Evaluation of the Urotest AB antibacterial substance detection test

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Abstract

The Urotest AB was used to detect antimicrobial substances in urine samples. Of 1022 urine specimens evaluated, Urotest AB detected inhibitors in 38.9%. Of 159 urine specimens from patients thought to be taking an antibiotic, inhibitors were detected in 80.5%. This test may help to explain culture negative urine samples from symptomatic patients, and could help elucidate treatment failures and the epidemiology of antibiotic resistance.

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Previous investigations have shown that a high percentage of urine samples submitted to the laboratory contain antimicrobial substances.^{1,2} Two reports^{3,4} noted that the prevalence of antimicrobial substances in urine was 16.1 and 19.94%, respectively.

Millar and Langdale developed a procedure for identifying antimicrobial substances in urine, which may be used to investigate specimens demonstrating pyuria without bacterial growth.⁴ Although, in principle, such testing would be ideal, it may exceed the level of testing that is either necessary or desirable for many microbiology laboratories. Instead, a simplified method of determining the presence of inhibitors may be of value. We evaluated the Urotest AB (Merck, Darmstadt, Germany) for its ability to detect the presence of inhibitors in urine.

Methods

The Urotest AB is a filter strip containing spores of *Bacillus subtilis* ATCC 6051, culture media, and an enzymatic substrate. To perform the test, the Urotest strip is dipped in urine, placed in a plastic incubation sachet, and then incubated for either five to seven

hours (rapid test) or 16 to 24 hours (routine test). In the presence of inhibitors the spores of *Bacillus* will not germinate and, therefore, enzymatic conversion of the substrate will not occur and a colour change will not be observed. If inhibitors are not present, germination of the spores results in enzymatic conversion of the substrate, producing a colour change.

Results

Urine specimens (n = 1022) were tested. Of these, documentation on the presence or absence of antibiotics was not available for 229 (22.4%). These specimens were excluded from further analysis. Of the remaining 793 urine samples, 372 (46.9%) were found to have mixed or pathologically irrelevant micro-organisms present, 269 (33.9%) had no visible growth (NVG), and 152 (19.2%) had clinically relevant findings (table 1). Of 641 urine samples with NVG or mixed/irrelevant growth, 252 (39.3%) had inhibitors detected; reported antibiotic use accounted for 116 (46%). Of 152 urine specimens with clinically important growth, 57 (37.5%) had inhibitors detected and reported antibiotics accounted for 12 (21.1%). Overall, Urotest AB detected inhibitors in 309 of 793 (38.96%) of all urine samples tested. As shown in table 1, at least 66 of 82 Urotest AB positive urine specimens with mixed or irrelevant findings, 70 of 170 Urotest AB positive urine specimens with no visible growth, and 45 of 57 Urotest AB positive urine samples with clinically important findings did not have antibiotics present. Likewise, 12 of 290 negative urine samples with mixed or irrelevant findings, six of 99 negative urine specimens with no visible growth, and 13 of 95 negative urine specimens with clinically important findings were from patients with reported antibiotic use. Overall, reported antibiotics accounted for 42.8% of detectable substances in urine that

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Table 1 Number of Urotest AB positive and negative urine specimens with and without antibiotics

Culture results	No of specimens	UAB*	Antibiotics	
			Prescribed	Not prescribed
Mixed/irrelevant	82	Pos	16	66
	290	Neg	12	278
No visible growth	170	Pos	100	70
	100	Neg	6	94
Clinically important	57	Pos	12	45
	95	Neg	13	82

* Urotest AB

Table 2 Summary of documented antibiotics on requisition accompanying urine specimens

Amoxycillin	Doxycycline
Ampicillin	Erythromycin
Cefazolin	Gentamicin
Cefotetan	Metronidazole
Cefotaxime	Nitrofurantoin
Cefuroxime	Norfloxacin
Cephalothin	Tetracycline
Cephalexin	Tobramycin
Ciprofloxacin	Trimethoprim/sulfamethoxazole
Clindamycin	Vancomycin
Cloxacillin	

may affect the growth of *B subtilis*. These substances may differentially affect Gram positive and Gram negative micro-organisms.

There were 12 urine samples with mixed or irrelevant culture results, six with NVG, and 13 with clinically important findings that were Urotest AB negative but which were from patients with documented antibiotic use. All of the documented antibiotics associated with these urine specimens were of the same mixture as those listed in table 2 and documented as Urotest AB positive.

The sensitivity, specificity, positive, and negative predictive values of Urotest AB compared with antibiotics were 80.5, 71.5, 41.4, and 93.6%, respectively.

Discussion

The Urotest AB test detected inhibitors in 128 of 159 (80.5%) urine specimens from patients with documented antibiotic use. There are several possible explanations as to why the remaining 31 (19.5%) were not detected. Additionally, 181 of 635 (28.5%) urine specimens without documented antibiotic use were positive. First, the documentation of antibiotics on the ordering requisition may have been incorrect—that is, instead of a patient being treated with antibiotics—the patient was not being treated or was going to be treated. This would mean that there were no antibiotics present at the time the specimen was collected. However, we believe the reported antibiotic information was accurate. Second, the concentration of antibiotic present may have been too low to be detected. Previously, Millar and Langdale⁴ found that antibiotic concentration in urine affected the pattern of inhibition zones for antibiotics used for treating urinary tract infections. Although their screening test was positive, the antibiotic concentration did affect this test. Third, the Urotest did not detect the presence of some antibiotics. This is the most unlikely reason as a comparison of the documented antibiotics in Urotest AB positive and negative urine specimens showed that similar types and fre-

quency of the same antibiotics were used. Millar and Langdale⁴ reported that antimicrobial substances were found in 19.94% of 1514 urines tested. This is lower than the findings of this current study. The differences may be explained by the sensitivities of the two methods for detecting inhibitors. To our knowledge, such a comparison has not been carried out.

The value of a test that can detect inhibitors present in urine is considerable. It might explain why an individual with symptoms of urinary tract infection has negative urine cultures. From a different perspective, it may be of value in testing the success of a treatment and patient compliance. The ease of use and interpretation of the Urotest AB test would make this product easy to introduce into the clinical laboratory.

An important finding in this study is that the Urotest AB seems to detect inhibitors that were not accounted for by known antibiotics. The antibacterial activity of substances other than antibiotics has been reported.^{5,6} We have no way of knowing if these inhibitors were, in fact, antimicrobial agents that were self administered or previously prescribed and not revealed during the current investigation. This information may help to explain treatment failure and the epidemiology of antimicrobial resistance.

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