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Original Research Article

Rapid Urine Cultures - Revolutionizing UTI Management for Faster and Better Patient Care

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Abstract:

Purpose: Rapid reporting of urine cultures is important so as to avoid unnecessary antibiotic use in absence of infection. Also, use of broad spectrum antibiotics for treatment of urinary tract infections (UTI) in hospitalised patients without de-escalation should be curtailed. We aimed to provide negative result for urine cultures within 6-24 hours as well as antimicrobial susceptibility testing (AST) result within 24 hours for positive monomicrobial urine cultures with use of rapid automated system HB&L Light.

Methods: The prospective observational study was carried out in Department of Microbiology between October 2022 and May 2023. Total number of urine specimens tested was 432. Growth results by both conventional culture (CC) and Uro-Quick (UQ) methods were compared. Antimicrobial susceptibility testing results by both CC and UQ isolates were compared wherein UQ isolates were directly used from either subculture agar or positive Monomicrobial pellet with single and double wash technique.

Results: Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of rapid automated urine culture system was found to be 97.7%, 100%, 100% and 100% respectively. 100% agreement was found in comparison of AST result amongst UQ isolates (processed from solid agar subculture) and corresponding CC isolates (n=20). Agreement for fluoroquinolones and nitrofurantoin ranged from 85% to 90% when UQ isolates (bacterial pellet from positive vial obtained after one wash technique) and corresponding CC isolates were compared for AST results (n=27). More than 90% agreement was observed for fosfomycin, beta lactam antibiotics except for 3rd generation cephalosporins, aminoglycosides and colistin. However, after double wash technique, agreement for AST results increased upto 97% to 100% (n=31).

Conclusion: HB&L Light system can be used for rapid reporting of negative as well as positive urine cultures. Antimicrobial susceptibility testing results can be reported within 24 hours for positive monomicrobial urine cultures on rapid automated system.

Keywords: Rapid, Automated, Urine, Culture, Monomicrobial, Antibiotic.

Introduction

Urinary tract infections (UTIs) are the most common cause of community acquired infections second only to respiratory infections. Also, UTIs are the fifth most common type of healthcare-associated infection virtually caused by instrumentation of the urinary tract. [1]

The incidence increases with presence of diabetes, malformations of urinary tract and with age. Definitive diagnosis of UTIs is by urine cultures. Urine is the most common specimen received in the clinical microbiology laboratory for bacterial culture and antimicrobial susceptibility testing. However, negative reports are obtained after 48 hours of incubation and positive report with identification and susceptibility of causative organisms can take 48 to 96 hours. Significant numbers of urine specimen sent for cultures are either negative or grow insignificant amounts of bacteria. [2]

Thus, a rapid and reliable urine screening procedure will be useful to decrease turnaround time for negative cultures as well as to increase efficiency of laboratory to predict treatment for patients on same day in case of positive cultures. The turn-around time for antimicrobial susceptibility report can also be decreased by 24 hours.

Materials and Methods

The prospective observational study was carried out in Department of Microbiology between October 2022 and May 2023. Total number of urine specimens tested was 432. The specimens were tested by both conventional culture (CC) and Uro-Quick (UQ) rapid automated method and the performances were compared.

Conventional Urine Culture: Urine samples (n=432) were centrifuged at 3500 rpm for 10 minutes. Wet mount for presence of pus cells and Gram stain for presence of micro-organisms was carried out from centrifuged deposit. Specimens were inoculated on MacConkey Agar for colony count and CHROM Agar (Biomerieux, France) for presumptive identification of colonies using (0.01ml) calibrated loop.

Uro-Quick Screening system: Uro-Quick system (Alifax, Italy) is an automated rapid method for screening of bacteriuria by laser nephelometry (light scattering). The presence of microorganisms causes light deviation which is detected by extremely sensitive detectors placed around the tube. 30° detector is sensitive and detects all particles present in culture vial whereas 90° detector is specific for size and shape of replicating microorganisms. Interference from non-replicating substances (erythrocytes, leucocytes, dead cells and salts) is eliminated during initial zero reading.

500 µl of well mixed urine (n=432) was inoculated into urine culture vial containing 4ml of eugonic broth (containing peptone, NaCl, Dextrose, Yeast extract, Animal Tissue infusion) by calibrated system offering real representative volume of original specimen. Broths are in sterile vials with pierceable hermetic seals which reduce chance of contamination.

The inoculated vials were manually introduced into the Uro-Quick reading unit. Vials were incubated at 37˚C, constantly mixed avoiding sedimentation and flotation with the help of magnetic stirrer in individual broths. Specimen were read every 5 minutes.

The signals are processed by software which monitors the growth curves and calculates the microbial count as colony forming units (cfu/ml). The sensitivity depends on analysis time and ranges from 20,000,000 cfu/ml in 70 minutes to less than 50 cfu/ml at 6 hours. [3]

Workup of positive urine cultures was performed according to general interpretive guidelines for urine cultures. [4]

Gram-stained smears from centrifuged urine specimen were prepared and presence of organisms in absence of pus cells was correlated with history of patient.

Evaluation for use of a positive vial on Uro-Quick system for identification and antimicrobial susceptibility testing of the microorganisms

Gram stained smear was prepared from 50µl of Alifax positive broth.

In presence of mixed growth, broth was subcultured on MacConkey agar and antimicrobial susceptibility testing was performed from pure culture isolates deemed significant depending on gram stain result of primary urine smear and smear from positive vial.

In presence of monomicrobial bacterial flora broth was subcultured on CHROM Agar (Biomerieux, France) for identification depending on colour of colonies produced and other morphological characteristics.

2.4.4 Whole volume of the positive vial was centrifuged at 4500 g for 10 minutes. Supernatant was discarded, pellet was resuspended in 3ml of 0.9% Sodium chloride, washed at 3500 g for 10 minutes, supernatant was discarded. (One wash technique)

Whole volume of the positive vial was centrifuged at 4500 g for 10 minutes. Supernatant was discarded, pellet was resuspended in 3ml of 0.9% Sodium chloride, washed at 3500 g for 10 minutes, and supernatant was discarded. The washing step is repeated one more time. (Two wash technique)

The pellet (UQ isolates) was resuspended in 0.45% Sodium chloride and adjusted to 0.5McFarland to inoculate AST card to be read on Vitek2C automated system.

Subculture on CHROM Agar and inoculation of AST card was also done from corresponding growth on MacConkey agar plate from conventional urine culture (CC isolates) of same specimen and results compared with those of automated rapid urine culture system.

Susceptibility testing results of UQ isolates and CC isolates were evaluated for agreement, minor errors (mE), major errors (ME) and very major errors (VME). Agreement represented similar results by both culture methods. Minor error represented susceptibility or resistance for a particular antimicrobial by one method which showed intermediate result by another method. Major error happened when a particular antimicrobial showed resistance towards UQ isolates whereas susceptibility towards CC isolates. Very major error was defined as susceptibility towards a particular antimicrobial by UQ isolates whereas resistance was shown by CC isolates.[5]

Results

Table 1: Comparison of automated (Rapid) urine culture results with conventional urine culture method.

Total no. Of specimen (n)	Concordant positive (n=127)	Concordant negative (n=211)	Discordant negative
338	90	211	37
Percentage	70.8%	100%	29.2%

Table 2: List of variables leading to discordant negative result

Sr. No.	Confounding Factor	n	%	Protocol for correction of confounding factor
1.	Boric Acid Containers without adequate volume (28ml) of urine.	17	5	Boric acid containers less than 28 ml of urine are rejected OR processed by conventional urine culture method.
2.	Consumption of antibiotics prior to collection of urine specimen (evident as post antibiotic effect on graphs)	20	5.9	1. If history of antibiotic consumption is available, urine specimen is processed by conventional method. 2. If antibiotic effect is evident on graph, negative vial is subcultured. Also, in presence of pus cells/ bacteria on wet mount/ Gram's stain, conventional culture is performed.

Table 3: Result after removal of confounding variables

Total no. Of specimen	Concordant positive (n=88)	Concordant negative (n=6)	Discordant negative
94	86	6	2
Percentage	97.7	100	2.3

Table 4: Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of rapid automated urine culture system

Sensitivity	**97.7%**
Specificity	100%
Positive Predictive Value	100%
Negative Predictive Value	100%

Table 5: Sensitivity and Specificity of concording positive and negative samples at 3 and 4.5 hours

Sensitivity		Specificity	
At 3 h	At 4.5 h	At 3 h	At 4.5 h
86.3%	97.7%	100%	100%

Table 6: Uropathogens isolated simultaneously from both rapid and conventional urine culture methods.

Organisms Isolated	Percentage (%)
Escherichia coli (n=100)	54.34
Klebsiella pneumonia (n=42)	22.82
Pseudomonas aeruginosa (n=5)	2.71
Citrobacter koseri (n=5)	2.71
Enterobacter cloacae (n=2)	1.08
Proteus mirabilis (n=1)	0.54
Serratia marscecens (n=1)	0.54
Serratia rubidea (n=1)	0.54
Morganella morganii (n=1)	0.54
Providentia rettgeri (n=1)	0.54
Acinetobacter baumannii complex (n=1)	0.54
Pseudomonas fluorescens (n=1)	0.54
Burkholderia cepacia (n=1)	0.54
Enterococcus fecalis (n=7)	3.8
Enterococcus faecium (n=2)	1.08
Staphylococcus aureus (n=1)	0.54
Staphylococcus saprophyticus (n=1)	0.54
Candida albicans (n=2)	1.08
Candida tropicalis (n=7)	3.8
Candida guillermondii (n=1)	0.54
Trichosporon inkin (n=1)	0.54
Total (n=184)	**100%**

Table 7: Comparison of VITEK2 antibiotic susceptibility testing results of Uro-Quick (solid agar subculture from positive vial) and conventional culture bacteria (n=20)

Antibiotic Name	Agreement (%)	Minor error (%)	Major Error (%)	Very Major Error (%)
Ampicillin	100	0	0	0
Amoxycillin-clavulanic acid	100	0	0	0
Ticarcillin	100	0	0	0
Piperacillin-tazobactam	100	0	0	0
Cefalothin	100	0	0	0
Cefoxitin	95	0	0	5
Cefixime	100	0	0	0
Ceftriaxone	100	0	0	0
Ceftazidime	100	0	0	0
Cefepime	100	0	0	0
Cefoperazone-sulbactam	100	0	0	0
Imipenem	100	0	0	0
Meropenem	100	0	0	0
Ertapenem	100	0	0	0
Amikacin	100	0	0	0
Gentamicin	100	0	0	0
Nalidixic Acid	100	0	0	0
Ciprofloxacin	100	0	0	0
Norfloxacin	100	0	0	0
Ofloxacin	100	0	0	0
Colistin	100	0	0	0
Tigecycline	100	0	0	0
Nitrofurantoin	100	0	0	0
Fosfomycin	100	0	0	0
Cotrimoxazole	95	5	0	0

Table 8: Comparison of VITEK2 antibiotic susceptibility testing results of Uro-Quick (bacterial pellet from positive vial obtained after one wash technique) and conventional culture bacteria. (n=27)

Antibiotic Name	Agreement (%)	Minor error (%)	Major error (%)	Very Major Error (%)
Ampicillin	86.6	6.7	6.7	-
Amoxycillin clavulanic acid	92.6	3.7	-	3.7
Ticarcillin	93.3	-	6.7	-
Piperacillin tazobactam	96.3	3.7	-	-
Cephalothin	73.3	13.3	6.7	6.7
Cefoxitin	100	-	-	-
Cefuroxime	100	-	-	-
Cefixime	80	6.6	6.7	6.7
Ceftazidime	86.7	-	6.7	6.6
Ceftriaxone	85.2	-	7.4	7.4
Cefepime	91.7	8.3	-	-
Cefoperazone sulbactam	91.7	-	8.3	-
Imipenem	100	-	-	-
Meropenem	91.7	-	8.3	-
Ertapenem	100	-	-	-
Amikacin	96.3	-	3.7	-
Gentamicin	96.3	-	3.7	-
Nalidixic acid	80	-	13.3	6.7
Ciprofloxacin	85.2	7.4	3.7	3.7
Norfloxacin	86.7	-	6.6	6.7
Ofloxacin	86.7	-	6.7	6.6
Fosfomycin	100	-	-	-

Nitrofurantoin	86.7	13.3	-	-
Trimethoprim-Sulfmethoxazole	92.6	-	3.7	3.7
Colistin	100	-	-	-
Tigecycline	100	-	-	-

A total of 27 isolates including Escherichia coli and Klebsiella pneumoniae isolated from corresponding manual and rapid cultures of same urine specimen were tested for antibiotic susceptibility by Vitek2C AST cards N235 and N405 and results were compared. Bacterial pellet from Uro Quick vial is obtained after one wash technique. Antibiotic susceptibility result from manual urine culture isolate was taken as standard.

Table 9: Comparison of VITEK2 antibiotic susceptibility testing results of Uro-Quick (bacterial pellet from positive vial obtained after two wash technique) and conventional culture bacteria. (n=31)

Antibiotic Name	Agreement (%)	Minor error (%)	Major Error (%)	Very Major Error (%)
Ampicillin	100	0	0	0
Amoxycillin-clavulanic acid	96.7	3.22	0	0
Ticarcillin	100	0	0	0
Piperacillin-tazobactam	100	0	0	0
Cefalothin	100	0	0	0
Cefoxitin	96.7	3.22	0	5
Cefixime	100	0	0	0
Ceftriaxone	96.7	0	3.22	0
Ceftazidime	100	0	0	0
Cefepime	90.3	3.22	3.22	3.22
Cefoperazone-sulbactam	96.7	3.22	0	0
Imipenem	100	0	0	0
Meropenem	100	0	0	0
Ertapenem	100	0	0	0
Amikacin	100	0	0	0
Gentamicin	100	0	0	0
Nalidixic Acid	100	0	0	0
Ciprofloxacin	100	0	0	0
Norfloxacin	100	0	0	0
Ofloxacin	100	0	0	0
Colistin	100	0	0	0
Tigecycline	100	0	0	0
Nitrofurantoin	93.5	6.45	0	0
Fosfomycin	100	0	0	0
Cotrimoxazole	96.7	0	0	3.22

Discussion

Urine samples comprise the largest number of samples received for culture and susceptibility testing in clinical microbiology laboratory. HB&L Light is the first automated system for rapid reporting of urine cultures with high sensitivity and specificity. Negative results can be reported within 6 hours and positive result with antimicrobial susceptibility test result can be reported within 24 hours of inoculation of specimen into broth.

We compared the results of HB&L Light with conventional urine culture method which is a standard protocol. 338 urine specimen were processed by both methods wherein 100% concordance (n=211) was noted for negative culture results. However, we found that only 70.8% (n=90) specimen showed concordant positive results by both methods. However, according to various publications, the agreement for HB&L Rapid and conventional urine culture results fell in range of 97-98%. [6,7,8,9] Discordant results amounted to 29.2% (n= 37) showing significant growth of uropathogens in conventional culture but no growth in automated system. Urine was processed from Boric acid containing sterile containers in 5% (n=17) of discordant results. Requirement of minimum 28 ml of urine in boric acid containers was reinforced. Lesser amounts were subjected to conventional culture method. When processing of urine specimen was not done within 3 hours of collection in preservative [8], colony counts at lower thresholds (10^3 & 10^4) were evaluated depending on presence of significant pus

cells, presence of bacteria on Gram stained smear and clinical complaints of patient. Also, in 5.9% (n=20) of urine specimen, antibiotic effect leading to false negative growth in automated system was evident on real time graphs. These findings were correlated with clinical history and history of antibiotic consumption in patients. A protocol to subculture the negative urine vial was added in standard operating procedures in such scenario.

After taking control of these variables, 94 urine specimen were subjected to culture by both methods. Out of these, 97.7% (n=86) showed results of significant bacteriuria by both methods. 2.3% (n=2) specimen showed discordant results, however, antibiotic effect was observed on graph and after following new protocol, growth was observed on plate culture method. Thus, sensitivity, specificity, positive predictive value and negative predictive value of automated urine culture on HB& L Uroquattro as compared with standard plate method was 97.7%, 100%, 100% & 100% respectively.

Sensitivity for concording positive specimen was found to be 86.3% at 3 hours and 97.7% at 4.5 hours. Specificity was found to be 100% at both 3 hours and 4.5 hours. High sensitivity reduces the number of false negatives; hence 100 cfu/ml is most ideal cut off for reducing false negatives.

E. coli was the most common uropathogen identified (54.34%) as evident in other studies. [6,10,11,12] Along with P. aeruginosa, other non-fermenters like Pseudomonas fluorescens, Acinetobacter baumannii complex as well as Burkholderia cepacia were isolated by both methods.

E. faecalis was the most common uropathogen isolated amongst gram positive. [6, 10] Yeasts were isolated from the rapid automated system too and comprised 5.96% of total uropathogens. [8] We compared antibiotic susceptibility results on Vitek2C for 20 gram negative bacteria isolated simultaneously from both automated (subcultured on solid agar) and conventional culture system. There was 100% agreement in results for all the antibiotics tested except cefoxitin (5% VME) and cotrimoxazole (5% mE), both of which showed 95% agreement.

However, our aim was to provide the rapid and reliable antibiotic susceptibility result, hence we evaluated antibiotic susceptibility testing results of Uro-Quick (bacterial pellet from positive vial obtained after one wash technique) and conventional culture bacteria on Vitek2C (n=27). More than 90% agreement was observed for fosfomycin, beta lactam antibiotics except for 3rd generation cephalosporins (cefixime, ceftazidime, and ceftriaxone), aminoglycosides and colistin. Agreement for fluoroquinolones and nitrofurantoin, which mostly encompass first line treatment for urinary tract infections, ranged between 85% to 90% whereas agreement for 3rd generation cephalosporins was between 80% to 87%. However, >90% agreement was observed in some studies for the tested antibiotics. [5,11,12] Major and very major errors were depicted in susceptibility to fluoroquinolones and cephalosporins by UQ isolates as compared to CC isolates, whereas only minor errors were observed in susceptibility to nitrofurantoin by UQ isolates.

As our quest to utmost reliability persisted, we introduced a double wash technique and compared VITEK2 antibiotic susceptibility testing results of Uro-Quick (bacterial pellet from positive vial obtained after two wash technique) and conventional culture bacteria (n=31). 97% to 100% agreement in susceptibility was observed for all the antibiotics tested except cefepime (90.3%). We obtained more than 90% agreement for nitrofurantoin susceptibility by UQ isolates.

Conclusion

HB&L Light system can be used to report negative urine cultures at as early as 6 hours. Positive results can be informed to clinicians at 4.5 hours so that empiric treatment can be started. Non-fermenting gram negative organisms along with yeasts were readily isolated from rapid system. Time to reporting of antimicrobial susceptibility testing can be decreased upto 24 hours in monomicrobial growth from rapid urine culture.

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