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Diagnosis of Urinary Tract Infections by a Modified Culture Method

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Abstract: Urinalysis is a valuable diagnostic tool for many common disease states. Urinary Tract Infection (UTI) is one of the most commonly diagnosed infections in both outpatient and inpatient populations. In order to make an accurate diagnosis, it is essential for practitioners to understand the value and limitations of urinalysis and urine culture. Use of these tests in conjunction with an assessment of urinary symptoms will yield a diagnosis of either asymptomatic bacteriuria or symptomatic UTI. Microbiologists can play a key role in recommending the withdrawal of antibiotic when it is not indicated, by providing guidance on appropriate antibiotic selection when treatment is warranted. Urinalysis is the most frequently used test for the evaluation of potential UTI. In addition, it can provide useful information related to screening and diagnosis of other conditions, including malignancy, proteinuria, glycosuria, ketonuria, and renal calculi. Accurate interpretation of urinalysis results is a key concept for health care providers in order to diagnose and treat patients appropriately. This article presents a simple and reliable urine culture technique for the diagnosis of UTI by identifying various infecting microbes and pathogens. Further studies are required to develop methods for urine culture techniques to identify more resistance pathogens and microbes.
Keywords: Culture, UTI. Ecoli, Urinanalysis, Bacteria, Pathogens.

INTRODUCTION

Escherichia coli (E.coli) from both outpatients and hospitalized patients are highly susceptible (>93%) to cefalothin, mecillinam and nitrofurantoin, and more than 75% of E. coli isolates are also sensitive to ampicillin.

Bacteria causing UTIs in Norway are less resistant to antibacterial medication than in other western countries and the reason for this may be the low consumption of antibacterial by the Norwegian population [1]. Significant rise in the resistance of E.coli to gentamicin and ciprofloxacin was detected in outpatient isolates. In the hospital, gram-negative urinary pathogens demonstrated increased resistance to ampicillin, cefuroxime, gentamicin and ciprofloxacin during the study period. The changing etiology of urinary tract infections and the increasing resistance of organisms indicate that periodic monitoring and possibly also modification of empirical therapy are required [2]. Resistance of E.coli isolated to trimethoprim-sulfamethoxazole varied significantly according to geographic region, ranging from a high of 22% in the western United States to a low of 10% in the Northeast. There were no clinically significant age-related differences in the susceptibility of E. coli to any of the study drugs, but the susceptibility to fluoroquinolones of non-E. Coli isolates that were recovered from women who were aged >50 years was significantly lower than that of isolates recovered from younger women. The in vitro susceptibility of uropathogens in female outpatients varies according to age and geographic region [3].

High rates of antimicrobial resistance in UTI pathogens, especially in non-European Union (EU) countries, where Pseudomonas aeruginosa presented rates of aminoglycoside resistance as high as 72% to gentamicin, 69.2% to tobramycin and 40% to amikacin have been observed. Nosocomial UTI accounts for an important proportion of the workload in microbiology laboratories. The levels and patterns of resistance of UTI pathogens must be a serious cause for concern and a clear reason for stricter guidelines and regulations in antimicrobial policy are required [4]. The incidence of

Nosocomial UTI (NAUTI) in a large European population is 3.55/1000 patient-days. There is clearly room for improvement in the area of bladder catheterization, catheter care and medical management of NAUTI [5]. Bacterial UTIs are an important cause of septicemia resulting in high mortality rates, prolonged hospital stays and increased healthcare costs. Periodic reviews of pathogen frequency and susceptibility patterns impact on appropriate antimicrobial usage are required for effective prescribing practices. There is need for continued surveillance studies for common infections which establish baseline resistance patterns by geographic areas, and have the potential to detect epidemics or direct local epidemiologic interventions [6].

Dysuria is one of the most common presenting complaints of young women, and urinalysis is one of the most common laboratory tests performed. Despite the fact that the midstream clean-catch technique is commonly used for urine collection, contaminated urine cultures occur with distressing regularity. The midstream clean-catch technique is time-consuming to explain, frequently not performed correctly by patients, costly for suppliers, often embarrassing for patients and staff, and of unproven benefit. Comparing the no-cleansing group with the combined cleansing, midstream groups also showed no difference in contamination rates. In young, outpatient women with symptoms suggestive of a UTI, the midstream clean-catch technique does not decrease contamination rates [7]. Culture of urine within four hours of voiding is likely to give a true indication of the presence or absence of infection. With further delay the interpretation of a heavy growth of bacteria in urine becomes progressively more unreliable, even if that growth is in pure culture [8]. Results of a study with large population indicated that an average of 18 bacteria per oil immersion field were observed in the urine of patients with significant bacteriuria, and an average of <1bacterium per oil immersion field was found in the urine of patients without significant bacteriuria. Direct susceptibility testing by Autobac proved to be rapid (3 h versus 24 h) and reliable (0.5 to 1.2% discrepancies) [9].

The 10-µL loop technique can be used as an alternative to the 50-µL drop technique for presumptive diagnosis of UTI in bacteriological practice, with the advantages of greater rapidity and ease of performance [10]. Centrifugation with Gram stain of a urine specimen offers excellent sensitivity but very poor specificity compared with microscopic urinalysis for the detection of asymptomatic bacteriuria and is not an acceptable screening test in an obstetric population. The false-negative rates of urinalysis (19.4%) and reagent strip testing (52.8%) preclude these from being excellent screening tests for asymptomatic bacteriuria. Given the potential sequelae of undiagnosed asymptomatic bacteriuria in an obstetric population, it has been concluded that urine cultures should be used for all pregnant patients to detect asymptomatic bacteriuria [11]. Noninvasive localization techniques continue to be explored as possible alternatives to invasive localization procedures, but they remain largely research tools that are not readily available to the practicing clinician. Understanding the applicability and appropriate use of newer technologies in the evaluation of patients with UTIs and how these technologies complement the standard diagnostic techniques will lead to better, more efficient, and less costly patient care [12].

General procedures for urine cultures

Urine cultures are performed to detect organisms that are the causative agents of UTI**.** Normally the urinary tract is sterile above the urethra. However, during noninvasive collection techniques, urine is potentially contaminated with normal flora of the urethra and genitourinary tract. For this reason, urine cultures utilize a colony count (quantitation of growth) to aid in determining if dealing with contamination, colonization, or infection. Infections are associated with counts of 100,000 (105) or more organisms per mL. However, low counts can be clinically significant in symptomatic patients. Selection of media and incubation requirements are based on the potential pathogens and bacterias isolated by culture techniques.

The common pathogens include

Enterobacteriaceae, nonfermenting gram negative rods, Staphylococcus saprophyticus, Enterococcus, Group B Streptococcus and yeast. Based on potential pathogens, in general media includes a nutrient agar along with a selective Gram Negative Rod (GNR) media is used. UTIs are not usually associated with fastidious organisms.

Cystitis/Lower UTI Symptoms

The most common symptoms associated with lower UTI include dysuria or acute pain, frequent urination, urgency, and incontinence. Occasionally, hematuria, cloudy urine, or foul-smelling urine may be present

Pyelonephritis/Upper UTI Symptoms

Compared with cystitis, pyelonephritis often has a more severe, systemic presentation. In addition to the urinary symptoms seen in cystitis, patients may also present with suprapubic pain, costovertebral angle tenderness (flank pain), fever, chills, elevated WBC count, nausea, and vomiting. Bacteria usually originate from the bowel, vagina, or skin as normal flora of the host.

Gram-positive organisms

Staphylococcus saprophytic us *(causative organism in 5% to 15% of UTIs)*
Enterococcus facials

Gram-negative organisms
Escherichia coli (causative organism in 85% of community-acquired infections)
Klebsiellapneumoniae
Proteus and Providencia species
Pseudomonas aeruginosa
Enterobacter and Serrati species

Some rare causes include
Salmonella species
Mycobacterium tuberculosis
Chlamydia trachomatis

Candida species (more common in immunocompromised patients, patients with diabetes, and patients who have recently received antibiotics)

Multiple microbial organisms causing infection may be found in patients with renal calculi, chronic renal abscesses, indwelling urinary catheters, or a fistula between the bladder and either the bowel or the vagina

Serious causes
Staphylococcus aureus *(*commonly a result of bacteremia, sometimes producing renal or perinephric abscesses in addition to bacteriuria)

Candida species (found in critically ill, immunosuppressed, and chronically catheterized patients)

MATERIALS AND METHODS

Sample collection

There are several different ways that may be used to collect a sample of clean urine. Urine may be collected by urinating in a cup using the clean-catch technique, or by collecting urine from an existing urinary catheter. Generally, there are no risks when collecting a clean urine sample using either of these methods. Another way to collect a clean urine sample is by inserting a temporary urinary catheter. Risks of using this method include bleeding, infection, catheter misplacement, and damage to the urethra or bladder. If they have a medical condition, or are using a medication or supplement that causes excessive bleeding, at a higher risk of bleeding. The person doing this test may need to perform it more than once

Urine collection

Blood agar plate brought from JC diagnostics was used for this study

Urine sterile container supplied by MB lab consumables was used for urine collection

- Early morning urine samples are preferred.
- Use the sterile screw-lid urine container provided by the laboratory.

Wash and dry your hands thoroughly. Remove the lid on the container and keep it upside down. Do not touch the inner surface of the lid or the container.

For women, keep the legs apart and hold the skin folds apart while voiding. For men, retract the foreskin (if uncircumcised) while voiding.

Clean genital area with novelette prior to voiding. Pass a small amount of urine into the toilet.

Midway through urination, fill the container to half full. Finish voiding in the toilet.

Replace the lid and tighten firmly. Wash and dry your hands thoroughly after collection

Label the container with the patient's first and last name, MCP, date of birth, and the date and time of collection of the sample.

For sanitary reasons, the container must be enclosed in a plastic biohazard bag.

Refrigerate the urine sample IMMEDIATELY and deliver to the Laboratory as soon as possible after completion of the collection.

Urine refrigerated for more than 24 hours cannot be used for Culture it will be rejected by the Laboratory.

Generally used media

- Blood Agar Plate (BAP)
- MacConkeyAgar (MAC)
- Muller-Hinton agar (MHA)

METHOD USED FOR THIS STUDY

Preparation of MacConkey Agar
Suspend 55.04 grams of MacConkey Agar (HiMedia) medium in 1000 ml purified/distilled water.
Heat to boiling to dissolve the medium completely
Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
Cool to 45-50°C.
Mix well before pouring into sterile Petri plates.

Preparation of Mueller-Hinton agar
Suspend 38 g of Mueller Hinton Agar (HiMedia) in 1 liter of purified water.
Mix thoroughly.
Heat with frequent agitation and boil for 1 minute to completely dissolve the components.
Autoclave at 121°C for 15 minutes
Cool to 45°C.
Mix well before pouring into sterile Petri plates.

The Techique

Wire loop that can hold 0.001 mL urine
Inoculated on MAC and BLOOD agar
Incubated at 35ºC overnight
Aerobically at 37ºC for 18-24hrs
After the incubation Colony count

Culture work up

Perform gram stain. Gram positive cocci (pairs, chains and clusters); Perform catalase. Catalase positive Perform; Coagulase testing. For identification and further Bio chemical tests; Catalase negative; Observe hemolysis pattern. Beta hemolytic (potential pathogens) Positive (further tests Streptococcaceae). Alpha hemolytic Positive (further tests Enterococcus) .Gamma hemolytic Positive (further tests Enterococcus)

Gram negative rods

Observe Mac Conkey Growth Lactose fermenter; Perform oxidase Negative further tests motility, Triple Sugar Iron (TSI) agar, indole, Mannitol, citrate, and Catalase

Non lactose fermenter

Perform oxidase; If negative further tests motility, Triple Sugar Iron (TSI) agar, indole, Mannitol, citrate, and Catalase

Yeast

Perform Germ tube Positive (Candida albicans)

Gram positive rods

Gram positive rods; Perform catalase Positive-Diptheroids

Long thin gram positive rods

Alpha hemolysis – probable lactobacillus

RESULTS

Identification of uropathogens

Identification of the isolated bacterial pathogens was done on the basis of gram staining, morphology and biochemical characters. [Catalase, TSI agar, indole reaction, citrate, urease, Mannitol, oxidase and motility agar].

Antimicrobial Susceptibility Testing

The positive, Negative cultures, antibiotic sensitivity discs were put on the Muller Hinton agar plates. The plates were incubated at 37ºC for 18-24 hours. The results of sensitivity plates were read after 24 hours. Negative cultures were reinsulated for another 24 hours and report was given as no growth at the end of 48 hours of incubation.

Antimicrobial sensitivity of the isolated pathogens was determined by using Kirby Bauer Disc Diffusion method the antibiotics tested were

Amikacin (AIK)
Aztreonam AT
Nitrofurantoin FU
Cefdinir CN
Cefixime FX
Cefotaxime CX
Ceftazidime CZ
Ceftriaxone FR
Cefuroxime sodium CR
Ciprofloxacin CI
Gentamicin GEN
Nalidixic acid NA
Norfloxacin NF
Ofloxacin OFL

The samples were analyzed for isolation and identification of bacterial isolates. Maximum samples were found to have significant bacteriuria and remaining samples were found to have either non-significant bacteriuria or very low bacterial count or sterile urine. Among the isolated pathogens the most common microbes isolated was E.coli followed by Klebsiellaspp, Streptococcus spp, Acinetobacterspp, Staphylococcus spp, Candida, Pseudomonas and Proteus. Antimicrobial sensitivity testing of the E.coli was done by using Kirby Bauer Disc Diffusion method. At the end of incubation period, the diameter of the zones of inhibition around each disc was measured with vernier calipers on the back of plate, with reflected light against a dark non-reflected background. Fourteen antibiotics commonly used were tested against the isolated E. coli. Amikacin was found to be the most effective drug (97.61%) followed by nitrofurantoin (90%).

Distribution of isolated urinary tract pathogens

- Escherichia coli
- Klebsiellaspp
- Streptococcus spp
- Acinetobacterspp
- Staphylococcus spp
- Candida spp
- Pseudomonas spp
- Proteus spp

Antibiotics sensitivity pattern

Amikacin -97.61%
Nitrofurantoin -90 %
Ofloxacin OFL-80%
Gentamicin GEN -75%
Cefuroxime sodium CR -60%
Cefdinir CN -50%
Ceftriaxone - 45 %
Ciprofloxacin CI – 40%
Cefotaxime CX - 35 %
Aztreonam AT– 30%
Norfloxacin NF – 25%
Cefixime FX – 20%

Ceftriaxone FR – 15%
Nalidixic acid NA -5%

DISCUSSION

Laboratory test results may vary depending on age, gender, Exhibiting symptoms of a UTI, Previous antibiotic therapy, health history, the method used for the test, and many other factors.

If the CFU was more than 10,000 it was considered significant bacteriuria. Such urine samples were further processed for identification and antibiotic susceptibility pattern was determined. If the CFU was less than 10,000 it was considered as negative for culture or non-significant bacteriuria. Mixed growth of two or more organisms especially with gram positive bacilli, Lactobacilli, Gardnerella vaginalis, diphtherias were considered to be urinary contamination. Repetition of urine examination was advised with early morning fresh urine specimen. Previous studies have founded out the importance of urine collection as bacterial UTIs are important cause of septicemia and we have taken precautionary measures in collecting urine sample [4,5]. Culture should be set up as soon as sample collection is over, as delay beyond 4 hours is unlikely to give correct result and this point have been taken into account in our study [7,8]. We followed 10-µL loop techniques instead of 50-µL which has greatly improved the culture result and our observation are in agreement with the previous study [10,11]. This research article will be very usefull to identify large number of pathogens and bacteria with the techniques established and shown in this article.

CONCLUSION

This article present a simple and inexpensive method to identify pathogens and bacteria causing UTIs in a simple microbiology lab set up. The preparation of the culture media is easy and the chemicals are locally available. The culture technique presented is simple to follow and the results obtained cover a wide range of pathogens and microbes. Both gram positive and negative organisms could be identified. A wide range of diseases causing pathogens and microbes could be easily identified. The sample collection techniques presented is an important aspect of this work which enabled to minimize the contamination. The contents of this research article will be very useful for setting up such technique in microbiology laboratories. More works should be done in this field to identify some missing pathogens and microbes not identifies in this study.

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