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"Analysis of Antibiotic Susceptibility Tests for Bacterial Strains Linked to Urinary Tract Infections in Pregnant Women"

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KEYWORDS

UTI infection, Pregnant women, E. coli, Klebsiella pneumonia, Antibiotic susceptibility pattern.

ABSTRACT:

Urinary tract infections (UTIs) pose a significant health concern for pregnant women, especially in underdeveloped regions, being a primary cause of pregnancy-related illnesses globally. Causative agents include E. coli, Klebsiella species, Staphylococcus aureus, Staphylococci, Proleusmirabilis, Enterococcus species, Pseudomonas aeruginosa, Enterobacter species, streptococci, and Citrobacter species. The prevalence of UTIs among pregnant women ranges approximately from 13% to 18%. Bacterial identification revealed both gram-positive and gram-negative forms, with gram-negative bacteria accounting for 85% and gram-positive for 15%. Notably, E. coli emerged as the most frequently found bacterium in this study.Antimicrobial susceptibility testing demonstrated high resistance among these isolates to nitrofurantoin, ciprofloxacin, and gentamicin, while displaying greater sensitivity to antibiotics like amoxiclav and cefuroxime. Additionally, pregnant women with asymptomatic UTIs, caused by Klebsiella pneumonia, E. coli, Proteus mirabilis, Acinetobacter baumanni, Pseudomonas aeruginosa, Staphylococcus arlettae, and Enterococcus faecalis, showcased resistance to multiple drugs.

Understanding the prevalence of these isolates in urine samples and their resistance profiles becomes crucial in guiding empirical treatments, aiming to minimize adverse effects on pregnant women. Ongoing research endeavors focus on combatting UTIs in pregnant women, particularly addressing drug resistance through novel technologies and identification methods to manage these infections effectively

INTRODUCTION

Urinary tract infection (UTI) is a condition in which bacteria develop and multiply within the urinary tract [1]. UTI is a common bacteriological infection that affects various areas of the urinary system and can affect both males and females. The infection which occurs in the bladder and urethra. The urinary system is divided into two parts: the upper and lower urinary tracts. The upper urinary system contains the kidneys and ureters. The lower gastrointestinal system includes the bladder and urethra. The bladder and urethra are part of the lower urinary system. Additionally, it is a condition that all women will experience at some point in their lives, with the prevalence being higher among women during pregnancy [2]. There are two types of urogenital infection in pregnant women: asymptomatic and symptomatic. Asymptomatic bacteriuria is described as the presence of substantial bacteria in the urine without the presence of signs and symptoms of a UTI.

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Infection with symptoms. Symptomatic infection is divided into upper tract infection (acute pyelonephritis) and lower tract infection (acute cystitis) [3]. The kidneys, ureters, bladder, urethra, and associated structures make up the urinary system, which collects and stores urine before releasing it from the body. A UTI is diagnosed when two consecutive midstream urine samples contain 105 germs or a single bacterial strain per ml. Urine is a sterile fluid that serves as an ideal environment for bacteria to flourish [4]. Infectious organisms move from the perineal area to the vagina in females, causing UTI to ascend in nature. Females are three times more prone than males to have UTI due to their shorter catheter, which emerges closer to the anus, the nature of sexual intercourse, pregnancy, simple intestinal flora infection of the urinary system, and rapid hormonal fluctuations. UTI is amongst the most common infectious diseases, affecting individuals of all ages and resulting in over 150 million cases each year, costing the global economy over $6 billion (about $18 per person in the US) in treatment expenditures. Infections of the urinary system are quite prevalent 2 in pregnant women and can be very dangerous. The potential of significant consequences makes this a therapeutic challenge to both the mother and her kid, and the stakes are quite high. Pregnant women are more prone to get a UTI, which affects one out of every five women, according to the World Health Organization. Pregnant women are four times more likely to develop diabetes. When compared to non-pregnant women, pregnant women have a higher rate of UTI [5]. The predominant UTI-causing organism identified is Escherichia coli. Other commonly found bacteria include Klebsiella pneumoniae, Citrobacter, Salmonella group A, and Enterobacter cloacae. Asymptomatic bacteriuria accounts for most UTI cases. Risk factors encompass low birth weight, preterm delivery, stillbirth, pre-eclampsia, maternal anemia, sepsis, and amnionitis. It's crucial to note that the virus may not elicit symptoms despite its presence. [6]. UTIs are primarily caused by commonly found bacteria such as E. coli, Klebsiella spp, and Enterococcus spp. Specifically, Escherichia coli, a rod-shaped bacterium from the Enterobacteriaceae family, stands out as the leading cause of UTIs. [7]. E. coli was the most prevalent bacterium found in pregnant women [8]. The rise in antibiotic resistance has impacted the effectiveness of practical treatments, underscoring the importance of conducting susceptibility tests. Among pregnant women, the most frequently isolated bacteria were E. coli. [9]. The increase in resistance of antibiotics in the progress rate of pragmatic treatments and hence it is essential to carry out susceptibility tests. UTI can either be symptomatic or asymptomatic. The bacteria E. coli is the most common cause of clinical and subclinical infections in the urinary system during gestation [10, 11]. Pregnant women who are infected with bacterial must be medicated, and the antimicrobials used in pregnancy therapy must be safe for both the foetus and the mother [12]. During pregnancy, drugs are used to treat uncomplicated and complicated urinary tract infections [13]. The targeted bacteria will become resistant to effective antibiotics. The purpose is to study the isolation and identify the bacteria which cause UTI and their development in antibiotic susceptibility pattern. Additionally, pregnant women`s are associated with physiological changes and because of their immune-compromised UTI host. These modifications raise the risk of infections, both symptomatic and asymptomatic. The application of UTI 3 chemotherapy increases drug resistance in microorganisms that cause UTI [14]. UTIs are caused by bacteria invading and growing in any region of the urinary system, including the renal pelvis, kidney, urethra, and bladder. It is one of the most well-known chronic disorders, with over 150 million cases reported each year worldwide. All males and females will become affected with UTI because of this; however, females are more vulnerable to UTI than men due to their small urethra and anus, that allows for easy contamination of the urinary system, triggering hormonal abnormalities in pregnancy. The most prevalent bacterial infection during pregnancy is UTI, which has been associated to premature births, hypertension, early delivery, and pregnancy problems. There are two types of urinary tract infections: During pregnancy, bacteriuria can be asymptomatic or symptomatic. Asymptomatic bacteria are bacteria that develop microbiologically significant in pregnant women's urine but do not induce UTI symptoms [15]. It affects 2-15 percent of pregnant females if left untreated, giving it a significant serious threat for pyelonephritis. Age and the presence of genitourinary disorders (dialysis, bladder stones, urethral, tumors, sexual activity, anemia, impaired immunity, and a history of UTI) are among the factors linked to an elevated likelihood of bacteriuria. For 60-80 percent of pregnant women, Escherichia coli is the most common pathogenic bacterium linked with both clinical and subclinical bacterial infection, which mostly causes UTI. According to (WHO) world health organisation surveillance data, low-income nations with high levels of bacterial illness are recognized with Escherichia coli and K. pneumonia as the most resistant pathogens. As a result, there is a growing concern in the state about the bacterial composition and antibiotic resistant characteristics of UTI amongst pregnant women [16]. Some of the symptoms of

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UTI include high frequency of urination and burning sensation with pain association when urine is discharged, and rigorous urinary infection that also cause nausea, fever, chills and vomiting. The infected pregnant women are treated with antibiotics with the special emphasis of antibiogram results [17] .

AIMS AND OBJECTIVES:

1. The goal was to find out how often urinary tract infections are in pregnant women and what antibiotics they are susceptible to.
2. Study the common organisms involved in UTI
3. And their molecular study

REVIEW OF LITERATURE

Urinary Tract Infection (UTI) can affect various parts of the urinary system, including the kidneys, urethra, ureters, and bladder. Typically, UTIs primarily involve the lower tract, affecting the urethra and bladder. Women tend to be more susceptible to UTIs due to their shorter urinary tract, which can result in discomfort and pain. In severe cases, if the infection progresses, it can extend to the kidneys. UTIs are commonly treated with antibiotics by healthcare providers. During pregnancy, UTIs are categorized into asymptomatic and symptomatic types . (Figure 1)

Asymptomatic UTI indicates significant bacteriuria, presenting as an acute urinary tract infection without noticeable symptoms.

Symptomatic UTI, comprising acute cystitis affecting the lower tract and acute pyelonephritis impacting the upper tract, involves inflammation in the renal parenchyma, pelvis, and calices. Cystitis is characterized by invasion of the bladder mucosa. [18].

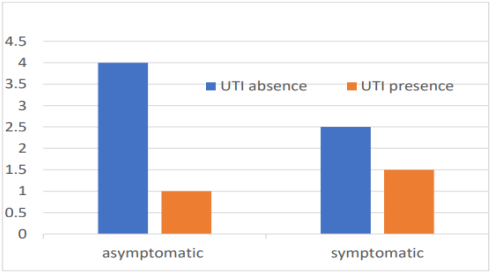

Fig. 1: Predominance of asymptomatic and symptomatic infection in UTI

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Globally, there are an estimated 150 million cases of UTIs annually, leading to over 6 billion dollars in direct healthcare costs. Among pregnant women, UTIs are the most prevalent medical concern, with an estimated 40% experiencing this infection, also known as bladder infection or cystitis, at some stage during their pregnancy. These infections typically occur when bacteria enter the bladder through the urethra and multiply. The urinary tract houses various microorganisms that can lead to bacterial infections (UTIs), a primary cause of premature births. While approximately 40-50% of women worldwide are affected by this condition, only 5% of men experience UTIs. Common bacterial culprits responsible for these infections include Escherichia coli, Klebsiella pneumoniae, Proteus, Saprophyticus, Streptococcus Group B, Staphylococcus, and Pseudomonas aeruginosa. Among these, the most frequently encountered bacteria are E. coli and Klebsiella pneumoniae. (Fig. 2)

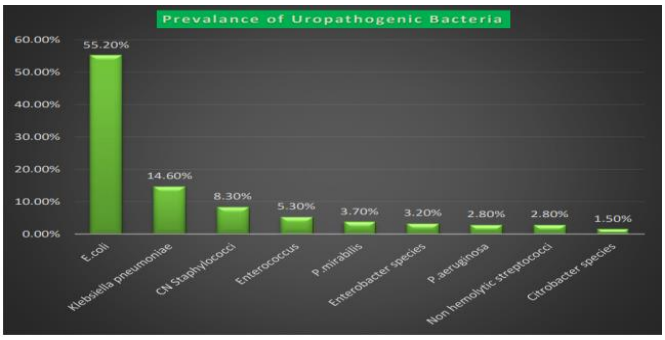

Fig. 2: Shows that prevalence of uropathogenic bacteria

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Epidemiology:

Urinary tract infections (UTIs) are incredibly common, with over six million affected individuals seeking medical attention annually. The prevalence of complex urine infections has risen due to the frequent occurrence of UTI-related abnormalities. Among the elderly population, UTIs stand out as the most common bacteremia infection. Several risk factors contribute to urinary tract infections, including sexual intercourse, a history of previous UTIs, and hygiene practices. Research indicates a notable association between the use of spermicide and changes in vaginal flora, potentially leading to increased colonization of uropathogens in the urethra and the urinary tract. Recurrence of UTIs is quite common after the initial infection. Among pregnant women experiencing these recurring infections, Escherichia coli (E. coli) is the most prevalent bacterium, accounting for up to 55% of cases in comprehensive studies [19]. In Karnataka, UTI cases among the population range from approximately 5-10%. In pregnant women, prior UTI history and increased risk factors during pregnancy, such as low socioeconomic status, sickle cell trait, anemia, and inadequate prenatal care, contribute significantly to UTI occurrences. Studies have indicated that acute pyelonephritis and acute cystitis affect about 1-2% of pregnant women, showcasing a microbial spectrum akin to asymptomatic infections. Reports from Mysore suggest that 2 to 10% of pregnant women experience asymptomatic urine abnormalities.

Its scientific name is Escherichia coli.

Domain: Bacteria

Order: Enterobacterales

Higher classification: Escherichia Family: Enterobacteriaceae Phylum: Pseudomonadota

Rank: Species

It's interesting how certain bacteria play a significant role in causing UTIs during pregnancy. Research has shown that during the sixth week of pregnancy, UTIs can peak around 22 to 24 weeks (approximately 2 months) of gestational age. Escherichia coli belongs to the family Enterobacteriaceae and is one of the commonly encountered bacteria during UTIs in pregnancy.

Another bacterium often observed in UTIs among pregnant women is Klebsiella pneumoniae. This bacterium is Gram-negative, has lactose-fermenting capabilities, is encapsulated, and appears as a non-motile rod-shaped organism. It typically presents as a mucoid lactose fermenter when cultured on MacConkey agar. Its scientific classification places it in the species Klebsiella pneumoniae within the family Enterobacteriaceae, belonging to the order Enterobacterales in the phylum Pseudomonadota. These bacteria are frequently identified in cases of urinary tract infections during pregnancy.

Pathogenesis:

Absolutely, UTIs during pregnancy, especially when they progress to pyelonephritis (a kidney infection), pose significant risks to both the mother and the fetus. Pyelonephritis can increase morbidity and pose dangers to both the expectant mother and the developing baby. To manage this condition effectively, studying the virulence factors and conducting cultures of the uropathogenic bacteria are strongly recommended. In cases where antibiotic treatment fails, it's often due to concurrent lower genital tract infections associated with pyelonephritis.

Regular screening and urine cultures for every pregnant woman are highly advised. This proactive approach allows for timely detection and appropriate treatment with antibiotics, ensuring the management of UTIs during pregnancy [20]. Asymptomatic bacteriuria, if left untreated, can progress into more severe conditions like pyelonephritis or cystitis. Preterm birth remains a significant contributor to neonatal mortality each year. Approximately 15 million newborn babies are born prematurely annually, with complications from prematurity affecting around 11 million births worldwide.

Preterm infants often face increased risk factors that can impact their long-term health and development. These risks include challenges in school learning, behavioral issues, chronic lung disease, hindered growth, and hearing impairment.

There are interventions available that can effectively help prevent preterm birth and its associated complications. These interventions play a crucial role in safeguarding both the health of the mother and the well-being of the newborn [21]. Preventing premature births is a crucial goal, especially in low-income countries, where maternal infection treatments play a significant role. Around 40% of women worldwide suffer from urogenital tract infections, and remarkably, 60-80% of infected pregnant women show no symptoms.

In healthy women, many uropathogens originate in the intestinal flora and travel to the bladder via the urethra. There, they settle temporarily around the urethra and the base of the urethral opening. Symptomatic UTIs occur when these uropathogens in the bladder or kidney trigger the release of cytokines, resulting in an inflammatory response and noticeable symptoms.

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UTIs are particularly prevalent in women and can lead to significant morbidity due to their frequent occurrence. The pathogenesis of UTIs is intricate and influenced by various factors, including biological and behavioral aspects of the host, as well as characteristics specific to the infecting pathogen. Understanding these complexities is vital in managing and preventing the impact of UTIs, especially during pregnancy.

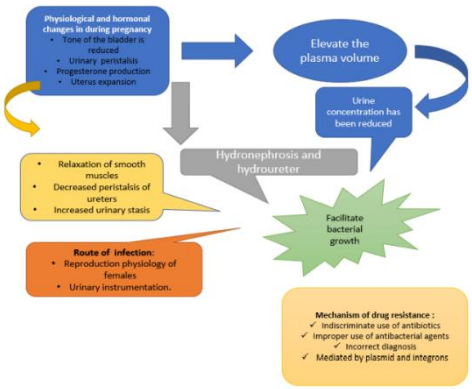

Figure 3: Pathogenesis and risk factors of UTI among pregnant women.

Risk Factors:

Urinary tract infections (UTIs) are prevalent clinical complications that can affect various parts of the urinary system, including the urethra, kidneys, and bladder. In pregnant women, UTIs rank as the second most common issue following anemia. If left untreated, UTIs can significantly impact the health of the expectant mother [22]. Here are some key complications associated with UTIs in pregnant women:

Urinary tract infections (UTIs) pose a significant concern across various age groups, with pregnancy rendering the urinary tract notably susceptible to contamination from intestinal flora. In females, the absence of prostatic secretions heightens this vulnerability [10]. During pregnancy, physiological changes, such as increased plasma volume and reduced urine concentration in about 80% of women, lead to glucosuria, fostering bacterial growth in urine. This period makes women more prone to urogenital symptoms, including intensified bladder indications that start early in pregnancy and escalate with gestational age. Lower urinary tract symptoms prevail globally in antenatal care, reflecting alterations in the urethra, urinary bladder, urethritis, or cystitis. Post-delivery improvement in gastrointestinal excretion in pregnant women is attributed to detrusor instability. While asymptomatic UTIs in pregnancy are easily treatable, symptomatic bacteriuria raises the risk of low birth weight and preterm delivery. Untreated asymptomatic bacteriuria can progress to cystitis and, if left unchecked, to pyelonephritis, potentially leading to anemia. Screening for UTIs during pregnancy is pivotal for timely intervention and management. Socioeconomic factors like low income, household wealth, maternal undernutrition, and the absence of obstetric care correlate with a higher UTI risk. Educating women, especially those with higher education, about UTI infections and encouraging spousal involvement in preventive measures is crucial [23- 24].

Modes of Transmission: Urinary Tract Infection (UTI) occurs when pathogenic microorganisms invade and multiply in the urinary tract, caused by bacteria, fungi, or viruses. Prolonged hospital stays, financial constraints, and a lack of awareness among the population contribute to its prevalence. The majority of UTIs stem from bacteria typically residing in the normal flora of the bowel and perineal areas, transmitted through fecal matter.

These infections can affect both the lower and upper urinary tracts, with pathogens having a propensity to invade damaged tissues. Severe morbidity from UTIs, particularly in public health settings, can lead to renal function loss and prolonged illness. UTI development involves the multiplication and colonization of uropathogens in epithelial cells, sometimes leading to tissue invasion.

UTIs are common occurrences, particularly among pregnant women, posing risks despite the availability of potent medications. Bacterial resistance persists, varying in

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causative agents and their antibiotic resistance patterns worldwide, potentially leading to life-threatening complications and even mortality.

Culturing urine samples remains the most effective method for diagnosing and treating UTIs. Symptomatic bacteriuria in infected patients often presents clinically as pyelonephritis or cystitis, primarily caused by bacteria commonly found in UTIs. Symptoms may include a burning sensation in the bladder, nausea, fever, dysuria, flank pain, increased urination, and urine with a foul odor and milky appearance. [25].

Microbiology: Urinary Tract Infection (UTI) is a prevalent, uncomplicated bacterial infection commonly caused by uropathogenic strains, notably Escherichia coli (E. coli) as the primary culprit. Other organisms like Klebsiella, Enterobacteriaceae, Proteus, and Enterobacter species are also frequently encountered. Occasionally, gram-positive bacteria such as Enterococcus and S. saprophyticus can be involved.UTIs during pregnancy occur in nearly 10% of cases, with higher frequencies observed in the summer and autumn seasons. Enterococcus-induced UTI poses complications, especially when patients have urinary catheters or have been prescribed broad-spectrum antibiotics for other bacterial infections. Staphylococcus aureus is the primary organism causing UTIs through secondary sources or bloodstream infections in primary urine infections. Antibiotic-resistant or unusual bacteria often lead to complicated UTIs. Various organisms, including mycobacteria, yeast, and viruses, have been isolated from the urinary tract, indicating the diverse range of pathogens that can contribute to UTIs. [26]. In UTI causing patients, it was recently reported that when their structures differ, it has Actinomyces bernardiae, Nacardia astroides, Mycobacterium terrae and urethralis causing agents. Infrequently, the other enteral pathogen is nontyphoidal Salmonella which cause urinary tract infection among pregnant women. Enterococcus faecalis strain which causes UTI is reported that vancomycin drug will be the antibiotic resistant bacteria for complicated UTI infection [27].

Pathophysiology: UTIs are indeed complex interactions between bacteria and the host's defense mechanisms. The virulence of these infections is greatly influenced by the bacteria and their specific traits. While a lot of research has focused on understanding the virulent factors in Escherichia coli (E. coli), similar theories and investigations are also connected to other gram-negative bacteria like Klebsiella. Studying these virulence factors helps in comprehending how these bacteria interact with the host's body and cause infections, aiding in the development of targeted treatment strategies. [28]. Certainly, most E. coli strains belong to specific serotypes and possess organelles called fimbriae. In certain cases of asymptomatic infection, bacterial strains containing P fimbriae are frequently associated with acute pyelonephritis. These P fimbriae play a crucial role in mediating the adherence of E. coli to uroepithelial cells, contributing to the progression and severity of acute pyelonephritis. [29]. Those children who carry the intestinal strain of E. coli with P fimbriae cause a high risk of emerging UTI [30]. Absolutely, various factors contribute to the development of urinary tract infections (UTIs). In healthy young women, behavioral changes play a significant role in UTI development. Activities like sexual intercourse, the use of spermicides, and specific voiding practices can lead to urethral colonization by bacteria, paving the way for UTI occurrence. These behaviors might facilitate the transfer of bacteria into the urinary tract, increasing the likelihood of infection. [31]. UTIs tend to be more challenging for elderly individuals compared to younger women. Around 3-10% of the global population relies on urethral catheterization, which poses an increased risk of UTIs due to the presence of a foreign body and potential bacterial colonization.

In the elderly, various factors contribute to complex UTIs. Medical conditions, such as prostatic hypertrophy, nosocomial infections acquired in healthcare settings, and neurogenic bladder (often associated with conditions like spinal cord injury or neurological disorders), can all increase the complexity and severity of UTIs in this population. These factors make managing UTIs for the elderly more intricate and demanding. [32].

MATERIALS AND METHODS 3.1 Sample collection Mysuru is located at 12.30°N 74.65° E and has an average attitude of 770 meters (2,526 ft.) it is spread over an area 128.42 km sq. (50 sq. MT) at the base of southern part of Karnataka, Kuvempunagar is a residential layout in Mysuru, city in southern India. 3.1.1 Sample area: The study was conducted in the Kamakshi hospital, Kuvempunagar in a residential layout in Mysuru. Over a duration of December 2021 to March 2022. During this period 25 UTI infected pregnant women were considered. 3.1.2. Study population: This study includes 302 UTI pregnant patients, women are aged between 18 to 35 were selected for the study, which includes urine routine tests in the Kamakshi hospital, Mysuru. 3.1.3 Sample collection: Among 302 UTI infected pregnant women, the sample was

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from mid -stream urine was collected in sterile container for routine test and culturing. 3.1.4 Sample processing: All the samples were tested by some following methods: 1. Urine routine 2. Sample plating 3. Gram`s staining 4. Microscopy 5. Biochemical tests 6. Antibiotic susceptibility test 7. Minimum Inhibitory concentration 14 3.2.0. Media used: 1. MacConkey agar 2. Blood agar 3. Nutrient agar MacConkey agar: Is connected to gram-negative rods and used for the selection of Enterobacteriaceae. Gram-positive bacteria and several fastidious gram-negative bacteria are inhibited by the bile salts and crystal violet in this medium. Lactose-fermenting bacteria form colonies that are different shades of red or pink because lactose is the only carbohydrate, whereas non-lactose fermenters produce colourless colonies. Blood agar: This is a highly useful enriched media for isolating and studying the haemolytic characteristics of most pathogenic bacteria. It's made by mixing 5-10% citrate human, rabbit, or sheep blood with 1.3 percent nutritional agar, then solidifying the mixture into slopes and pouring it onto Petri plates. Nutrient agar: It's a clear pale-yellow medium. It's one of the most often used solid basal media in laboratories. The addition of 1.3 percent agar solidifies the nutritional soup. It can be poured into test tubes as slopes, stands, or deep wells, as well as Petri plates. It is utilised as a substrate for antibiotic sensitivity testing and for colony morphology studies of nutritionally non-demanding organisms. 3.2.1. Preparation and maintenance of bacterial isolates: Around 25 Isolates labelled with S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18, S19, S20, S21, S22, S23, S24 and S25 were isolated and sub cultured in 2ml Luria-Bertani (LB) broth and incubated at 37degree C for24hours and used for further studies. 3.2.2. Phenotypic characterization: 1. Morphology of colony 2. Cell and its arrangement 3. Gram's staining and biochemical test was done for its phenotypic characterization based on their Shape, size, colour and consistency of the colony was also noted. 15 3.2.3. **GRAM`S STAINING:** This test is used to differentiate Gram positive and Gram-negative bacteria by observing morphology under the microscope by observing the gram`s reaction and cells shape (rod, cocci or coccobacilli). Procedure: 1. Took a clean, grease-free glass slide. 2. Prepared a smear of suspension with a loop full of sample on a fresh glass slide. 3. Allowed to air dry before applying heat to the suspension on the slide. 4. Pour the crystal violet and let for 30 seconds to 1 minute before rinsing with water. 5. Fill the gram's Iodine with water and let it sit for 1 minute. 6. Rinse the slides with water after washing them with 95 percent for 10-20 seconds. 7. After about 1 minute, added Safranin and washed with water. 8. Allowed the slide to air dry before examining it under a microscope. Interpretation: 1. Gram positive -purple colour 2. Gram negative-pink colour 3.3.1. BIOCHEMICAL TESTS: The tests are conducted to distinguish distinct bacterial species based on differences in biochemical activity. 3.3.2. Catalase test: Hydrogen peroxide is converted to water and oxygen gas by the enzyme catalase. The test is used to determine whether bacteria have the enzyme catalase or not, and it aids in the differentiation of morphologically identical bacteria. Procedure: 1. Spread an inoculum of culture on a clean microscope slide. 2. Place a 3 percent H2O2 drop on a slide. 3. Keep an eye on the development of the oxygen bubbles. Interpretation: 1. Catalase positive: Bubbles observed 2. Catalase negative: No bubble formation 16 3.3.3. Triple Sugar Iron test: It is a microbiology test named for its ability to test a microorganism which ferments sugars and to produce hydrogen sulphide. Procedure: 1. Took a bunch of colonies that are well-separated. 2. On a TSI agar slant tube, a loop was poked from the medium's centre to the tube's bottom. 3. Streaking was done on the slant's surface. 4. Cotton was inserted into the tube tip and incubated for 20 to 24 hours at 35-37 degrees. 5. Keep an eye on the colour changes in the tube. 3.3.4. Citrate test: This test identifies bacteria that use sodium citrate as their sole supply of carbon and inorganic ammonium hydrogen phosphate as their sole source of nitrogen, as well as determining their fermenting capacity. Procedure: 1. The isolate was used to inoculate a needle. 2. Stabbed a needle into citrate agar on a slope from the top to the bottom of the medium. 3. On an agar slant, the isolate was streaked. 4. The tip of the tube was plugged with cotton and incubated for 20-24 hours at 35-37 degrees. 5. Slant was detected after the incubation hours. Interpretation: 1. Citrate positive: Colour change from green to intense blue along the slant. 2. Citrate negative: No growth, no colour change and slant remain same. 3.3.4. Indole test: Test helps to evaluate organism`s ability to digest amino acid tryptophan and create Indole. Procedure: 17 1. In a sterile tube, 4ml tryptophan broth was taken. 2. The isolate was introduced to the tube aseptically and cultured for 24 hours at 35- 37 degrees. 3. The soup was spiked with 0.5 mL Kovac's reagent, and the tube was inspected for the presence or absence of a ring. **Interpretation**: 1. Indole test positive: Formation of red colour ring 2. Negative result: Absence of ring 3.3.5. Urease test: The urease test detects organisms that can hydrolyse urea and produce ammonia and carbon dioxide. Procedure: 1. A urea agar slant was infected with 1 to 2 drops of bacteria from an overnight brain-heart infusion broth culture. 2. Incubated the tube at 35°-37°C for 48 hours. 3. For colour alteration, a slant was noted.

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Interpretation: 1. Positive result: colour changes to pink 2. Negative result: no colour change 3.3.6. Coagulase test: In the presence of the enzyme coagulase, the plasma clots, converting fibrinogen to fibrin. The test can be performed using either a slide or a tube. Procedure: 1. In sterile test tubes, 0.5ml of plasma was extracted. 2. A loopful of cultivated colony was collected and put into the tubes after 24 hours. 3. The tubes were incubated at 37 degrees for 24 hours. Interpretation: 1. Coagulase positive: Clots can be observed 2. Coagulase negative: no clot 18 3.4.0. ANTIBIOTIC SUSCEPTIBILITY TESTING (AST)ASSAY: AST is a laboratory test that identifies which medicine, particularly an antibiotic, will be most effective in treating a bacterial infection in vivo. On MH medium, the test was conducted using the Kirby-Bauer disc diffusion technique. Current clinical and Laboratory Standard Institute (CLSI) criteria were used to identify the outcomes. Principle: Antibiotic susceptibility tests determine whether or not an antibiotic or another antimicrobial agent can decrease bacterial growth in vitro. This ability may be estimated using either the dilution technique or the diffusion method. Antibiotic-laden tiny wafers were put on a plate containing microorganisms. CLSI guidelines were used to evaluate the results. The findings of this test aid in the selection of the most effective antimicrobial drugs for treating pathogenic organisms. Procedure: 1. The isolate's pure culture plate was chosen. 2. A sterile swab was used to take the colony aseptically. 3. For a lawn of growth, a swab was streaked on a sterile MH medium plate. 4. Using sterile forceps, the vancomycin medicine was put on the surface together with additional antibiotic discs such as Ampicillin, Penicillin, Nitrofurantoin, and Linezoid (gently press it) 5. For 24 hours, the plate was incubated at 37 degrees. 6. The inhibition zone was measured and compared to the diameter zone, with the results reported according to CLSI rules. Interpretation: Zone was observed and compared with standard size of the zone then it can be considered into sensitive, intermediate and resistant to antibiotics. 3.5.0. MINIMUM INHIBITORY **CONCENTRATION (MIC) ASSAY**: Throughout an overnight incubation period, the test is characterized by a low dose of antibiotics that will suppress the development of bacteria. The MIC test is a quantitative approach for determining the most effective antibiotic class. It aids in the selection of appropriate antibiotics for treating illnesses and aids in the battle against antibiotic resistance. 19 The MIC Assay was carried out using the disc diffusion technique and various concentrations of drugs: Procedure: 1. Using sterile water, a sterile selected antibiotics was diluted into 10 percent, 20 percent, 50 percent, 60 percent, 80 percent, and 100 percent, and each concentration was studied. 2. A sample of bacteria was isolated in LB broth and streaked on an MH plate with a sterile cotton swab for a lawn of growth. 3. Using sterile forceps, autoclaved discs were dipped in various concentrations of drugs and put on a plate. 4. For 24 hours, the plate was incubated at 37 degrees. 5. There was a zone around the discs that was seen. Interpretation: Zone around the different concentration discs was compared with standard guidelines. 3.5. MOLECULAR CHARACTERIZATION: 1. Isolation of DNA 2. DNA purity check 3. Polymer chain reaction 4. Gel electrophoresis 5. 16s RNA sequencing and 6. Resistance gene identification 3.5.1. Isolation of DNA: Procedure: 1. 1ml of overnight culture was placed in Eppendorf tubes and centrifuged for 20 minutes at 5k rpm at 4°C. 2. The pellet was mixed with 270 l 1xTE buffer after the supernatant was discarded. 3. Tubes are centrifuged for 20 minutes at 5k rpm and 4°C. 4. The second and third steps were performed twice more. 5. 270 micro litres of 1X TE buffer + 20 microlitres of lysosome were added to the pellet, and tubes were incubated at 37°C for 1 hour. 20 6. 10 µl of 20 percent SDS + 10 µl Proteinase K were added to the tubes and incubated for 30 minutes at 55°C. 7. 250 µl of phenol + 250 µl of chloroform + 10µ l of isoamyl alcohol were added in a 25:24:1 ratio, and the pellet was dissolved using a vertex. 8. Upper layer was removed to fresh Eppendorf and DNA was precipitated with 10µl of CH3COCNa and 270µl of cold 100% ethanol after centrifugation at 5k rpm, 4°C for 30 minutes. 9. The tubes were maintained at -4°C in the freezer overnight. 10. The tubes were centrifuged at 5k rpm for 1 hour at 4°C, and then the pellet was rinsed with 50µl of cold 70% ethanol. 11. Tubes were centrifuged at 5k rpm for 30 minutes at 4°C. 12. The sediment was air dried for 5-10 minutes at 30-37°C before being suspended in 50µl of 1X TE buffer. 3.5.2 DNA purity check: In nanodrop, the absorbance ratios for accessing DNA purity were 260/280 and 260/230. In most molecular procedures, such as PCR and Gel electrophoresis, depend on on this technology. As a result, testing in nanodrop provided an accurate determination of DNA purity. In general, a ratio of 1.8 indicates pure DNA, whereas a ratio of 1.6 indicates DNA polluted with proteins, phenols, and other contaminants. Procedure: 1. A DNA sample was obtained and dissolved in 1XTE buffer. 2. Nanodrop 2000/2000 c was used to test DNA purity. 3. A nano drop machine was used to deposit 0.1l of DNA sample. 4. Absorbance was measured at 260/230 and 260/280. Interpretation: The absorbance of pure DNA is 1.8 or near to 1.8. It's likely that only 1.6 percent of the DNA has been contaminated.

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Polymer Chain reaction (PCR): Requirements: 1. Buffer (10X) to working 1X buffer 2. dNTPs :0.5mM 3. Mgcl2 :1.5mM 4. Taq DNA Polymerase :0.3U 5. Template 6. Autoclaved water Reaction mixtures:

Table 1: PCR mixtures

Table 1: PCR mixtures

PCR reaction mixture	15µl
1X PCR buffer	2µl
1.5mM $Mgcl_2$	2µl
0.5mM dNTP's	0.5µl
Taq DNA polymerase	0.4µl
10µM forward primer	0.5µl
10µM reverse primer	0.5µl
Template DNA	5µl
Nuclease free water	4.1µl

PCR programme:
Lead temperature:

Table 2: PCR programme

Temperature	Time	Process
95	10 minutes	
94	30 seconds	Denaturation
55	1minutes	Annealing
72	10minutes	Extension
4	5minutes	Final extension
35 CYCLES		

GEL ELECTROPHORESIS: Materials required for the gel electrophoresis were 1. TAE buffer 2. Ethidium bromide 3. Agarose 4. DNA loading dye (bromophenol blue, xylene cyanol, glycerol), 5. Distilled water 6. Electrophoresis apparatus 7. Micropipettes 8. Microtips 9. Conical flasks 10. Oven 11. Stirrer 12. UV transilluminator Preparations of Gel: 1. Ethidium Bromide (EtBr) ❖ Ethidium Bromide -10mg ❖ Distilled Water -1ml 2. Agarose gel (0.8%) ❖ Agarose - 0.8g ❖ 1X TAE - 100ml ❖ EtBr - 3µl 3. DNA loading dye: The dye should be prepared in distilled water and stored at 4°C. ❖ Bromophenol Blue - 0.25% ❖ Xylene cyanole - 0.25% ❖ Glycerol - 30% Preparation of the Gel: 1. Ethidium bromide was used to create 0.8 percent agarose gel. 2. When heated in 100mL TAE buffer, the agarose dissolves entirely and turns transparent. 3. Using hand gloves, take the conical flask from the oven and wait for it to cool down enough to handle. 4. The ethanol was used to clean the gel casting tray and the comb, and the tray was set up to pour the gel. 23 5. Allowed 10 to 15 minutes for the gel to harden in the tray after it was poured. Preparation of DNA Samples: In an Eppendorf tube, a DNA sample was combined with 50µl of TE buffer. Loading Of DNA Samples: 1. A tiny pipette was used to combine 3µl of DNA sample and 3µl of DNA loading dye. 2. Tank was filled with TAE buffer and agarose gel was placed in the electrophoresis tank wells were placed towards the cathode 3. Samples were loaded in the gel carefully using suitable micro pipettes 4. Samples were run until it reached half area of the gel 5. Gel was removed from electrophoresis tank and observed under UV transilluminator Observations and Results: The concentration of Agarose used to separate DNA fragments was 0.8 percent. It was carried out in order to assess the quality of DNA. Because of the particular binding of EtBr(1l) to the DNA molecule, the gel under UV light exhibits a band. 3.5.5 16sRNA SEQUENCING: Amplification of 16sRNA is the best molecular method for identifying bacterial isolates at the species level. In a sequencing bacterium, the 16sRNA gene is a highly conserved unique sequence that encodes for 16sRNA. This tool assists in identifying bacteria from closely related species. As a result, the identification of bacterial isolates in this investigation was done using 16sRNA amplification. In addition, 16sRNA gene sequencing was used to identify the genus of the selected E. coli isolate. Below is the protocol for 16s rDNA amplification: 1. As a template, the entire DNA isolated from each isolate was employed 2. 0.3U Taq DNA polymerase, 1X PCR buffer, 0.5Mm dNTPs, 1.5Mm MgCl2, and 0.4mmM Universal Primers MAGSPIN- 21 were used in each reaction mixture 24 (27F AGAGTTTGATCCTGGCTCAG 1492R CGGTTACCTTGTTACGACTT). 3. The PCR amplified products were visualised using a trans-illuminator on a 2% agarose gel in TAE buffer at 75V. 3.5.6 Resistant gene

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identification: After the identification of the 16sRNA the genomic DNA was further subjected for the identification of the specific resistant gene from E. coli isolates. The gene selected for resistant gene identification was CTX-M-1. The specific primers of CTX-M-1 sequence was blaCTX-M-1F (5-AACCGTCACGCTGTTGTTAG-3) and blaCTX-M-1R (5-TTGAGGCTGGGT GAAGTAAG-3).

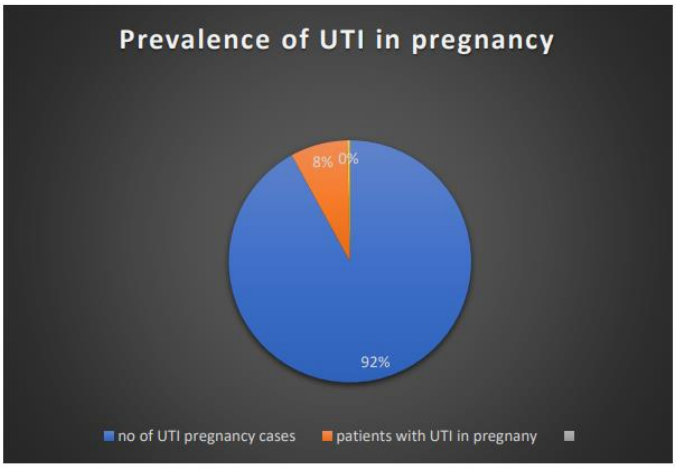
Instrument Used: Biorad- thermocycle

PCR mix (25µl): 1. 10X PCR buffer : 2 µl 2. MgCl2 : 1.5 µl 3. dNTP's : 5 µl 4. Primer F : 0.32 µl 5. Primers R : 0.32 µl 6. Taq polymerase : 0.3 µl 7. DNA template : 4 µl Instrument Programme: ❖ Initial denaturation 95˚C for 5 min, ❖ followed with 35 cycles of 94˚C for 1 min, 57˚C for 1 min and 72˚C for 1 min and final extension at 72˚C for 5 min. Observation and results: The bands were observed on the gel electrophoresis and base pair range was noted through the ladder 1Kb.

RESULTS AND DISCUSSION

Incidence: Between December 2021 to March 2022, 302 infected pregnant women's urine samples were obtained, out of that 25 samples were sent to urine culture which was studied. Distribution of patients according to incidence of UTI in pregnancy.

Total number of pregnancy cases	Patients with UTI in pregnancy	Percentage
302	30	30%



Phenotypical characterization: The samples were streaked on MacConkey agar plate and incubated at 37 °C for 24 hours and growth of bacterial colony was observed and helps to characterize the isolates colony characteristics labelled with S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18, S19, S20, S21, S22, S23, S24, 25

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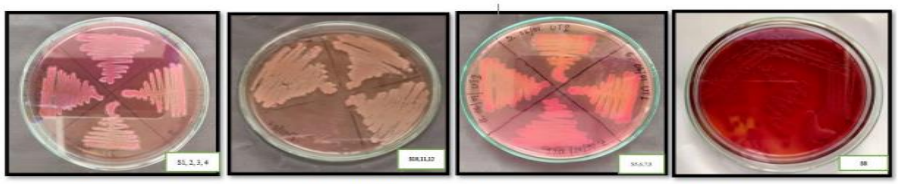

Figure 4: sample 1, 2, 3, 4, 5, 6, 7, 8, 10, 11 and 12 shows cultures on MacConkey agar media.

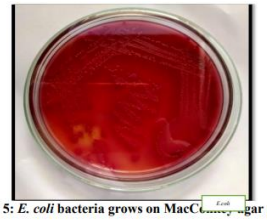

Figure 5: *E. coli* bacteria grows on MacConkey agar media.

Figure 6: Klebsiella pneumonia bacteria grows on MacConkey agar

MICROSCOPIC OBSERVATION:

E. coli Klebsiella 28 Under a light microscope, the isolates are discovered to be Gram's negative bacteria, which looked pale reddish in colour. As their cell membranes are unable to maintain the crystal violet dye, the safranin counterstain is used to colour them. Bacteria with rod-like shapes were discovered.

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Table 5: Microscopic observation of isolates.

isolates	Gram's reaction	Shape of the bacteria
S1	Negative	Rod shape
S2	Negative	Rod shape
S3	Negative	Rod shape
S4	Negative	Rod shape
S5	Negative	Rod shape
S6	Negative	Rod shape
S7	Negative	Rod shape
S8	Negative	Rod shape
S9	Negative	Rod shape
S10	Negative	Rod shape
S11	Negative	Rod shape
S12	Negative	Rod shape
S13	Negative	Rod shape
S14	Negative	Rod shape
S15	Negative	Rod shape
S16	Negative	Rod shape
S17	Negative	Rod shape
S18	Negative	Rod shape
S19	Negative	Rod shape
S20	Negative	Rod shape
S21	Negative	Rod shape
S22	Negative	Rod shape
S23	Negative	Rod shape
S24	Negative	Rod shape
S25	Negative	Rod shape

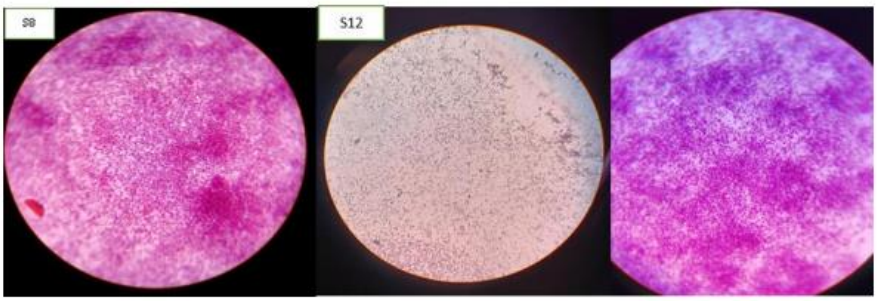

Figure 7: microscopic observation of isolates under 40X magnification.

BIOCHEMICAL TESTS:

The biochemical test for 25 isolates was done and their results were mentioned below.

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Fig 8: TSI before

Fig 9: TSI result,

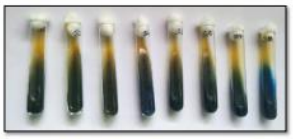

Figure 10: showing citrate negative and positive results

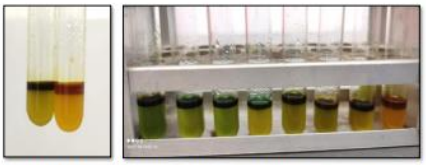

Figure 11: Indole results, green ring indicates the *klebsiella pneumonia* and red ring shows *e. coli* positive results

Figure 12: showing catalase positive results for both organisms

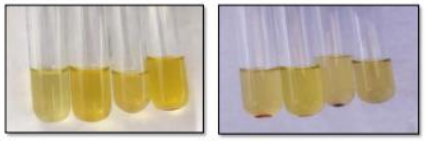

Fig13: Coagulase test before

Fig14: Coagulase test after

30

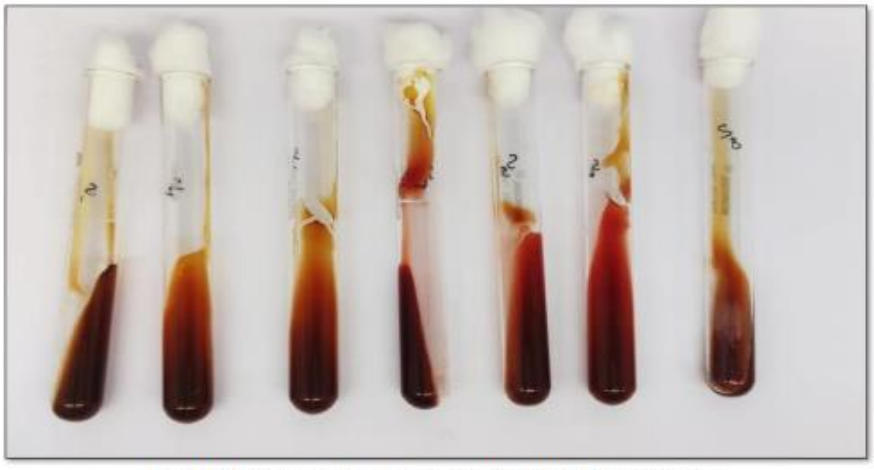

Fig 15: Urease negative results for both organisms.

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Table 7: Standard zone interpretation for nitrofurantoin, gentamicin and cefuroxime.

Antibiotics	Interpretation for	Resistance (mm /≤)	Intermediate (mm)	Sensitive (mm /≥)
Nitrofurantoin (300mcg)	*Escherichia coli*	14	15-16	17
Gentamicin (20mcg)	*E. coli and klebsiella*	12	13-14	15
Cefuroxime (30mcg)	*E. coli and klebsiella*	14	15-17	18

Zone diameter for Nitrofurantoin, gentamicin and cefuroxime

S=Sensitive, I=Intermediate, R=Resistance.

Out of 25 UTI infected pregnant women samples, 8 were resistant and some are susceptible also intermediate to other antibiotics on the basis of disc diffusion method.

ANTIBIOTIC SUSCEPTIBILITY TESTING (AST): The zone of size was interpreted according to CLSI guidelines and zone was examined using metric ruler. E. coli and klebsiella pneumonia in the study showed that more resistant towards antibiotics is nitrofurantoin (300mcg), gentamicin (20mcg) and cefuroxime (30mcg).

Table 8: Zone measurement for 25 samples in cm.

Samples	Gentamicin (GEN)	Amoxyclav (AMC)	Cefuroxime (CXM)	Nitrofurantoin (NIT)	Cefotaxime (CTX)	Ciprofloxacin (CIP)
S1	0.7	0.4	R	1.0	0.4	1.4
S2	0.4	0.2	R	R	0.9	0.3
S3	0.7	2.1	R	0.9	1.8	1.2
S4	0.7	0.8	0.9	1.0	0.6	0.7
S5	0.8	1.0	1.2	0.6	2.2	1.8
S6	0.9	1.6	1.6	0.6	1.8	1.5
S7	1.0	1.6	1.9	0.9	1.8	1.7
S8	1.0	1.6	1.5	0.6	1.9	1.4
S9	0.7	1.5	1.8	0.5	1.7	1.0
S10	1.4	1.0	0.6	0.4	1.0	0.6
S11	0.5	0.8	0.4	0.3	0.9	0.4
S12	0.5	1.0	0.5	0.3	1.0	0.7
S13	0.4	0.9	0.8	0.3	1.0	0.6
S14	0.5	10.	0.8	0.3	1.0	0.5
S15	0.8	1.4	1.3	0.6	1.7	0.9
S16	0.5	1.0	1.0	0.3	1.3	0.6
S17	1.5	1.7	1.6	0.5	1.5	1.3
S18	1.6	1.5	1.3	0.2	1.7	1.6
S19	0.8	1.9	1.4	0.3	1.3	0.5
S20	0.7	1.4	0.5	0.3	1.9	1.2
S21	1.4	1.8	2.2	0.7	1.5	1.4
S22	0.3	1.5	0.6	0.2	2.0	0.7
S23	0.8	1.9	08	0.5	1.6	0.9
S24	1.0	2	1.2	0.6	1.8	1.4
S25	0.6	1.3	1.5	0.3	1.0	1.5

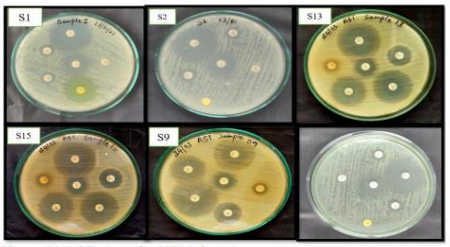

Figure 16: AST plates for UTI infected pregnant women.

Minimum Inhibitory Concentration Assay: Nitrofurantoin, Cefuroxime, and Gentamicin showed resistance to some Enterobacteriaceae species which are subjected to MIC assay and its results was shown below.

Genomic DNA Extraction:

The Isolation of DNA was according to conventional procedure, and the nanodrop absorption ratio was recorded for the different samples in the table below. The extracted DNA was quantified for the quantitative and qualitative analysis and subjected to the 16S -rDNA.

Table 10: DNA purity of samples.

Samples	Concentration ng/µl.	260/230
S1	611.579	1.89
S2	1185.025	1.67
S3	1571.424	1.65
S4	1273.207	1.72
S5	995.900	1.43
S6	924.711	2.05
S7	955.264	1.86
S8	1444.597	1.64
S9	605.511	1.35
S10	703.817	1.92
S11	872.082	1.78
S12	643.633	2.00

Fig 19: DNA in Eppendorf's

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Fig 20: DNA purity of S1, S4, S8 and S11 at nanodrop.

16S rRNA SEQUENCING: The genomic DNA was extracted from all the 12samples of the UTI and subjected for the molecular analysis of PCR. For the selected 2 E coli isolates and were used as template for the universal primers (AGAGTTTGATCCTGGCTCAG-27F) and (CGGTTACCTTGTTACGACTT-1492R) in the PCR analysis. The DNA was analysed for both qualitatively and quantitively. The DNA after the amplification was subjected for the agarose gel electrophoresis with 0.8% of the agarose gel, which displayed sharp single band and then confirmed the selected and isolated E coli bacteria form the samples. The reaction conditions for the 16S rDNA for Universal primer pairs using the primers for the PCR and steps are annealing temperature was set to different condition of the gradient, and Pre-heating temperature was 95°C for 5 minutes, Denaturation was 95℃ for 50 seconds, and annealing for 55℃ with primer annealing was 1.30 seconds, Extension temperature were 25℃ for 5 minutes, Final Extension 4℃ for the 5 minutes with run of 35 cycles. BLAST for the E. coli sample: The sequence of amplified PCR product of 16sRNA was obtained from Sakala Enterprises (Bangalore, India). These sequences were used as a query sequence for the identification of respective Escherichia coli bacteria. It was performed using Basic Local Alignment Search Tool (BLAST), and the FASTA sequence is added below;

>0422_610_001_PCR_3_16SF_A06.ab1

AGGGTCGATGTCGACTTGGAGGTTGTGCCCTTGA
GGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGAC
CGCCTGGGG
AGTACGGCCGCAAGGTTAAAACTCAAATGAATTG
ACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTT
TAATTCGAT
GCAACGCGAAGAACCTTACCTGGTCTTGACATCC
ACGGAAGTTTTCAGAGATGAAAATGTGCCTTCGG
GAACCGTGA
GACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTT
GTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCA
ACCCTTATCC
TTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGG
AGACTGCCAGTGATAAACTGGAGGAAGGTGGGG
ATGACGTCA
AGTCATCATGGCCCTTACGACCAGGGCTACACAC
GTGCTACAATGGCGCATACAAAGAGAAGCGACCT
CGCGAGAG
CAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGA
TTGGAGTCTGCAACTCGACTCCATGAAGTCGGAA
TCGCTAGTA
ATCGTGGATCAGAATGCCACGGTGAATACGTTCC
CGGGCCTTGTACACACCGCCCGTCACACCATGGG
AGTGGGTTG
CAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGC
GCTTACCACTTTGTGATTCATGACTGGGGTGAAGT
CGTAACAA GGTAACCGGAGA

>0422_610_002_PCR_3_16SR_A06.ab1

TGGGTCTGCAGGCGGTCGACTTACGCGTTAGCTC
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CGACATCGT
TTACGGCGTGGACTACCAGGGTATCTAATCCTGTT
TGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTT
CGTCCAGG
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TACGCATTTCACCGCTACACCTGGAATTCTACCCC
CCTCTACG
AGACTCAAGCTTGCCAGTATCAGATGCAGTTCCC
AGGTTGAGCCCGGGGATTTCACATCTGACTTAAC
AAACCGCCT
GCGTGCGCTTTACGCCCAGTAATTCCGATTAACG
CTTGCACCCTCCGTATTACCGCGGCTGCTGGCACG
GAGTTAGCC

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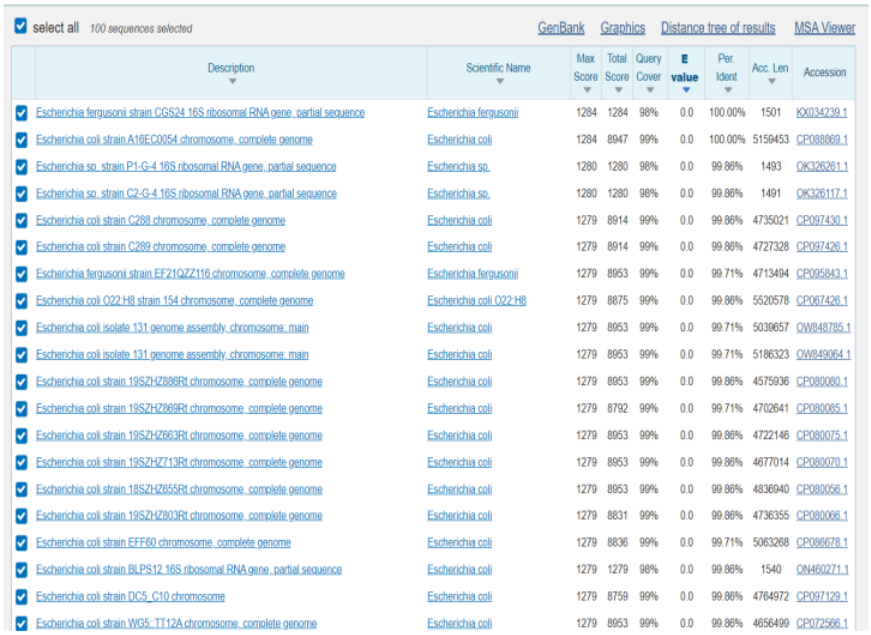
GGTGCTTCTTCTGCGGGTAACGTCAATGAGCAAA
GGTATTAACTTTACTCCCTTCCTCCCCGCTGAAAG
TACTTTACA
ACCCGAAGGCCTTCTTCATACACGCGGCATGGCT
GCATCAGGCTTGCGCCCATTGTGCAATATTCCCCA
CTGCTGCCT CCCGTAGGAGTCTGGA

Basic Local Alignment Search Tool (BLAST) for bacteria: The resulting hits of BLAST results viz, E-valve were <0.0, Query Coverage was found to be 99% for Escherichia. coli, the percentage of identity were found to be 99.86% similarity with exiting NCBI database.



select all 100 sequences selected — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Escherichia fergusonii strain CGS24 16S ribosomal RNA gene, partial sequence	Escherichia fergusonii	1284	1284	98%	0.0	100.00%	1501	KX034239.1
Escherichia coli strain A16EC0054 chromosome, complete genome	Escherichia coli	1284	8947	99%	0.0	100.00%	5159453	CP088869.1
Escherichia sp. strain P1-G-4 16S ribosomal RNA gene, partial sequence	Escherichia sp.	1280	1280	98%	0.0	99.86%	1493	OK326261.1
Escherichia sp. strain C2-G-4 16S ribosomal RNA gene, partial sequence	Escherichia sp.	1280	1280	98%	0.0	99.86%	1491	OK326117.1
Escherichia coli strain C288 chromosome, complete genome	Escherichia coli	1279	8914	99%	0.0	99.86%	4735021	CP097430.1
Escherichia coli strain C289 chromosome, complete genome	Escherichia coli	1279	8914	99%	0.0	99.86%	4727328	CP097426.1
Escherichia fergusonii strain EF21QZZ116 chromosome, complete genome	Escherichia fergusonii	1279	8953	99%	0.0	99.71%	4713494	CP095843.1
Escherichia coli O22:H8 strain 154 chromosome, complete genome	Escherichia coli O22:H8	1279	8875	99%	0.0	99.86%	5520578	CP067426.1
Escherichia coli isolate 131 genome assembly, chromosome: main	Escherichia coli	1279	8953	99%	0.0	99.71%	5039657	OW848785.1
Escherichia coli isolate 131 genome assembly, chromosome: main	Escherichia coli	1279	8953	99%	0.0	99.71%	5186323	OW849064.1
Escherichia coli strain 19SZHZ886Rt chromosome, complete genome	Escherichia coli	1279	8953	99%	0.0	99.86%	4575936	CP080080.1
Escherichia coli strain 19SZHZ869Rt chromosome, complete genome	Escherichia coli	1279	8792	99%	0.0	99.71%	4702641	CP080085.1
Escherichia coli strain 19SZHZ663Rt chromosome, complete genome	Escherichia coli	1279	8953	99%	0.0	99.86%	4722146	CP080075.1
Escherichia coli strain 19SZHZ713Rt chromosome, complete genome	Escherichia coli	1279	8953	99%	0.0	99.86%	4677014	CP080070.1
Escherichia coli strain 18SZHZ655Rt chromosome, complete genome	Escherichia coli	1279	8953	99%	0.0	99.86%	4836940	CP080056.1
Escherichia coli strain 19SZHZ803Rt chromosome, complete genome	Escherichia coli	1279	8831	99%	0.0	99.86%	4736355	CP080066.1
Escherichia coli strain EFF60 chromosome, complete genome	Escherichia coli	1279	8836	99%	0.0	99.71%	5063268	CP086678.1
Escherichia coli strain BLPS12 16S ribosomal RNA gene, partial sequence	Escherichia coli	1279	1279	98%	0.0	99.86%	1540	ON460271.1
Escherichia coli strain DC5_C10 chromosome	Escherichia coli	1279	8759	99%	0.0	99.86%	4764972	CP097129.1
Escherichia coli strain WG5::TT12A chromosome, complete genome	Escherichia coli	1279	8953	99%	0.0	99.86%	4656499	CP072566.1

sFigure 22: BLAST image of E. coli sample.

RESISTANT GENE IDENTIFICATION: PCR with specific primers was done followed by gel electrophoresis CTX-M-1 gene was identified

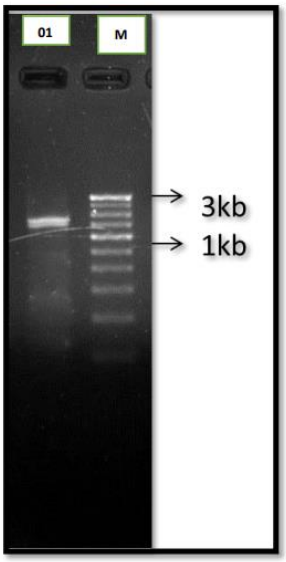


Figure 23: CTX-M-1 gene amplified 863bps in the gel electrophoresis.

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Discussion

Urinary tract infections (UTIs) stand as the most widespread bacterial infections globally, prevalent across all age groups and particularly prominent in underdeveloped nations. Notably, UTIs rank among the primary causes for hospitalizations related to infections in elderly patients and pregnant women. Additionally, they constitute the most common reason for antibiotic prescriptions in primary care settings and contribute to over 30% of infectious complications post kidney transplantation. However, diagnosing and treating both upper and lower urinary tract infections pose challenges due to their frequent occurrence, recurrence rates, and the escalating global issue of antibiotic resistance.

Given these complexities, urine culturing and antimicrobial susceptibility testing play a pivotal role in definitively diagnosing and treating UTIs. The objective of this study was to ascertain the antibiotic susceptibility profiles of bacterial isolates and identify their resistance genes from the urine samples of UTI patients. The research involved 302 samples from pregnant women with UTIs, with 25 samples specifically analyzed. Microscopic observations and biochemical tests—such as indole, TSI, citrate, coagulase, urease, and catalase—confirmed the presence of E. coli and Klebsiella pneumoniae.

Subsequent antibiotic susceptibility testing included drugs like gentamicin, amoxiclav, cefuroxime, nitrofurantoin, cefotaxime, and ciprofloxacin. Findings indicated resistance among some bacterial samples to nitrofurantoin, gentamicin, and cefotaxime. The Minimum Inhibitory Concentration (MIC) assays were conducted to determine appropriate antibiotic dosages, revealing resistance rates of 10%, 20%, 50%, 80%, and 100% for specific drugs. Nitrofurantoin, gentamicin, and cefotaxime were the prescribed antibiotics.

Moreover, molecular characterization identified resistant genes like CTX-M (group 1) for the E. coli strain, aligning with studies conducted by Bahati Johnson et al. (2021) and Bhushan P. Bhusare et al. (2021), who explored similar aspects of UTI in pregnant women, emphasizing the prevalence of gram-negative bacteria like Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, and Citrobacter freundii.

Furthermore, research by Yeva Rosana et al. (2020) detailed various antibiotics—such as fosfomycin, co-amoxicillin/clavulanate, trimethoprim, amoxicillin, cephalexin, and nitrofurantoin—used to treat asymptomatic bacteriuria in pregnant women. Elaf Sameer et al. (2020) highlighted the virulence of uropathogenic E. coli isolates through polymerase chain reaction assays, identifying virulence genes like fimH, kpsMTII, iroN, and hly genes in 90 local uropathogenic E. coli isolates.

This collective research underscores the complexity of UTI diagnosis and treatment in pregnant women, emphasizing the necessity for tailored antibiotic therapies in the face of increasing antibiotic resistance.

Summary and Conclusion

Urinary tract infections (UTIs) pose a significant health concern for pregnant women, especially in underdeveloped regions, being a primary cause of pregnancy-related illnesses globally. Causative agents include E. coli, Klebsiella species, Staphylococcus aureus, Staphylococci, Proleusmirabilis, Enterococcus species, Pseudomonas aeruginosa, Enterobacter species, streptococci, and Citrobacter species. The prevalence of UTIs among pregnant women ranges approximately from 13% to 18%. Bacterial identification revealed both gram-positive and gram-negative forms, with gram-negative bacteria accounting for 85% and gram-positive for 15%. Notably, E. coli emerged as the most frequently found bacterium in this study.

Antimicrobial susceptibility testing demonstrated high resistance among these isolates to nitrofurantoin, ciprofloxacin, and gentamicin, while displaying greater sensitivity to antibiotics like amoxiclav and cefuroxime. Additionally, pregnant women with asymptomatic UTIs, caused by Klebsiella pneumonia, E. coli, Proteus mirabilis, Acinetobacter baumanni, Pseudomonas aeruginosa, Staphylococcus arlettae, and Enterococcus faecalis, showcased resistance to multiple drugs.

Understanding the prevalence of these isolates in urine samples and their resistance profiles becomes crucial in guiding empirical treatments, aiming to minimize adverse effects on pregnant women. Ongoing research endeavors focus on combatting UTIs in pregnant women, particularly addressing drug resistance through novel technologies and identification methods to manage these infections effectively

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