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Original Research Article

Rapid Nitrite Dip Stick Vs Urine culture for diagnosis of Urinary tract Infections (UTI): Laboratory prospective

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Abstract

Background: Urinary tract infection common bacterial disease with all age group causing lower to complicated UTI leading to life threatening condition urosepsis. Urine culture is gold standard however urine dipstick test can help in early detection of UTI by avoiding the complication of UTI. The study aims to compare the diagnostic accuracy of rapid nitrate dip stick test with urine culture and identify the common uropathogens and their antimicrobial susceptibility pattern.
Materials and Methods: Clean catch mid stream urine samples from suspected cases of UTI were evaluated by rapid nitrate dip stick and culture. The culture isolates were identified and antimicrobial susceptibility test was performed following Clinical and Laboratory Standards Institute (CLSI) guidelines.
Results: Total of 202 urine specimens evaluated, out of which 46(22.77%) were positive for rapid nitrate dip stick test, but 42(20.79%) were culture positive. Among the culture positives, nitrate test was positive in 29(69.04%). Gram-negative bacilli were predominant (95.25%), *Escherichia coli* (21) being most frequent isolate followed by *Enterobacter* (6), *Klebsiella* (5) and *Acinetobacter* (3). The GNB were mostly susceptible to imipenam (75%), amikacin (62.5%) and cotrimoxazole 45%. The overall sensitivity, specificity, positive and negative predictive values of nitrite test in relation to culture were calculated to be 69.04%, 89.4%, 63.0% and 91.6%, respectively but the value of pyuria in relation to culture was 36%, 60%, 68% and 55%, respectively.
Conclusion: The study reveals that the rapid nitrate dip stick test can be used in conjunction with urine culture for diagnosis of UTI but cannot replace it entirely.
Keywords: UTI, Urine culture, dip stick.

1. Introduction

Urinary tract infection (UTI) is the most common bacterial infections encountered in once in life span either in community or, hospital settings and also a common cause of nosocomial infection among hospitalized patients [1]. UTI is associated with multiplication of organisms in the urinary tract. It can be defined as the microbial invasion of any of the tissues of the urinary tract extending from the renal cortex to the urethral meatus. In most instances, growth of $>10^5$ colony forming unit per milliliter (CFU/mL) from a properly collected midstream "clean-catch" urine sample indicates infection and the most common etiological agents of UTI are *Escherichia coli* accounts for 80 to 90% of infections, and other gram-negative rods such as *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas species*, also *Streptococci*, *Staphylococcus species* alone with certain viruses, fungi and parasites [2]

In the community, women are more prone to develop UTI. It has been observed that about 20% of the women experienced a single episode of UTI during their life time, and 3% of women had more than one episode of UTI

per year. UTI is also commonly seen in children and it is estimated that at least 1% of boys and 3% of girls develop urinary tract infection during first ten years of life, mainly due to the ascending infection from the urethra. Similarly, catheter-associated UTI in hospital setting, a trenchant problem with about 10% of the patients develops bacteriuria [2].

The diagnosis of UTI should be based initially on the clinical aspects presented by the patient but the definitive diagnosis depends on positive bacterial culture of urine (gold standard). The method is expensive, and time consuming because the result usually takes 24 -48 hours to be available for the clinician and patients are often treated before results are available. Different rapid urine dip stick tests are now available like leukocyte esterase and nitrites tests are useful for early detection of UTI [3].

A dipstick nitrite test is highly sensitive and specific when properly used in first morning urine, because bladder incubation for the organism to convert nitrate to nitrite takes a minimum of four hour [3, 4, 5]. So random specimens collected at any time and urine from patients with draining catheters do not show good correlation between significant bacteriuria and nitrite test. False positive results commonly occur with poorly collected or stored specimens resulting to contamination and post collection bacterial proliferation. False negative results may be due to low pH (<6), ascorbic acid or urobilinogen [6-8].

The microscopic examination of urine for the presence of pyuria with nitrite test is taken as highly reliable marker for bacterial UTI [8]. Thus purpose study was aimed to find out the common uropathogens and its antimicrobial susceptibility patterns in our local setting and also to correlate of rapid urine dip stick test with urine culture.

2. Materials and method

Urine specimens that were routinely sent for culture and sensitivity in microbiology laboratory, B. P. Koirala Institute of Health Sciences (BPKIHS), from May 2012 to November 2012 were included. During that period of time 202 urine specimens were evaluated and they were subjected for microscopy, dipstick. This study had been conducted on the approval of academic division of the Institute. The freshly midstream urine (MSU) specimens were collected under sterile conditions as far as possible. For this a sterile, dry, wide-necked, leak proof container was given to the patient with an advice to collect the urine with as little contamination as possible, i.e. a 'clean catch' specimen. In infants, the application of an adhesive, sealed, sterile collecting bag after disinfecting of the skin of the genitals was suggested for obtaining urine and from toilet-trained children, a midstream urine samples were taken.

Then, the collected samples were labeled with patient identification number and serial number. Patient who had taken antibiotics in the past 72 hours, or had indwelling Foley catheters, symptomatic vaginal discharge, diabetes mellitus, or immunodeficiency disorders should be informed in prescription form and with no delay urine samples were transported to the microbiology laboratory of BPKIHS. A complete urinalysis included physical, chemical, and microscopic examinations, so urine samples were divided into 3 sterile aliquots, 2 for urinalysis and 1 for urine culture.

2.1 Urine dipstick chemical analysis

Urine dipstick is a useful and commonly used test because of its rapidity and low cost. "CYBOW" reagent strips were chosen for urine analysis. The strips include reagent pads for semi-quantitative assessment of nitrite, pH, specific gravity, protein, glucose, ketones, urobilinogen, bilirubin, and blood.

2.1.1 Test procedure:

The strip was dipped into the urine up to the test area for no more than 60 seconds. The edge of the strip along the brim of the vessel was drawn to remove excess urine because excessive urine on the strip may cause the interaction of chemicals between adjacent reagent pads and results were recorded.

2.2 Microscopic examination for pus cells

Urine was examined directly under microscope for pus cells. A count more than 5 pus cells per high power field was considered as pyuria.

2.2.1 Gram's staining

Smears were prepared using 2 drops of centrifuged or sediment urine on a slide within the standardized marked area (1.5 cm in diameter), air-dried, fixed, and Gram-stained. Then, the morphology and Gram stain of bacteria were recorded.

Urine culture was performed by Calibrated loop direct streak method. Using a 4 mm diameter Nichrome loop delivering 0.001 ml, one biconvex loop full of well mixed uncentrifuged urine specimen was inoculated on Cysteine lactose electrolyte deficient (CLED) media.

2.2.2 Isolation and culture

Plates were streaked by passing loop through inoculum downwards to the lower edge of the plate in a T pattern from the inoculum site then incubated overnight at 37^0 C and reading was done next morning. Colonies were examined and counted on all the plates. Colony forming units were estimated from blood agar plate. In each case colonies were multiplied by 1000, to give an estimate of the number per milliliter of urine. One hundred colonies on agar plate i.e. 10^5 bacteria per ml were taken as significant bacteriuria.

After determining the colony forming units, we processed for further identification and the susceptibility to antimicrobial. Mixed growth of three or more organisms was considered as contamination and repeated for sample. In case of absence of growth, specimens reported as no growth after 24 hrs [9].

Bacterial pathogens were identified by conventional biochemical methods according to the microbiological technique. The biochemical tests which were performed were catalase, Coagulase, Oxidase, Citrate utilization test, Urease test, Sulphide indole motility and Triple sugar iron test. Antimicrobial susceptibility of the isolates was determined by Kirby- Bauer Disc Diffusion method on Mueller Hinton agar according to Clinical Laboratory Standards Institute (CLSI) guidelines [10].

3. Results

The study was performed in 202 samples of UTI suspected patients at B.P.Koirala Institute of Health Sciences (BPKIHS) in the microbiology laboratory. Out of 202 UTI suspected cases, 42 (20.8%) were cultures positive. Of that culture positive cases 30 were females (72. %), 11 males (26. %) and 1 boy (2%). Among them the study shown that age groups 20-40 were found to be highly suffered from UTI.

Figure 1: Distribution of culture positive cases according to sex

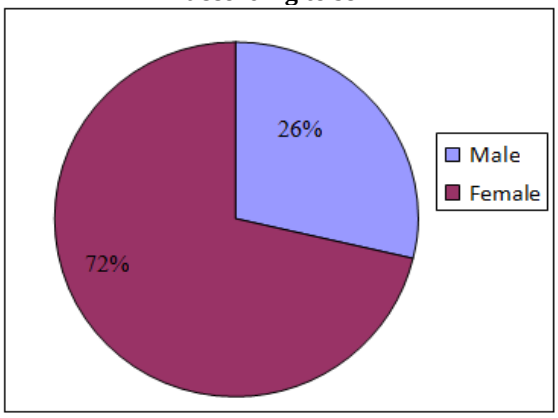


Table 1: Comparison between the result of nitrite test and culture

	Culture positive	Culture negative	Total
Nitrite positive	29	17	46
Nitrite negative	13	143	156
Total	42	156	202

Of total samples (n=202), 46 specimens were found nitrite test positive, of which 29 specimens (63.04%) shown culture positive, while of 156 negative nitrite test, 143 specimen (91.6%) shown culture negative Shown in Table 1.

Table 2: Microscopic detection of pus cells in urine specimen

Culture results	Pus cell ≤5/HPF	Pus cell >5/HPF	Total
Positive	12	30	42
Contamination	1	7	8
Negative	109	43	152
Total	122	80	202

Table 3: Correlation of nitrite and urinalysis with urine culture results

Culture results	Nitrite		Pyuria	
	No.	%	No.	%
Positive (n=42)	29	69.04	30	71.42
Contamination (n=8)	4	50	7	87.5
Negative (n=152)	17	11.18	43	28.28

The microscopic urinalysis and its correlation with nitrite test and culture shown that, among 42 positive cultures, the nitrite was present in 29 (69.04%) with a significant pyuria in 30 (71.42%) of urine culture positive, having pus cell counts >5/hpf. Seven specimens had significant pyuria (87.5%) among contaminated cultures and 43 specimens (28.28%) had negative cultures, having pus cell > 5/hpf as shown in the Table 3 and 2.

Table 4: Comparison of pyuria with gram stain

	Gram staining	
Wet mount (Pus cells>5/hpf)	Gram positive organisms	Gram negative organisms
30	2	25

Among 202 urine specimens, 30 samples showed presence of pus cells >5/hpf where only 27 organisms were seen in which two were gram positive cocci and 25 were gram negative bacilli shown in Table 4.

Table 5: Reliability of nitrite, pyuria, and culture in the laboratory diagnosis of UTI

	Nitrite (%)	Pyuria (%)
Sensitivity(n=42)	69.04	36.0
Specificity(n=160)	89.4	60
Positive predictive value	63	68
Negative positive value	91.6	55

The dipstick nitrite test and pyuria had a sensitivity of 69.04% and 36.0% respectively and specificity of 89.4% and 60.0%respectively.Similarly, positive predictive value of nitrite test and pyuria were 63.0% and 68.0% and negative predictive value were 91.6% and 55.0% respectively, as shown in the Table-5.

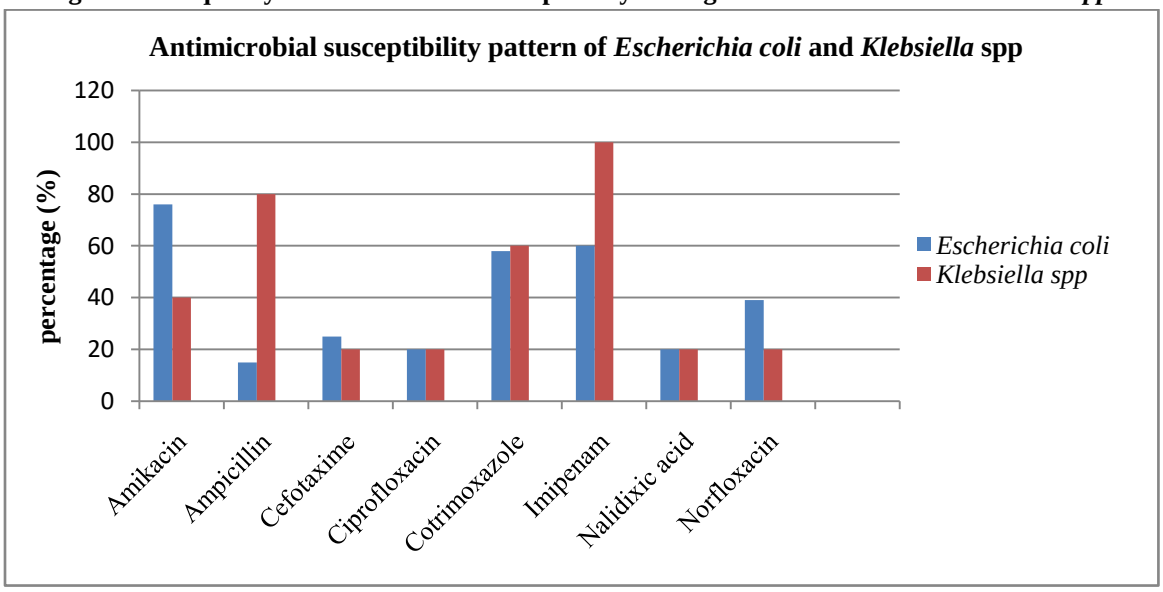
Among total culture positive cases, 42 (95.2%) gram negative bacteria of which *Escherichia coli* (21), *Enterobacter spp* (6), *Klebsiella spp* (5**)**. The details are given in Table-6.

Table 6: Growth based on the culture medium (total=202)

Growth based on the culture medium (total=202)	
Culture Sterile	15
Contamination	8
Enterococcus Spp	2
Escherichia coli	21
Enterobacter Spp	6
Klebsiella pneumoniae	5
Acinetobacter Spp	3
Citrobacter Koseri	2
Proteus mirabilis	1
Pseudomonas aeruginosa	2

It was found that *Escherichia coli* were susceptible to amikacin (76.2%) followed by imipenam (61.9%). The details are shown in Figure 2.

Figure 2: Frequency of Antimicrobial Susceptibility among *Escherichia coli* and *Klebsiella spp.*



4. Discussion

Urinary tract infection (UTI) exists when pathogenic microorganisms are detected in the urine, urethra, bladder, kidney, or prostate. In most instances, growth of $>10^5$ colony forming unit per milliliter (CFU/mL) from a properly collected midstream "clean catch" urine sample indicates infection [2]. However, several rapid screening tests are used commonly to make a presumptive diagnosis of UTI, including dipstick biochemical analysis of urine for nitrites or leukocyte esterase, as well as microscopic examination of urine for formed elements including white blood cells or bacteria. Numerous studies have been published concerning the usefulness of these tests in diagnosing UTI [2].

In this study UTI was found in 42 of 202 outpatients, among them females (71%) were more infected than males (29%) and age groups 20-40 were found to be highly suffered from UTI. Of the 42 positive culture, 30 presented with pyuria (71.4%) and 29 with nitrite positive (69.04%). The nitrite test was found to have high sensitivity (69.64%) and its specificity (89.4%). The positive predictive value (PPV) and negative predictive value (NPV) for nitrite test were 63.0% and 91.6% which concordance to our findings reported by Mustafa *et al* who found the sensitivity and specificity for nitrite 61.7% and 96.9% respectively and the positive predictive value for nitrite was 95.4% [1]. Also Carias MJ B *et al* had found sensitivity for nitrite test as 78.6% [5]. In contrast, May Mohamed Ali [8] found that sensitivity for nitrite test was 39.3% while specificity and PPV were 87.6% and 74% respectively and also in study conducted Subramanian R *et al* [3]by reported sensitivity 36.6% which is lower to our findings where as specificity 99.9% positive predictive value 98.3% negative predictive value 87.8% were higher than ours. This difference may be because of different sample population for these studies or because of the different brands of strips used for urinalysis. It may also be due to improper techniques for collection or transportation to the laboratory, allowing the colonizing bacteria to multiply, which result in false positive nitrite test [1, 8].

In the present study, pyuria (>5pus cell /hpf) was detected in 80 specimens however only 30 specimen were culture positive and the sensitivity, specificity, PPV and NPV for pyuria were 36%, 60%, 67% and 55% respectively. Mustafa *et al* [1] reported detection of pyuria had low accuracy, compared to test for nitrite. Furthermore, the study conducted by Mac Dermott found that there was no correlation between the degree of pyuria and a significant urine culture [11].

Most of urinary tract infections are caused by gram-negative bacteria like *Escherichia coli, Klebsiella species, Proteus mirabilis, Pseudomonas aeruginosa, Acinetobacter, and Serratia.* 90% of UTI cases are caused by gram-negative bacteria while only 10% of the cases are caused by gram positive bacteria.

Taneja N *et al* [12] also reported that organisms implicated in culture positive UTI were *Escherichia coli* (62.2%), *Klebsiella pneumoniae* (9.2%), *Enterobacter aerogenes* (5.1%), *Enterobacter cloacae* (1%), *Citrobacter diversus* (2%), *and Pseudomonas aeruginosa* (5.1%). MA Mazed reported that Gram-negative bacteria were the commonest organisms isolated, among which *Escherichia coli* was the principal urinary pathogen [13]. Sharma A *et al,* also reported *Escherichia coli* was the most common (67.5%) bacterial isolate followed by *Klebsiella spp* (20.0%) and *Proteus spp* (10.0%) [7]. The higher number of *Escherichia coli* in UTI may be related to hygienic practice leading to their acquisition through anterior urethra and it is especially higher among females due to contamination of perineum through fecal flora. Adherence properties of some organisms prevent the normal washout these organisms by bladder emptying and mucosal host defense mechanisms. Although *Escherichia coli* being a normal flora of a colon is virulent due to the presence of *P. fimbriae*, which may attach or adhere on specific receptors of uroepithelial cells and interfere with the washout of bacteria. The hospital-acquired infections following instrumentation and catheterization are mostly caused by *Pseudomonas* and *Proteus spp* [2, 4, 6, 13].

The antimicrobial susceptibility patterns of isolates were variable. The study had shown that imipenam and amikacin were found to be the most effective antimicrobial agents among the gram negative bacteria. Among the *Escherichia coli* isolates, 76.2% were highly sensitive for amikacin followed by imipenam (62%) where as *Klebsiella spp* isolates were 100% sensitive for impenam. Subedi M *et al* [6] suggested that amikacin as highly effective against gram negative bacteria, reported 64.2% of *Escherichia coli* being susceptible to amikacin. Similarly, A Sharma *et al* also reported *Escherichia coli* were more than 80% sensitive to amikacin [7]. MA Mazed, reported that members of the *Enterobacteriaceae* were 80%-100% sensitive to imipenam and 75% were sensitive to amikacin also added that the clinicians should use imipenam selectively in cases of un-responsiveness to commonly used antibiotics [13].

5. Limitation of the study

This study limits to find out the relationship between bacterial colony counts with the count of the pus cells on microscopy, since low bacterial count might give negative result on microscopy. And also the nitrate urine dipstick only used could miss out the organisms that do not utilize nitrate to nitrite.

6. Conclusion

Among 42 culture bacterial growth, 69.04% were nitrite test positive and 71.42% showed significant pyuria. The negative predicate value of nitrite test was 91.6%, and specificity 89.4% and sensitivity was 69.04%. The study reveals that the rapid nitrate dip stick test can be used in conjunction with urine culture for diagnosis of UTI but cannot replace it entirely.

Although, confirmatory diagnosis can be achieved by urine culture method, the combination of Nitrite test with pyuria appears to be rapid test and very useful markers for bacterial UTI in district laboratories where culture facilities are rare.

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