MOLECULAR CHARACTERIZATION AND ANTIBIOGRAM STUDIES OF URINARY TRACT INFECTION BACTERIA ISOLATED FROM URINE SAMPLES OF PREGNANT WOMEN

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Abstract. Urinary tract infection is a frequent ailment during pregnancy that creates critical complications for the mother and fetus requiring immediate detection and prevention. In the current study, we aimed to investigate the microbial diversity of microbes associated with urinary tract infection by randomly selecting urine samples from 100 patients exhibiting UTI symptoms. We characterized patients' bacterial isolates by differential culture media, following the identification of an isolate by the *16s rRNA* gene. Drug susceptibility testing revealed that Gram-negative bacteria demonstrated higher resistance to the drugs as compared to Gram-positive organisms. Overall, in this study, six isolates were identified as *E. coli* and designated as the main causative agent of UTIs and diagnosed in the third-trimester, which is the most dangerous stage of pregnancy for mothers. Appropriate and receptive antibiotics and therapeutic drugs with no side effects and those that are inexpensive and widely available are needed for the treatment of UTIs. Appropriate use of antibiotics can reduce the complications of infected mothers and fetuses.
Keywords: *microbial diversity, drug susceptibility, therapeutic drug, infected mothers*

Introduction

Urinary tract infections (UTI) are bacterial infections with a worldwide annual occurrence of about 150 million cases and a cost of more than 6 billion dollars (Patton et al., 1991). Approximately 40% of women and 12% of men will have at least one symptomatic UTI in their lifetime, with up to 40% of affected women experiencing recurrent UTI (Foxman and Betsy, 2010). UTI is an infection that primarily affects the urinary system, which includes the ureters, kidneys, bladder, and urethra. While UTIs

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can be caused by various factors, the most common type is a bacterial infection (Pereira et al., 2013**)**. UTIs are one of the most common problems in pregnant women, following anemia. If left untreated, UTIs can indeed lead to complications that can harm the health of both the pregnant mother and the developing fetus (Schieve et al., 1994; Mittal et al., 2005). During pregnancy different hormonal and mechanical changes occur in the body (Schnarr et al., 2008). Some factors that contribute to increased urine stasis and ureterovesical reflux include urethral dilation (which occurs in week 6), increased bladder volume (weeks 22-24), and decreased urethral tone (which occurs in week 6) (Chaliha et al., 2002). Glycosuria, a bacterial growth-promoting condition in the urine, affects up to 70% of pregnant women (Al-Issa et al., 2009; Patton et al., 1991). Short urethra and trouble with hygiene increase UTI risk in pregnant women... In the case of UTI, a single bacterial species is responsible for 95% of the cases. In cases of acute infection, *E. coli* is the most common infecting organism (Ronald et al., 2002).

Enterobacter, *Staphylococci*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Enterococci* species were isolated from patients with a higher prevalence of *E. coli* in an outpatient population (Bronsema et al., 1993) while 80-85% UTI cases are implicated by *E. coli* (Nicolle and Lindsay, 2008) and *Staphylococcus saprophyticus* causes 5-10%. The colonization of the vaginal mucous by certain bacteria, such as *E. coli*, can be a precursor to UTIs and leads to, pyelonephritis, kidney damage, high blood pressure, and death (Surgers et al., 2013).

Molecular techniques such as Pulsed-Field Gel Electrophoresis (PFGE), phylogenetic typing, Multi-Locus Sequence Typing (MLST), and whole-genome sequencing can be used to characterize Escherichia coli (*E. coli*) strains. These techniques provide valuable insights into the genetic relatedness, phylogenetic classification, and diversity of *E. coli* isolates. It is crucial to note that the increasing prevalence of antibiotic-resistant *E. coli* strains, including those with extended-spectrum beta-lactamase (ESBL) or carbapenemase production, pose challenges to UTI treatment.

By addressing the major causes and risk factors associated with UTIs in pregnant women, this research can contribute to improved preventive strategies, optimized treatment guidelines, and the overall management of UTIs in this population. It can also provide valuable insights into the social determinants and implications of UTIs, facilitating a holistic approach to patient care in Bangladesh.

Materials and methods

Selection of research region and period

For six months, July–December 2017, urine samples were obtained from pregnant women at three hospitals in the Dinajpur district of Bangladesh (M Abdur Rahim Medical College and Hospital, Saint Vincent Hospital, and Sheba Diagnostic and Consultation Center) and taken to the Department of Microbiology at Hajee Mohammad Danesh Science and Technology University for microbiological analysis.

Collection of clinical specimens

A total of 100 urine samples were randomly collected from pregnant women (Age 17-35). Among them, 50 samples were collected from M Abdur Rahim Medical College and Hospital, 40 from Saint Vincent Hospital, and the remaining 10 from Sheba

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Diagnostic and Consultancy Center. Regarding the age of patients 60% of samples were collected from age group under 25, 35% were collected from age group 25-30 and 5% were form age group above 30.

Sample preparation, bacteria isolation, and identification

Bacterial culture preparation, and enumeration was carried out a described by Saraf et al. (2022) and the local Laboratory Manual of Microbiology. Prepared samples were diluted and spread on plate count agar (PCA) to determine total viable counts. Then incubated at 37°C for 24 h and the colony was counted. For primary culture nutrients, agar, and nutrient broth were used. Then, used for subculturing media such as MacConkey agar, eosin methylene blue agar (EMB), Mannitol salt agar (MSA), Blood agar, Cetrimide agar, Tryptic soy agar (TSA), Baird parker agar, and Mitis salivary agar base. Then incubate all culture plates at 37°C for 24 h, and finally, pure cultures were derived following the published protocols (Saraf et al., 2022). A group of biochemical tests was performed to identify UTI-resourced bacteria based on biochemical and physical analysis bacteria followed by Holt et al. (1994) and CLSI (2013).

Sensitivity test for antibiotics of isolated bacteria

Commercially available antibiotics from HiMedia Laboratories were used for antibiotic sensitivity tests of isolated bacteria. According to CLSI (2013), The Kirby-Bauer disk diffusion method, as described by Jan Hudzicki in 2009, was employed and the interpretation of results was done using Muller-Hinton agar plates. The following total 18 commercially available antibiotics from HiMedia Laboratories were used for the sensitivity tests, each with their respective concentration such as ampicillin 25 µg, amoxicillin 30 µg, amikacin 30 µg, chloramphenicol 30 µg, ciprofloxacin 5 µg, gentamycin 10 µg, kanamycin 30 µg, penicillin 10 µg, tetracycline 30 µg, vancomycin 30 µg, erythromycin 15 µg, levofloxacin 5 µg, neomycin 30 µg, norofloxacin 10 µg, novobiocin 30 µg, ofloxacin 2 µg, cefotaxime 30 µg, cliostin 10 µg were applied for the measuring of zone of inhibition.

Molecular detection of E. coli

Sample collection, extraction, and purification of genomic DNA

Keeping in view the antibiotic sensitivity tests, physical characteristics at various culture media, and the importance of *E. coli*. An isolate of potential *E. coli* was processed for identification by *16s rRNA* genes The isolation and purification of the genomic DNA of *E. coli* were extracted as described previously (Rumi et al., 2019). Briefly, the chloroform-isoamyl process extracted genomic DNA from *E. coli* isolates cultured in sodium thioglycolate broth. The bacterial cells were centrifuged for 10 min at 2400 *g* to pellet cells. The supernatant was removed and resuspended the pellets in 100 µl Tris EDTA buffer, 0.01 M Tris –HCL, pH 5.2, 0.001 M EDTA. Then add 10% SDS and proteinase K (Ambion) into the suspension and incubated in an oven (Biometra OV2, Menachem, UK) at 65°C for 60 min. 100 µl of 5 M NaCl and 100 µl CTAB/NaCl were added into the solution and incubated for 20 min at 65°C inertly. Remove from the incubator and cool at 25°C for 5-7 min. Chloroformisoamyl alcohol (Sigma- Aldrich) 24:1 was added into the solution and centrifuge at 1300 *g* for 15 min. Finally, supernatants were collected and treated with 5 µl RNase A and incubated at

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37°C for 30 min. DNA quality and quantity were examined using the scientific Nano Drop 2000 spectrophotometer machine (Thermofifher Scintific USA) by measuring the DNA concentration (ng/µL) and absorbance ratio (260 nm/280 nm) of DNA that express the actual purity and concentration of DNA. Finally, we stored DNA at -20°C for further use.

Amplification protocols of DNA, sequencing, and phylogenic tree

Keeping in view the antibiotic susceptibility testing results, molecular confirmation of *E. coli* was carried out by using *16S rRNA* gene with forward primer-27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer -1492R (5'-TACCTTGTTACGACTT-3') (McCabe et al., 1999) to randomly selected isolate. A total of 2.5 µl of buffer, 2.5 µl of dNTPs, 2.5 µl of $MgCl_2$, 1 µl of forward primer (27F), and 1 µl of reverse primer (1492R) were used in the PCR reaction and to make a final volume of 25 ml, 2 µl of DNA sample, 1 µl of Taq DNA polymerase, and 12.5 µl of nanopore water were added. The cycling parameters for *E. coli* PCR were started denaturation at 95°C for 10 min, followed by 35 cycles of 94°C for 1 min, 53°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min (Kumar et al., 2016) and PCR reaction run by Sanger sequencing method with an applied biosystem (Foster City, CA, USA) automatic DNA sequencer (ABI3130xl genetic analyzer) at National Institution of Biotechnology (NIB), Savar, Dhaka. The phylogenic tree was analyzed using molecular evolutionary genetic analysis software resulting from edited and analyzed sequences (Kumar et al., 2016) using the neighbor-joining method (Saitou et al., 1987). Using the BLASTN (http://www.ncni.nih.gov/BLAST) algorithm, the gene sequence was uploaded to the GeneBank database.

Statistical analysis

All data were input a spreadsheet and analyzed by SPSS version 20 (Chicago, USA). Chi-square tests were used to analyze the relationship between categorical variables such as study area, age, education, and stage of pregnancy with UTI occurrence. The P-value < 0.05 indicates the relationship between each variable and UTI occurrence is statistically significant.

Results

Isolation and identification of bacteria

The results of various tests such as morphological, staining, cultural, biochemical, and antibiotic sensitivity patterns as well as the frequency of isolated microorganisms. *E. coli, Klebsiella* species, *Proteus* varieties, and *Pseudomonas* species are shown in the figures and tables as below. *Staphylococcus* spp. and *Streptococcus* spp. shows different cultural characteristics in different selective media, which are represented in *Figures 1, 2,* and *3*. Biochemical test results are shown in *Figure 4* respectively.

Morphological features of the *E. coli* were as follows at NA media, the shape was circular with grayish-white color, at MacConkey agar it was pink, at EMB agar it was Green metallic sheen and at SS agar media it was brown-yellowish (*Fig. 1*).

Morphological features of the *Klebsiella pneumoniae* were as follows at NA media, the shape was circular with grayish-white color, at MacConkey agar it Pink – Red, at EMB agar it was Pink purple and at blood agar media it was pink-red (*Fig. 2*).

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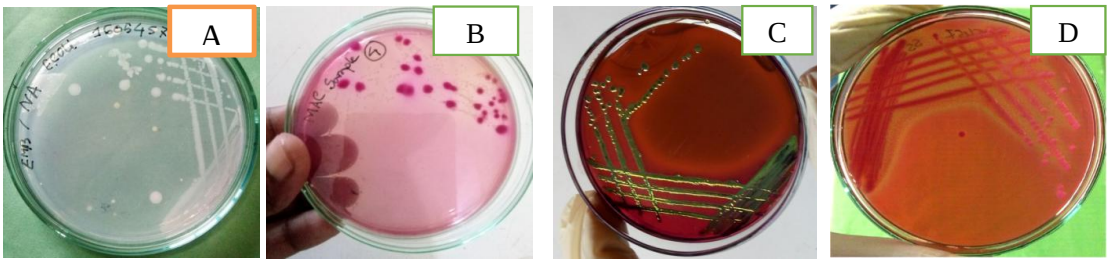

Figure 1. *Growth of Escherichia coli on nutrient agar (A), MacConkey agar (B), EMB agar (C), and SS agar (D)*

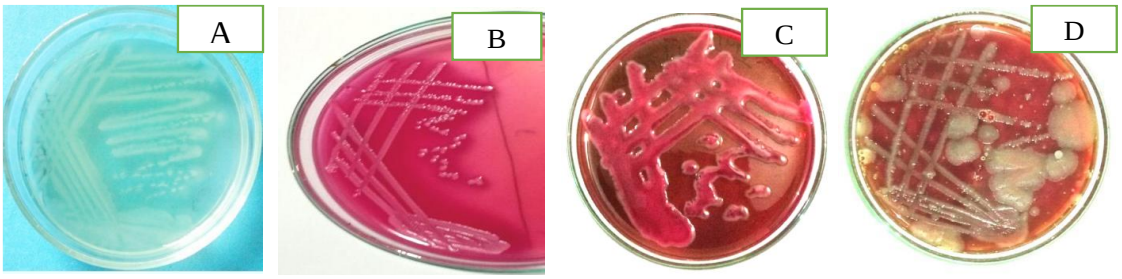

Figure 2. *Klebsiella spp. growth on nutrient agar (A), MacConkey agar (B), EMB agar (C), and Blood agar (D)*

Morphological features of the Staphylococcus spp. were as follows at MSA media, small, circular, and smooth gray-white or yellowish colonies shape was observed, at TSA yellow-pigmented colonies, at BP agar dark gray and black colonies were observed, at Blood agar zones of clear beta-hemolysis with golden appearance was observed (*Fig. 3*).

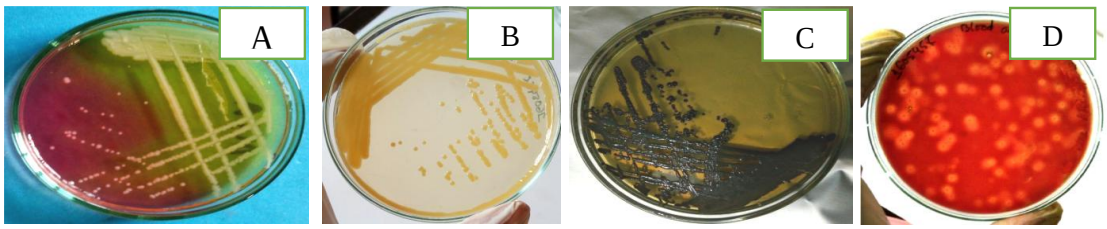

Figure 3. *Staphylococcus spp. growth on MSA agar (A), TSA agar (B), BP agar (C), and Blood agar (D)*

Differential media which is a type of culture medium used in microbiology to distinguish and identify different microorganisms based on their biochemical and metabolic characteristics and are valuable in clinical settings as they help researchers and healthcare professionals to isolate and identify specific pathogens or microorganisms present in patient samples. These findings have elucidated that the samples resourced from 100 patients included with key pathogenic bacteria such as *E. coli*, *Staphylococcus* spp and *Klebsiella* spp (*Fig. 4*).

Biochemical tests play a crucial role in the identification and differentiation of various microorganisms. These biochemical tests, along with other differential and

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selective media, contribute to the identification and characterization of bacteria in clinical and research settings. The isolates were further characterized by different Biochemical tests such as Methyl Red, Voges-Proskauer test, Indole test, TSI test, MIU test, and Simmons citrate test which has further confirmed the identification of *E. coli*, *Kleibsella Sp.* and *Stapphylocous* Sp. (*Table A1*).

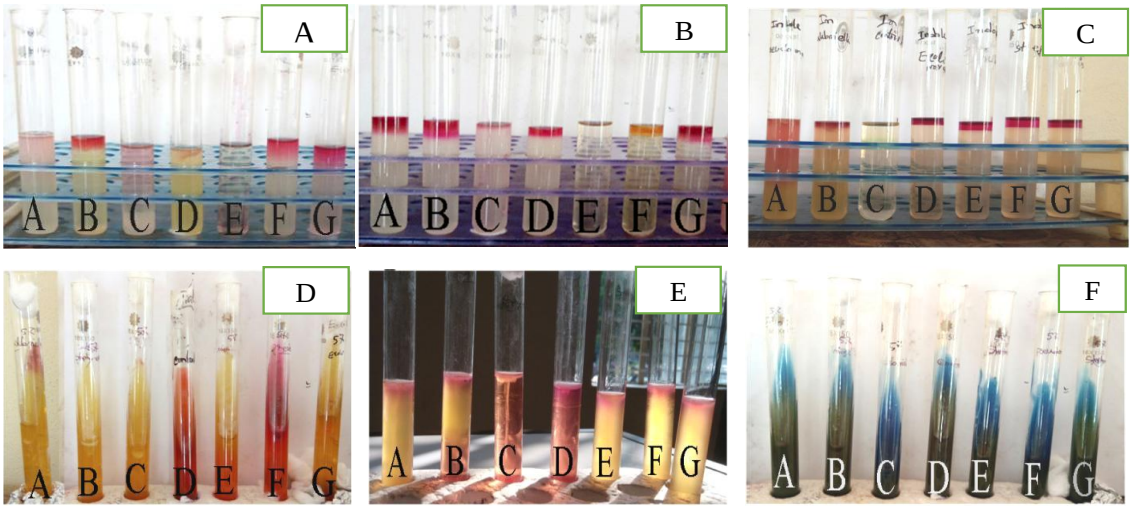

Figure 4. *Biochemical test analysis. Methyl Red (A), Voges- Proskauer test (B), Indole test (C), TSI test (D), MIU test (E), and Simmons citrate test (F)*

Distribution of isolates from urine samples in different categories

75.76% of positive patients were identified as less than 25 years of age, 18.18% of positive patients were aged between 25-30 years, and 6.06% were found in more than 30 years of age (*Table 1*).

Table 1. *Distribution of UTI associated Isolates of in pregnant women according to age*

Patient age	Number of samples	Positive cases	% of positive case
Less than 25 years	60	25	75.76
25-30 years	35	6	18.18
More than 30 years	5	2	6.06
Total	100	33	100

In the case of education level highest number (66.67%) of positive patients were identified in primary education which is shown in (*Table 2*). Importantly, in the stage of the third trimester, 45.46% of UTI positives were found whereas in the first trimester was identified only 24.24% were (*Table 3*). It was reported that the highest number of *E. coli* (73.33%) and lowest number of *E. coli* (6.67%) were found in < 25 years of age. Only 2(6.67%) E. coli was isolated in above 30 years of aged patients (*Table 5*).

Notably, we collected 100 urine samples from pregnant women and among the total isolates, 75 bacterial isolates were recovered. Sixty (80%) of the 75 bacteria isolates were gram-negative, while 15 (20%) were gram-positive, as shown in *Table 4*.

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Table 2. *Distribution of UTI associated Isolates in pregnant women according to level of education*

Level of education	No. examined	No. positive	Percentage (%)
Primary	50	22	66.67
Secondary	25	7	21.21
Higher	25	4	12.12
Total	100	33	100

Table 3. *Percentage of UTI by stage of pregnancy*

Stage of pregnancy	No: Tested	No: positive (%)
First trimester	45	8(24.24)
Second trimester	25	10(30.30)
Third trimester or above	30	15(45.46)
Total	100	33(100)

Table 4. *Gram-positive and gram-negative bacterial percentages (%)*

Bacterial isolates	Number of isolates	% of total bacteria	Specific % of gram (-) and gram (+) bacteria
Gram-negative *E. coli* *Klebsiella* spp.	60 (80%) 30 16	40 21.33 12 6.67	50 26.67 15 8.33
Gram-positive *Staphylococcus* spp.	15 (20%) 12	16 4	80 20
Total	75	100	100

Antibiotic sensitivity test findings

Antibiotic sensitivity pattern for gram-negative bacteria

The antibiotics showed that *E. coli* were sensitive to gentamicin, chloramphenicol, ciprofloxacin (80%), neomycin, and colistin (70%) and resistant to penicillin, amoxicillin, and ampicillin (100%) ensued by vancomycin (90%) and cephalexin (80%). Chloramphenicol, Ciprofloxacin, and Levofloxacin were all 100% susceptible against *Klebsiella spp.*, led by Norfloxacin (60%) where Amikacin, Amoxicillin, and Cloxacillin were observed resistance 100%. *Proteus spp.* were sensitive to neomycin and Gentamycin (66.67%) and resistant to Ciprofloxacin, Chloramphenicol, Penicillin, Amoxicillin, Ampicillin (100%), followed by Cloxacillin and Amikacin (66.67%). *Pseudomonas spp.* were sensitive to Colistin (100%) and resistant to all other antibiotics (100%) except Ciprofloxacin and Amikacin, shown in *Table 5* and *Figure 5* and *6*.

Percentage of antibiotic sensitivity pattern for gram-positive bacteria

The antibiotic study of all isolates *of Staphylococcus spp.* (5) were sensitive to levofloxacin, ofloxacin, chloramphenicol, and Gentamycin (100%), followed by

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novobiocin, kanamycin, and tobramycin (80%). The isolates were resistant to cephalexin, amoxicillin, and ampicillin (100%), followed by norfloxacin and Colistin (60%). Antibiotic sensitivity test showed in *Table 9* and *Figures 10* and *11*.

Table 5. *Resistant and susceptibility percentage for isolated gram-negative pathogens*

Antibiotics with disc concentration (µg/disc)	***E. coil* (10)**		***Klebsiella* spp. (5)**		***Staphylocosus* spp. (3)**	
	%R	**%S**	**%R**	**%S**	**%R**	**%S**
Ciprofloxacin (5)	2(20)	8 (80)	0(0)	5(100)	2(66.67)	1(33.33)
Chloramphenicol (30)	2(20)	8 (80)	0(0)	5(100)	3(100)	0(0)
Penicillin (10)	10(100)	0(0)	NT	NT	3(100)	0(0)
Cloxacillin (1)	NT	NT	5(100)	0(0)	NT	NT
Kanamycin (30)	5(50)	2 (20)	0(0)	5 (100)	3(100)	0(0)
Gentamycin (10)	2(20)	8 (80)	0(0)	5(100)	3(100)	0(0)
Vancomycin (30)	9(90)	1 (10)	5(100)	0(0)	3(100)	0(0)
Neomycin (30)	1(10)	7 (70)	2(20)	4(80)	3(100)	0(0)
Amoxicillin (30)	10(100)	0(0)	5(100)	0(0)	3(100)	0(0)
Ampicillin (25)	10(100)	0(0)	4(80)	2(20)	3(100)	0(0)
Amikacin (30)	6(60)	4 (40)	5(100)	0(0)	2(66.67)	1(33.33)
Norfloxacin (10)	NT	NT	2(40)	3(60)	NT	NT
Levofloxacin (5)	NT	NT	0(0)	5(100)	NT	NT
Colistin (10)	2(20)	7 (70)	NT	NT	0(0)	3(100)
Erythromycin (15)	NT	NT	0(0)	5(100)	NT	NT
Cephalexin (30)	8(80)	2 (20)	4(80)	0(0)	3(100)	0(0)
Cefotaxime (30)	NT	NT	3(100)	0(0)	NT	NT

S = Sensitive, R = Resistant, % = Percentage and NT = Not tested

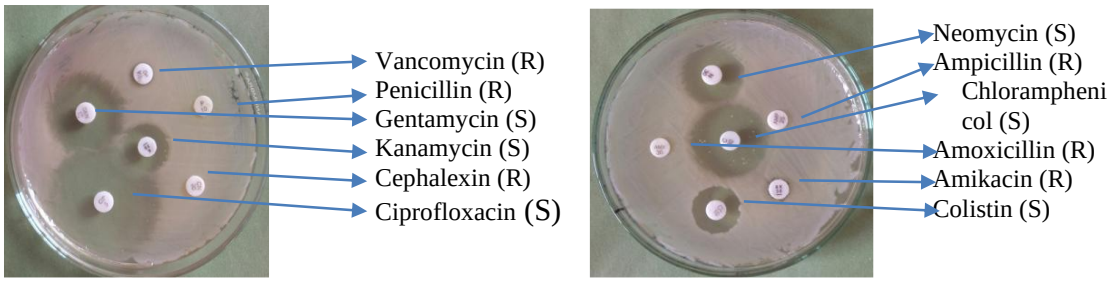

Figure 5. *Antibiotic sensitivity test for E. coli*

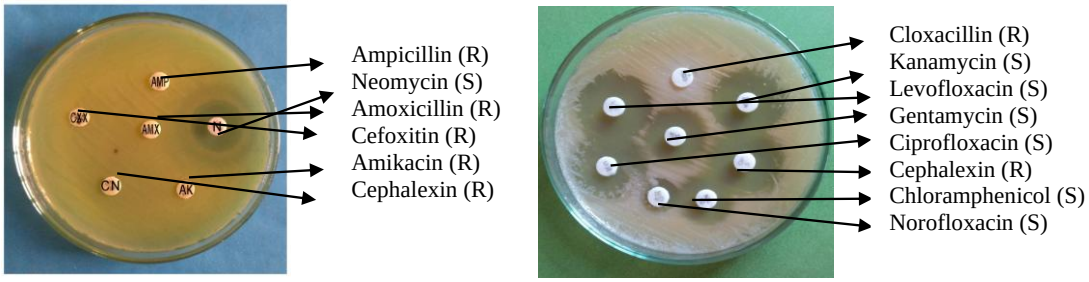

Figure 6. *Antibiotic sensitivity test for Klebsiella spp.*

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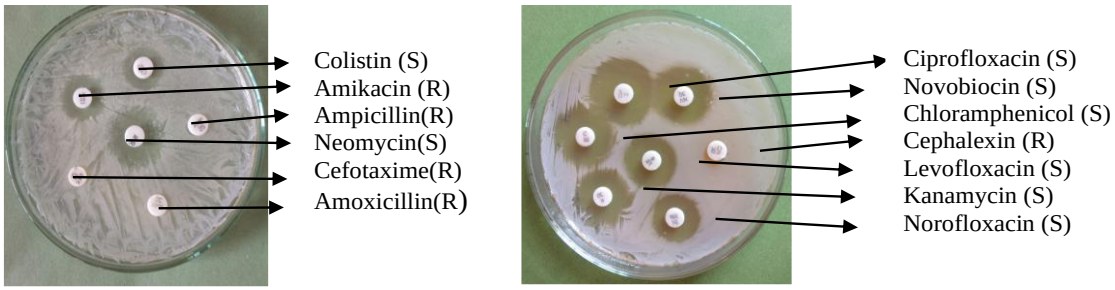

Figure 7. *Antibiotic sensitivity test for Staphylococcus spp.*

Graphical presentation of gram-negative bacteria such as *E. coli*, *Klebsiella* spp., and *Pseudomonas* spp and *Staphylococcus* spp. are shown in *Figure 8*.

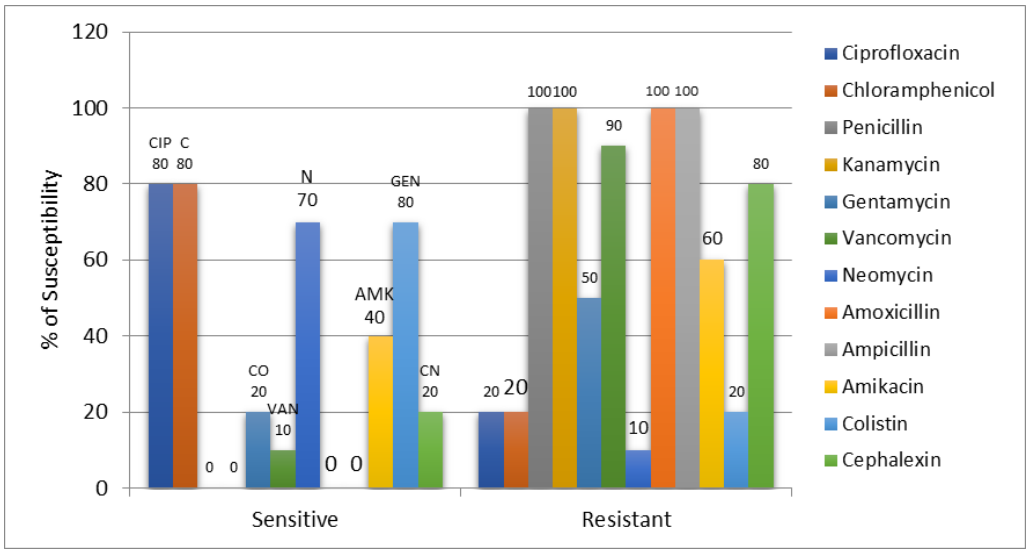

Figure 8. *Antibiotic susceptibility pattern of E. coli from urine sample of pregnant women*

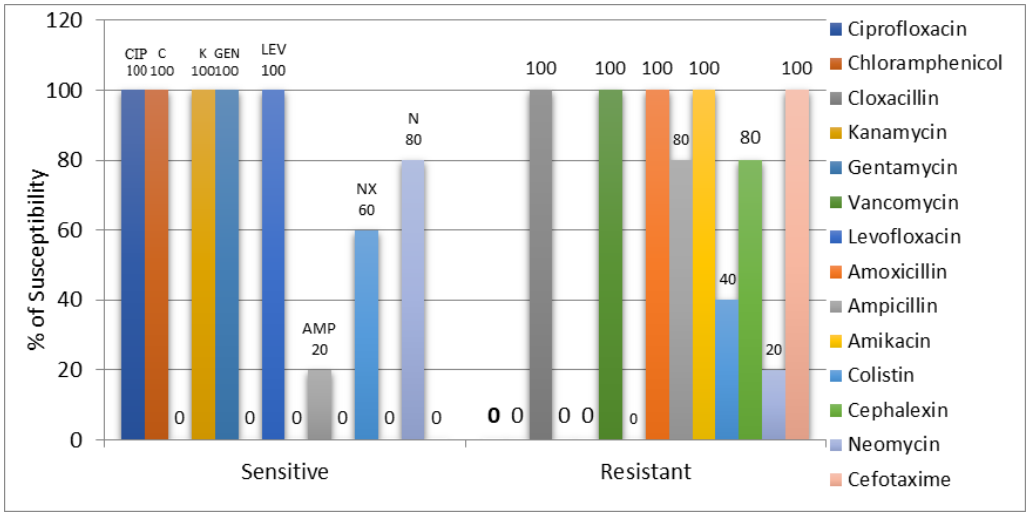

Figure 9. *Antibiotic susceptibility pattern of Klebsiella spp. From urine sample of pregnant women*

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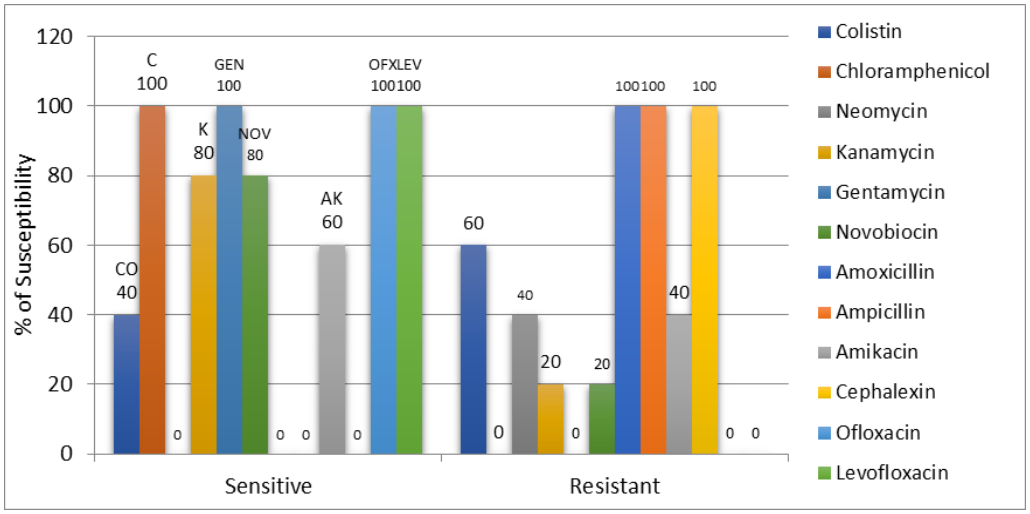

Figure 10. *Antibiotic susceptibility pattern of Staphylococcus spp. from urine samples of pregnant women*

Molecular identification of E. coli

To verify our findings by molecular base studies, the use of universal forward primer -27F (5'AGAGTTTGATCCTGGCTCAG3') and reserve primer-1492R (5' TACCTTGTTACGACTT3') was carried out to amplify the *16S rRNA* gene and found 1465 bp in PCR amplification. The resulting PCR product was sequenced following analysis by NCBI BLAST search. To determine the genetic diversity and evolutionary relationship between *E. coli* strain BD1 and those in other publicly available databases (n = 1,465), we conducted phylogenetic analyses using *16S rRNA* most comparable strains based on BLAST search similarity. The tree derived from *16S rRNA* from NCBI has traditionally been used to define prokaryotic taxonomy (*Fig. 11*). The *16S rRNA* tree revealed that *E. coli* strain BD1 seemed genetically more identical to *E. coli* isolates such as *E. coli* strain MLI108K2, *E. coli* strain B2207, and *E. coli* strain RHB45-C21 than those from the public database (*Fig. 2*). The *16S rRNA* sequence data has been submitted to GenBank/DDBJ/ENA under accession no. OQ984066 and strain was designated as *Escherichia coli* strain BD1.

Discussion

This research was to characterize the different types of bacteria from urine specimens of gestating women from different hospitals in the Dinajpur district.. *E. coli* in urine samples showed greyish, white, smooth, and opaque colonies in nutrient agar and metallic sheen blue to green colonies with light reflection in EMB agar and rose pink color colonies in MacConkey agar which are similar to the previous studies (Carter and Gordon, 1967; Buxton et al., 1977). The cultural characterization of *Klebsiella* spp. from urine samples of pregnant women revealed a large colony on Nutrient Agar, large, red, mucoid on Mac-Conkey's agar and convex, pink-purple, and translucent opaque on EMB agar medium, which was similar to Carter and Gordon (1967) and Buxton et al. (1977). *Pseudomonas* spp. were observed in different culture media, agreed to Buxton et al. (1977) *Proteus* spp. forms different colonies in different cultural media and close to Carter and Gordon (1967) and Buxton et al. (1977). Gram-positive *Staphylococcus*

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spp. in different culture media were very near Buxton et al. (1977). On Blood agar, *Streptococcus* spp. is a bêta hemolytic colony, a dark bluish color colony on Mitis Salivarius agar, and a beta-hemolytic colony on TSA. Out of 100 samples, 33 patients were UTI-positive, and 75 isolates were isolated. There were gram-negative isolates 60 (80%) and gram-positive isolates 15 (20%). 80% of gram-negative bacteria are responsible for UTIs. Angoti et al. (2016) agree with these findings.

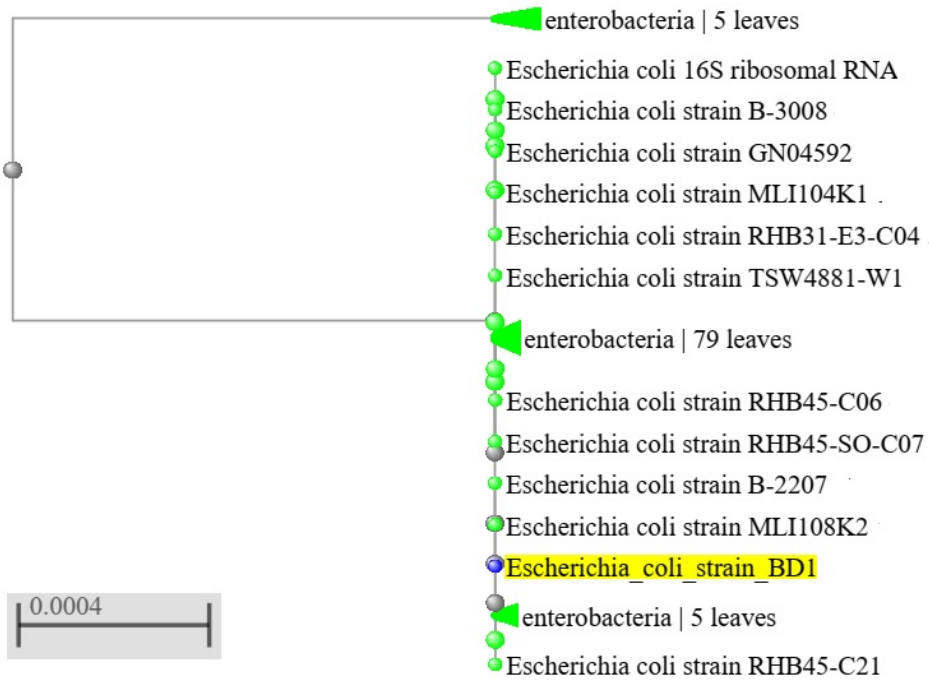

Figure 11. *Escherichia coli strain BD1 was analyzed by phylogenic tree*

In the present study, overall, 40% of *E. coli* was observed which is consistent to previous studies which have reported that *E. coli* is the most frequently identified causative agent in hospital-acquired UTIs (Gales et al., 2002) following *Klebsiella* spp. (21.33%), *Proteus* spp. (12%), *Pseudomonas* spp. (6.67%), similar to Ranjan et al. (2017) *Staphylococcus* spp. (16%) and *Streptococcus* spp. (4%). Out of 100 samples, 60 were from less than 25 years old pregnant women, and the highest number of positive cases such as 24(72.72%), were found. We found the lowest number of positive cases, 2(6.06%) from up to 30 ages, pregnant women. Ranjan et al. found that 60% of pregnant women UTI infected were those whose age was less than 25 years old. In the present study, 60.60% of pregnant women were highly infected and were similar to Ranjan et al. (2017). As for as education is concerned, 57.58% of pregnant women were at a primary education level and were highly infected with UTIs. Moreover, based on the current findings, the 3rd trimester has the highest rate of UTI (45.46%), followed by the 2nd trimester (30.30%), and the 1st trimester has the lowest rate of UTI (24.24%).

Our findings were not significant at ($P < 0.05$) where the P value was 0.13, in the case of the study area, and the result was significant at $P < 0.05$ in the case of an age difference, education level, and stage of pregnancy where P value was 0.047, 0.043 and 0.01 (*Table A2*) and this result were agree to Ranjan et al. (2017). The most frequent cause of UTI is *E. coli*, which has a high proportion of resistance to Penicillin,

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Amoxicillin, and Ampicillin (100%), whereas sensitive to Ciprofloxacin, Chloramphenicol, and Gentamycin (80%). The result of the antibiotic sensitivity test was nearly close to Shigemura et al. (2005). In this research, Levofloxacin, Ofloxacin, Chloramphenicol, and Gentamycin were more susceptible to gram-positive bacteria. In contrast, gram-negative bacteria were strongly resistant to most antibiotics, and the results are related to Shigemura et al. (2005). The resistance activity of microorganisms that causes UTI infections in pregnant women depends from place to place, and the findings are similar.

Using *16S rRNA* gene sequencing, *Escherichia coli* strain BD1 was identified, and greater than 99% sequence similarity was analyzed by NCBI blast search. 1465 bp PCR band was confirmed by using CLC drug discovery workbench 1.02 software. The 16s studies have not only verified our findings but have also endorsed that *E. coli* is a potential causative agent of UTI.

Conclusion

Our research strongly indicated that the relationship between UTIs and patients age, education level and stage of pregnancy and the study statistically significant. Therefore, we strongly suggested that pregnant mother should take of their health seriously.

UTI-positive patients need to screen for urine culture every month of pregnancy and treatment is taken by the physician. Early diagnosis and treatment can prevent UTIs and are also helpful for both mother and fetus. We strongly recommended antibiotics such as Ciprofloxacin, Ampicillin and Chloramphenicol to treat UTIs only when the patients in critical condition. Antibiotics should be used under close supervision and administered in sufficient doses for the appropriate amount of time to avoid or minimize antibiotic resistance. Our research will be helpful for pregnant mothers and doctors.

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Conflicts of interests. The authors have no conflict of interests.

Data availability statement. The 16S rRNA sequence data has been submitted to GenBank/DDBJ/ENA under accession no. OQ984066.1

Ethical approval. This study was approved by the ethical committee, Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh. Notably, an informed consent was obtained from all the subjects involved in this study.

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APPENDIX

Table A1. *Results of biochemical test*

Name of isolates	OX	CT	IN	MR	VP	SC	TSI		MIU		
							Slant	Butt	M	I	U
E. coli	-	+	+	+	-	-	A (yellow)	A (yellow)	+	+	+
Klebsiella spp.	-	+	+	-	+	+	K (red)	A (yellow)	+	-	+
Pseudomonas spp.	+	+	+	-	-	+	K (red)	K (red)	+	+	+
Proteus spp.	-	-	+	+	-	-	A (yellow)	A (yellow)	+	+	+
Staphylococcus spp.	-	+	+	+	+	+	A (yellow)	AG	-	-	-
Streptococcus spp.	-	-	-	-	+	+	A (yellow)	NC	+	-	+

+ = positive, - = negative, A = acid, K = alkaline, G = gas, NC = no color change vOX = oxidase, CT = catalase, IN = indole, MR = methyl-red, VP = voges-proskauer, SC = simmon's citrate, TSI = triple sugar iron, MIU = motility indole urease

Table A2. *Prevalence of UTI based on study area, age, education and stage of pregnancy*

Parameter		No. examined	No. positive	Prevalence (%)	P value
Study area	M Abdur Rahim Medical College	50	18	36	0.13
	Saint Vincent Hospital	40	12	30	
	Sheba Diagnostic Center	10	3	30	
Age	< 25 years	60	25	41.67	0.047
	25-30 years	35	6	17.14	
	> 30 years	5	2	40	
Education	Primary	50	22	44	0.043
	Secondary	25	7	28	
	Higher	25	4	16	
Stage of pregnancy	1st trimester	45	8	17.78	0.01
	2nd trimester	25	10	40	
	3rd trimester	30	15	50	

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