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http://www.banglajol.info/index.php/BJID/index
**Original Article**
**Bangladesh Journal of Infectious Diseases**
***December 2024, Volume 11, Number 2, Page 121-130***
ISSN (Online) 2411-670X
ISSN (Print) 2411-4820
DOI: https://doi.org/10.3329/bjid.v11i2.70222


# Assessment of Human Urine and Sputum Samples for Multiple Antibiotic Resistance Patterns and Genomic DNA Analysis of *Escherichia coli*

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## Abstract

**Background:** Antimicrobial resistance (AMR) surveillance is inadequate in low-and middle-income countries, and first-choice antibiotic therapy recommendations need to be confirmed by precise data on antibiotic susceptibility. **Objective:** This study was about isolating and identifying *Escherichia coli (E. coli)* from human urine and sputum samples and evaluating the antibiotic sensitivity pattern. **Methodology:** This retrospective study investigated antimicrobial resistance (AMR) in *E. coli* isolated from 200 human urine and sputum samples collected from patients who showed symptoms of urinary tract infections at Kurmitola General Hospital, Dhaka, Bangladesh, during February and March 2019. The samples were selectively screened to assess their antibiograms and plasmid profiles. Laboratory analysis was performed using staining techniques, culture methods, and biochemical characterization, followed by polymerase chain reaction (PCR). The antibiotic susceptibility of the isolates was determined using the disc diffusion method." **Results:** We have screened 25 urine and sputum samples for antibiotic resistance. Here about six selective *E. coli* from Urine and two sputum samples were discussed for further Plasmid DNA analysis. The antibiotics sensitivity patterns and plasmid profiling were done to find out the possible correlation between plasmids and antibiotic sensitivity patterns of *Escherichia coli* isolates. Antibiogram study revealed that imipenem 12.0%, meropenem 3.0%, ciprofloxacin 7.0%, norfloxacin 7.0%, levofloxacin 12.0%, amoxicillin 11.0%, chloramphenicol 1.0%, gentamicin 6.0%, kanamycin 9.0%, cefuroxime 11.0%, erythromycin 7%, vancomycin 7.0%, tetracycline 7% resistance. Out of the eight isolates tested, 25.0% showed the presence of plasmid and 55.0% of *E. coli* isolates positive for different primers. NDM (24.0%) has shown maximum gene expression. SHV (4.0%), and BlaTEM (4.0%) showed minimum gene expression out of the eight primers. **Conclusion:** *Escherichia coli* isolates from different samples have increased antimicrobial resistance, particularly to levofloxacin, imipenem, amoxicillin, cefuroxime and kanamycin. [*Bangladesh Journal of Infectious Diseases, December 2024;11(2):121-130*]

**Keywords:** Antimicrobial resistance; *Escherichia coli;* DNA; Bangladesh

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## Introduction

Antimicrobial compound discovery and administration have revolutionized modern medicine and have become one of the most important medical interventions required for the development of complex medical approaches. Unfortunately, the rise in antimicrobial resistance (AMR) among common bacterial pathogens is jeopardizing this therapeutic breakthrough, putting critically ill patients' survival at risk specifically in low and middle-income countries[1].

AMR is on the upswing across the world, and the World Health Organization deems it to be one of the top three public health dangers of the 21st century[2]. The impact on morbidity, mortality and treatment costs is anticipated to be larger in developing nations, where the burden of infectious diseases is higher and the population is poorer and is estimated that around 300 million premature deaths will be occurred due to AMR by 2050, with a loss of up to $100 trillion to the global economy[3].

AMR in clinically important gram-negative bacteria like *Escherichia coli*, the most common cause of hospital-acquired infections is resistant to all first-line antibiotics[4]. In most regions, antimicrobial resistance to older generations is also high, and resistance to most newer antimicrobials has emerged in community-acquired infections[5]. In vitro testing of *Escherichia coli* urine isolates from outpatients in the United States revealed significant increases in antimicrobial resistance to a variety of routinely used antibiotics[6]. As a result, acquiring AMR genes is significant because they have the ability to give cross- or co-resistance to several therapeutic classes, resulting in multidrug resistance (MDR)[7].

Therefore, exploring antimicrobial bacterial resistance patterns is important for antimicrobial drug development and improvement. The objective of this study was to isolate and identify *Escherichia coli*, from urine and sputum samples and to evaluate the antibiotic sensitivity pattern.

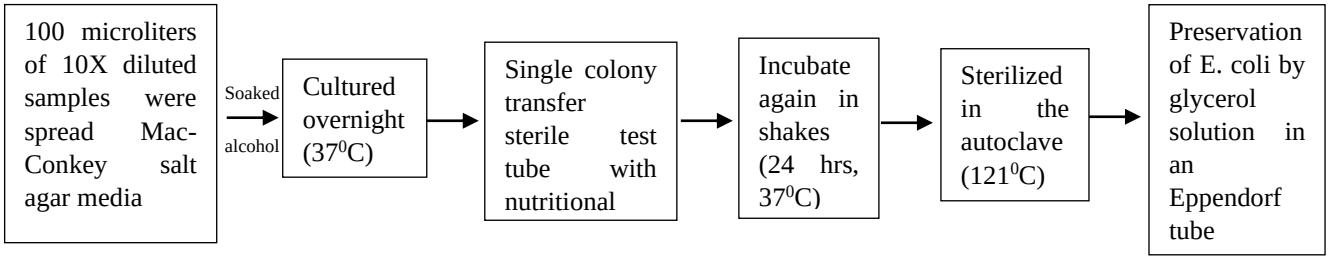
## Methodology

**Study Settings and Population:** This was retrospective study and was conducted in the Department of Pharmacy at North South University, Dhaka, Bangladesh from January 2019 to June 2019 for a period of six months. All the suspected cases of urinary tract infection (UTI) patients were selected in this study.

**Collection and Selection of *Escherichia coli* Samples:** *Escherichia coli* isolated from 200 human urine and sputum samples collected from patients who showed symptoms of urinary tract infections at Kurmitola General Hospital, Dhaka, Bangladesh, during February and March 2019. People of all ages and sexes who showed symptoms of urinary tract infections were taken into consideration for the study. We were able to analyze a subset of 20 samples that were lab-isolated from a total of 200 samples that we collected for our thesis. Here, a few samples of 6 out of 20 are discussed.

**Isolation of *Escherichia coli*:** Using the standard sterilization procedures, 100 microliters of the 10X diluted sample were distributed on the MacConkey salt agar media plate and grown overnight at 37 $^0$ C. After overnight incubation, one colony from the plate was placed into a sterile test tube with nutritional broth media. The test tube was once more incubated for 24 hours at 37 $^0$ C in a shaker incubator. Following that, the nutritional agar medium was made in accordance with the directions on the labels of the products and sterilized in an autoclave at 121$^0$ C. Preservation of *Escherichia coli* bacteria was done by glycerol solution in an Eppendorf tube.

**Multiple Drug Resistance:** In this research, the antibiotic susceptibility test was done using the Disc diffusion method following the Kirby-Bauer technique. First, we took a large (120mm) culture media plate which was also sterile then the plate was divided and marked using a permanent marker to keep track of the drug and its results. Spread 100µL diluted sample on a sterilized nutrient agar media plate and incubated overnight at 37$^0$ C.



Transfer a single colony into 2ml nutrient broth media and incubate in a shaker for 24 hours. Spread the culture in a MacConkey media plate using a loop following the sticking method. Take a single colony in 2ml nutrient broth media and incubate overnight at $37^0$ C. Spread 100µL *Escherichia coli* culture in a Muller Hinton agar media and place the multiple antibiotic discs using sterilized forceps and incubate for 24 hours at $37^0$ C.

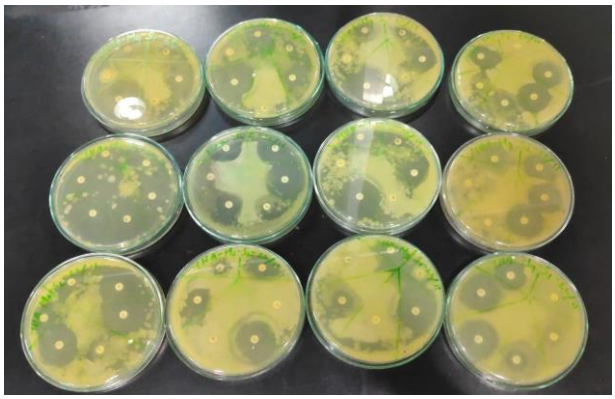


**Figure I: Petri Dishes used in the Antibiotic Resistance Test Indicating that the Bacteria are Sensitive to the Antibiotic Drugs**

Take 180µl bacterial culture and 820µl of 87% glycerol in an Eppendorf to make glycerol stock got long time preservation. Measure the zone of inhibition of different antibiotics using a ruler. Then plasmid isolation is needed for isolating plasmid DNA from samples. After that, gel electrophoresis is used for separating the DNA of *Escherichia coli* bacteria samples.

**Plasmid Isolation by Boiling Lysis:** There are many different protocols for isolating plasmid DNA from bacterial cultures. In our study, plasmid DNA from samples of Staphylococcus and E coli were isolated. One common and reliable chemical procedure, which is used, is known as the boiling lysis method. This method is based on exactly the same principles as the alkaline lysis method. First, we need to make lysozyme Preparation: Lysozyme (10mg/m1) was mixed in 10mM Tris-Cl (pH 8.0), a freshly prepared solution.

**Method (Boiling Lysis):** The culture was taken in an Eppendorf tube first, centrifuged and then the supernatant was discarded. The pellets were re-suspended in STET buffer by vortexing. The lysozyme solution was added to it and mixed by vortexing at room temperature. The top of the Eppendorf tube was pierced and then placed in a boiling water bath. The bacterial lysate was centrifuged at room temperature too. The pellets of bacterial debris were removed from the Eppendorf tube with a sterile toothpick. The supernatant was taken and put into agarose gel. Then agarose gel electrophoresis was carried out. After boiling lysis, we need to use gel electrophoresis to separate the DNA of *Escherichia coli* bacteria.

**Glycerol Stock:** Bacterial glycerol stocks are important for the long-term storage of plasmids. Although we can store plasmid DNA at -20°C, many labs also create bacterial glycerol stocks of their plasmids.

**Agarose Gel Electrophoresis:** Gel electrophoresis is used for separating the DNA of *Escherichia coli* bacteria by size (e.g., length in base pairs) for visualization and purification. Shorter DNA fragments have migrated through the gel more quickly than longer ones. Thus, the determination of the approximate length of a DNA fragment can be done by running it on an agarose gel alongside a DNA ladder (a collection of DNA fragments of known lengths). In this case, agarose gel electrophoresis was used to determine the presence of plasmid in the sample where pUC 19 was used as the control. During the procedure of Gel Preparation, 0.8% agarose gel was prepared using agarose powder and 1xTAE buffer. The mixture was heated in the oven till it turned clear and the liquid was poured into a gel electrophoresis tray. During the procedure for running the gel, the isolated solution was mixed with bromophenol blue dye (2µ1 dye + 10 µl isolated solution).

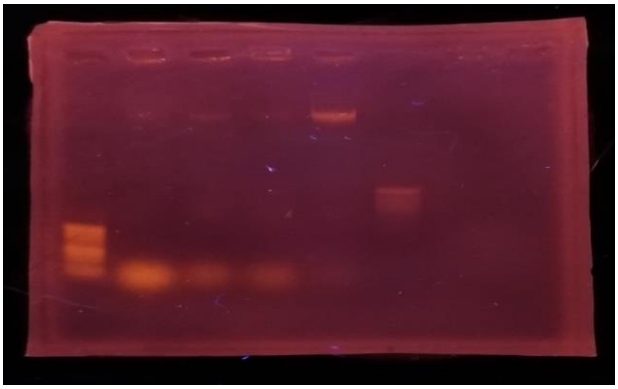


**Figure II: picture taken under UV light representing bands of plasmid DNA of *E. Coli* isolates (**Lane 1: For the ladder, which was used as the control, the plasmid was obtained; Lane 2: For SP E 20 S1, the plasmid was obtained; Lane 3: For SP E 20 S2, the plasmid was obtained); For other isolates, no plasmids were detected.

The mixture was poured into wells of the gel using a 0.5-10µ1 micropipette. The plug was attached to the power source and the gel was run for 15 minutes. After completion of travel by the expected segments, the gel was separated from the tray and

dipped in ethidium bromide solution for 15 minutes. Then, the gel was visualized under UV light in a dark room.

**Plasmid DNA Isolation:** The following picture depicts the results obtained after carrying out Gel Electrophoresis on the clinical isolates after boiling lysis had been done-

**Observation after Boiling Lysis:** The following picture depicts the results obtained after carrying out gel electrophoresis on the clinical isolates after boiling lysis had been there:

**Table 1: Plasmid Result in Clinical Isolates of *Escherichia coli* and Sputum after Gel Electrophoresis**

| Number | Sample | Plasmid Result |
|---|---|---|
| 1 | U - 75 S1 | NO |
| 2 | U - 75 S2 | NO |
| 3 | U - 85 | NO |
| 4 | U - 87 S1 | NO |
| 5 | U - 87 S2 | NO |
| 6 | U - 89 | NO |
| 7 | SP - 20 S1 | YES (F-1, C - 2) |
| 8 | SP - 20 S2 | YES (F-1, C - 3 ) |

**Genomic DNA Extraction:** Now, after gel electrophoresis, Wizard® DNA Purification Kit (*Promega Corporation,USA*) is used to extract Genomic DNA extraction from samples. Genomic DNA was extracted by Wizard® DNA Purification Kit. Some materials were supplied from the laboratory while others were included in the kit.

About 1 ml bacterial culture was taken in an Eppendorf tube and centrifuged at 10,000rpm for 3 minutes and the supernatant was discarded. The mixture was then incubated for 60 minutes at 37ºC followed by centrifugation at 10.000rpm for 3 minutes and the supernatant was removed. 600µL of Nuclei Lysis Solution was then added to both *E. coli* cells and resuspended by gently pipetting. The cells were then incubated at 80ºC for 60 minutes to lyse the cells and then cooled to room temperature.3µL of RNase solution was then added to the cell lysate and mixed by inverting the tubes and then incubated for 60 minutes at 37ºC.

Cell lysates were then cooled to room temperature and 200µL of Protein Precipitation Solution was added and mixed through vigorous vertexing for 20 seconds. When mixed properly the tubes were put in an icebox and incubated on ice for 5 minutes. After incubation, the samples were centrifuged for 5 minutes at 10,000rpm and the supernatant was transferred to a new Eppendorf tube containing 600µL isopropanol and mixed by inverting the tubes until a visible mass is formed by the thread-like DNA strands.

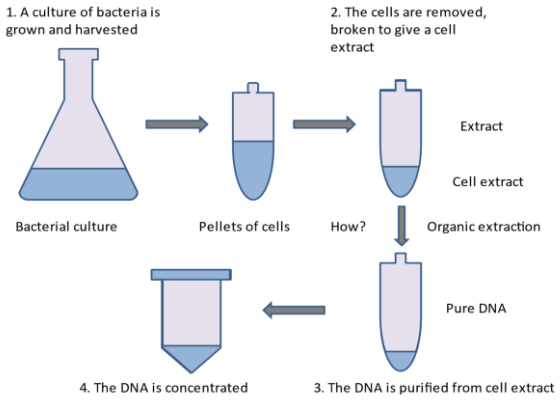



**Figure III: Process of Genomic DNA Extraction**

The mixture is again centrifuged for 5 minutes at 10,000rpm and the supernatant was drained carefully on an absorbent paper followed by the addition of 600 µL of 70.0% ethanol to wash the DNA pellets. The tubes containing samples were then centrifuged for one last time at 10,000rpm for 5 minutes and the ethanol supernatant was aspirated and poured on a clean absorbent tube and left to air dry for 15 minutes. To rehydrate the DNA, 100µL of rehydration solution was added and kept at $4^0$ C for overnight and then stored at $-20^0$ C for a long time of preservation. The extracted DNA was then run on 0.8% agarose gel to confirm the isolation and quality of the DNA and later subjected to PCR for further investigation. Using gel electrophoresis again to visualize the results of PCR.

**Principle of PCR:** PCR makes it possible to obtain, by in-vitro replication, multiple copies of a DNA fragment from an extract. Matrix DNA can be genomic DNA as well as Complementary DNA obtained by RT-PCR from a messenger RNA extract (poly-A RNA), or even mitochondrial DNA. It is a technique for obtaining large amounts of a specific DNA sequence from a DNA sample. This amplification is based on the replication of a double-stranded DNA template.

It is broken down into three phases: a denaturation phase, a hybridization phase with primers, and an elongation phase. The products of each synthesis step serve as a template for the following steps, thus exponential amplification is achieved.

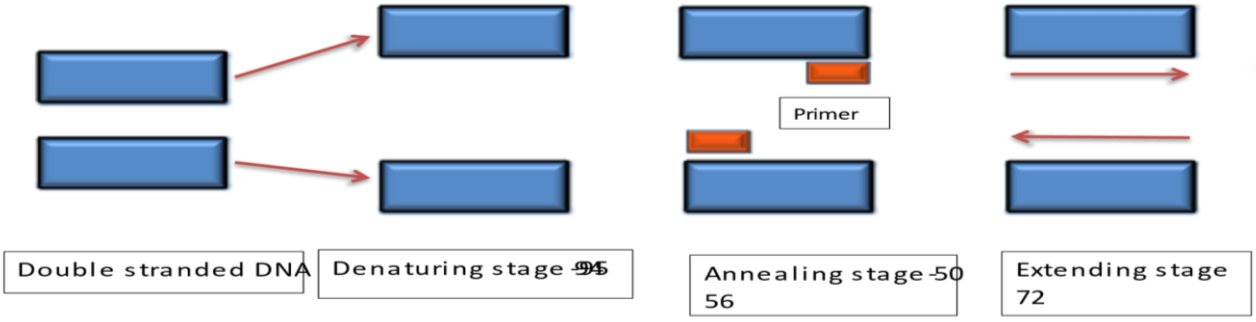



**Figure IV: Process of Polymerase Chain Reaction**

**Table 2: Sequence of Primers Selected for PCR Analysis**

| Target | Primer | Sequence | Annealing Temperature |
|---|---|---|---|
| *bla*OXA | OXAF OXAR | ACACAATACATATCAACTTCGC<br>AGTGTGTTTAGAATGGTGATC | $56^0$ C |
| *bla*OXA-47 | OXA-47F<br>OXA-47R | TCAACTTTCAAGATCGCA<br>GTGTGTTTAGAATGGTGA | $47^0$ C |
| $bla_{TEM}$ | TEMF TEMR | TCGGGGAAATGTGCGCG<br>TGCTTAATCAGTGAGGACCC | $58^0$ C |
| $bla_{CTX}$ | CTXF CTXR | CACACGTGGAATTTAGGGACT<br>GCCGTCTAAGGCGATAAACA | $56^0$ C |
| $Bla_{SHV}$ | SHVF SHVR | CACTCAAGGATGTATTGTG<br>TTAGCGTTGCCAGTGCTCG | $56^0$ C |
| Tet C | TetCF TetCR | CTTGAGAGCCTTCAACCCAG<br>ATGGTCGTCATCTACCTGCC | $56^0$ C |
| *bla*NDM | NDMF NDMR | GGTGCATGCCCGGTGAAATC<br>NDM-R: ATGCTGGCCTTGGGGAACG | $56^0$ C |
| ampC | ampCF ampCR | TGAGTTAGGTTCGGTCAGCA<br>AGTATTTTGTTGCGGGATCG | $56^0$ C |

**PCR Analysis:** PCR technique was used to amplify the DNA and detect the presence ESBLs in the selected samples. Primers selected for analysis are as follows.

**Statistical Analysis**

Statistical analysis was performed by Windows based software named as Statistical Package for Social Science (SPSS), versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Continuous data were expressed as mean, standard deviation, minimum and maximum. Categorical data were summarized in terms of frequency counts and percentages.

**Ethical Consideration:** All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration 2013) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local ethics committee. All data were collected and were recorded anonymously and were analyzed using the coding system.

**Results**

**Antibiotic Resistance Pattern of Isolated from Clinical Samples:** Antibiotic resistance profile of collected *Escherichia coli* from different urine sources and sputum samples.

**Selected sample (For further Plasmid DNA Analysis) from the result:** Antibiotic resistance profile of 8 selected *Escherichia coli* from different urine sources and sputum sources:

**Table 3: Antibiotic Resistance Pattern of Isolated from Clinical Samples**

| Sample | Imipenem | Meropenem | Ciprofloxacin | Norfloxacin | Levofloxacin | Amoxicillin | Chloramphenicol | Gentamicin | Kanamycin | Cefuroxime | Erythromycin | Vancomycin | Tetracycline | Mar Index |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UE 15 | R | R | R | R | R | R | 15R | 8R | 9R | R | R | 10I | R | 0.84 |
| UE 16 S1 | 24S | R | 25S | 25S | R | R | 40S | 18S | R | 31S | 38S | R | 11R | 0.42 |
| EU 16 S2 | 38S | R | 28S | 35S | 17I | 38S | 17I | 26S | R | 30S | 11R | R | 30S | 0.33 |
| EU 20 S1 | 19S | 10R | R | R | R | R | R | R | R | R | R | R | 15I | 0.79 |
| EU 20 S2 | 17I | 9R | R | 18S | R | R | R | R | R | R | R | R | 11R | 0.83 |
| EU 27 S1 | R | R | R | 21S | R | 20S | 8R | R | R | R | R | 16I | 20S | 0.75 |
| UE 69 S1 | 20S | 20S | 32S | 22S | R | R | 35S | 21S | 12R | 16I | R | R | 11R | 0.46 |
| UE 75 S1 | 7R | 20I | 23S | R | R | R | 17I | 11R | 13R | R | R | R | 13R | 0.76 |
| UE 75 S2 | R | 20I | 38S | R | R | R | 16I | 15S | 12R | R | R | R | 13R | 0.69 |
| UE 81 S1 | 17I | R | R | 13I | 7R | R | 7R | 11R | 15I | 10R | R | 20I | 13R | 0.69 |
| UE 81 S2 | 15R | R | 11R | R | 9R | R | R | 20S | R | R | R | 10I | R | 0.84 |
| UE 83 S1 | 8R | 16S | R | 9R | 9R | R | 25S | 20S | 13R | 12R | R | 11I | 10R | 0.69 |
| UE 83 S2 | 24S | 26S | 11R | 12R | 7R | R | 20S | 15I | 8R | 20I | 20I | 10I | 32S | 0.38 |
| UE 85 | R | 20I | R | 15I | 7R | R | 15I | 8R | 9R | R | 30S | R | 15I | 0.61 |
| UE 87 S1 | R | 15R | 10R | R | 10R | R | 16I | 20S | 15I | R | R | 10I | 17I | 0.53 |
| UE 87 S2 | R | 20I | 11R | R | 9R | R | 23S | 20S | 13R | R | R | 11I | 16I | 0.61 |
| UE 89 | R | 25S | 31S | R | R | R | 19S | 16S | R | R | R | R | 5R | 0.76 |
| UE 91 S1 | 17I | 12R | R | 13I | 10R | R | 8R | 10R | R | 10R | R | 12S | 20S | 0.69 |
| UE 91 S2 | 9R | 9R | 7R | 20S | 9R | R | 21S | 20S | 15I | 11R | R | 11I | 10R | 0.61 |
| UE 94 | R | R | R | 15S | 7R | R | 16I | 16S | R | R | R | 10I | 17I | 0.61 |
| Sputum Specimen (*E. coli*) | | | | | | | | | | | | | | |
| E (Sp 20)S1 | R | 19R | R | 22S | 10R | R | 10R | 8R | 12R | 8R | 20I | 12S | R | 0.69 |
| E (sp 20)S2 | R | 27S | 7R | 21S | 9R | R | 23S | 12R | 14I | R | 19I | R | 11R | 0.61 |
| E(SP 21)S1 | R | 20S | R | 20S | R | R | 10R | R | R | R | R | R | 33S | 0.76 |
| E(SP 22) S2 | R | 23S | R | R | 9R | R | 21S | 22S | R | R | R | R | R | 0.76 |
| E(SP35) S1 | R | 20S | R | 20S | R | R | 10R | R | R | R | R | R | 33S | 0.76 |

Note: E = (*E. coli*), U= (Urine), SP = Sputum, S1/S2= (Sample 1/Sample 2), S = (Sensitive), I = (Intermediate), R = (Resistance)

**Resistance Pattern of *Escherichia coli* (Percentage):** Given below the result - the chart of the percentage of the resistant pattern of *E. coli*: In chart, imipenem 2.0%, meropenem 3.0%, ciprofloxacin 7.0%, norfloxacin 7.0%, levofloxacin 12.0%, amoxicillin 11.0%, chloramphenicol 1.0%, gentamicin 6.0%, kanamycin 9.0%, cefuroxime 11.0%, erythromycin 7.0%, vancomycin 7.0%, tetracycline 7.0% (Figure V).

**Plasmid Isolation:** From the result of Gel Electrophoresis of selected clinical *Escherichia coli* isolates: We have found the presence of plasmid in 2 samples from 8 clinical samples.

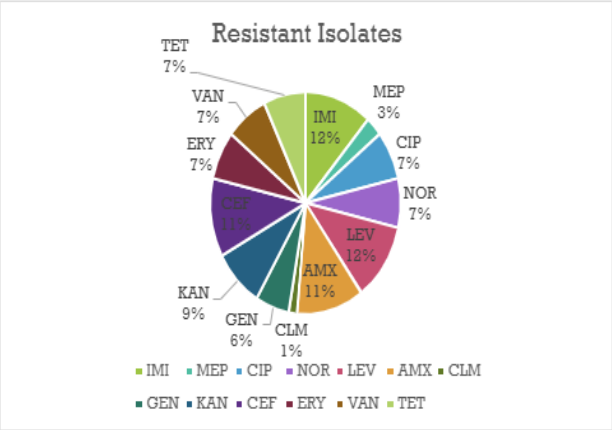



**Figure V: Percentage of the Antibiotic-Resistant Pattern of *Escherichia coli* isolates**

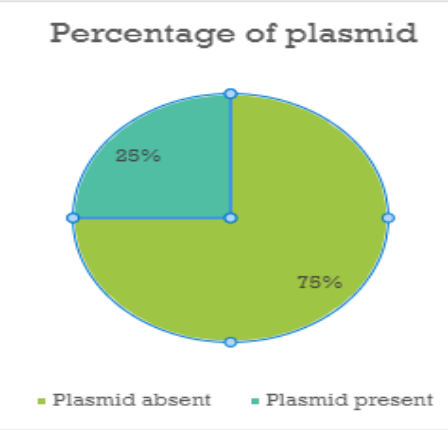



**Figure IV: Showing the Status of Plasmid**

**PCR Analysis:** The following table depicts the results obtained after carrying out Gel Electrophoresis on the clinical isolates after PCR analysis had been done.

The result of gel electrophoresis shows that 25% of the clinical isolates showed the presence of resistant plasmid while 75% showed the absence of the plasmid

**Table 5: This depicts that the Positive and Negative Results of Plasmids are Obtained after carrying out Gel Electrophoresis on the Clinical Isolates after PCR analysis**

| Number | Sample/ Primer | BL | BLT | BLC | AMP | SHV | NDM | TET | BLX - 47 | MAR index | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | U-75 S1 | +ve F-3 C-5 | -ve F-8 C-5 | -ve F-2 C-16 | +ve F-2 C-7 | +ve F-2 C-5 | +ve F-4 C-8 | +ve F-5 C-2 | +ve F-1 C-5 | 0.76 | 6 |
| 2 | U-75 S2 | -Ve F-9 C-2 | -ve F-9 C-3 | -ve F-9 C-4 | +ve F-9 C-5 | -ve F-9 C-6 | +ve F-9 C-8 | -ve F-9 C-7 | -ve F-12 C-7 | 0.69 | 2 |
| 3 | U-85 | +ve F-10 C-2 | -ve F-10 C- 3 | -ve F-10 C-4 | -ve F-10 C-5 | -ve F-10 C-6 | +ve F-10 C-8 | -ve F-10 C-7 | +veF-12 C- 8 | 0.61 | 3 |
| 4 | U-87 S1 | -ve F- 6 C-11 | -ve F-6 C-15 | -ve F-6 C-16 | -ve F-6 C-12 | -ve F-6 C-13 | -ve F-6 C-14 | -ve F-6 C-10 | -ve F-12 C-9 | 0.53 | 0 |
| 5 | U-87 S2 | -ve F-5 C-4 | -ve F-5 C-8 | +ve F-6 C-2 | +ve F-5 C-5 | -ve F-5 C-6 | +ve F- 5 C-7 | +ve F-5 C-3 | -ve F-12 C-10 | 0.61 | 4 |
| 6 | U-89 | +ve F-3 C-2 | +ve F-8 C- 2 | -ve F-8 C-6 | +ve F-1 C-6 | -ve F-2 C-2 | +ve F-4 C-2 | +ve F-4 C-3 | +veF-1 C-2 | 0.76 | 6 |
| 7 | SP-20 S1 | +ve F-9 C-9 | -ve F-9 C-10 | +ve F-9 C-11 | +ve F-9 C-13 | -ve F-9 C-15 | +ve F-9 C-15 | -ve F-9 C-14 | +veF-12 C-11 | 0.69 | 4 |
| 8 | Sp-20 S2 | -ve F-11 C- 9 | -ve F-11 C-10 | +ve F-11 C-11 | -ve F-11 C-12 | +ve F-11 C-14 | +ve F-11 C-15 | -ve F-11 C-14 | +ve F-12 C-12 | 0.61 | 3 |

Note: U= Urine, S1/S2= Sample 1/ Sample 2, (-VE) = Negative, (+VE) = Positive, F= Figure, C= Column

**Percentage of Antibiotic Resistance Gene Analysis:** The following chart shows the results obtained after antibiotic resistance gene analysis of selected *Escherichia coli*: The following information acquired from the results of Gel Electrophoresis depicts that the percentage of PCR isolates showed positive of genomic plasmid (+ve) accordingly in graphical representation -

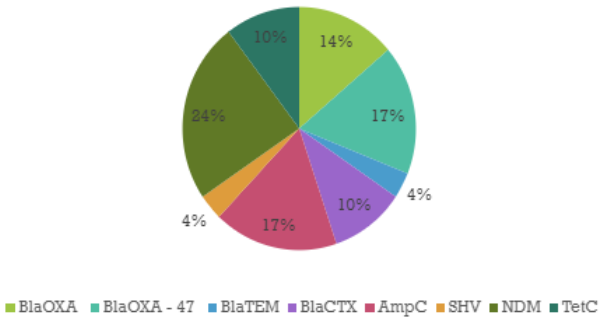



**Figure VII: The Following Chart Shows the Results Obtained after Antibiotic Resistance Gene Analysis of selected** *Escherichia coli* **(**Percentage of Genomic Analysis of selected *E. coli*: Primer - BlaOXA:14%, BlaOXA – 47: 17 %, BlaTEM: 4%, BlaCTX: 10%, AmpC: 17%, SHV: 4%, NDM 24%, TetC: 10%)

**Percentage of *Escherichia coli* isolates +ve and –ve for different primers:** The following chart acquired from the results of Gel Electrophoresis depicts that the percentage of PCR isolates which showed positive and negative findings of genomic plasmid in graphical representation-

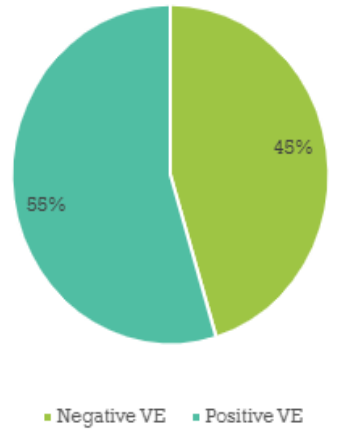



**Figure VIII: Showing 55.0% of** *Escherichia coli* **Isolates Positive for Different Primers**

## Discussion

This study demonstrates a significant incidence of first-line antibiotic resistance among patients presenting to hospitals in Dhaka, Bangladesh with symptoms suggestive of UTIs. Our findings demonstrated that multiple antibiotics such as imipenem, levofloxacin, amoxicillin, and cefuroxime had the highest resistance. Although Bangladesh has a National Action Plan 2017-2022 for AMR prepared by the Disease Control Unit of the Director General of Health Services, MoHFW with a road map for its implementation in accordance with the global plan, the high resistance of those antibiotics suggests the irrational use of antibiotics cannot be controlled or minimized[8]. The document emphasizes the rational use of antibiotics across all sectors through the improvement of standard treatment guidelines, antibiotic stewardship, the introduction of reference laboratories, Good Manufacturing Practices, Good Pharmacy practices, infection prevention and control, and the implementation of extensive surveillance.

The reason for its failure might be attributed to a variety of things. First off, a significant percentage of Bangladeshi patients self-medicated used medication without seeking medical advice[9]. Besides, the standard treatment guidelines were found to be followed by some hospitals at the secondary and tertiary levels[10], but not by all hospitals in Upazilas (sub-districts) where treatment guidelines were occasionally unavailable[11-12]. This is why, antimicrobials were frequently administered in cases of fever, the common cold, cough, diarrhoea, and ARI without proper indication[11,13,14].

Additionally, we discovered significant levels of resistance to commonly used antibiotics like Ciprofloxacin, Norfloxacin, Tetracycline, and Kanamycin. These findings point to a lack of policymakers' and practitioners' knowledge of AMR, a lack of resources to implement measures to address AMR, and a lack of a comprehensive national surveillance system to track the spread of AMR. In Bangladesh, prescribing antibiotics without doing laboratory tests was relatively widespread due to the lack of appropriate testing facilities or financial issues[14-16]. For our clinician, this poses a major challenge. However, we are fortunate in that competent medical professionals occasionally had the confidence to choose the best antibacterial and give the right dose and duration based on a clinical diagnosis[17]. However, patients' non-compliance with the recommended dosage, which promoted the development of AMR, was a prevalent phenomenon in our nation[15].

These findings also revealed a significant level of resistance to broad-spectrum antibiotics likes Meropenem as well as the resistance primer. This

indicates that the National One Health Strategy in our nation is not working as intended. Considering that it is a coordinated multi-sectoral, multi-disciplinary, and multi-institutional response using a comprehensive approach to improve human health, animal health, and the environment[18].

Because coordination between various sectors and raising professional and practitioner awareness for consensual activities across sectors continues to be a major obstacle to progress. The high prevalence of first-line antimicrobial drug resistance emphasizes the critical requirement for actual data on AMR prevalence to guide antimicrobial therapeutic interventions. To ensure that treatment recommendations are adapted to the local environment and epidemiology, enhancing laboratory capacity, enhancing AMR surveillance, and encouraging research in AMR in LMICs are essential.

## Conclusion

The effectiveness of antibiotics in treating common infections like fever, the common cold, and diarrhoea in Bangladesh is significantly threatened by antimicrobial resistance. This study shows that *Escherichia coli* is becoming increasingly resistant to widely administered antibiotics. Increased antimicrobial resistance has been found in *Escherichia coli* isolates from various samples, particularly levofloxacin, imipenem, amoxicillin, cefuroxime, and kanamycin. However, the development of drug resistance to other antibiotics is equally concerning. Antimicrobial drug resistance highlights the urgent need for accurate surveillance of AMR prevalence to properly guide antimicrobial therapeutic measures.

**Acknowledgments**
We express our gratitude to the laboratory personnel at North South University's Microbiology Unit.

**Conflict of Interest**
The authors have no relevant conflicts of interest to declare.

**Financial Disclosure**
This research did not receive any grant from funding agencies in the public, commercial or not-for-profit sectors.

**Contribution to authors**
G M, A K B: Conception and design, or design of the research; G M, A K B: the acquisition, analysis, or interpretation of data; conceptualized and designed the overall study; G M, E I: involved in data collection; G M, A K B, M M: Drafting the manuscript or revising it critically for important intellectual content; G M, M M: involved in data input and data cleaning. M M: conducted data analysis; G M, A K B: drafted the manuscript. All authors reviewed and approved the final manuscript.

**Data Availability**
Any questions regarding the availability of the study's supporti ng data should be addressed to the corresponding author, who can provide it upon justifiable request.

**Ethics Approval and Consent to Participate**
The Institutional Review Board granted the study ethical approval. Since this was a retrospective study, formal informed consent was no needed. Each method followed the appropriate rules and regulations.

**How to cite this article:** Mehbuba G, Barman AK, Marma M, Iqbal E. Assessment of Human Urine and Sputum Samples for Multiple Antibiotic Resistance Patterns and Genomic DNA Analysis of *Escherichia coli*. Bangladesh J Infect Dis 2024;11(2):121-130



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**Article Info**
Received on: 14 August 2024
Accepted on: 20 November 2024
Published on: 1 December 2024

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