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Detection of bacteriuria by microscopy and dipslide culture in general practice

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Background: Patients presenting with symptoms of urinary tract infections account for 2-5% of contacts in general practice, but only about half of them have significant bacteriuria. A definite diagnosis depends on a microbiological test demonstrating a significant number of bacteria. In general practice the diagnosis is often reached by a microscopic analysis or a dipslide culture test. Only a few studies have looked at the validity of urine examinations when performed in general practice, and the results are diverging. We need more knowledge about the validity of tests for detection of uropathogenic bacteria in general practice.
Aim: To validate detection of bacteriuria by urine microscopy and dipslide culture in general practice.
Method: Urine specimens with a known quantity of bacteria (*Escherichia coli, Proteus mirabilis, Enterobacter cloacae, Staphylococcus epidermidis* and *Enterococcus faecalis*) were sent to 25 general practices for microscopic examination and dipslide culture. No prior instruction in testing procedure was given. The results of a standardised culture method performed by skilled bacteriologists at the bacteriological laboratory were used as gold standard.
Results: Significant bacteriuria was identified by microscopy with a sensitivity of 95% and a specificity of 83%. The corresponding figures for urine culture were 95% and 96%, respectively. The morphology of bacteria was interpreted correctly in 80% of microscopic examinations, and 60% of the bacteria strains were classified correctly concerning their motility.
Conclusion: Microscopy and dipslide are valid methods for detecting significant bacteriuria in general practice.
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Key words: urinary tract infections, diagnosis, bacteriuria, microscopy, family practice

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Introduction

Patients presenting with symptoms of urinary tract infections (UTI) account for about 2-5% of contacts in general practice.[1-3] The diagnosis of UTI is generally based on culture of urinary specimens, and traditionally, significant bacteriuria is defined as a growth of more than 100,000 colony-forming units (CFU) per ml urine.[4] However, this criterion has been a matter of dispute and there are still differences of opinion on how to define significant bacteriuria in general practice.[5,6] Usually only about half of the patients with symptoms of UTI in general practice are found to have more than 100,000 CFU per ml, but rates exceeding 80% have been reported.[3,7]
In general practice the diagnosis of UTI should be based on a simple, rapid and reliable testing procedure that can be performed by the general practitioner, giving the result while the patient is still waiting in the practice.[5,8,9] Most studies evaluating the validity of methods for diagnosing UTI were performed in bacteriological laboratories by skilled bacteriologists or laboratory technicians.[9-11] Only a few studies have examined the validity of urine examinations for UTI in daily practice and results are disappointing. Winkens found that UTI could neither be confirmed nor excluded sufficiently on the outcome of a urinary sediment or a test strip.[12]
In Denmark, many GPs routinely perform urine microscopy and culture,[13] but our knowledge about the

Table 1. Results of microscopy and dipslide culture testing performed in 25 practices. The table shows the sensitivity and the specificity for significant bacteriuria (more than 100,000 colony forming units per ml urine) when analysed by microscopy and dipslide culture.

	Microscopy		Dipslide culture	
	Significant bacteriuria	*Non-significant bacteriuria*	*Significant bacteriuria*	*Non-significant bacteriuria*
Number of tests performed	191	42	247	46
Number of correct results	181	35	236	44
Sensitivity (%, 95% confidence interval)	95 (92-98)	-	95 (93-98)	-
Specificity (%, 95% confidence interval)	-	83 (72-95)	-	96 (90-100)

validity of urine analysis in general practice is scarce. This study was carried out in order to examine the validity of urine microscopy and culture based on the examination of standardised urine specimens in general practice.

Material and methods

Selection of practices

The study was carried out in 1995 as a multi-practice investigation in the County of Funen, Denmark (174 general practices, 300 physicians). A random sample of 25 practices (14 single-handed and 11 group practices) participated in the study.

Microbiological testing procedures

Urine specimens were produced in the bacteriological laboratory at Odense University Hospital, by adding a known quantity of typical uropathogenic bacteria to a sterile urine specimen. Bacteria were obtained from patients with UTI and included strains of *Escherichia coli*, *Proteus mirabilis*, *Enterobacter cloacae*, *Staphylococcus epidermidis*, and *Enterococcus faecalis*. Overnight cultures were diluted and a suitable volume was transferred to a tube with 10 ml of sterile urine yielding a bacterial concentration of 10,000 to 10,000,000 bacteria per ml. Three hundred samples, of which 250 contained more than 100,000 bacteria per ml, were sent to the practices. Specimens were transported refrigerated and urine microscopy and culture were performed at the surgeries on the same day they received the specimen. Each practice received 12 specimens for analysis.

Eleven practices used a traditional light microscope and 14 used a phase-contrast microscope. If the presence of bacteria was determined by microscopy, the practitioners were asked to quantify the number of bacteria seen per field of vision, determine the morphology of the organisms (rod or cocci), and describe their type of motility (non-motility, polar or non-polar motility). As microscopic criteria for significant bacteriuria we used the occurrence of one or more bacteria per field of vision.[14]

For culture testing, practices used one of the dipslide methods commercially available in Denmark (Uricult, Uricult Trio, or Urotube). A dipslide is a simplified culture consisting of a 3x9 cm double-coated agar plate, on one side covered with a non-selective Cled agar (cystein-lactose-electrolyte-deficient) to detect the number of colony-forming units (permitting growth of all types of organisms) and on the other side covered with one or two selective agars to detect gram-negative rods (McConkey agar).[15-17] Uricult Trio, furthermore, contains a selective agar which indicates growth of *E. coli* by a colour change (black).[10]

As gold standard we used the results of a standardised culture method performed by skilled bacteriologists at the bacteriological laboratory.[5,11]

Results

Table 1 shows the results of microscopy and culture in general practice. By means of microscopy, the practitioners correctly identified 181 of 191 samples with significant bacteriuria (sensitivity 95%) and 35 of 42 samples without significant bacteriuria (specificity 83%). With culture, the corresponding figures were 95% and 96% respectively. The evaluation of significant bacteriuria when using the culture method was based on counting of the number of colony-forming units on the Cled agar, which permits growth of all bacteria. The percentage of correct results based on interpretation of culture on the selective MacConkey agar (permits growth of gram-negative bacteria only) was 95%. However, only 69% of specimens cultured on the coli-specific MacConkey agar on Uricult Trio were identified correctly with regard to the presence of *E. coli*.

Eighty percent of samples with significant bacteriuria were classified correctly by microscopy concerning the bacteria morphology (rods or cocci) and 60% were interpreted correctly concerning the motility (non-motility, polar or non-polar motility). Microscopic analysis in practices that used a phase-contrast microscopy showed a slightly higher percentage of correct answers than microscopic analysis in practices that used light microscope, but the difference was not significant.

Discussion

A presumptive diagnosis of UTI may be based on the presence of typical symptoms of UTI (dysuria, stranguria). However, the prevalence of significant bacteriuria among patients with symptoms of cystitis in general practice is only about fifty percent and a definite diagnosis depends on a microbiological test demonstrating a significant number of bacteria. In this study nearly all specimens (95%) containing a significant number of bacteria were identified correctly by both culture and microscopy. Seventeen percent of specimens without significant bacteria were, however, interpreted as positive (false positive) by microscopy and 4% by culture.

A rather high percentage of false results were found when diagnosing the morphology and the motility of bacteria based on the microscopic examination. About one quarter of the strains were interpreted wrongly with regard to their morphology (rod or cocci) and nearly half of bacteria were misclassified with regard to their type of motility (non-motile, polar or non-polar). This should, however, be seen in the light of the fact that the participants were not trained in microscopy before the project started, and no previous experience in microscopy was required to participate in the study. For the trained investigator it may be easier to identify the morphology and motility of the bacteria in question. This information may be useful when deciding on the therapy. For example, a microscopic examination that shows chains of cocci indicates an infection caused by enterococci. This strain of bacteria is obligate resistant to sulphamethizole and in such cases ampicillin is a better choice than sulphamethizole, which has been the recommended drug for UTI in Denmark for many years.

A limitation of this study is the fact that the microscopic examination and the culture test were performed on the same specimens and the result of one analysis could therefore have an influence on the interpretation of the other analysis. Indeed, the interpretation of the microscopic examination could be influenced by knowing the result of a culture test. In order to reduce this potential bias, we asked the participants to perform the microscopic examination on the same day they received the specimens and fill them in before they were aware of the culture results.

A limitation of the generalisability of our results is the fact that the urine samples were artificially produced and did not contain an increased number of leukocytes or other cellular elements as often found in urine samples from patients with UTI. However, leukocyturia is an unspecific indicator of UTI and the predictive value of leukocyturia has been a matter of dispute.[18]

Another limitation of the generalisability is the lack of an appropriate gold standard for UTI in general practice. The only available criterion for UTI that is generally accepted is the one based on Kass's paper from 1957 but it may, by modern standards, be considered as anecdotal. Some authors have proposed other criteria based on a lower number of bacteria, but the number of bacteria remains a matter of dispute, and there are no standard criteria for significant bacteriuria for UTI in general practice. This has the effect that the sensitivity and specificity of tests may vary considerably according to the spectrum of disease and population tested.

Another limitation concerning generalisability is the fact that this study only included practices where microbiological analysis is routinely performed. In Denmark, however, most general practitioners have a microscope,[13] and nearly all practices perform urine culture.

Other studies have shown that the validity of microscopy is diverging.[19,20] Frimodt-Møller et al. found, like us, that phase-contrast microscopical examination of non-centrifuged urine had a high validity, but in their study examinations were performed by skilled laboratory technicians in a bacteriological laboratory.[21] Jenkins et al. also found that urine microscopy for bacteria was a useful and valid method for evaluation of urinary tract infection.[14] However, in that study the authors proposed oil-immersion microscopy of Gram-stained centrifuged urine sediment, which is a rather complicated method to use in general practice. Vickers et al. examined the validity of urine microscopy and culture in 342 children with UTI.[22] They found that microscopy had a higher validity compared with culture. However, Vicker et al.'s study was based on examinations performed by skilled laboratory technicians in a hospital setting and the results can not be transferred to a general practice setting. Vucina et al. examined 214 urine samples by microscopy and culture and proposed that phase-contrast microscopy should replace the culture method as the initial test in patients with UTI.[23] Ditchburn et al. also found that microscopy of a drop of urine was the most useful aid in diagnosing UTI.[24]

In our study, urine culture was most often performed by the Uricult test and growth on the non-selective agar (Cled) and selective agar (MacConkey) was interpreted correctly in nearly all cases. However, only about two thirds of results indicating the presence of *E. coli* based on culture by using Uricult-Trio (dipslide containing an *E. coli* specific agar) were correct. This is in contrast to the result of a Finnish study examining Uricult Trio.[10] In that study, which was performed by skilled bacteriologists in a clinical microbiological department, no false positive results were observed. We found that most of the false results were caused by false positive classification of coli, due to a minor colour change of colonies cultured on the selective MacConcey agar.

The ideal method for detection of bacteriuria in general practice should be quick, inexpensive, accurate, and easy to perform. Both microscopic examination and culture methods meet most of these criteria. However, culture methods are valid, but not rapid. The result of a culture is not available for 24 to 48 hours and, if positive, necessitates a new contact with the patient. Furthermore, the cost is substantial. At a price of £1.5 - £2 for a dipslide, a general practice could more cost-effectively perform a microscopic examination. Microscopic urine analysis can be performed while the patient is in the clinic and treatment can be initiated if indicated.

Nitrate and leucocyte esterase dipstick tests are often used for the diagnosis of UTI, particularly at home visits and out-of-hours consultations. These methods are quick, simple and inexpensive, but the validity may be limited. Not all types of uropathogenic bacteria result in a positive nitrate reaction and a patient with symptoms of UTI and a negative nitrate test should therefore be examined further. A positive leucocyte esterase test should also be interpreted cautiously because genital contamination may lead to a false positive reaction. An advantage of dipstick tests is that they only take a few minutes to perform. However, the extra time for possible further analyses should be considered when comparing with other methods. An experienced doctor can do a phase-contrast urine microscopy as quickly as a dipstick test.

Simple microscopy of the urine is recommended in several textbooks for the diagnosis of UTI in primary healthcare. By using phase-contrast microscopy at 400 times magnification of non-centrifuged urine, observation of one or more bacteria per field of vision indicates significant bacteriuria.[5,23,25] Buying a phase-contrast microscope is a big investment for a small general practice and a considerable routine in use is imperative for a valid result. However, routine in microscopy may be obtained as a microscope in general practice can also be used for several other diagnostic issues, such as gynaecological wet smears, fungal infections and blood smears.[26,27]

Conclusion

This study suggests that the validity of diagnosing significant bacteriuria by dipslide and microscopy performed in general practice is sufficient. ■

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