



Review Article

PREVALENCE OF BACTERIAL ISOLATION IN URINE SAMPLES AND THEIR ANTIBIOTIC SUSCEPTIBILITY: A REVIEW

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ABSTRACT

Urinary tract infections (UTIs) can be complicated if not diagnosed early and treated. Bacteriological analysis of urine samples collection, urine samples were collected and classified and analyzed for urinary tract infection (UTI) using pour plate method. The bacterial organisms isolated from the urine samples were characterized and identified using their colony descriptions, morphological and biochemical characteristics. The isolates were subjected to sensitivity test against conventional antibiotics using disc diffusion method. The presence of bacterial isolates with very high resistance to the commonly prescribed drugs leaves the clinicians with very few alternative options of drugs for the treatment of UTIs. So Culture and sensitivity of the isolates from urine samples should be done as a routine before advocating the therapy.

KEYWORDS: *Urinary tract infections, antimicrobial susceptibility, E. coli, Broth cultures, Urine analysis.*

INTRODUCTION:

Urinary Tract Infection is classified as the most common and occurring nosocomial bacterial infection in human populations around the world¹⁻³ UTI is a condition caused by pathogenic invasion of the epithelium, which lines the urinary tract from the minor calyx to prostatic urethra. The proliferation of bacteria in the urothelium can be asymptomatic or symptomatic, which causes inflammatory response and symptomatic case characterized by a wide range of symptoms including, fever, lethargy, anorexia and vomiting⁴⁻⁹ However, both genders are susceptible to this type of infection, but women are more, as their reproductive anatomy and physiology are more sensitive. Half of all women by 32 years age had experienced at least an infection history⁷⁻¹⁰

Normally, urinary tract urine mostly dominated by *E. coli* 75%- 80%, followed by *S. saprophyticus* 10-15%¹¹⁻¹⁵. While, Anatomy or physiological factors cause abnormality of urinary tract and lead to localize infectious bacteria, such as different species of *Klebsiella*, *Proteus*, *Enterobacter*, *Enterococcus*, *Staphylococcus* and *Pseudomonas aeruginosa*. Those bacteria are more common in most of the cases, and infrequently cause to uncomplicated cystitis and pyelonephritis^{11,12,16}. Furthermore, pathogenesis of Urinary tract is more complicated and influenced by other factors, such as vaginal ecosystem especially *Lactobacillus spp.*, intestinal population, genetic and behavioral factors, virulence properties of uropathogens and host defense factors¹⁷⁻¹⁹. The presence of factors

will increase opportunity for uropathogens to colonize and invade urothelium²⁰⁻²².

More than 95% of urinary tract infections are caused by a single bacterial species. *E. coli* is the most frequent infecting organism in acute infection^{23,24}. *Enterobacter*, *Staphylococci*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Enterococci* species are more often isolated from inpatients, whereas there is a greater preponderance of *E. coli* in an outpatient population²⁵.

2. MATERIAL & METHODS:

2.1 Study Population/collection Urine samples:

Urine samples were collected from the patients, with age ranging from 18 to 26 years. The collections were randomly selected on everyday basis within the periods of 6.30am to 8.00am. Those patients who were on antibiotic treatment prior to the sampling period were excluded from the study. The urine samples collected were classified based on age, marital status and field of study.

2.2 Culturing of the urine Samples:

According to **Obirikwurang et al., 2012**, we can use these particular method for culture the urine samples- The urine samples were cultured using pour plate method (1.0 ml) on Nutrient agar (for total heterotrophic aerobic bacteria count), MacConkey agar (for *Enterobacteriaceae* family) and Mannitol Salt Agar (For *Staphylococcus* species). Inoculated plates were incubated inverted at 37°C aerobically for 24 hrs. After incubation, the total heterotrophic aerobic

bacterial counts were carried out, and then the plates were sub cultured for further identification.

2.3 Colony counts:

Colonies were counted on Nutrient agar using electric colony counter. A bacterial count of 10⁵ per ml was considered significant for urinary tract infection (UTI) and counts of 10² - 10⁴ per ml were considered as suspected bacteriuria while counts less than 10² per ml were considered as non-significant bacterial growth (**Obirikwurang et al., 2012**).

2.4 Preparation of the Test Organisms for Sensitivity Test:

This was carried out using the method of **Obirikwurang et al.(2012)**. The isolates were sub cultured on nutrient broth and incubated aerobically at 37°C for 24hrs. Broth cultures of the isolates were centrifuged at 3000 rpm for 10 minutes. The sediments were diluted with sterile phosphate buffer saline (PBS) and adjusted to the 10⁸ CFU/ml using McFarland matching standard (mixture of 0.6ml of 1% BaCl₂.H₂O and 99.4ml of 1% conc. H₂SO₄) using spectrophotometer at 540nm.

2.5 Bacterial isolation and antimicrobial susceptibility testing:

According to (**Bunchanan and Gibbons, 1974**) A significant bacterial count was taken as count equal to or in excess of 10⁵ per milliliter. Identification of pure isolates was done by observing morphological, cultural and biochemical characters according to **Cheesbrough (2002-2004)**.

The isolates were identified by **Bergey's Manual** for Determinative Bacteriology.

2.6 Antibiotic Sensitivity Testing:

According to **IP 2010**, the disc diffusion method was used to carry out the antibiotic sensitivity testing. The test organism was seeded on Mueller Hinton agar using pour plate method, and allowed to solidify. A sterile forceps was used to place the antibiotic sensitivity disc on the surface of the medium. The set-up was incubated aerobically at 37°C for 24 hrs. The inhibition zone diameters were measured using meter rule after 24 hrs incubation and recorded.

We can also use the another method- Antibiotic sensitivity testing was performed using the **Kirby Bauer disc diffusion method**, determining sensitive and resistant bacteria to antibiotics by measuring the diameter of inhibition zone by mm and then compared with the standard diameters that installed in the standard scales. Antimicrobial drug susceptibility testing for Ampicillin 10 g, Amoxicillin/ clavulanic acid (augmentin)20/10 g, Gentamicin 10 g, Cefotaxime 30 g, Ceftriaxone (30 g), Ceftazidime(30mg), Cotrimoxazole 25 g, Ciprofloxacin (5 g), Amikacin 30 g, Nitrofurantoin (300 g)and Norfloxacin (10 g) was done on all bacteria isolated. Interpretation of results was done based on the diameter of the zone.

Bacterial uropathogen isolates from patients with UTIs revealed the presence of high levels of single and multiple antimicrobial resistances against commonly prescribed drugs *E.coli*, which is the predominant cause of UTI, showed high

percentage of resistance to ampicillin, cotrimoxazole, cefotaxime, ciprofloxacin, ceftriaxone and norfloxacin and low resistance to Augmentin and gentamicin, nitrofurantoin but all were sensitive to amikacin. *Klebsiella* spp. which is the second most prevalent pathogen of UTI displayed a similar resistance pattern as of *E.coli* and showed hundred percent resistant to ampicillin however, and all other gram negative isolates were similarly resistant to most of the antibiotics as that of *E. coli*.²⁶

Bacterial infection of the urinary tract is one of the common causes for seeking medical attention in the community. Micro-organisms causing UTI vary in their susceptibility to antimicrobials from place to place and from time to time. So identification of the etiological agent and the selection of an effective antibiotic agent to the organism in question is very important for effective management of patients suffering from bacterial UTIs. UTIs are caused by a variety of microorganisms, including both gram positive and gram negative ones. In our study *Escherichia coli* was predominant isolate followed by *Proteus* spp. and *Klebsiella* spp. respectively. This finding is similar to many reports which indicated that gram negative bacteria mostly *E.coli* and *Proteus* spp. are the commonest pathogens isolated in patient with urinary tract infections.²⁷

3. CHARACTERIZATION AND IDENTIFICATION OF THE ISOLATES:

The growth on the mixed culture plates were sub cultured on Nutrient agar and incubated

aerobically at 37°C for 24 hrs. Growths on the culture media were identified using the colony descriptions of the isolates, morphological characteristics.²⁸

4. CONCLUSION:

The systematic representation of urine analysis and isolation of bacteria were identified from patients. Their sensitivity to antibiotics was performed and the activity of antibiotics for inhibiting bacterial growth was at different levels, According to their ability.

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