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ISOLATION, BIOCHEMICAL CHARACTERISATION AND MULTI DRUG POTENTIAL OF URINARY TRACT INFECTIOUS BACTERIA

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ABSTRACT

One of the most common infectious diseases, urinary tract infections (UTIs) impact millions of people worldwide, especially women and those with weakened immune systems. Numerous bacteria that colonise the urinary system and cause inflammation, discomfort, and other symptoms are the main cause of UTI pathogenesis. Following Escherichia coli as the most commonly isolated pathogen are other Gram-negative bacteria, including Pseudomonas aeruginosa, Klebsiella spp., and Proteus spp. In order to ascertain the therapeutic potential of the bacterial pathogens that cause UTIs, this study will isolate and identify them, describe their virulence characteristics, and assess their antibiotic susceptibility profiles. Collected and processed three patient urine samples. Selective media were used for bacterial isolation, and Gramme staining, biochemical testing, and antibiotic susceptibility testing were used for identification. High sensitivity rates to widely used antibiotics were found through antibiotic susceptibility testing utilising the Kirby-Bauer disc diffusion method. Interestingly, a large number of strains showed multidrug sensitivity.

KEYWORDS: Urinary tract infections, susceptibility, *Escherichia coli*, *Pseudomonas aeruginosa*, bacterial isolation.

INTRODUCTION

Pathogenic invasion of the urinary tract results in an inflammatory reaction of the urothelium, which causes urinary tract infection (UTI). Urinary tract infections are brought on by the growth of bacteria in the urinary tract. The part of the urinary tract affected, the causative organism or organisms, the severity of the infection, and the patient's capacity to

generate an immune response all influence the clinical symptoms of UTI (Foxman and Brown, 2003). Fever, chills, dysuria, urgency, frequency, and murky or foul-smelling urine are some of the symptoms. Bacteria in the distal urethra and periurethral flora are the primary cause of infections, which nearly invariably have an ascending origin. These bacteria colonise the perineal region and live in the distal gastrointestinal system. A child's first infection is typically caused by E. coli, but other gram-negative bacilli and Enterococci can also cause infection (Brkic et al., 2010). Urinary tract infections in female adolescents are frequently caused by Staphylococcal infections, particularly those caused by Staphylococcus saprophyticus (Assel et al., 2009). Mammals have sterile kidneys, uteruses, and bladders under normal conditions. Additionally, the urine in the bladder is sterile. However, the distal part of the urethra often contains a few germs in both males and females. Few things can survive in the kidney medulla because it is so hypertonic.

To get rid of any infections, the lower urinary tract is flushed four to five times a day with pee and some mucus. A barrier that keeps microbes out of the bladder is provided by the urethra's anatomical length of 20 cm in males. On the other hand, girls have a 14-fold higher prevalence of general urinary tract infections than males due to the fact that bacteria can more easily pass through their shorter urethra (5 cm). Oestrogen causes the vaginal epithelium to create more glycogen, which is then broken down by acid-tolerant Lactobacillus acidophilus bacteria known as Doderlein's bacilli to make lactic acid. An acidic environment (pH 3.5) is unfavourable to the majority of established organisms since normal vaginal secretions include up to 108 Doderlein's bacilli per millilitre.

Additionally, cervical mucous has certain antimicrobial properties. The majority of UTIs are believed to be brought on by bacteria that come from the patient's own intestines. One of the most prevalent and frequent nosocomial illnesses is urinary tract infection. Numerous Gram-positive and Gram-negative bacteria are typically the cause of UTIs. Staphylococcus species, Streptococcus species, and Enterococcus species are examples of Gram-positive bacteria. Numerous aerobic bacilli, including Salmonella, Escherichia, Klebsiella, Enterobacter, Citrobacter, Proteus, Serratia, and Pseudomonas, are classified as gram-negative. E. Coli is responsible for 80–90% of UTIs (H.G.I. Rushton, 1997), whereas Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, and Enterococcus faecalis are the most commonly isolated bacteria in nosocomial infections and ambulatory individuals.

Urinary tract infection symptoms vary depending on the age and location of the patient. Both acute and chronic urinary tract infections cause kidney damage, elevated blood pressure, and even death. Acute and chronic pyelonephritis, which is a disease process caused by infection of the kidney's pelvis and parenchyma, cystitis, renal carbuncle, urethritis, and prostatitis are chronic symptoms of UTIs. The sensitivity of bacteria to several antibiotics, including trimethoprim-sulfamethoxazole (TMP-SMX), determines how UTIs are treated. Prolonged use of antibiotics, however, can have negative consequences on patients and generate plasmid and/or mutational changes in the pathogens that lead to resistance.

The steps involved in conducting a thorough investigation of microorganisms in a urine sample are as follows: aseptic specimen collection, quantitative evaluation, pathogen isolation, Pathogen identification and Testing for antibiotic sensitivity The bacteria that cause UTIs determine how to treat them since each bacterium has a unique susceptibility to different antibiotics. Significant changes in the susceptibility patterns of the major urinary pathogens have occurred in recent years, including a steady rise in infections brought on by enterobacteria that produce extended spectrum beta-lactamases (ESBLs) or even bacteria that produce carbapenemase.[6,7] As a result, the empirical treatment of these

infections has changed. However, the rise in resistance must result in initiatives that support empirical therapy as well as improvements to it.[2]

In rural hospitals in underdeveloped nations, the issue of antibiotic resistance is made worse by the limited supply of antibiotics and, in many cases, the lack of a reliable etiological diagnosis, which is further compounded by the paucity of antibiotic sensitivity testing.[8] The economy and health of these cultures suffer as a result of the overuse and uncontrolled use of broad-spectrum antibiotics, which can result in extended hospital admissions and ineffective treatment, among other consequences.[1,8] Inadequate laboratory staff and material resources hinder the ability to accurately diagnose infectious diseases using microbiology and to administer appropriate therapy.[8,9]

People in developing nations buy antibiotics on the black market without a prescription due to limited availability to high-quality antibiotics; at the moment, these patients are not tracked by surveillance programs. The overuse and abuse of antibiotics are exacerbated by their unregulated selling.[1] While ciprofloxacin and amoxicillin are used as second-line empirical treatments for UTIs, nitrofurantoin has lately been established as the preferred treatment.[10] More thorough investigation is required[8,11,12] since second-line medications have not shown the same results as nitrofurantoin, despite recent trials demonstrating its great effectiveness.[8] Lastly, it is important to emphasise that not much study has been done on the frequency of common diseases and how susceptible they are to antibiotics in rural Uganda. The purpose of this study was to identify and characterise these infections' antibiotic susceptibility patterns. We believe that we can effectively treat diagnostic urine infections by performing a microbiological examination of the bacteria involved, in which the pathogens' antibiotic susceptibility is unknown. The most frequent bacterial infection is urinary tract infection (UTI). These days, they are among the most prevalent illnesses that can affect people of any age, from newborns to adults.1 According to data, 150 million people globally suffer from UTIs in poor nations.[2,3]

A 1997 evaluation of National Ambulatory Medical Care and National Hospital Ambulatory Medical Care found that UTIs are responsible for about 100,000 hospital admissions, 1 million cases in emergency rooms, and 7 million OPD visits. Women are more likely than men to get UTIs. Almost one-third of women will have experienced at least one UTI episode requiring antibiotic usage by the time they are 24 years old.[2,3] Nearly 20% to 40% of women will have recurrent infections, and about 50% of women will have at least one UTI episode at some point in their life.[1,4]

UTIs are more common in infants, pregnant women[2,5], the elderly, and individuals with multiple sclerosis, AIDS/HIV, diabetes mellitus, spinal cord injuries, urologic abnormalities, or catheters. Over a million UTI cases are recorded annually in hospitals and nursing homes due to the increased risk of UTIs associated with long-term catheterisation.[3,5] Approximately 25% of all infections in the elderly are UTIs, making them the second most prevalent infection.[2,3] Gender, age, hospitalisation, catheterisation, and prior antibiotic exposure are among the variables that affect the bacterial uropathogens.[6] Numerous research indicate that the most common uropathogens are *Escherichia coli and other Enterobacteriaceae, Staphylococcus, and Enterococcus. Klebsiella pneumoniae, Klebsiella oxytoca, Streptococcus agalactiae, Streptococcus viridans, Proteus mirabilis, Pseudomonas aeruginosa, Citrobacter freundii, Enterobacter cloacae, and Staphylococcus aureus* are other etiological agents of UTIs.[7] Today, drug resistance of bacterial uropathogens is a significant problem in medical settings[8], especially in developing nations where poverty, poor hygiene, and illiteracy compound the problem.[1,9] Despite the fact that antibiotic resistance is a global health concern[6], developing nations are particularly affected because of the high incidence of infectious diseases and the potential that patients with resistant infections won't have access to the right drugs.

However, the creation and rapid spread of resistant diseases are facilitated by variables like poor sanitation, contaminated water, internal conflicts, and an increase in the number of immunocompromised HIV-positive people.[9,10] It is challenging to accurately determine the frequency of UTIs and bacterial resistance in hospitals in impoverished nations like Afghanistan due to the absence of hospital surveillance systems and computerised data. Given the prevalence of multidrug-resistant bacteria in Afghanistan, the goal of this study was to ascertain the prevalence of UTIs among patients at the French Medical Institute for Children, identify the bacteria causing the infection, and look into their patterns of antimicrobial resistance.

MATERIALS AND METHODS

Sample collection: Three samples of urine were collected from three different patients. These collected urine samples were mid-stream collected in sterile bottles and were stored in a refrigerator. The physical parameters of the collected urine samples were analysed, such as the pH, colour, appearance and odour (Fig. 1; Table 1).

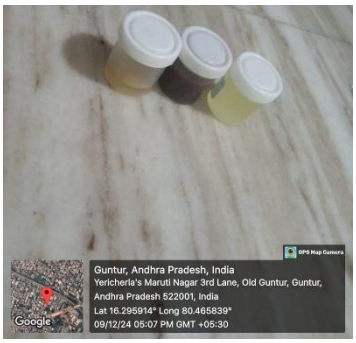

Fig. 1: Sample collection.

Table 1: Urine Sample Analysis.

S. No.	Sample Code	pH	Colour	Appearance
1	380	6-7	Reddish brown	Turbid
2	317	9-10	Pale yellow	Turbid
3	368	6	Pale yellow	Turbid

Gram staining: The technique of serial dilution was carried out after the culture specimen from culture plates was inoculated into test tubes containing 9 millilitres of distilled water to create the bacterial suspension solution. In order to better examine the bacterial colonies, this was done. Each test tube was filled with a loop of sample, which was then applied to the glass slide and heated. The slides were placed over a staining tray or basin. For one minute, crystal violet dye was poured over the streaks. Using a wash bottle and distilled water, the discolouration was removed from the basin. A blotting paper was used to blot dry the slides. Gram's iodine was applied to each smear for a duration of one minute. Distilled water was used to remove any excess iodine from the gramme.

After soaking the slides in alcohol for a minute, the extra alcohol was emptied into the basin for ten seconds. Distilled water was used to remove the alcohol. For 20 to 30 seconds, safranine was poured over the streaks. After rinsing with distilled water and blot drying, the slides were inspected under a microscope.

IMVIC REACTIONS: The IMVIC reactions are used to determine the biochemical characteristics of an unknown bacterium. The five reactions are the Indole test, Methyl red test, Voges-proskauer test, Citrate test, catalase test.

Indole Test: The bacterium to be examined is inoculated in peptone broth containing NaCl and the amino acid tryptophan, and it is cultured at 37°C for two days. The inoculated tryptone broth is then treated with a few drops of Kovac's reagent. Con. HCl, isoamyl alcohol, and para-dimethyl aminobenzaldehyde make up Kovac's reagent. When it comes to identifying indole synthesis in anaerobes and non-fermenters, Ehrlich's reagent is more sensitive. It is considered favourable when a ring of red or pink forms at the top.

Methyl Red (Mr) Test: The bacterium to be tested is added to glucose phosphate broth, which is made up of glucose and a phosphate buffer. The soup is then cultured for 48 hours at 37°C. The organism that produces mixed acids must generate enough acid over the course of 48 hours to overcome the phosphate buffer and acid. Five drops of MR (methyl red) reagent are added to the medium to evaluate its pH. Red colour development is viewed favourably. Yellow is produced by MR negative organisms.

Voges Proskauer (Vp) Test: The test bacterium is added to glucose phosphate broth and allowed to incubate for a minimum of 48 hours. The test broth is mixed with 0.6 cc of alpha-naphthol and shaken. The broth is mixed with 0.2 cc of 40% KOH and shaken. For fifteen minutes, the tube is left to stand. If the colour becomes red, the test is considered successful. Since the maximum colour development happens within an hour of the reagents being added, the negative tubes must be kept for an hour.

Citrate Utilisation Test: Simmon's citrate agar slope is injected with bacterial colonies taken from a straight wire, and the colonies are incubated at 37°C for the entire night. The medium turns blue instead of green if the bacterium can use citrate.

Catalase test: Bacterial colonies are added to the nutrient broth and allowed to incubate at 37C for at least 48 hours. Next, hydrogen peroxide is gradually introduced to the sample. If bubbles occur shortly after hydrogen peroxide is added, the test is catalase positive; if not, the test is catalase negative.

RESULTS AND DISCUSSION

Isolation and characterization of bacteria

Ten bacterial isolates in all were found, and ten distinct strains were subcultured and kept for additional examination. The isolates' microscopic and cultural traits were noted. The isolates belonged to the genera Pseudomonas, Marinispora, Bacillus, and Brevibacillus. Tests were conducted to determine how sodium chloride affected the growth of isolated bacteria. Every isolate was grown at concentrations of sodium chloride ranging from 1% to 10%. In the NaCl-containing media, every colony was growing well (fig. 1).

Features of the bacterial strains' morphology

After plating the bacteria on NAM and incubating them for seven days, the bacterial isolates were first identified by morphological characterisation. Growth and the existence or lack of pigmentation were observed. By examining their mycelium arrangement, spore development, and cultural traits, the bacteria were identified. Lacto-phenol Cotton Blue (LPCB) reagent was used in the tease mount procedure to produce the slides.

The majority of urine bacteria are motile, Gramme negative, and found in lower numbers in water and larger amounts in bottom sediments. The structural variety of the cell wall and its constituents, especially lipopolysaccharide (LPS), allows gram-negative urinary tract bacteria to flourish in hostile marine environments. The three primary components

of LPS—an O-antigen, lipid A, and a core region—all exhibit significant structural differences between various bacterial species. These elements not only maintain cell integrity but also trigger an immunological response in the host, which can be humans or other marine species.

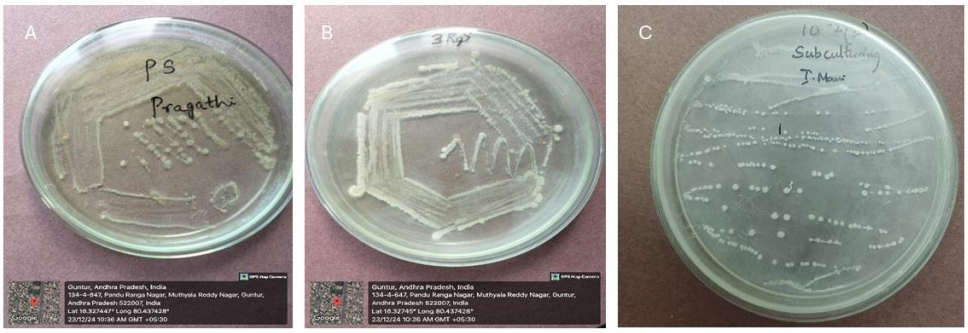

Fig. 2: Subculturing of isolated from Urine samples.

Fig. 3: Pure-cultures of bacteria isolated from urine samples.

Anti-biotic sensitivity testing (Kirby-Bauer method)

The Kirby-Bauer Method was used to examine the isolated organisms' overall susceptibility to specific antibiotics that are typically used to treat uropathogens. Different antibiotic discs were chosen and sterile nutrient agar plates were made. The lawn streak method was used to inoculate the surface of nutrient agar plates with incubated test organisms using sterile cotton swabs. The cultures were left on the plate to dry at room temperature for five to ten minutes. For improved contact and efficient diffusion of the antibiotics into the medium, different antibiotic discs were gently pressed into the agar medium's surface using sterile forceps. Make sure that contact is made between the antibiotic disc and the culture. The plates were incubated in an inverted position for 2-3days at normal room temperature (Table 2; fig. 4).

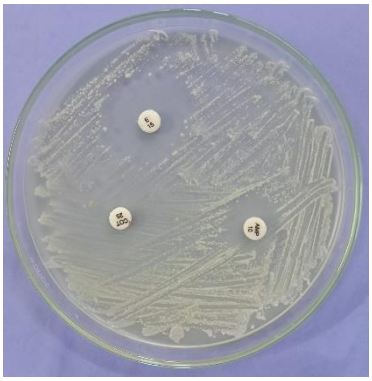

Fig. 4: Disc diffusion assay.

Table 2: Antibiotic susceptibility test (Disc diffusion method).

S.NO	Plate no.	Ampicillin (AMP10)	Chloramphenicol (C30)	Novobiocin (NV5)	Erythromycin (E)	Co-trimoxazole (COT25)
1.	20	10mm	0.5mm	0(R)	0(R)	0(R)
2.	22	15mm	15mm	0(R)	0(R)	20mm
3.	23	10mm	15mm	15mm	12mm	0(R)
4.	24	0(R)	15mm	15mm	10mm	0(R)
5.	25	0.5cm	18mm	10mm	10mm	10mm
6.	26	0(R)	0.5cm	15mm	0(R)	0(R)
7.	27	0(R)	0.5cm	0(R)	0(R)	0(R)
8.	29	10mm	15mm	15mm	15mm	0(R)

IMVIC REACTIONS (fig.5)

Indole Test: *Escherichia coli*: Positive; *Klebsiella pneumoniae*: Negative.

Methyl Red Test: Example: *Escherichia coli*: Positive; *Klebsiella pneumoniae*: Negative.

Voges Proskauer (Vp) Test: Examples: *Escherichia coli*: Negative; *Klebsiella pneumoniae*: Positive.

Citrate Utilization Test: Examples: *Escherichia coli:* Negative*; Klebsiella pneumoniae*: Positive.

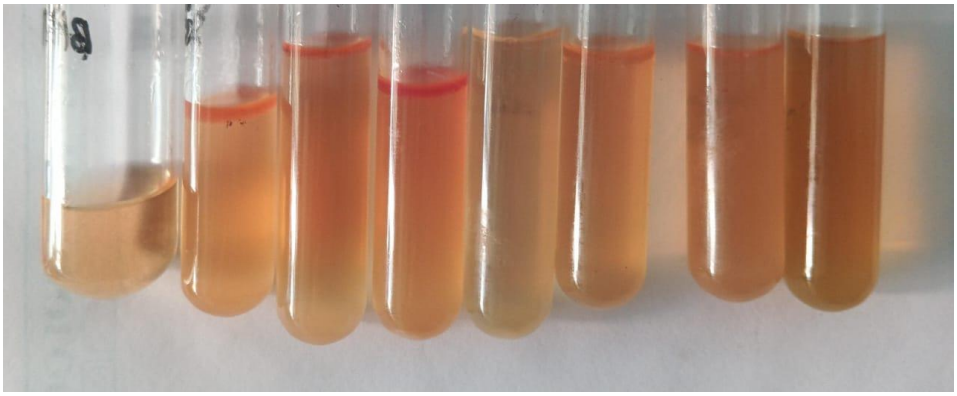

Fig. 5: Biochemical reactions of the isolated bacteria.

CONCLUSION

Drink plenty of water daily. Maintaining hygienic conditions in the wash rooms and at the genital organs is mandatory because these are the major routes to cause and pass the infection. Wipe from front to back as to avoid the contact of microbes from the anus to the genital areas. Stop smoking. Reduce the usage of sprays and scents for those areas. Antibiotics could be used for only minor infections that too up to 1-2 days only, major infections need complete treatment of antibiotics for several weeks.

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CONFLICT OF INTEREST: Declared none.

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