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Isolation and identification of targeted uropathogenic bacteria from urinary tract infections

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Abstract

Urinary Tract Infections (UTIs) are common bacterial infections that affect the urinary system, primarily caused by pathogenic microorganisms. The objective of this study was to isolate and identify the microorganisms responsible for UTIs from urine samples obtained from patients exhibiting symptoms of infection. Urine samples were collected midstream to minimize contamination, and bacterial isolation was performed using standard microbiological techniques. Samples were cultured on selective and differential media, including Nutrient agar, Salmonella Shigella Agar (SS), Eosin Methylene Blue Agar (EMB), Mannitol salt agar, MacConkey agar and King's B medium followed by incubation at 37°C for 12-24 hours. The bacterial colonies were then characterized based on their macroscopic appearance, Gram-staining properties, and biochemical tests, including catalase tests. Results indicated that the most common uropathogens were Escherichia coli, Klebsiella sp., Pseudomonas sp., Salmonella sp., and Staphylococcus sp. These findings contribute to a better understanding of the microbial etiology of UTIs.

Keywords: Uropathogen, Bacterial isolation, Microbial identification, UTI etiology

Introduction

Urinary tract infections rank as the second most prevalent infectious issue in community healthcare. Each year, approximately 150 million individuals around the globe receive a UTI diagnosis, with these infections categorized as either uncomplicated or complicated (Stamm *et al*., 2001). Uncomplicated UTIs typically occur in sexually active, healthy females who have normal urinary tracts both structurally and functionally. On the other hand, complicated UTIs are linked to underlying health conditions that either extend the duration of treatment or raise the risk of treatment failure. Such conditions may include urinary tract abnormalities that hinder urine passage, the presence of foreign objects, or infections caused by multidrug-resistant organisms. UTIs among males are considered complicated. Even when the upper urinary tract is involved, pyelonephritis may still be classified as uncomplicated if it develops in a healthy individual (Hooton *et al.*, 2000; Stapleton *et al*., 2003). Urinary tract infections can affect solely the lower urinary tract or involve both the upper and lower tracts. The term cystitis refers to the syndrome characterized by painful urination, increased frequency, and sometimes tenderness in the suprapubic area. Acute pyelonephritis describes a clinical syndrome marked by discomfort or pain in the flank, fever, and often accompanied by dysuria, urgency, and frequent urination (Mandell *et al*., 2005). Over 95% of urinary tract infections are attributed to a single type of bacteria. *E. coli* is the most common pathogen responsible for acute infections (Jellheden *et al*., 1996; Ronald *et al*., 2002). In patients are more likely to have isolates of *Klebsiella, Staphylococci, Salmonella, Pseudomonas,* while *E. coli* is more predominant among outpatient (Bronsema *et al*., 1993). *Corynebacterium urealyticum* is recognized as a notable pathogen in nosocomial settings. Rarely do anaerobic bacteria act as pathogens in the urinary tract (Jacobs *et al*., 1996). Reports indicate that Coagulase Negative *Staphylococci* often cause urinary tract infections (Mandell *et al.*, 2005). *Staphylococcus* saprophyticus is particularly associated with infections in young, sexually active women (Schneider *et al*., 1996). The objective of this study is to identify and isolate the bacteria responsible for UTIs from the urine samples of patients showing infection symptoms. By recognizing the most prevalent uro pathogens and analyzing their resistance profiles, we aim to enhance diagnosis, treatment methods, and patient outcomes in UTI management.

Urinary tract infection indicates the existence of microbial pathogens within the urinary system. Any condition recognized to be present at the location of disturbance, the kidneys, or

urine can manifest as a UTI, which may be either symptomatic or asymptomatic, characterized by a spectrum of reactions ranging from irritative voiding to bacteremia, sepsis, or other complications (Foxman *et al.*, 2003). UTI is recognized as the most frequently encountered bacterial illness (Nicolle *et al.*, 2001). Contamination of the urinary tract is arguably among the most prevalent bacterial infections, affecting around 150 million individuals globally every year (Flores-Mireles *et al.*, 2015). It affects all age groups and both genders. However, women are generally more susceptible than men due to factors like the shorter urethra, absence of prostatic growth, pregnancy, and the prevalence of strong fecal flora contaminating the urinary tract (Hooton *et al.*, 2012) Additionally, the physiological increase of plasma during pregnancy leads to reduced urine concentration, and up to 70% of pregnant women experience glucosuria, which aids in bacterial growth in the urine (Nielubowicz *et al.*, 2010). Pregnant women face a heightened risk of UTIs. From week 6 of pregnancy, peaking around weeks 22 to 24, about 90 percent of pregnant women encounter urethral dilation, which persists as pregnancy progresses (Hannan *et al.*, 2012). Increased bladder volume and reduced bladder tone, alongside diminished urethral tone, contribute to heightened urinary frequency and ureterovesical reflux (Levison *et al.*, 2013). The entire structure of the human urinary tract hosts normal flora. This typical flora, often referred to as normal commensals, can comprise significant or contradictory amounts of live microorganisms or parasites from outside or within the body (endogenous). Commensalism within a human host environment can be influenced by factors such as the capacity to counteract pathogenic invasion, pain response, immunity, genetic factors, diet, stress, age, or other physiological components (Findley *et al.*, 2014). UTIs can occur due to voiding urine, given that the genitourinary tract passage opens. Microorganisms that naturally inhabit the urinary tract have the potential to cause illness but typically do not result in severe harm.

Materials and Methods

Sample Collection: The urine sample was collected from Jana Laboratory at Virudhungar.

Isolation of bacteria: The urine sample was streak into Nutrient agar, Salmonella Shigella Agar (SS), Eosin Methylene Blue Agar (EMB), Mannitol salt agar, MacConkey agar and King's B medium. Incubate the plates at 37ºC for 12-24 hours. After the incubation the colonies were observed on agar plates. Then the colonies are sub-culture into nutrient broth.

Identification of bacteria: The isolated strains are streaked onto the SS agar, EMB agar, Mannitol salt agar, Simmon citrate agar, MacConkey agar and King's B medium. Then the colonies are identified by Morphology, Gram Staining and Biochemical tests.

Gram Staining: Gram staining is a widely used microbiological technique that helps differentiate bacterial species into two major groups: Gram-positive and Gram-negative, based on the structure of their cell walls. The procedure involves several steps and relies on differences in the bacterial cell wall's ability to retain a violet dye (crystal violet) during the staining process.

Biochemical Test: To identify and differentiate microorganisms based on their metabolic properties. The test includes Indole, Methyl red, Voges-Proskauer, Citrate utilization, Triple sugar iron, catalase and Starch hydrolysis.

Indole test: Tryptophan is converted to indole by bacteria that have the enzyme tryptophanase. A crimson ring is created when indole and Kovac's reagent react.

Methyl Red Test: Detects mixed acid fermentation. A red color indicates a low pH, showing strong acid production.

Voges-proskauer test: Detects acetoin production from glucose fermentation. A color change (red) indicates acetoin presence.

Citrate Utilization Test: Some bacteria use citrate as their sole carbon source, producing alkaline by-products. This changes the medium color from green to blue.

Triple Sugar Iron Test: The Triple Sugar Iron (TSI) test is used to identify enteric bacteria based on sugar fermentation and hydrogen sulfide (H_2S) production.

Catalase Test: The catalase enzyme breaks down hydrogen peroxide into water and oxygen. When hydrogen peroxide is added to the bacteria, bubbling indicates a positive result.

Oxidase test: In this test, tetramethyl-p-phenylenediamine disc was used. A disc was placed on a clean slide, and added to it to determine the presence or absence of the oxidase enzyme. A color change to dark purple within 10-30 seconds indicated a positive result.

Results

Sample Collection

Urine sample were collected in sterile container from Jana Laboratory (9.5876° N, 77.9523° E) located at Virudhunagar. It was collected in the sterile container (Fig. 1).

Fig 1: The urine sample was aseptically collected in a sterile container.

Isolation of bacteria

After 24 hours of incubation, Microorganisms were identified from collected urine samples (Fig 2 to 6) in selective media's

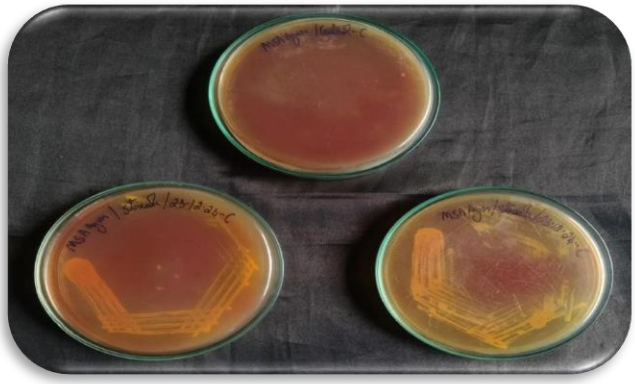

Fig 2: Isolation of *Staphylococcus* sp. on Mannitol Salt Agar.

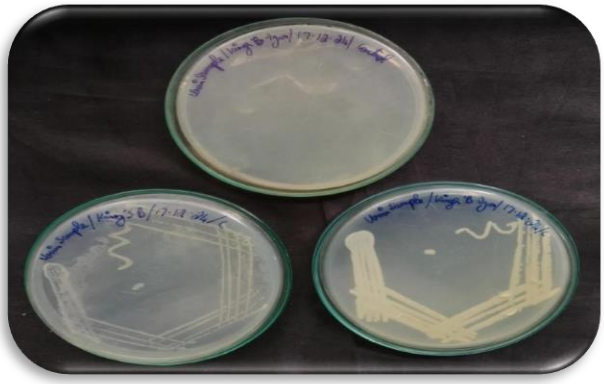

Fig 3: Isolation of Pseudomonas sp. on King's B medium.

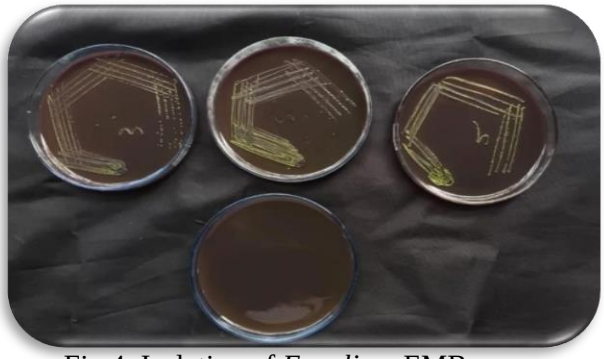

Fig 4: Isolation of *E. coli* on EMB agar.

Fig 5: Isolation of *Salmonella* sp. on SS agar.

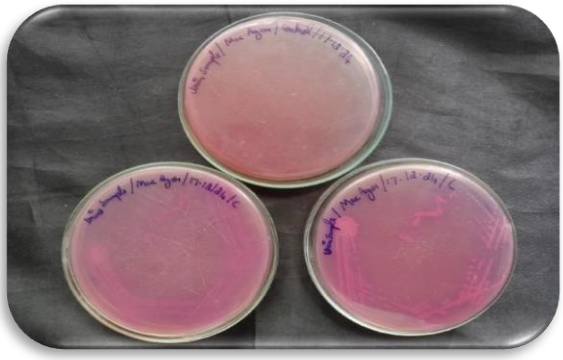

Fig 6: Isolation of *Klebsiella* sp. bacteria on MacConkey agar.

Gram Staining & Biochemical test

After 24 hours of incubation, results for Gram staining and biochemical tests were observed (Fig: 6-10) tabulated (Table 1). Based on morphological and biochemical results and reference to Bergey's Manual, the isolates were identified.

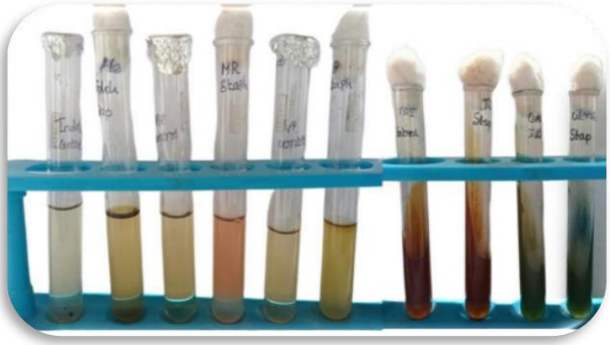

Figure 7: *Staphylococcus* sp.

Figure 8: *Salmonella* sp.

Figure 9: *Pseudomonas* sp.

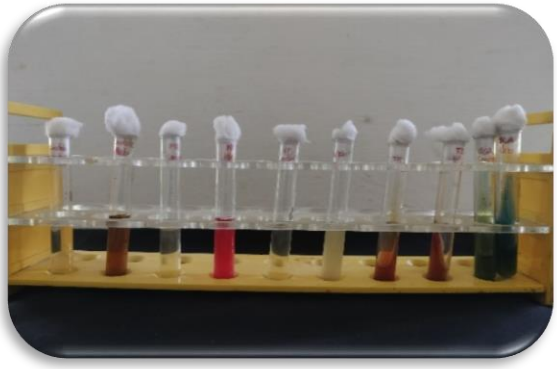

Figure 10: *Klebsiella* sp.

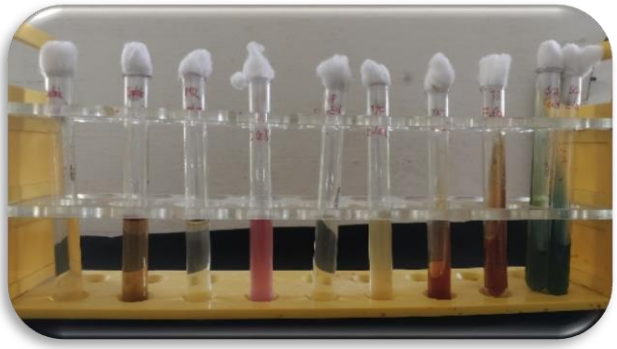

Figure 11: *E. coli*

Table 1: Gram staining & Biochemical Characterization of isolated microorganisms

Gram staining & Biochemical Test	***E. coli***	***Staphylococcus* sp.**	***Salmonella* sp.**	***Pseudomonas* sp.**	***Klebsiella* sp.**
Gram staining	Gram negative	Gram-positive	Gram negative	Gram negative	Gram negative
Catalase	Positive	Positive	Positive	Positive	Positive
Oxidase	Negative	Negative	Negative	Positive	Negative
Indole	Positive	Negative	Negative	Negative	Negative
Methyl red	Positive	Positive	Positive	Negative	Negative
Vogus proskauer	Negative	Positive	Negative	Negative	Positive
Citrate utilization	Negative	Positive	Negative	Positive	Positive
Triple sugar iron	Acid/acid with no gas	Acid/acid with no gas	Alkaline/ acid with gas	Alkaline/Alkaline with no gas	Acid/acid with gas

Discussion

Urinary tract infections (UTIs) are commonly caused by bacterial pathogens, with *Escherichia coli* being the most prevalent uropathogen. The accurate isolation and identification of bacteria from urine samples are critical for effective diagnosis and treatment. Traditional methods, such as culturing on selective media, remain the gold standard. However, they are often time-consuming and may not detect all potential pathogens, especially non-culturable or fastidious bacteria. Advances in molecular techniques, such as PCR-based identification, have significantly improved the sensitivity and specificity of UTI diagnostics, allowing for faster and more accurate detection of pathogens directly from urine samples. Additionally, next-generation sequencing (NGS) offers a comprehensive approach by analyzing the microbial community in urine, providing insight into both culturable and non-culturable microorganisms.

Conclusion

In conclusion, while traditional methods remain valuable, the incorporation of molecular, AI, and next-generation technologies will revolutionize the diagnosis and treatment of UTIs, improving patient outcomes and reducing the burden of bacterial infections globally.

References

Flores-Mireles, A. L., Walker, J. N., Caparon, M., and Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature reviews. Microbiology*, *13*(5), 269-284.

Bronsema, D. A., Adams, J. R., Pallares, R., and Wenzel, R. P. (1993). Secular trends in rates and etiology of nosocomial urinary tract infections at a university hospital. *The Journal of urology*, *150*(2 Pt 1), 414-416.

Foxman B. (2003). Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Disease-a-month: DM*, *49*(2), 53-70.

Nielubowicz, G. R., and Mobley, H. L. (2010). Host-pathogen interactions in urinary tract infection. *Nature reviews. Urology*, *7*(8), 430-441.

Hooton T. M. (2000). Pathogenesis of urinary tract infections: an update. *The Journal of antimicrobial chemotherapy*, *46 Suppl 1*, 1-65.

Jacobs L. G. (1996). Fungal urinary tract infections in the elderly: treatment guidelines. *Drugs & aging*, *8*(2), 89-96.

Jellheden, B., Norrby, R. S., and Sandberg, T. (1996). Symptomatic urinary tract infection in women in primary health care. Bacteriological, clinical and diagnostic aspects in relation to host response to infection. *Scandinavian journal of primary health care*, *14*(2), 122–128.

Zumla A. (2010). Mandell, Douglas, and Bennett's principles and practice of infectious diseases. *The Lancet. Infectious Diseases*, *10*(5), 303-304.

Levison, M. E., and Kaye, D. (2013). Treatment of complicated urinary tract infections with an emphasis on drug-resistant gram-negative uropathogens. *Current infectious disease reports*, 15(2), 109–115.

Nicolle L. E. (2005). Catheter-related urinary tract infection. *Drugs & aging*, *22*(8), 627–639.

Ronald A. (2002). The etiology of urinary tract infection: traditional and emerging pathogens. *The American journal of medicine*, *113 Suppl 1A*, 14S–19S.

Findley, S., Matos, S., Hicks, A., Chang, J., and Reich, D. (2014). Community health worker integration into the health care team accomplishes the triple aim in a patient-centered medical home: a Bronx tale. *The Journal of ambulatory care management*, 37(1), 82-91.

Schneider, P. F., and Riley, T. V. (1996). *Staphylococcus saprophyticus* urinary tract infections: epidemiological data from Western Australia. *European journal of epidemiology*, *12*(1), 51–54.

Stamm, W. E., and Norrby, S. R. (2001). Urinary tract infections: disease panorama and challenges. *The Journal of infectious diseases*, *183 Suppl 1*, S1–S4.

Del Mar C. (2010). Urinary tract infections in healthy women: a revolution in management?. *BMC family practice*, *11*, 42.

Hooton T. M. (2012). Clinical practice. Uncomplicated urinary tract infection. *The New England journal of medicine*, *366*(11), 1028-1037.

Hannan, T. J., Totsika, M., Mansfield, K. J., Moore, K. H., Schembri, M. A., and Hultgren, S. J. (2012). Host-pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic *Escherichia coli* bladder infection. *FEMS microbiology reviews*, 36(3), 616–648.