A Review on Antibiotic Resistant Bacteria Found in Dhaka Water Supply

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled “A Review on Antibiotic Resistant Bacteria Found in Dhaka Water Supply” submitted by Tasnim Tabassum, of spring,2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

The study does not involve any kind of animal or human trial.

Abstract

The unsafe water supply of Dhaka city causes concern for public health. This review work aims to identify whether antibiotic resistant bacteria are available in Dhaka water supply. According to the collected 25 articles that is reviewed in this paper, Escherichia coli bacteria was isolated from tap water samples taken from several places in Dhaka city. They were characterized for their antibiotic resistance, pathogenic characteristics, and genetic diversity in order to gain a better understanding of the degree of the public health risk that is attributed to the supply water in Dhaka city. It was identified that there is a widespread presence of E. coli that is resistant to many antibiotics in the Dhaka public water supply. The distribution of antibiotic resistant bacteria through municipal water supplies poses a significant risk to the general population's health in urban settings.

Keywords: β-lactamase, E. coli, ESBL-E, horizontal gene transfer, Multidrug resistance

Dedication

Dedicated to my parents who could do anything for my happiness.

Acknowledgement

I would like to begin by thanking Almighty Allah for keeping me in good health and blessing me with the capability, strength and assistance needed to complete this project. However, this research paper would not have been completed without the support of several individuals and I would like to express my gratitude to all of them.

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List of Acronyms

E. coli: Escherichia coli

MRSA: Methicillin resistant staphylococcus aureus

AMR: Antimicrobial resistance

Bla: Beta lactamase

ESBL-E: β-lactamase-producing Enterobacteriaceae

EPEC: Enteropathogenic E. coli

EIEC: Enterinvasive E. coli

STEC: Shiga toxin-producing E. coli

EAEC: Enteroaggregative E. coli

ETEC: Enterotoxigenic E. coli

DAEC: Diffusely adherent E. coli

NDM: New Delhi Metallo-lactamase

HGT: Horizontal gene transfer

FC: Fecal coliforms

MFC: Membrane fecal coliform

CFU: Colony forming unit

CLSI: Clinical Laboratory Standards Institute

MDR: Multi-drug resistance

PFGE: Pulsed field gel electrophoresis

Chapter 1

1.1 Introduction

Dhaka Water Supply and Sewerage Authority (WASA) is a Bangladesh government organization under the Ministry of Local Government, Rural Development and Co-operatives responsible for water and sewage in Dhaka. Several sources of water supplied by the Dhaka Water Supply and Sewerage Authority have been found to be contaminated with bacteria and other contaminants. (Talukdar et al., 2019). Researchers discovered that Escherichia coli (ESBL-Ec) infected 74% of healthy babies in rural Bangladesh (Islam et al., 2019). Antibiotics are widely accessible and often used in lower middle-income countries (LMICs), where there is a high population density and inadequate access to clean water and sanitation (Ghafur, 2010). 1.8 billion people consume polluted water worldwide, with Southeast Asia and Africa having the greatest rates of pollution (Bain et al., 2014). More than 80% of sewage or wastewater produced in LMICs discharges directly into the environment (UN Water, 2018). Additionally, hospital wastewater, agricultural run-off from animal husbandry, and pharmaceutical waste with high antibiotic concentrations are all directly discharged into the environment (Lübbert et al., 2017; Rozman et al., 2020; Zhang et al., 2020). All of these wastes include a combination of bacteria of human, animal, and environmental origins as well as drug and antimicrobial residues that can lead to antimicrobial resistance (Woerther et al., 2013).

1.1.1 Mechanism of Antibiotic Resistance

Antibiotics are medications used for the prevention and treatment of bacterial infections. When bacteria mutate in reaction to the use of antibiotics, this phenomenon is known as antibiotic resistance. (WHO,2020).

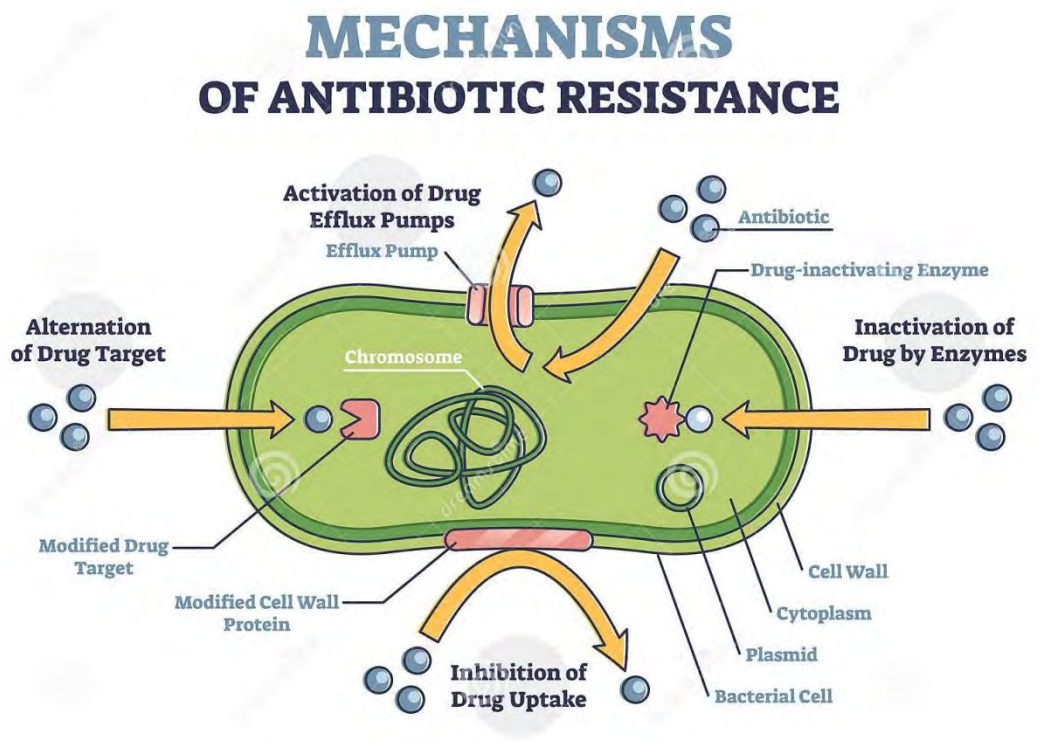

Figure 1 Different antibiotic resistant action in bacterial cell. (Blanco et al,.2016)

Antibiotic resistance can happen in two ways, one is the natural way in which the bacteria transfer in the vertical way. Another is acquired or horizontal. Acquired antibiotic resistance can happen in four different ways:

1.1.2 Reduce The Entry or Absorption of Anti-biotic

An antibiotic can generally kill the bacteria by targeting a specific site. But in this case the bacteria will not let the antibiotic enter inside the bacteria and hit the target site.

1.1.3 Modification of Antibiotic through Bacterial Enzyme

The bacteria try to modify or break down the antibiotic. for example, $\boldsymbol{\beta}$-lactamase. There are antibiotic resistance genes in the plasmid of the bacterial cell. They secrete antibiotic degrading enzyme such as $\boldsymbol{\beta}$-lactamase. This enzyme is the key ingredient to breakdown the $\boldsymbol{\beta}$-lactam ring. penicillin contains the $\boldsymbol{\beta}$-lactam ring.

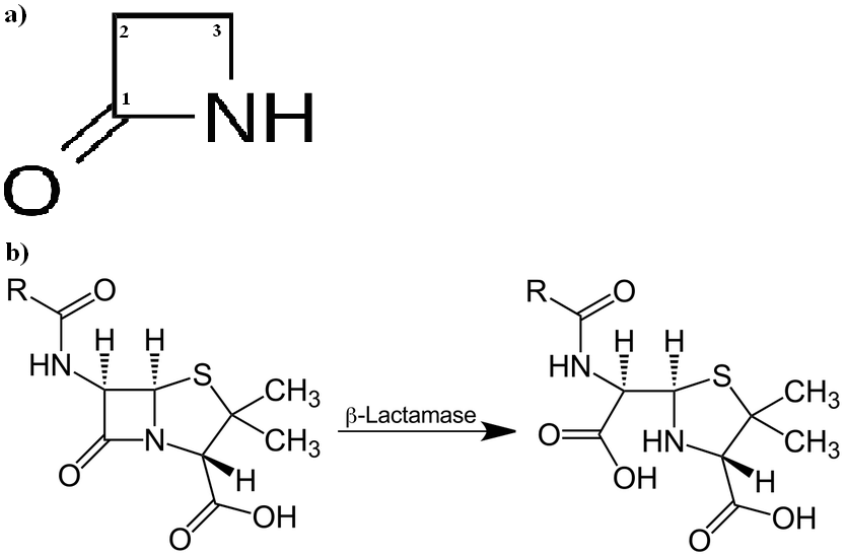

*Figure 2: a) Active **β**-lactam ring structure b) Breakdown of **β**-lactam ring structure by **β**-lactamase enzyme in penicillin. (susann et al., 2012)*

The ring is actively working and has its antibiotic effect while it is closed (figure 2). But when it encounters ***β***-lactamase enzyme it will break down and the penicillin will be inactive. The C1-N bond of the β-lactam ring is broken by the β-lactamase, and the β-lactam antibiotics' properties are deactivated (figure 2). In this case, the penicillin will have no effect on bacteria. For example, the E.coli has ***β***-lactamase enzyme.

1.1.4 Modification of Target Site

If the bacteria could not change the antibiotic then it will change the target site so that the antibiotic could not attach to the target site as a result the bacteria could not be killed. For example penicillin has ***β***-lactam ring and targets penicillin binding protein in bacteria which are found in the peptidoglycan layer of the bacteria. If the bacteria has the genes to modify the penicillin binding proteins, the penicillin is unable to bind to the protein anymore because the protein is changed. The target modifying genes change the structure of the protein. Example: MRSA bacteria is resistant to antibiotic Methicillin.

1.1.5 Efflux Pump and Reduced Permeability of Bacteria

The cell wall of the bacteria is made of peptidoglycan layer. It also has an efflux pump. The bacteria will use energy through the efflux pump and throw the antibiotic out of its cell. For example, the Tetracycline antibiotic will not be effective because it's pumped out using the efflux pump. Equally the permeability of the cell can be altered by the acquisition of mutations in porins. These mutations can include porin loss, a modification of the size or conductance of the porin channel, or a lower expression level of a porin. Ultimately both mechanisms, efflux pumps and reduced permeability, lower the intracellular antibiotic concentration in the bacterial cell by either exporting the antibiotic or by not allowing its importation (figure 3).

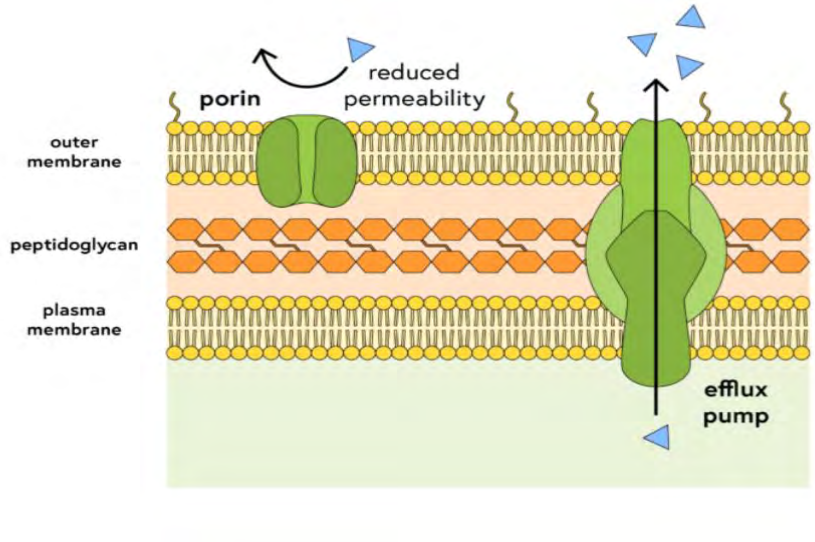

Figure 3: Outer membrane, peptidoglycan cell wall, and plasma membrane. A porin channel sits in the outer membrane, and an antibiotic cannot enter the cell from the channel labeled reduced permeability. A large multi-subunit efflux pump crosses all three layers and an arrow shows the antibiotic being removed from the cell. (Wanda et al.,2018)

1.2 Effect of Antibiotic Resistance

Low- and medium-income countries (LMICs), where AMR rates are significantly higher than in high income nations, account for a sizable part of mortality from AMR (Murray et al., 2022). The global projected yearly growth rate of extended spectrum β-lactamase-producing

enterobacteriaceae (ESBL-E) is 1.5%, with Southeast Asia reporting the greatest prevalence in a prior investigation (Bezabih et al., 2021).

Furthermore, diarrhea illnesses are responsible for an estimated 4.1% of the daily global disease burden and the deaths of 1.8 million people annually, 90% of whom are children under the age of 5. According to estimates, 88% of this burden is attributed to inadequate water supply, sanitation, and hygiene and is mainly concentrated among children in impoverished countries such as Bangladesh (WHO, 2012). Escherichia coli is commonly employed as an indicator of the microbiological quality of water and is also a significant cause of diarrhea and other enteric illnesses. Certain strains of E. coli may cause life-threatening illnesses, although the majority of E. coli usually are innocuous (KT and Gopal,2008). Six known pathotypes of E. coli can cause diarrhea in humans. Enteropathogenic E.coli(EPEC), enteroinvasive E.coli (EIEC), Shiga toxin-producing E.coli (STEC), enteroaggregative E.coli (EAEC), enterotoxigenic E.coli (ETEC), and diffusely adherent E.coli (DAEC). In recent years, there have been reports of increased antimicrobial resistance in enteropathogens, particularly E. coli (Tucker et al. ,2006).

Occasionally resulting in instances when no antibiotic therapy alternatives exist. In underdeveloped nations, where enteropathogens are commonly found and cause life-threatening illnesses, especially among youngsters, these conditions are of grave concern (Rolain J. 2014). Recent appearance and dissemination of a new carbapenemase, New Delhi Metallo -lactamase (NDM)-producing organisms are an illustration of a circumstance in which existing antibiotics are useless (cooper et al.,2006). This new enzyme and other antibiotic resistance components are transported by mobile genetic elements like plasmids and transposons. Horizontal gene transfer (HGT) is one of the most prevalent routes through which antibiotic resistance characteristics are transmitted from one organism to another. In Enterobacteriaceae, plasmids are the predominant HGT vectors. In a natural environment, in vivo transfer of resistance characteristics across Enterobacteriaceae has been documented

(Toleman MA. 2011). Because they are the most prevalent commensal enteric bacteria in humans and animals, can be cultured easily and affordably, and can acquire and maintain resistance genes from other organisms in the environment and animal populations, generic E. coli are frequently used as indicator bacteria to monitor the trends in antimicrobial resistance. E. coli is also a useful indication of the selection pressure produced by the use of antibacterials in food-producing animals. The prevalence of diarrhea illnesses is prevalent in Bangladesh. In 2008, an estimated 20,000 children less than 5 years old perished in Bangladesh from diarrhea illnesses (Talukdar et al., 2012).E. coli is one of the primary causes of enteric infections in Bangladesh, with ETEC being the most prevalent pathotype, followed by EPEC, EAEC, and STEC. In addition, the spread of ESBL and carbapenemases (CARBase) that confer resistance to life-saving b-lactams is a cause for worry (Sultana R andIslam F, et al.,2004).

The vast majority of E. coli infections are transmitted by water since the bacteria is prevalent in surface water. The triggering factors are poor sanitation and hygiene, overcrowding, and lack of access to safe drinking water. In this investigation, the antibiotic resistance, pathogenic, ESBL phenotype, presence of main ESBL genes, and acquisition of transferable plasmids of E. coli bacteria isolated from household water supplies in Dhaka, Bangladesh, were studied (Aminul, et al.,2017).

1.3 Aim of the Project

The aim of this review is to conduct a comprehensive review on antibiotic resistant bacteria found in Dhaka water supply.

1.4 Objective

- Investigate whether there are any antibiotic resistance bacteria in Dhaka water supply.
- The type of bacteria found in the water system.

Chapter 2

Methodology

Relevant literature was selected, analyzed and summarized for this review work. The information and data for this review were compiled from relevant articles. To gather the journals connected to this topic, an electronic search has been done. After scrutinizing data from the selected recent articles an outline was created to present the information as the requirement of the project objectives. According to the aim of the work, it was important to explore the presence of antibiotic resistant bacteria in the supplied water of Dhaka.

In order to gather as much essential information as possible regarding antibiotic resistant bacteria a thorough search of several journals, review articles and research papers from official websites and research databases was carried out. Utilizing well-known and reliable sources including PubMed, Google Scholar, SCOPUS and ScienceDirect, the articles for this review study were collected. Relevant literatures were gathered using appropriate keywords, such as Selected articles were connected to E.coli, β-lactamase-producing enterobacteriaceae (ESBL-E), Antibiotic resistance bacteria. 60 articles have been assessed based on the title and keyword content. Then, 40 papers were reduced after reading the abstracts. 28 papers were selected after going through the entire text, which made up this review paper. Mendeley software was used for accurate and fair referencing in order to show respect for the writer's original works.

Chapter 3

Result

3.1 Introduction of the Experiments

Three different experiments have been conducted by Talukdar and his team under surveillance of International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) in 2012 on water samples collected from different areas of Dhaka and 233 isolates were prepared to investigate and identify the presence of life threatening antibiotic resistance bacteria. The experiments are described below:

3.2 Estimation of Fecal Coliform Bacteria and Isolation of E.coli

Fecal coliforms (FC) concentrations in water samples were determined using membrane filtration (APHA, 2019). After passing the water through a membrane filter with a 0.2 mm pore size, 100 ml of the filtered solution was plated onto membrane fecal coliform (MFC) agar (BD, Maryland, United States) (SartoriusStedim, Goettingen, Germany). The MFC plates were incubated at 44 C for 18-24 hours. Following incubation, the number of blue colonies indicative of coliforms was counted and reported as CFU per milliliter of water. A 100 ml sample of water was filtered in accordance with the technique to detect E. coli. Eighteen to twenty-four hours at 37 degrees Celsius, the filter was submerged in EE broth (Oxoid ltd, Basingstoke, UK). We grew the enrichment broth in a TBX agar medium for 18 to 24 hours at 37 degrees Celsius (Oxoid). MacConkey agar medium and Eusine Methylene Blue agar (Oxoid) were used to cultivate typical E. coli colonies recovered on TBX plates (Oxoid). Three E. coli colonies were isolated from each sample and frozen at -70 degrees for further analysis (Talukdar et al.,2013).

3.3 Antimicrobial Susceptibility Tests

Agar diffusion testing using antimicrobial agent impregnated paper discs (Oxoid), as stated in Clinical Laboratory Standards Institute (CLSI) standards, was used to assess susceptibility to

antimicrobials (CLSI,2009). Antibiotics such as Ampicillin (10µg), Ceftriaxone (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Trimethoprim-sulfamethoxazole (25µg), Gentamicin (10µg), Mecillinam (25µg), Meropenem (10µg), Nalidixic acid (30µg), Tetracycline (30µg), Norfloxacin (10µg), Imipenem (10µg), Kanamycin (30µg), Erythromycin (15µg), Cefotaxime (30µg), Cefixime (5µg), Aztreonam (30µg), Ceftazidime (30µg), Cefoxitin (30µg) and Piperacillin-tazobactam (110µg) were used. E. coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were used as positive and negative controls, respectively. Results were interpreted using CLSI cutoffs (CLSI,2009). The double disc synergy test was used to look for ESBL in isolates that were resistant or intermediately susceptible to Cephalosporins (DDST). Discs containing 30g of Ceftazidime, Cefotaxime, or Aztreonam were used in the DDST, which was performed on MuellerHinton agar (Difco Laboratories, Detroit, MI, USA) with a disc containing Amoxicillin-clavulanic acid (20 g/10 g) in the center of the plate (Talukdar et al.,2013).

3.4 Detection of Antibiotic Resistance Genes in ESBL Producing Organisms

ESBL-producing isolates were analyzed for the presence of blaESBL genes (blaTEM, blaSHV, blaCTX-M-1-group, blaCTX-M-15, blaCTX-M-2-group, blaCTX-M-8-group, blaCTX-M-9-group), Carbapenemase genes (blaOXA-1-group, blaOXA-47, and blaNDM (Islam et al.,2012). To verify the gene's specificity, PCR products using blaCTX-M-15 primers were sequenced on an ABI PRISM 310 sequencer (Applied Biosystems). Isolates were further examined for the presence of 16sRNA methyltransferase genes (rmtA, rmtB, and armA) and qnr genes (qnrA, qnrB, and qnrS) using the methods outlined earlier (Islam et al.,2012). All of the PCR reactions' primer sequences and annealing temperatures are recorded in.

3.5 Plasmid Profile Analysis and Conjugation Experiment

Horizontal electrophoresis on 0.7% agarose gels was used to examine plasmid DNA that was extracted using the fast alkaline lysis method (chang H et al.,2008). Unknown plasmid molecular weights were determined using gel electrophoresis by comparison to known plasmid molecular weight standards. The researchers used the E. coli V517 plasmids (1.4, 1.8, 2.0, 2.6, 3.4, 3.7, 4.8, and 35.8 MDa) and the Sa plasmid (23 MDa), RP4 plasmid (34 MDa), R1 plasmid (62 MDa), and pDK9 plasmid (140 MDa) as benchmarks (chang H et al.,2008). The MDR water isolates were the donors, and E. coli MC1061 (SmR, F2, non-lactose fermenting) and E. coli J53 (AziR, F2) were the recipients. The researchers used 30uC for both our broth and filter mating assays. In order to select for transconjugants, E. coli MC1061 was grown on MacConkey agar supplemented with 50 mg/L Ampicillin, whereas E. coli J53 was grown on MacConkey agar supplemented with 100 mg/L sodium azide and 20 mg/L Cefotaxime/Cefoxitin. This conjugation took 18 hours. Transconjugant colonies were demonstrated through antibiotic susceptibility testing. The previously established alkaline lysis technique was used to retrieve plasmid DNA from transconjugants (chang H et al.,2008). The rate of conjugation was determined by dividing the total number of transconjugants by the total number of original recipients.

Actual results of conducted experiments are described below:

3.6 Enumeration of Fecal Coliform Bacteria and Isolation of E.coli

Fecal coliform bacteria have an 80% positivity rate in the water and some of the samples have fecal coliform count more than 100 CFU/ml water. On the other hand, E.coli was isolated from 63% of the samples.

3.7 Antimicrobial Susceptibility Tests of Isolates

Among the 233 isolates tested, 57% (n = 133) were resistant to Ampicillin. Tetracycline was next with 45% (n = 105) resistance, followed by Nalidixic acid (37%, n = 87), Trimethoprim-Sulfamethoxazole (36%, n = 83), Ciprofloxacin (17%, n = 39), Ceftriaxone (9%, n = 22), Mecilinam (The isolates were classified as multi-drug resistance because more than 73% (n = 171) of them were resistant to at least one antibiotic and 36% (n = 84) of them were resistant to three or more classes of antibiotics (MDR). The double disc synergy test confirmed that all 22 Ceftriaxone resistant isolates were ESBL-producing after further testing. All 22 isolates were resistant to these following drugs: Cefotaxime and Cefixime, Erythromycin, Aztreonam, Ciprofloxacin/Norfloxacin, Kanamycin, and Ceftazidime, as well as Piperacillin-Tazobactam, Ciprofloxacin/Norfloxacin, 55%, and 55%. None of the isolates, including Imipenem and Meropenem, were resistant to Carbapenem drugs.

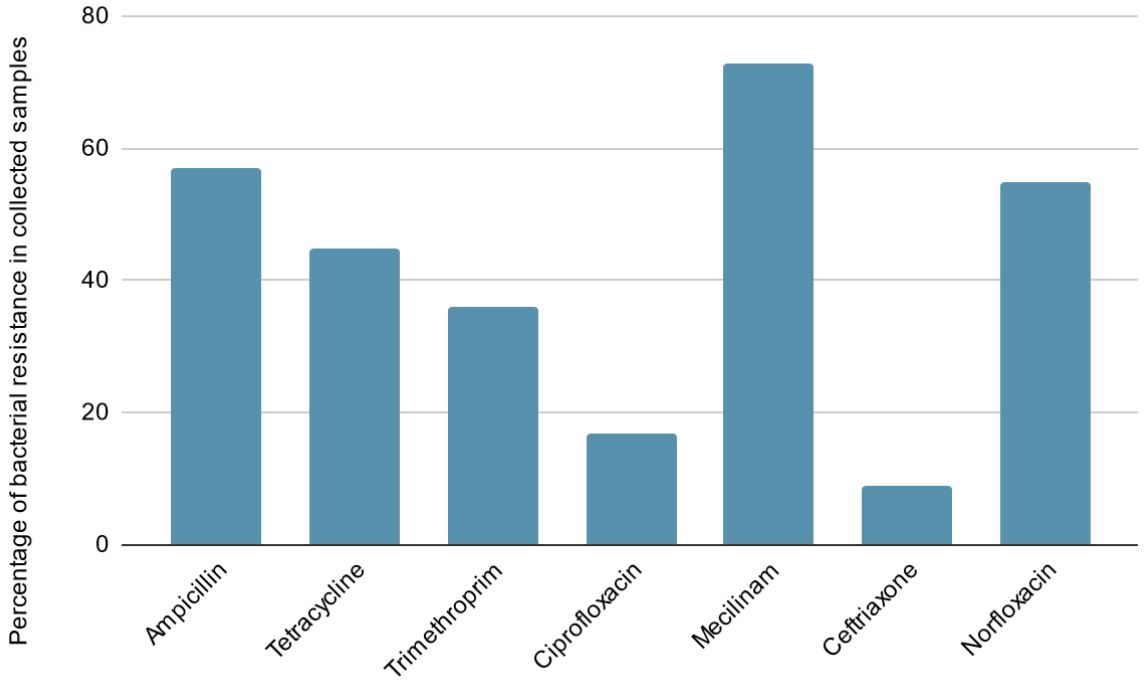

Figure 4: Percentage of bacterial resistance (%) in selected Antibiotics. (Islam et al.,2012)

3.8 Detection of Antibiotic Resistance Genes in ESBL Producing Organisms

90% ESBL-producing isolates, blaCTX-M-1-group specific gene and blaCTX-M-15 were found to be positive. The PCR result was sequenced to confirm the presence of the blaCTX-M-15 gene. One isolate tested positive for a gene particular to the blaCTX-M-9 group, but none of the isolates tested positive for genes specific to the blaCTX-M-2 group or the blaCTX-M-8 group. Nearly 41% of the isolates were positive for blaTEM, but none for blaSHV. In 32% of the isolates, the Carbapenemase genes blaOXA-1-group and blaOXA-47 were found. A test for the Metallo-lactamase gene blaNDM-1 was negative for all of the isolates. In 9% of the isolates, blaCMY-2 plasmid-type b-lactamases were found. Both qnrS and qnrB, which are Quinolone resistance genes, were found in 27% and 9% of the isolates, respectively.

Serial no.	Strain ID	Antibiotic resistance pattern[a]	Presence of ESBL genes	Plasmid pattern (in MDa)
1	4C3	Amp, Cro, Cfm, Ctx	$bla_{CTX-M-15}$, *qnrS*	36
2	24C2	Amp, Cip, Cro, Sxt, NA, Te, Cfm, Ctx, Nor, K, E	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{OXA-47}	90,3.2,3
3	24C3	Amp, Cip, Cro, Sxt, NA, Te, Mel, Atm, Cfm, Ctx, Nor, E,	$bla_{CTX-M-15}$, bla_{TEM},	90
4	28C2	Amp, Cip, Cro, Sxt, NA, Te, C, Atm, Caz, Cfm, Ctx, Nor, K, E	$bla_{CTX-M-15}$, bla_{TEM}, bla_{OXA-1}, bla_{OXA-47}	105,90,17,2
5	88mf2	Amp, Cro, Mel, Atm, Cfm, Ctx, Tzp	$bla_{CTX-M-15}$, bla_{TEM}	105,90
6	90C1	Amp, Cip, Cro, NA, C, Atm, Cfm, Ctx, Nor, E	$bla_{CTX-M-15}$, bla_{TEM}	62
7	102C1	Amp, Cip, Cro, Sxt, NA, Te, Cfm, Ctx, Nor, E	$bla_{CTX-M-9}$	90,8.6,7.4,3.4
8	112C2	Amp, Cip, Cro, Sxt, NA, Te, Fox, Atm, Caz, Cfm, Ctx, Nor, E	$bla_{CTX-M-15}$, bla_{TEM}, bla_{CMY-2},	90,35.8,3.1
9	123C4	Amp, Cip, Cro, Sxt, NA, Te, C, Mel, Atm, Caz, Cfm, Ctx, Nor, K, E	$bla_{CTX-M-15}$, bla_{TEM}, bla_{OXA-1}, bla_{OXA-47}	140,70
10	134C1	Amp, Cro, Cfm, Ctx, E	$bla_{CTX-M-15}$, *qnrS*	140
11	145C2	Amp, Cro, Cfm, Ctx,	$bla_{CTX-M-15}$	No Plasmid
12	146C2	Amp, Cro, Cfm, Ctx, E	$bla_{CTX-M-15}$, *qnrS*	100
13	156C1	Amp, Cro, Cfm, Ctx, E	$bla_{CTX-M-15}$, *qnrS*	140, 62, 27
14	169C1	Amp, Cro, Cfm, Ctx, E	$bla_{CTX-M-15}$,	70,2.7
15	169C3	Amp, Cro, Sxt, Te, Atm, Cfm, Ctx, E	$bla_{CTX-M-15}$, bla_{TEM}, *qnrS*	62
16	174TC1	Amp, Cip, Cro, Sxt, NA, Te, C, Cn, Atm, Cfm, Ctx, Nor, K, E	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{OXA-47}	105, 2.7,2.1,1.4,1.2
17	174FC1	Amp, Cip, Cro, NA, Te, Atm, Caz, Cfm, Ctx, Nor, K, E, Tzp	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{OXA-47}	140,62
18	177TC1	Amp, Cro, Sxt, Te, Atm, Cfm, Ctx, E	$bla_{CTX-M-15}$	62
19	185C2	Amp, Cro, Te, Atm, Cfm, Ctx, E	$bla_{CTX-M-15}$, *qnrS*	200,100,35.8
20	186C2	Amp, Cip, Cro, Sxt, NA, Te, C, Cn, Atm, Caz, Cfm, Ctx, Nor, K, E, Tzp	$bla_{CTX-M-15}$, bla_{TEM}, bla_{OXA-1}, bla_{OXA-47}, *qnrB*	70
21	199C5	Amp, Cip, Cro, NA, Mel, Fox, Atm, Caz, Cfm, Ctx, Nor, E	bla_{TEM}, bla_{CMY-2},	62,23,9
22	200C2	Amp, Cip, Cro, Sxt, NA, Te, Atm, Caz, Cfm, Ctx, Nor, K, E	$bla_{CTXM-15}$, bla_{OXA-1}, bla_{OXA-47}, *qnrB*	No Plasmid

Table 1: Antibiotic resistance pattern, presence of antibiotic resistance genes and plasmid patterns of ESBL-producing E. coli isolated from water samples (Talukdar et al.,2013)

3.9 Plasmid Profile Analysis and Conjugation Experiment

One hundred and sixty-six (80%) of the 233 isolates included plasmids of various sizes, resulting in heterogeneous inter-isolate plasmid profiles. Plasmid numbers varied from 2 to 8, and their molecular weights ranged from 1.2 MDa to.120 MDa. Conjugation experiments with representative isolates with various plasmid patterns showed that plasmids of 50 to 105 MDa were capable of self-transmission to an Ampicillin-resistant strain of E. coli. Transconjugant cultures' antibiotic susceptibility assays showed that Ampicillin resistance-granting plasmids also co-transferred resistance to Trimethoprim-sulfamethoxazole, Tetracycline, and

Ceftriaxone. However, conjugative plasmids did not transmit Cefoxitin resistance. No conjugative plasmids were used to transmit Quinolone resistance genes. Except for a 50 MDa plasmid carrying Ampicillin, Trimethoprim-sulfamethoxazole, and Tetracycline resistance, which displayed a relatively higher transfer frequency (1.9561022) than that of the other plasmids (4.429.061024), the frequency of plasmid transfer was nearly the same for all conjugative plasmids.

Strain no.	**Parent strain**		**Transconjugant**		**Transfer frequency**
	Resistance pattern	**Plasmid-pattern (MDa)**	**Resistance pattern**	**Plasmid pattern**	
25C3	Amp-Sxt	140,62,45,2.3,2.0	Amp-Sxt	62	9×10^{-4}
51C1	Amp-Cip-Na-Sxt-Mel	90,62,4.8,3.7	Amp	62	5.2×10^{-4}
88mf2	Amp-cro-Mel	105,70	Amp-cro	70	4.8×10^{-4}
133C4	Amp-Sxt-Te	50	Amp-Sxt-Te	50	1.95×10^{-2}
174TC1	AMP-cip-cro-sxt-na-te-c-cn	105,2.7,2.1,1.4,1.2	Amp-cro	105	4.4×10^{-4}

Table 2 Results of conjugation assays between antibiotic resistant E. coli isolates obtained from water samples and the recipient E. coli MC-1061 strain (Talukdar et al.,2013)

Chapter 4

Discussion

For the millions of residents residing in the Dhaka metropolitan area, household water supply provided by the municipal government is a crucial shared resource. Water sources, particularly the city of Dhaka's municipal water supply, are frequently isolated for having E. coli [Islam et al.,2010].

In this review, it was identified from various literary sources that 63% of the water samples included E. coli, and that 38% of the water samples contained high concentrations of fecal coliform bacteria around 100 CFU per ml (Talukdar et al.,2013).

E. coli contamination in the water supply system and the presence of other microorganisms that may be potentially harmful to human health are both indicated by the presence of the bacteria in the water sample. When these E.coli show resistance to several antibiotics and have pathogenic traits that make people who drink this polluted water sick, it becomes a severe hazard.

In the review, it was observed that more than 73% of the E. coli were resistant to at least one of the 10 antibiotics examined and almost half (49%) of these isolates were multidrug resistant, defined as resistant to three or more classes of antibiotics. Also, it was shown that the isolates in this investigation exhibited a higher incidence of b-lactam, Quinolone, and Fluoroquinolone antibiotic resistance [cloeman et al,.2012]. Nine percent of the E. coli isolates examined for the study produced ESBLs. This could be as a result of the antibiotics' aftereffects from their extensive usage in the human population and food chain, which has led to selective antibiotic pressure in the environment. The majority of ESBL producers tested positive for clinically important class A b-lactamases, particularly the blaCTX-M-15 and blaCTX-M-1-group. It is interesting to note that while none of the isolates in our analysis were positive for blaSHV, the bulk of them were for blaTEM. Many enterobacterial species, including E. coli, have been

found to carry the quinolone resistance gene (qnr), which is mediated by plasmids (poirel et al.,2008). Two strains tested positive for plasmid-mediated qnr genes of the qnrS and qnrB kinds in the current study, it is discovered. The qnrB-carrying isolated co-hoarded genes from other classes of b-lactamases, such as blaCTX-M-15 and blaOXA-47, and was susceptible to 13 medications, including Ciprofloxacin.

The isolate with the qnrS gene, in contrast, co-harbored blaCTX-M-15 and blaTEM and was resistant to 8 antibiotics, with the exception of Ciprofloxacin. Many studies have been conducted on E. coli isolates from clinical samples that include Metallo b-lactamases and several types of b-lactamases (Oteo et al.,2010). The adaptability and fitness of clinically significant E. coli are proved to allow them to acquire the majority of b-lactamase gene variations.

In regions of the world where enteric infections are endemic, pathogenic E. coli makes a sizable contribution to the burden of infectious diseases. In this review it is identified that a sizable portion (7% of isolates) of E. coli from supply water sources belonged to the pathogenic kinds, including EPEC and ETEC. Diarrheal infections are a significant public health issue in Bangladesh, and pathogenic E. coli is the second most common cause of diarrhea after rotavirus. 20% of all diarrheal cases in children under 2 years old are caused by ETEC [Qadri et al.,2005]. The presence of ETEC in Dhaka's drinking water and environmental water, as well as its continued viability following prolonged water incubation, have been demonstrated in the past [Begum et al.,2005], suggesting that water may be a significant mode of transmission. According to a recent study, ETEC creates biofilms in home drinking water throughout the year, with summer and wet seasons seeing an increase [Begum et al.,2005]. Due to the advent of MDR organisms, there is currently no vaccine for E. coli diarrhea and the available therapeutic options, including antibiotic therapy, are not particularly effective [Begum et al.,2005].

The majority (80%) of isolates were found to have several plasmids, and there was some degree of pattern similarity across the isolates, showing their clonal diversity, according to plasmid profile analysis. 32 isolates, or 14% of the total, had a.120 MDa plasmid present. For several enteropathogens, such as Shigella spp. and Enteroinvasive E. coli (EIEC), it is known that plasmids of this size transmit invasive qualities [Honna et al., 2005]. In general, the ipaH and ial genes, which are used as stand-in markers for the test of invasiveness, are positive in all invasive Shigella spp. and EIEC strains. The ipaH and ial genes were not present in any of the study's big plasmid-containing isolates. A substantial percentage of isolates included plasmids in the range of 50 to 100 MDa, according to analysis of the plasmid profile (middle-ranged). Previous research has shown that plasmids of these sizes are typically self-transmissible and carry the antimicrobial resistance factors in Enterobacteriaceae, particularly in Shigella spp. and E. coli [Talukdar et al., 2006]. In this investigation, it is also discovered a relationship between the isolates' characteristics of multiple drug resistance and the presence of middle ranged plasmids. We discovered self-transmissible plasmids carrying Ampicillin resistance in several strains with weight sizes ranging from 50 to 105 MDa.

Although in these experiments the conjugative plasmids based on Ampicillin resistance, these plasmids also co-transmitted additional antibiotic resistances, most notably Trimethoprim Sulfamethoxazole, Tetracycline, and Ceftriaxone, with a variable transfer frequency.

Despite the fact that both isolates (112C2 and 199C5) were positive for blaCMY-2, a plasmid-mediated AmpC beta-lactamase, neither isolate had the ability to conjugate its Cefoxitin resistance to the recipient E. coli J53. No Ciprofloxacin or Nalidixic acid transfer was seen, proving that conjugative plasmids cannot transfer quinolone groups. For other isolates, conjugation proved unsuccessful in transferring resistance plasmids. These isolates may contain non-conjugative plasmids or chromosomally encoded plasmids that carry the plasmid containing the resistance gene.

The isolates' PFGE patterns revealed a high level of polymorphism. 16 pathogenic isolates produced a total of 15 unique profiles, demonstrating the genetic diversity of the group. Only two ETEC isolates' PFGE profiles were identical. Surprisingly, the plasmid and antibiotic susceptibility patterns of both isolates were identical. After tracing the organisms' origins, it is discovered that they were separated from water samples taken from two distinct points of use within the same region, where water is provided from the same point of source via a single pipeline. This finding suggests that harmful organisms are spreading clonally via a community's water supply system. From this particular experiment it is determined that the water source is supplying antibiotic resistance bacteria to various locations that are spreading the resistance to antibiotics at a higher rate.

Chapter 5

Conclusion

People typically drink the water from their homes without any pre-treatment, though it is frequently advised to boil the water beforehand. Hence, the prevalence of multi-drug resistant ESBL-producing pathogenic E. coli in Dhaka's household water supply has significant effects on the population's health (Islam et al,2013). The MDR E. coli may serve as a significant source of genetic antibiotic resistance determinants that are easily transmitted to prospective human pathogens through horizontal gene transfer (HGT). Appropriate preventive measures are required to reduce the spread of these germs in the community and the danger posed by the emergence of antibiotic resistance in different enteric bacterial infections.

The improper and excessive use of antibiotics, in addition to ineffective measures used to prevent and treat infections, are major contributors to the acceleration of antibiotic resistance. At any level of society, the necessary actions can be made to take necessary efforts to lessen the impact and limit the spread of resistance.

Individuals should take preventative measures and implement control measures to limit the spread of antibiotic resistance. Antibiotics should only be taken if a doctor or other trained medical expert has prescribed them. One should never demand antibiotics if a healthcare provider has informed them not to require any. When taking antibiotics, one should always follow the advice of a health worker. Never allow anyone else to use antibiotics or share them. Infections can be avoided by thoroughly washing one's hands on a regular basis, preparing food in a sanitary manner, avoiding prolonged contact with ill persons, and maintaining current vaccines. First and foremost, prepare food in a sanitary manner by adhering to the WHO's Five Keys to Safer Food (keep clean, separate raw and cooked food, cook thoroughly, keep food at safe temperatures, use safe water and raw materials), and select foods that have been produced without the use of antibiotics for the purpose of promoting growth or preventing disease in animals that are otherwise healthy.

Policymakers have the ability to ensure that a solid national action plan to combat antibiotic resistance is in place. This is a role that policymakers may play that would have the greatest significant influence on efforts to prevent and control the spread of antibiotic resistance. They should cut off the water supply that is polluted with bacteria that are resistant to antibiotics, and they should regularly examine the water supply for germs that are resistant to antibiotics. In addition to that, they need to construct a water filtering system that is secure. They might be able to enhance the monitoring of illnesses that are resistant to antibiotics as well as the way of disposal used in hospitals and other medical facilities. Increase the effectiveness of infection prevention and control methods through improving relevant policies, programs, and practices. In addition, there should be regulations in place to promote the responsible use and disposal of high-quality medications. Last but not least, ensure that information is easily accessible regarding the effects of antibiotic resistance.

Members of the medical community Health workers may prevent infections by ensuring that their hands, instruments, and environment are clean. Because they have the greatest responsibility for preventing and controlling the spread of antibiotic resistance, they are best positioned to do so. According to the most recent rules, they are only permitted to prescribe and dispense antibiotics when such treatment is clinically necessary. They should immediately notify the surveillance teams about any infections that are resistant to antibiotics. Have a conversation with your patients about the correct way to take antibiotics, the risks associated with misusing medications, and the issue of antibiotic resistance. Discuss the importance of infection prevention with their patients. (For example, vaccination, hand washing, and covering nose and mouth when sneezing). Most importantly, they should implement appropriate disposal practices for antibiotic medications, vaccinations, injections, and tablets so that they do not risk contaminating natural sources such as water, soil, and a variety of other natural resources. The

Healthcare industry should invest in research and development of new antibiotics, vaccines, diagnostics, and other tools.

When it comes to the agricultural industry, the only way to ensure that the development of antibiotic resistance is prevented and kept under control is for farmers to only administer antibiotics to animals under the watchful eye of veterinarians. Do not provide antibiotics to healthy animals in order to speed up their growth or protect them from disease. Vaccinate animals to lessen the need for antibiotic treatment, and where possible, make use of treatment methods other than antibiotics. Encourage the use of effective procedures at each stage of the manufacturing and processing of foods derived from animal and plant sources, and put these procedures into effect. Increase biosecurity on farms and reduce the risk of infection by making improvements to hygiene and the treatment of animals. (WHO,2020).

In the end it can be said that everyone from every sector of work should come forward and play an active role to prevent antibiotic resistance spread.

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