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Rapid method for determining the antimicrobial susceptibility on chromogenic agar for urine specimen

Abstract

Background: UTIs are the most common infections. Standard bacterial isolation and antimicrobial testing, usually takes no less than 2-3 days. This can often lead to inadequate empirical therapy. Determining an antibiogram directly from urine sample, may provide preliminary information that improves antimicrobial use and that has a very good correlation with standard procedures. A comparison between rapid method and standardized disk diffusion test was made.

Methods: The samples were preselected for testing. Uncentrifuged and well-mixed urine was Gram stained and examined microscopically (x 1000). Only those specimens containing ≥1 microorganisms/field were selected for the study. The rapid method was performed by directly applying three drops of urine over the surface of a CHROMagar orientation medium, the drops were spreaded with a cotton-tip swab all around the plate. These ones were air dried for 15 min, then the antibiotic disks were pressed on the agar surface and the plates were incubated at 35°C, after 6 hours the interpretation of the inhibition zone could be done. The zone diameters in both methods were measured. Interpretative categories were determined by using CLSI guidelines. The results were compared.

Results: 163 samples were chosen for comparison, the organism isolated was *E. coli*. Total agreement of category (S-S, I-I, R-R) was high overall at 94.4%. The lowest correlation 87% was found to occur with amoxicillin/clavulanic acid. The highest correlation between methods was with trimethoprim-sulfamethoxazole (99.1%) no errors S-R and R-S. The majority of discrepancies occurred when the difference was from 1 to 4 mm.

Conclusion: The rapid method is equivalent to the standard test when the urine being tested is infected with ≥105org/ml. This procedure allows the earlier reporting in six hours of susceptibility results and improves antimicrobials use.

Keywords: urine specimen, susceptibility, uncentrifuged, disk diffusion test, blood culture, antimicrobial agent's

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Introduction

The traditional basis for the evaluation of urinary tract pathogens is the urine culture and the antibiotic susceptibility testing. The major drawback of the current microbiology approach is the time lapse of 2-3 days between specimen collection and the availability of the result with an objective evidence for treatment selection. Antibiotic resistance is a significant problem not only among nosocomial complicated UTIs, where it has traditionally been recognized, but also in community acquired simple UTIs. Early microbiological diagnosis and the correct administration of the appropriate initial antimicrobial therapy have proven to be associated with decrease rates of morbidity. Direct susceptibility testing of blood culture is being used in some microbiology laboratories, methods for urine cultures have been evaluated, some do not recommend the procedure, and others advocate a variety of conditions for acceptance of results. We performed a comparison between rapid method and standardized disk diffusion method in urine specimen, determining the antimicrobial susceptibility on CHROMagar Orientation Medium, without having to wait for bacterial isolation, our method may provide preliminary information that improves antimicrobial use and it also has a very good correlation with standard procedures.

Methods

Urine specimens: Clean catch; midstream urine specimens were used for the present study.

Microscopy: The samples were pre-selected for testing, uncentrifuged, well-mixed urine was Gram stained and examined microscopically (x 1000). Only those specimens containing ≥1 microorganisms/field were selected for study.

Bacterial cultures: Standard methods for urine cultures were used. Sheep blood and MacConkey agar plates were streaked with 1µg of urine using a calibrated loop for bacterial quantification.

Rapid method: Directly applying the drops of urine over the surface of a CHROMagar orientation medium that were spreaded with a sterile- cotton tip swab. The plates were air dried for a maximum of 15 min, and individual antibiotic disks were pressed firmly on the agar surface. Gram negative bacteria were tested with the antimicrobial agent's ampicillin, amikacin, amoxicillin/clavulanic acid, cefuroxime, cefotaxime, ceftriaxone, cefepime, ceftazidime, imipenem, trimethoprim-sulfamethoxazole, and fosfomycin. Plates were incubated at 35°C for at least 6 hours, and then the interpretation of the inhibition zone could be done.

Standardized disk diffusion method: After 18 to 24 hours of incubation, standardized inocula of bacteria obtained from well-isolated, morphologically similar colonies were tested by disk diffusion method by the method recommended by CLSI.

Interpretation of results from disk diffusion tests: The zone diameters in the direct and standardized disk diffusion tests were measured. Interpretative categories were determined from zone sizes by using the CLSI guidelines, susceptible (S), Intermediate (I), and, Resistant (R). Results from rapid method were compared with results from standard tests.

Results

During a one-year period, 163 urine samples were observed by gram staining. These were chosen to make a comparison between the rapid method and the standard method, the organisms isolated was *E. coli*. Using this rapid method, identification and susceptibility data was available within 6 hours, compared with the standard methods in which the final report is available only in 48 hours. There was a total agreement of category (S-S), (I-I), (R-R), was high overall at 94.4% (154/163). There were no errors S-R, and R-S.

Zone Changes	Number of Cultures (%)
Susceptible to Intermediate	2 (1.25%)
Intermediate to Resistant	4 (2.45%)
Resistant to Intermediate	3 (1.85%)
TOTAL	9 (5.6%)

The discrepancies in the results were caused by zone diameters differences of only 1 to 4 mm.

Conclusion

In this study we present evidence that urine specimen containing ≥1 bacteria/field examined microscopically (x1000) and processed by a rapid method directly into a CHROMagar Orientation medium allows the rapid identification and determination of antimicrobial susceptibility equivalent to the standard method. In other investigations direct susceptibilities have been criticized for not being reliable in mixed cultures, on the contrary with CHROMagar Orientation Medium there is no problem because this medium minimizes the negative impact of mixed growth. In conclusion this procedure allows the earlier reporting in 6 hours of susceptibility results, improves antimicrobial use and it is a less expensive method for the identification of a variety of organisms.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

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