] We hypothesized that,the *E. coli* strains studied in this research might be resistant to common antibiotics due to the abuse of antibiotics in poor areas in Bangladesh. To test the hypothesis, antibiotic sensitivity test was done against 5 commonly used antibiotics. The isolates were either sensitive or resistant but not intermediate. The test result revealed that, all the isolates were multidrug resistant as they showed resistance against amoxicillin, ampicilin and Ceftriaxone (Table 1). However, ciprofloxacin and azithromycin seems to be effective against these *E. coli* strains (Table 1). We assume that, availability and overuse of amoxicillin, ampicilin and Ceftriaxone in poor hygienic areas might be the reason for this differentiation. Table 1 interprets the detailed antibiotic response of the test isolates. Figure 1A illustrates the disc diffusion test of sample EC-CRS1 and sample EC-CRS2.

Plasmid profiling

To investigate the genomic relation behind the antibiotic resistance, plasmid profiling was done for all the identified *E. coli* strains. All the isolates of *E. coli* harbored single plasmids. Of the 8 isolates, 5 isolates contained more than 1 plasmid. The plasmid sizes ranged from approximately 1.5 to 15 kb, the most common plasmid of size approximately 11 to 12 kb being detected in all the *E. coli* strains (Figure 1B), Table 2.

Discussion

Our Laboratory has been focusing on antibiotic resistant bacteria since last 10 years. Previously, our lab had identified severe occurrence of antibiotic pollution in and around hospital setting in Chattogram city, Bangladesh.[20] In this study we have shown antibiotic resistance profiling of eight *E. coli* isolates which have been previously isolated from 4 poor hygienic areas of Chattogram city, Bangladesh. We have recently reported the probiotic activity of these isolates against diarrheal pathogen *Shigella*.[15] However, all these isolates were identified from slum areas of Chattogram city, Bangladesh, where antibiotic misuses are common. In Bangladesh there are no guidelines of antibiotic uses, people can buy antibiotic from local shop without any proper prescription by doctor. As a consequence, people have access to get almost all common antibiotics and such activities influences us to conduct this research. In this study, we found all of the *E. coli* isolates were resistant to 3 of the 5 commonly used antibiotics. Surprisingly all the β-lactam ring containing antibiotics (ampicillin, ceftriaxone and amoxicillin) were resisted by the *E. coli* (Table 1). In an earlier research, our laboratory has been identified similar antibiotic resistance pattern in *E. coli* isolated from hospital and dairy wastes[21] but this research proven that antibiotic pollution has now been reached

Table 1. Measurement of zone of inhibition by *E. coli* isolates against antibiotics. Zone of inhibition observed in 3 independent experiments has been measured and shown. Average zone of inhibition obtained from 3 independent experiments was considered to determine whether bacteria belong to resistant or sensitive.

	TRIAL NUMBERS	CIP	AMP	CTR	AZM	AMX
Ec-CRS1	1st	30	0	11	20	0
	2nd	27	0	12	15	0
	3rd	28	0	12	17	0
	Average	28.33	0	11.6	20	0
	Comments	S	R	R	S	R
Ec-CRS2	1st	23	0	12	27	0
	2nd	19	0	12	25	0
	3rd	40	0	17	28	0
	Average	27.33	0	15	27	0
	Comments	S	R	R	S	R
Ec-JHT3	1st	29	0	13	13	0
	2nd	15	13	20	11	11
	3rd	17	12	19	12	0
	Average	20.33	12	17.33	12	11
	Comments	S	R	R	S	R
Ec-AKS4	1st	27.5	0	10	0	0
	2nd	25	0	20	13	0
	3rd	25	0	18	11	0
	Average	25.33	0	18	12	0
	Comments	S	R	R	S	R
Ec-AKS5	1st	30	0	8	20	0
	2nd	33	0	12	31	0
	3rd	35	0	10	26	0
	Average	32.6	0	11	29	0
	Comments	S	R	R	S	R
Ec-AKS6	1st	26	15	9	17.5	9
	2nd	22	0	0	21	0
	3rd	25	11	0	22	0
	Average	25	13	9	21	9
	Comments	S	R	R	S	R
Ec-KPM7	1st	15	0	10	27.5	0
	2nd	20	0	0	20	0
	3rd	20	0	12	22	0
	Average	28.33	0	0	20	0
	Comments	S	R	R	S	R
Ec-KPM8	1st	27	0	0	20	0
	2nd	24	0	0	20	0
	3rd	26	0	0	19	0
	Average	25.6	0	0	20	0
	Comments	S	R	R	S	R

Abbreviations: AMP, ampicilin; AMX, amoxicillin; AZM, azithromycin; CIP, ciprofloxacin; CTR, ceftriaxone; S, sensitive; R, resistant.

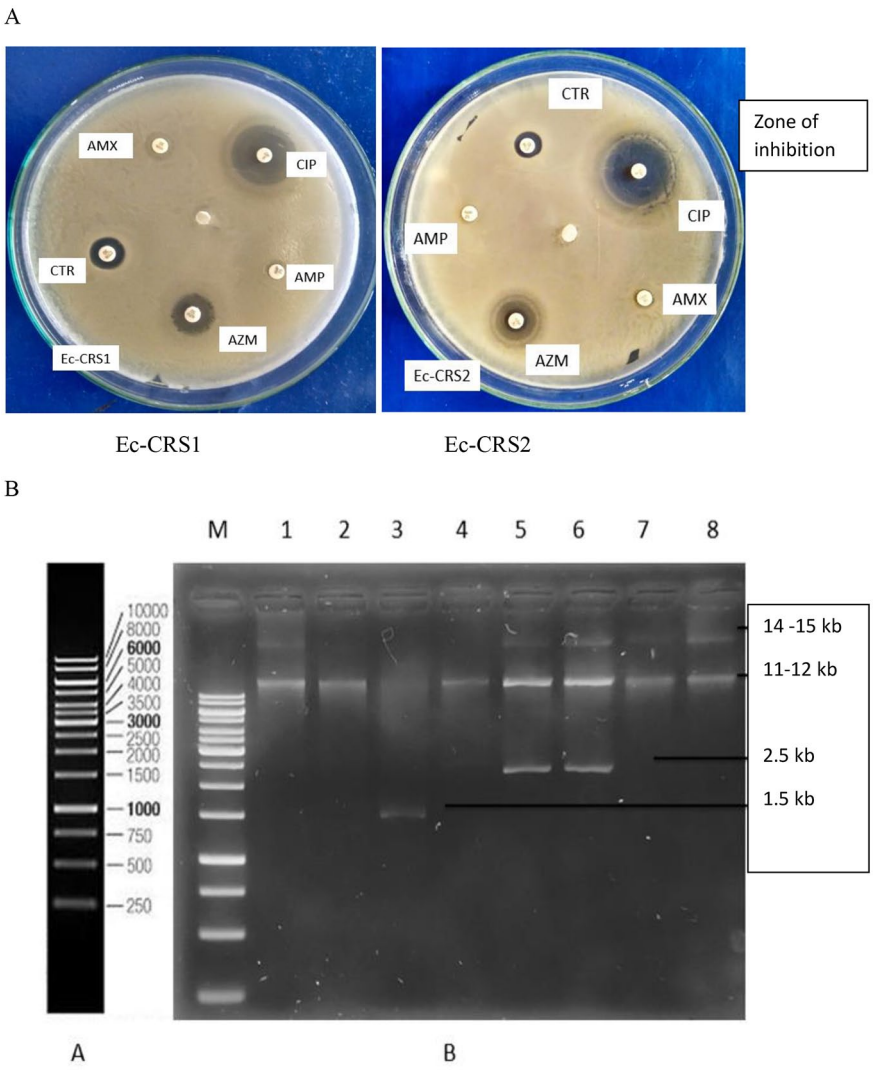

Figure 1. (A) Antibiotic sensitivity test by Kirby-Bauer Disk diffusion method. Picture of culture plates showed zone of inhibition by Ec-CRS1 and Ec-CRS2 against different antibiotics. (B) A, 1 kb DNA ladder; B, Identification of Plasmid DNA through gel electrophoresis (Lane M= DNA ladder, Lane 1-8 shows band for Ec-CRS1, Ec-CRS2, Ec-JHT3, Ec-AKS4, Ec-AKS5. Ec-AKS6, Ec-KPM7, Ec-KPM8, respectively).
Abbreviations: AZM, azithromycin; AMX, amoxicillin; AMP, ampicilin; CIP, ciprofloxacin; CTR, ceftriaxone.

Table 2. Antibiotic resistance and plasmid size of the *E. coli* isolates.

SAMPLE ID	RESISTANCE PATTERN	PLASMID SIZE (KB)
Ec-CRS1	AMP, CTR, AMX	14-15, 11-12
Ec-CRS2	AMP, CTR, AMX	11-12
Ec-JHT3	AMP, CTR, AMX,	1.5
Ec-AKS4	AMP, CTR, AMX	11-12
Ec-AKS5	AMP, CTR, AMX	14-15, 11-12, 2.5
Ec-AKS6	AMP, CTR, AMX	14-15, 11-12, 2.5
Ec-KPM7	AMP, CTR, AMX	11-12, 2.5
Ec-KPM8	AMP, CTR, AMX	11-12, 2.5

to the common human environmental situation in Chatogram city, Bangladesh.

The most fearful fact of growing antibiotic resistance is that the resistance is transferable. So, if the resistant bacteria are allowed to spread in the environment, there is a huge feasibility of transferring the phenotype to other bacteria. They might be of same or distantly related species. People of developing countries often bear antibiotic-resistant fecal commensal organism. As mention above the sources these *E. coli* isolates were human sewage samples of poor areas where people have no basic knowledge about antibiotic uses. If any individual became sick in those areas they have access to get antibiotic from local inexperience heath personnel. As a result, numerous antibiotics are consumed unnecessarily by ordinary people which are partially metabolized and are excreted into the municipal sewage system. Persistent exposure of various antibiotics to environmental setting expedites the development of superbug (multi drug resistant bacteria) which could be the greatest threat of public health in the 21st century.

Plasmid can mediate antibiotic resistance by several mechanisms. Some mechanisms are very popular among gram negative bacteria. *E. coli* produces 1 of the 3 enzymes (β-lactamase) that are responsible for antibiotic alteration and degradation which render the antibiotics inactive. This enzyme is coded by both chromosome and plasmid. Another popular mechanism is the efflux of antibiotics that is responsible for the presence of multicomponent pumps found in gram negative bacteria.[22] To find the causes of antibiotic resistance, we were analysed the presence of Plasmid DNA in all *E. coli* isolates and we found that all the isolates contain plasmid with different molecular weight (approximately 1.5-15 kb range, Figure 1B). Although, all the *E. coli* strains showed similar antibiotic resistance patterns some of their plasmids had different migration patterns on agarose gel electrophoresis. For example, -plasmid of lane 1 (EcCRS1) to lane-8 (Ec-KPM8) had similar migration pattern on agarose gel electrophoresis whereas plasmid in lane-3 (Ec-JHT3) migrate more quickly than others (Figure 1B). That means isolates Ec-JHT3 have low molecular weight plasmid (1.5kb). lane 1 (Ec-CRS1), lane 4 (Ec- AKS4), lane 5 (Ec-AKS5), lane 6 (Ec-AKS6) Showed more than 1 bands indicating that these isolates have more than 1 plasmid. Presence of multiple plasmids in these multi drug resultant *E. coli* may act as possible sources to transfer highly resistant genes to pathogenic organisms and human that could be a threat for the treatment of disease by commercially available antibiotics.

Conclusion

The study highlighted the bitter truth that antibiotic resistance is spreading faster day by day. It is a matter of great concern that the environmental samples are developing strong antibiotic resistance for various antibiotics. Antibiotics are used indiscriminately in Bangladesh due to lack of proper regulation and surveillance. This study will serve as a base that would recommend necessary initiatives to monitor and limit the practice of antibiotics, as well as proper surveillance following standardized protocol throughout the nation.

Acknowledgements

We are thankful to the Research and Publication Offices, University of Chittagong, Bangladesh, for research support.

Author Contributions

A. M. M. A. C designed this study. S. A and S. A. M performed the experiments; A. M. M. A. C analyzed the data; A. M. M. A. C., S. A and S. A. M. wrote the original draft. A. M. M. A. C and S. A. M reviewed the several versions of the manuscript. All authors read and approved the final manuscript.

ORCID iD

A. M. Masudul Azad Chowdhury https://orcid.org/0000-0002-1675-5075

Data Availability

All the data analyzed during this study are included in this manuscript.

REFERENCES

1. World Health Organization. Antimicrobial Resistance: Global Report on Surveillance 2014. http://www.who.int/drugresistance/documents/surveillancereport/en/. Accessed December 1, 2019.
2. Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States. Centers for Disease Control and Prevention; 2013.
3. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: causes and control strategies. *Antimicrobial Res Infect Control*. 2017;6:47.
4. Ahmed I, Rabbi MB, Sultana S. Antibiotic resistance in Bangladesh: a systematic review. *Int J Infect Dis*. 2019;80:54-61.
5. Sutradhar KB, Saha A, Huda NH, et al. Irrational use of antibiotics and antibiotic resistance in southern rural Bangladesh: perspectives from both the physicians and patients. *Annu Res Rev Biol*. 2014;15:1421-1430.
6. Biswas M, Roy DN, Tajmim A, et al. Prescription antibiotics for outpatients in Bangladesh: a cross-sectional health survey conducted in three cities. *Ann Clin Microbiol Antimicrob*. 2014;13:15.
7. Katouli M. Population structure of gut *Escherichia coli* and its role in development of extra-intestinal infections. *Iran J Microbiol*. 2010;2:59-72.
8. Odonkor ST, Ampofo JK. *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiol Res*. 2013;4:5-11.
9. Waturangi DE, Hudiono F, Aliwarga E. Prevalence of pathogenic *Escherichia coli* from salad vegetable and fruits sold in Jakarta. *BMC Res Notes*. 2019;12:247.
10. Elsas JDV, Semenov AV, Costa R, et al. Survival of Escherichia coli in the environment: fundamental and public health aspects. *ISME J*. 2011;5:173-183.
11. Johura FT, Tasnim J, Barman I, et al. Colistin-resistant *Escherichia coli* carrying mcr-1 in food, water, hand rinse, and healthy human gut in Bangladesh. *Gut Pathog*. 2020;12:5.
12. Szmolka A, Nagy B. Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. *Front Microbiol*. 2013;4:258.
13. Ochman H, Selander RK. Evidence for clonal population structure in *Escherichia coli*. *Proc Natl Acad Sci*. 1984;81:198-201.
14. Carattoli A. Plasmids and the spread of resistance. *Int J Med Microbiol*. 2013;303:298-304.
15. Chowdhury AMMA, Akter S, Mina SA. Isolation, identification and functional characterization of *Escherichia coli* as probiotic against Shigella in Bangladesh. *Indian J Microbiol Res*. 2020;7:313-321.
16. Bauer AW Kirby WMM, Sherris JC, et al.Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 1966;45:493-510.

17. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Testing for Bacteria that Grew Aerobically*. Clinical and Laboratory Standards Institute; 2009:M7-A10.
18. Sarker MS, Mannan MS, Ali MY, et al. Antibiotic resistance of *Escherichia coli* isolated from broilers sold at live bird markets in Chattogram, Bangladesh. *J Adv Vet Ani Res*. 2019;6:272.
19. Hossain A, Hossain SA, Fatema AN, et al. Age and gender-specific antibiotic resistance patterns among Bangladeshi patients with urinary tract infection caused by *Escherichia coli*. *Heliyon*. 2020;6:e04161.
20. Sikder MOF, Chowdhury AMMA, Uddin KN. Isolation of cefixime resistant *Salmonella* from hospitals waste and profiling multi-drug resistance pattern of the selected isolates. *Int Res J Biol Sci*. 2014;3: 86-92.
21. Naher N, Alam Z, Chowdhury AMMA. Isolation and characterization of ciprofloxacin resistant *E. coli* from hospital and dairy waste. *Chittagong Univ J Biol Sci* 2011;5:19-26.
22. Giedraitienė A, Vitkauskienė A, Naginienė R, et al. Antibiotic resistance mechanisms of clinically important bacteria. *Medicina*. 2011;47:137-146.