Rapid reporting of urine culture results: impact of the uro-quick screening system

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--- SUMMARY ---

This study evaluated the impact of the Uro-Quick (UQ) screening system (Alifax, Italy) for a rapid and accurate reporting of urine cultures, and whether it can provide bacterial yield to be used in identification and susceptibility testing. A total of 1480 urine samples collected between October 2006 and July 2008 were tested by conventional culture (CC) methods and UQ simultaneously. Sediments of positive UQ vials were used as bacterial yields for identification and susceptibility testing procedures. Of the 1480 samples, 999 revealed bacteria and/or leukocytes in direct microscopy. Among these 999 samples, positive growth was detected in 420 (42%) and 433 (43.3%) by UQ and CC, respectively. However, only 0.6% of samples without bacteria and leukocytes exhibited positive growth. When compared to CC, UQ demonstrated high levels of positive predictive value (95.9%), negative predictive value (94.8%), sensitivity (93%) and specificity (96.9%). Both CC isolates and UQ bacteria showed 81.3% concordance in identification results. Susceptibility testing of UQ bacteria displayed >90% agreement, when compared with standardized disk diffusion test. Our results indicate that UQ can reliably be used in routine laboratories giving microbial growth results in 3 hours. The most significant part of the study is that bacterial yields of UQ positive samples can be used in identification and susceptibility testing, allowing a rapid, same-day reporting of urine cultures.

KEY WORDS: Urine culture, Uro-Quick, Rapid reporting

INTRODUCTION

Urinary tract infections (UTIs) are among the most frequent bacterial diseases worldwide. The prevalence varies with age, sex and additional predisposing factors (Pezzlo, 1988). At least 10-20% of the female population can experience a symptomatic UTI at some time during their lives (Ronald *et al.,* 1991).

Furthermore, approximately 10% of men and 20% of women over 65 years of age suffer from bacteriuria (Nordenstam *et al.,* 1989; Baldassaere *et al.,* 1991). Nearly 5% of women and 1-2% of men also suffer from UTI during childhood (Waisman *et al.,* 1999; Viday *et al.,* 2008). Thus, urine samples constitute a large proportion of the

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specimens processed in most clinical microbiology laboratories.

Definitive diagnosis of UTI is achieved by urine cultures. However, reports are generally obtained after 18-24 hours of incubation and, can be delayed for an additional 24-48 hours if further testing is required (Graham *et al.,* 2001). Previous studies have also documented that up to 80% of urine samples submitted for culture to the laboratory are either culture negative or grow insignificant amounts of bacteria (Kellogg *et al.,* 1987; Okada *et al.,* 2000). Implementation of a rapid and reliable urine screening procedure for significant bacteriuria can increase the laboratory efficiency on empirical treatment of patients. There have been attempts to automate urinalysis, mainly functioning on biochemistry, microscopy and culture (Semeniuk *et al.,* 2002; Dimech *et al.,* 2002; Grosso *et al.,* 2008). UroQuick (UQ) (Alifax, Italy) is one of these automated systems for the screening of the urines and has been developed for rapid quantitative bacterial culture estimation wit-

hout identifying the pathogen. This study aimed to evaluate the impact of the UQ screening system on rapid but accurate reporting of urine cultures. To do so, we planned to investigate whether bacterial yields of UQ vials with positive growth can be used in identification and susceptibility testing procedures.

MATERIALS AND METHODS

This study was carried out in two steps. In the first step, urine samples collected between October 2006 and July 2008 were tested by both conventional culture (CC) and Uro-Quick (UQ) and system performances were compared. In the second step, the UQ system was evaluated to establish whether it can provide bacterial yield to be used in identification and susceptibility testing.

1. Conventional urine culture and the Uro-Quick screening system

a) Conventional Culture Methods
Urine samples were Gram stained for microscopic examination and inoculated on 5% blood agar (Biomerieux, France) using 10 µL calibrated loop quantitatively and MacConkey agar by the streaking technique. Cultures were incubated aerobically at 35-37°C for 18-24 hours.

b) 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 tubes. The signals are processed by software which monitors the growth curves and calculates the microbial count as colony forming units (cfu/ml) in 3 hours. The sensitivity depends on the preselected analysis time (i.e. $3x10^4$ cfu/ml in 3 hours) (Dimech *et al.*, 2002).
Well-mixed urine, 500 µl, was manually pipetted into a Uro-Quick vial containing 4 ml of broth. The inoculated vials were manually introduced into the Uro-Quick reading unit and incubated for 3 hours.

Detection of residual antimicrobial activity
UQ also provides a system called UQ-RAA (residual antimicrobial activity) for detection of an-

timicrobial agents, which may inhibit the bacterial growth in clinical samples and may also explain false negative results in standard plates. UQ-RAA vials contain 4 ml broth and *Staphylococcus epidermidis* ATCC 12228 to search for the presence of residual antimicrobial activity. Growth of this strain demonstrates the absence of antimicrobial agents in the urine, whereas lack of growth indicates the presence of these substances.
Two hundred µL of urine samples were inoculated into UQ-RAA vials and were incubated for three hours.

2. Evaluation of the Uro-Quick system: whether it can provide a bacterial yield to be used in identification and susceptibility testing
In the second part of our study, all CC positive cultures were undertaken for identification and susceptibility testing by VITEK2 (BioMerieux, France) as part of our routine practice. Meanwhile, randomly selected 102 UQ vials exhibiting positive growth were centrifuged at 4000 x *g*/5 min and sediments were held as bacterial yields to be used for identification and susceptibility testing by VITEK2. To compare the results of antibiotic susceptibility testing standardized disk diffusion method was also performed for those 102 isolates (CLSI, 2008).

Interpretation of results
Three different categories were established when identification results were analyzed:
1) *'agreement'* represents concordance between identification results of UQ bacteria and CC isolates at genus and species level;
2) *'misidentified'* represents the strain identification with UQ bacteria which showed discrepant results at genus level;
3) *'unidentified'* represents no identification at all.
Susceptibility testing results of UQ bacteria and CC isolates were evaluated for agreement, minor errors (mE), major errors (ME), and very major errors (VME) when compared to that of disk diffusion testing as a reference method. Agreement represents similar results between test method and reference method; mE means to be in the susceptible or resistant category for one system while intermediate for the other system. ME is identified when the results in the test system are

resistant whereas they are susceptible using the reference methods. VME is defined as results being in the susceptible category by test system, while they are resistant by the reference method.

RESULTS

Conventional culture results compared to the UroQuick system

A total of 1480 urine samples were analyzed and of these 999 (67.5%) revealed microorganisms and/or leukocytes and the remaining 481 (32.5%) samples lacked both. Both leukocyte and microorganisms were found in 600 (60%) out of 999 specimens, whereas 365 (36.6%) had leukocytes without microorganisms and only 34 (3.4%) had microorganisms without leukocytes on Gram stain. Positive growth was detected in 404 (67.3%) out of 600 samples with both microorganism and leukocyte, while it was detected in 24 (6.5%) out of 365 samples exhibiting presence of leukocyte without microorganism and in 5 out of 34 samples with microorganism but not leukocyte in Gram stain. In samples revealing no microorganism and leukocyte, only three (0.6%) were positive by conventional culture.

UQ and CC detected positive growth as $\geq 10^4$ cfu/ml in 420 and 433 of 999 samples, respectively. The performance of UQ compared to urine culture is shown in Table 1. As seen in Table 1, the concordant negatives were 256 and concordant positives were 403; 17 of the samples were considered as false positive since corresponding urine cultures were negative. Of the 425 negative results obtained from Uro-Quick, 21 were false negative and 148 were detected as mixed culture by CC. Although the specificity for all samples ($n = 1480$) was low as 73%, UQ had a sensitivity of 93%, and specificity of 96.9% with a positive (PPV) and negative predictive value (NPV) of 95.9% and 94.8%, respectively when calculated for the samples ($n = 999$) with bacteria and/or leukocytes.

TABLE 1 - *Comparison of conventional culture methods and Uro-Quick for samples revealing bacteria and/or leukocyte.*

Conventional culture	UroQuick			
	Growth	No Growth	Contamination	Total
	n	n	n	n
Growth	403	21	9	433
No Growth	-	256	23	279
Contamination	17	148	122	287
Total	420	425	154	999

TABLE 2 - *Analysis of the results in RAA positive samples according to direct microscopy results.*

Direct microscopy	RAA positive		
	Growth	No Growth	Total
	n (%)	n (%)	n (%)
Leukocyte &Microrganism	15 (35.7)	27 (64.3)	42 (100)
Leukocyte	2 (1.9)	106 (98.1)	108 (100)
Microrganism	-	2 (100)	2 (100)
Total	17 (11.2)	135 (88.8)	152 (100)

To explain one of the reasons for false negative results in samples with bacteria and/or leukocytes, we detected RAA in urine samples. For this, 604 of the samples (*n* = 999) with leukocyte and/or microorganism were randomly selected and 152 of them were found to be RAA positive; of these, 135 revealed no growth or contamination by CC. Most of the positive cultures, despite the presence of RAA, showed bacteria and leukocytes, whereas most of the RAA positive samples without growth was in the group of samples exhibiting leukocyte but not microorganism (Table 2).

Whether the Uro-Quick system can provide a bacterial yield for identification and susceptibility testing

As expected, *Escherichia coli (E.coli)* was the most frequent microorganism isolated, followed by *Klebsiella* spp. by conventional cultures. A total of 83 (81.3%) isolates were identified correctly when UQ bacteria were used as bacterial yields. Correct identification was highest (94.7%) for *Klebsiella spp*. with none in unidentified category. However, 3 of *E.coli* isolates were identfied as *Klebsiella* spp. and 12 of them could not be identified when UQ bacteria were used, resulting in an agreement level of 80% (Table 3).

Susceptibility test results of CC isolates revealed that agreement was >90% for tested antibiotics, with the exception of ampicillin/sulbactam between VITEK2 and disk diffusion results. Similar results, except for ampicillin/sulbactam and piperacillin/tazobactam, were obtained when UQ

bacteria were used as bacterial yield in VITEK2 system. VME were not detected either from the bacterial yield of CC or UQ in VITEK2 results. Compared to disk diffusion, VITEK2 susceptibility results of UQ isolates displayed 0-4.2% and 0-15.2% major and minor error rates, respectively, similar to that of CC results (Table 4).

DISCUSSION

Urine samples comprise the largest volume of workload in routine bacteriology laboratories, but almost 80% of these turn out to be culture negative. Despite the availability of several commercial screening systems, there is no agreement on which system seems to be ideal for routine microbiology laboratories. Ideally such a system should be in agreement with conventional cultures to a large extent with regard to accuracy. On the other hand, such system should be easy to perform, reproducible and have a considerably shorter turnaround time. This study evaluated one of those systems, the Uro-Quick screening system for its performance in urine culture results since the system was introduced to the market with a promise of giving results in a work time as short as 3 hours. First of all what we looked for was whether UQ accurately differentiates true pathogen growth from contamination and no growth. We detected 433 of urine samples with leukocytes and/or bacteria, as positive for pathogen growth by conventional cultures; UQ had signaled 403(93.1%) of which as positive. This result indi-

TABLE 3 - *Correlation of VITEK2 identification results for Uro-Quick bacteria and conventional culture isolates.*

Conventional Culture	Uro-Quick			
	Agreement	Misidentification	Unidentified	Total
	n (%)	n (%)	n (%)	n (%)
E.coli (n:75)	60 (80)	3 (4)	12 (16)	75
Klebsiella spp. (n:19)	18 (94.7)	1 (5.3)	0	19
Others* (n:8)	5 (65)	1 (10)	2 (25)	8
Total (n:102)	83 (81.3)	5 (4.9)	14 (13.6)	102

*Isolates (Enterobacter cloacae: 2, Enterobacter aerogenes: 3, Pseudomonas aeruginosa: 1, Proteus mirabilis: 1, Salmonella enteritidis: 1).

TABLE 4 - *Comparison of VITEK2 antibiotic susceptibility testing results of Uro-Quick and conventional culture bacteria by standard disk diffusion method.*

Antibiotics	UQ bacteria			CC bacteria		
	Agreement (%)	ME (%)	mE (%)	Agreement (%)	ME (%)	mE (%)
Ampicillin/sulbactam	80.7	4.1	15.2	84.8	0	15.2
Gentamycin	97.2	2.8	0	97.2	2.8	0
Cefuroxime	95.8	0	4.2	97.2	1.4	1.4
Ceftriaxone	94.5	0	5.5	97.3	2.7	0
Imipenem	100	0	0	100	0	0
Ciprofloxacin	97.2	1.4	1.4	97.2	1.4	1.4
Nitrofurantoin	90.2	2.8	7	95.8	0	4.2
Piperacillin/tazobactam	86	4.2	9.8	91.6	4.2	4.2
Trimethoprim sulfamethoxazole	100	0	0	97.2	2.8	0

cates that UQ may have the advantage of reporting the presence of infection within the same day for most of the patients with UTI, so that appropriate empirical treatment can be started in a reasonable time period within a sensitivity, specificity, PPV and NPV of 93%, 96.9%, 95.9%, and 94.8%, respectively. It seems likely that only 6.9% of positive cultures require awaiting for conventional culture results to give definite reporting. Similar statistical analysis results were obtained for all samples with the exception of a lower specificity rate of 73%, suggesting that the UQ screening system could be more reliably used if urine samples were first evaluated for the presence of leukocytes and/or microorganisms. This suggestion is also supported by the findings that only 0.6% of the 481 samples with no bacteria and leukocytes exhibited positive growth.

During the study, we also investigated the opportunity provided by the UQ screening system, that is, detecting residual antimicrobial activity in urine samples which might inversely affect microbial growth. As seen in Table 2, most of the samples (71%) with RAA were in the group of samples with leukocytes but without bacteria on direct microscopy and almost all of those samples (98.1%) exhibited no growth on routine culture plates. This RAA may explain the low ratio (6.5%)

of positive growth in those samples with relatively low numbers of bacteria that are undetectable on microscopy. Overall, it seems likely that direct microscopy is essential for preliminary examination of urine samples and combining this with the UQ screening system will eliminate the negative samples from the workload in microbiology laboratories.

The second part of our study evaluated the UQ system as to whether it can provide bacterial yields to be used in identification and susceptibility testing. When sediments of UQ vials were used as test microorganisms, VITEK2 detected the same phenotypes in relatively high concordance with that of CC isolates, especially in the case of gram-negative bacilli (Table 3). This means that a clinical microbiology laboratory can give information on both microbial identity and cfu/ml for a given infecting agent to the clinicians within the same day.

Antibiotic susceptibilities of CC results, performed by VITEK2 displayed >90% agreement with all tested antibiotics, other than ampicillin/sulbactam when compared to the disk diffusion results (Table 4). Ampicillin/sulbactam was the lowest in agreement, but all discrepant results (15.2%) were in the mE category. When UQ sediments were used as bacterial yields, antibiotic susceptibility

results were in concordance with CC results except for piperacillin/tazobactam. Imipenem was the only antibiotic that designated no error but full agreement by both methods and it was remarkable that at least for first choice antimicrobials used in UTI including ciprofloxacin, nitrofurantoin and trimethoprim-sulfamethoxazole demonstrated high (>90%) percentages of agreement. Similar results were evaluated in a previous study in which aliquots of urine were used directly for antibiotic susceptibility by adding antibiotics into the UQ vials and the results were compared by disk diffusion (Roveta *et al.,* 2006; Roveta *et al.,* 2004). Susceptibility results indicate that the bacterial yield of Uro-Quick system can be performed for the determination of the antibiotic susceptibilities of Enterobacteriaceae, especially for *E .coli* and *Klebsiella* spp. which are the most common pathogens in UTIs.

When the processing time was evaluated for all these procedures, it was found that UQ screening (3 h), identification and susceptibility steps using the UQ sediments as bacterial yield by VITEK2 (6+/-2 hrs time) together would take 7-11 h (Pérez-Vázquez *et al.,* 2001; Eigner, *et al.,* 2005). Therefore, there will be a time saving of 15-21 h for the positive samples, when compared with conventional culture methods. These observations altogether indicate that such systems can bring important advantages to clinical microbiology laboratories when handled by experienced personnel, provided that the system is used together with direct microscopic examination. These advantages include the following;

1) Diminishing workload (in clinical microbiology laboratory) by elimination of negative samples.
2) A chance of repeat request for contaminated samples on the same day.
3) Informing clinicians of positive growth in 3 hours.
4) Writing reports of identification and susceptibility results on the same day with great accuracy.

In conclusion, the UQ screening system, when used together with direct microscopy of urine samples seems to be a reliable instrument to obtain urine microbiological results in a timely manner. Both UQ screening and its bacterial yield used in identification and susceptibility procedures will also reduce the use of empirical and/or inappropriate antimicrobials, resulting in an appreciable cost-effectiveness to the medical care system.

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