

Comparison of Urine Dipstick Nitrite Test with Urine Culture in the Diagnosis of Urinary Tract Infection

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ABSTRACT

Introduction

Urine dipstick is a rapid, cost-effective test used as a marker for quick detection of bacterial Urinary Tract Infection (UTI). Nitrite test depends on the conversion of nitrate to nitrite by the action of Gram-negative bacteria present in urine. The purpose of this study was to determine the sensitivity, specificity, positive predictive value and negative predictive value of Nitrite test in relation to urine culture.

Methods

Two hundred fresh uncentrifuged urine samples with suspicion of UTI were collected and tested for nitrite by using urine dipstick strip (COMBI-10SL) prior to the culture. Nitrite was considered as positive if there was a change in color of dipstick from colorless towards pink within 60 seconds. Quantitative urine culture was performed by using the strips calibrated to deliver 0.02 µL of urine on Cystine Lactose Electrolyte Deficient (CLED) agar. All plates were incubated at 37°C and read after 24 to 48 hours. Culture was considered as the gold standard to evaluate the performance of the dipstick test. Antimicrobial susceptibility testing (AST) of the isolates was done using the Kirby-Bauer disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results

Out of 200 samples, 36 (18.00%) samples showed significant growth whereas 164 (82.00%) samples did not show significant growth. Out of 36 samples, 25 (69.44%) samples showed positive nitrite test while 11 (30.55%) showed negative nitrite test. The sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of the nitrite test were 69.44%, 89.63%, 59.52% and 93.03% respectively. The most frequently isolated bacteria were *Escherichia coli* 29 (80.55%) followed by *Enterobacter aerogenes* 3 (8.33%) and *Pseudomonas aeruginosa* 2 (5.56%). The antimicrobial profile of the isolates revealed that 14 (38.89%) isolates were MDR.

Conclusions

Dipstick test for the detection of nitrite in urine is sensitive and specific and can be used for the primary screening of UTI in a resource-limited setup. Confirmatory diagnosis should be followed by the urine culture method.

Keywords: MDR, nitrite test, urine dipstick, UTI.

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INTRODUCTION

Urinary tract infection (UTI) is defined as the presence of multiplying bacteria within the urinary tract. The presence of a significant number of bacteria in aseptically collected urine is an indication of urinary tract infection. Despite the presence of different host defense mechanisms against microbial infection in the urinary tract, it is an important health problem affecting millions of people annually from all age groups.¹

The urinary tract consists of the kidney, ureter, bladder and urethra. All of the urinary tract above urethra in a healthy human is sterile. Thus, urine is a sterile body fluid that gets contaminated as it passes through urethra. UTI is among the most frequently acquired infection in the community, but also in hospitals and other health care institutions, causing a huge amount of antibiotic treatment calling for an update of current trends.² UTI is caused by the presence of bacteria in urine, although fungi and viruses may be involved. Most often the bacteria involved in UTI are *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp., *Proteus* spp., *Staphylococcus saprophyticus*, *Staphylococcus aureus*, coagulase-negative *Staphylococcus* (CoNS) and beta-hemolytic Streptococci. Among these, *E. coli* is the most predominant uropathogens accounting for 80-85% cases.^{3,4}

In more complicated UTI, particularly in recurrent infections, the relative frequency of infection caused by *Proteus*, *Pseudomonas*, *Klebsiella* and *Enterobacter* spp. increases. Hospitalized patients are more likely to be infected by *E. coli*, *Klebsiella* spp., *Proteus mirabilis* and Enterobacteriaceae. In addition, UTIs are the leading cause of Gram-negative sepsis in hospitalized patients and are the origin of about half of all nosocomial infections caused by urinary catheters.⁵ *Candida* urinary infection

is usually found in diabetic patients and those with immune suppression.⁶ Risk factor includes female anatomy, sexual intercourse, diabetes, obesity and family history.⁷

Many prompt diagnostic methods are available including wet-mount microscopy, Gram stain, dipstick and automated assays, but the gold standard method for diagnosis of UTI is quantitative urine culture.⁸ Urine culture is an expensive procedure and needs a well-equipped microbiology laboratory with experienced technicians.⁹

A dipstick nitrite test is sensitive and specific when properly used for first-morning urine because bladder incubation for the organism to convert nitrate to nitrite takes a minimum of four hours.¹⁰ So random specimens collected at any time and urine from patients with draining catheters do not show a good correlation between significant bacteriuria and nitrite test. False positive results commonly occur with poorly collected or stored specimens resulting in contamination and post collection bacterial proliferation. False negative results may be due to low pH (<6), ascorbic acid or urobilinogen.¹¹

Rapid diagnostic tests can rule out urine infection, are inexpensive, less time-consuming and are useful in small laboratories having no culture facility. They are also more rapid than culture in diagnosing UTI. A sterile urine sample is not required for dipstick test, therefore, it is easy to collect samples especially in children by a noninvasive method.¹² For dipstick method of diagnosing UTI, there is no requirement of trained staff and well-equipped laboratory.¹³ Due to the clinical significance of early diagnosis, rapid urine tests such as urine dipsticks are used widely.¹⁴ In recent times, antimicrobial resistance among clinical isolates is ever-increasing and posing public health threat.¹⁵ UTI is often treated

with different broad-spectrum antibiotics when one with a narrow spectrum of activity may be appropriate because of concerns about infection with resistant organisms.¹⁶ Culture of urine for specific bacteria followed by antibiogram testing is still regarded as the best way to diagnose UTI.¹⁷

The major objective of the study was to evaluate the accuracy of dipstick nitrite tests for rapid screening of urine samples, keeping semi quantitative culture as the gold standard for the diagnosis of UTI. In addition, the study also investigated the common uropathogens and antimicrobial susceptibility patterns of the isolates recovered from the patients visiting Bharatpur Hospital.

METHODS

The study was a descriptive study carried out prospectively at the Microbiology Laboratory of Bharatpur Hospital, Chitwan. The study was conducted over a period of three months, from November 2018 to January 2019. A total of 200 samples were collected from patients suspected of UTI. Patients of all age groups and sex were included in the study. All midstream urine samples received in the Microbiology laboratory for culture were recruited in the study, whereas the samples showing insignificant growth, mixed growth, samples from the patients who had taken antibiotics before 72 hours or had indwelling Foley Catheters were discarded. Samples with improper labeling and inappropriate collection methods were also rejected. Clean catch, Mid-Stream Urine (MSU) samples with suspicion of UTI were collected in a sterile, wide-mouthed bottle and tested for nitrite by using dipstick nitrite test strip (COMBI-10SL, UK). Nitrite was considered as positive if there was a change in color of dipstick from colorless towards pink within 60 seconds. Urine culture was performed

by using the strips calibrated to deliver 0.02 μ L of urine on Cysteine Lactose Electrolyte Deficient (CLED) agar. All plates were incubated at 37°C and read at 24 and 48 hours. Culture was considered as the gold standard to assess the performance characteristic of dipstick test. Obtained isolates were analyzed for the antimicrobial resistance pattern. For this, a panel of 10 antibiotics [Amikacin (30 μ g) Amoxycylav (30 μ g) Ciprofloxacin (5 μ g) Cefixime (30 μ g) Levofloxacin (5 μ g) Nalidixic acid (30 μ g) Nitrofurantoin (300 μ g) Cotrimoxazole (25 μ g) Gentamicin (10 μ g) Cefalexin (30 μ g) Polymyxin-B (300 units) Colistin (10 μ g)] were used. Antibiotic sensitivity testing was performed by Kirby Bauer disc diffusion method and interpreted as per CLSI guidelines (2016).¹⁸ The turbidity of the inoculum was adjusted to the equivalent turbidity of 0.5 McFarland standards. Eighteen hours cultures of test organisms incubated at 37°C were standardized by diluting to 0.5 McFarland turbidity standards before spreading over the surface of Mueller-Hinton agar (Titan Biotech Ltd., Bhiwadi-301019, Rajasthan, India) plates using a sterile cotton swab and allowed to dry for 2 to 5 minutes. By using sterilize tweezers, antibiotic discs were placed 2 cm apart from each other. The plates were incubated at 37°C for 24 hours. Following incubation, the diameter of the inhibition zone was measured with a transparent ruler and expressed in millimeters (mm) as sensitive, intermediate and resistant based on CLSI guidelines. Resistance to at least one drug from 3 different antibiotics of different structural classes was considered MDR as described elsewhere.¹⁹ Data were analyzed by using Statistical Package for Social Sciences (SPSS) version 16. Diagnostic measures i.e., sensitivity, specificity, PPV and NPV were calculated by standard formulae using culture as gold standard.

RESULTS

Growth pattern

Out of 200 mid-stream urine samples, 36 (18%) samples showed significant growth whereas 164 (82%) samples did not show significant growth. Positive samples showed the growth of different Gram-negative organisms.

Comparison between nitrite test and culture

Out of total samples (n=200), 36 samples showed significant growth of which 25 samples showed nitrite test positive while 11 showed nitrite test negative. Among total of 42 nitrite test positive samples, 17 growth negative samples showed nitrite test positive while 147 growth negative samples showed nitrite test negative. The sensitivity and Specificity of the nitrite test were 69.44% and 89.63%. Also, positive predictive value and negative predictive value of nitrite test were 59.52% and 93.03% respectively considering culture as the gold standard (Table 1).

Nitrite test	Growth	No Growth	Total
Positive	25	17	42
Negative	11	147	158
Total	36	164	200

Sensitivity: 69.44%

Specificity: 89.63%

Positive predictive value: 59.52%

Negative predictive value: 93.03%

Bacteria isolated	Frequency	Number of nitrite positive isolates	Number of nitrite negative isolates
<i>Escherichia coli</i>	29	22	7
<i>Enterobacter aerogenes</i>	3	2	1
<i>Pseudomonas aeruginosa</i>	2	1	1
<i>Klebsiella pneumoniae</i>	1	-	1
<i>Proteus vulgaris</i>	1	-	1
Total	36	25	11

Distribution of pathogens

Out of 200 mid-stream urine samples, 36 (18.00%) Gram-negative bacteria were isolated. Among them, *Escherichia coli* 29 (80.55%) was predominant one followed by *Enterobacter aerogenes* 3(8.33%), *Pseudomonas aeruginosa* 2(5.56%), and *Klebsiella pneumoniae* 1 (2.78%).

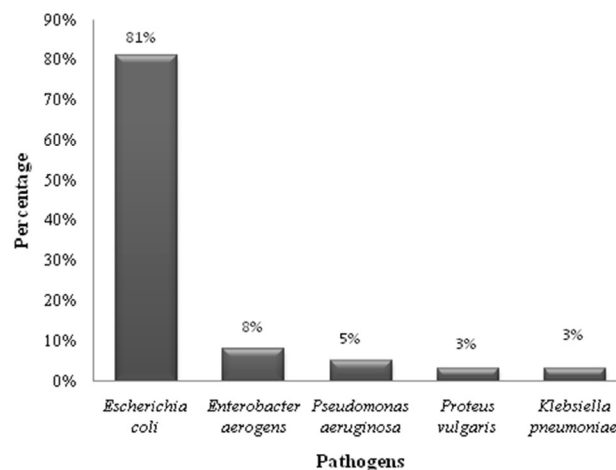


Figure 1. Distribution of pathogens

Comparison of isolates between nitrite test and culture

Escherichia coli 29(80.55%) were predominant with 22(88.00%)nitrite test positive and 7(63.63%) nitrite test negative followed by *Enterobacter aerogenes* 3(8.33%) with 2(8.00%) nitrite test positive and 1(9.09%) nitrite test negative and *Pseudomonas aeruginosa* 2(5.56%) with 1(4.00%) positive and 1(9.09%) negative nitrite test. The least isolated were *Klebsiella pneumoniae* and *Proteus vulgaris* (2.78%), both were nitrite test negative (Table 2).

Antibiotic susceptibility pattern of the isolates

Antibiotic susceptibility testing of the isolates revealed that isolates belonging to Enterobacteriaceae were susceptible to gentamicin (79.41%) and amikacin (76.47%). Antibiotics such as nalidixic acid (20.59%), cefalexin (35.29%) and amoxycylav (26.47%) were least effective drugs. In case of *P. aeruginosa*, polymyxin B, colistin and ciprofloxacin were the most effective antibiotics. Among 36 isolates, 14 (38.89%) were MDR of which *E. coli* 11 (78.58%) were the predominant one. Two (14.28%) isolates of *E. aerogenes* and a single isolate of *K. pneumonia* were MDR. Details of the antimicrobial patterns was shown in table 3.

of UTIs. Also, the study determined common uropathogens among the UTI-suspected patients visiting Bharatpur hospital.

The sensitivity and the specificity of nitrite dipstick have been found varying in different studies when compared to urine culture as the gold standard. In the current study, the sensitivity of urine nitrite dipstick test was 69.44% while specificity was higher (89.63%). This was however unparallel to other studies where sensitivity between 27.3% to 43.6% and high specificity from 99.6% to 100% was reported.^{20,21} Another study conducted in India showed the sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of nitrite test were 57.1%, 78.7%,

Table 3. Antibiotic susceptibility pattern of the isolates

Antibiotics	Antibiotic susceptibility pattern					
	Enterobacteriaceae (n = 34)			<i>P. aeruginosa</i> (n = 2)		
	S	I	R	S	I	R
Amikacin	76.47%	2.94%	20.59%	50.00%	50.00%	0.00%
Amoxycylav	26.47%	23.53%	50.00%	0.00%	0.00%	100.00%
Ciprofloxacin	50.00%	11.76%	38.24%	100.00%	0.00%	0.00%
Cefixime	26.47%	11.76%	61.76%	0.00%	0.00%	100.00%
Levofloxacin	50.00%	11.76%	38.24%	0.00%	100.00%	0.00%
Nalidixic acid	20.59%	8.82%	70.59%	NT	NT	NT
Nitrofurantoin	52.94%	5.88%	41.18%	100.00%	0.00%	0.00%
Cotrimoxazole	41.18%	52.94%	5.88%	NT	NT	NT
Gentamicin	79.41%	0.00%	20.59%	50.00%	0.00%	50.00%
Cefalexin	35.29%	8.82%	55.88%	0.00%	100.00%	0.00%
Polymyxin B	NT	NT	NT	100.00%	0.00%	0.00%
Colistin	NT	NT	NT	100.00%	0.00%	0.00%

S: Sensitive, I: Intermediate, R: Resistant, NT: Not Tested

DISCUSSION

The present study was aimed at evaluating the efficacy of a rapid urine dipstick test in comparison to urine culture for the diagnosis

42.7% and 86.8% respectively.²²

This difference in the values may be because of the different sample populations in different studies like high-risk populations, gender, old age population and because of different brands of strips used for dipstick biochemical analysis.

The variations in the results might also be attributed to improper techniques for collection or transportation to the laboratory, allowing the colonizing bacteria to multiply, which result in false positive nitrite test which may result in under treatment and as consequences could cause real damage or sepsis to the urinary tract system. Nitrite negative results may occur due to a low colony forming unit count or dilute urine. In addition, a nitrite test does not detect organisms unable to reduce nitrate to nitrite, such as Enterococci, Staphylococci species, *Acinetobacter*. Another disadvantage of the nitrite test is that the causative microorganism and its antibiotic susceptibility are not known.^{21,23}

Among 36 bacterial isolates recovered, all were Gram-negative bacteria. The result of this study is in tune with the previous studies where the prevalence was between 95-99.30%.²⁴ The reason behind obtaining the majority of Gram-negative isolates may be them being the normal habitat of the colon and their descent to the urinary tracts, urinary bladder and kidney caused urinary tract infection.²⁶

In this study, *Escherichia coli* (80.55%) was found to be predominant followed by *Enterobacter aerogenes* (8.33%), *Pseudomonas aeruginosa* (5.56%), *Proteus vulgaris* and *Klebsiella pneumoniae* (2.78%). Higher prevalence of *E. coli* in this study also resonates with the study done by Baral and co-workers.²⁶ Bhansali and his colleagues also reported a higher percentage of *E. coli* (56%) than other isolates.²⁷ Likewise, a previous study done in Western Regional Hospital, Pokhara also reported *E. coli* (50%) as the most predominant uropathogen.²⁸

E. coli is far most common pathogen isolated from urinary tract infection (UTI) and frequently originated from patient's intestinal flora. There are many components or products called virulence factor that helps the *E. coli* to

colonize the mucosal uroepithelium causing an inflammatory reaction and eventually helps to precede infection from the lower urinary tract to the renal cavities and tissues. The common virulence factors of *E. coli* are mainly two types; Surface virulence factors that are produced on the surface of the cell and exported factors those produced within the cell and exported to the site of action. Surface virulence factors are of fimbrial nature adhesions apparatus which includes Type 1 fimbriae, P fimbriae, S fimbriae, F1C fimbriae, curli, flagellum, capsule and lipopolysaccharide. Similarly, some of the exported virulence factors include alpha haemolysins of RTX family, Cytotoxic necrotizing factor I, Cytolysin A, Enterobactin etc. that helps *E. coli* to attach to the mucosal epithelium and tissue matrix, helps in motility, adaptation, bio-film production as well as cytokines induction.²⁹

In this study, the second commonest pathogen was *Pseudomonas aeruginosa* which accounted for 5% (2/36). A similar study also reported 5.01% *P. aeruginosa* among total isolates.³⁰ However, the result is in contrast with the study studied done in Nepal where the prevalence rate is less than 1%.^{25,31}

This study found that gentamicin (79.41%) and amikacin (76.67%) were the drugs of choice for infection caused by Enterobacteriaceae. Similar study done in Western Nepal, reported parallel result as 70.40% Enterobacteriaceae were susceptible to gentamicin.³² This study showed 70.59% resistivity towards nalidixic acid which resembles the study done elsewhere.^{33,34} All isolates of *P. aeruginosa* were completely susceptible to ciprofloxacin, colistin and polymyxin B. Mostly resistance to fluoroquinolones could be the generalized use of fluoroquinolones in animal feed (especially in poultry), and the subsequent transmission of resistant strains from animals to humans.³⁵ This study recorded 38.89% MDR isolates. This rate is

similar to the one done in Bharatpur hospital, in Nepal, which recorded 33.90% MDR isolates,³⁶ whereas Awasthi et al. (2015) reported a higher rate of MDR isolates (42.9%) as compared to this study.¹ The increasing rate of MDR may be attributed to excessive use of broad-spectrum antibiotics as observed in this study along with adherence to infection control measures.³⁷

CONCLUSIONS

For bacterial growth on culture media by standard Laboratory techniques upto 48 hours are required which means that diagnosis is undecided for a longer duration which leads to delay in treatment. Although culture is the gold standard test for diagnosis of UTI, dipstick

tests for the detection of nitrite in urine are sensitive and specific enough and can be used as a diagnostic test for detection of UTI in resource-poor setup, where facility of culture is not available. Regular monitoring of susceptibility patterns of antibiotics along with their careful usage are advised to avoid the dissemination of the drug resistant isolates.

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