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Application Anti Microbial Activity Test and Direct Inoculation of Urinary Specimen Test to Increase the Quality of Results and Decrease the Production Cost in Clinical Microbiology Laboratory, Sanglah General Hospital Hospital, Bali-Indonesia

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Objective: Urinary tract infection (UTI) is the most common bacterial infection in general practice and in hospitals. Fast and accurate urine culture and sensitivity test are needed for adequate therapy. Anti Microbial Activity test (AMA test) that is used to detect the presence of antibiotics in urine specimens is not commonly used in clinical microbiology laboratories. Some laboratories are still using indirect inoculation technique using enriched media before inoculating onto the agar media. The aim of this research is to compare results of urinary examination of direct inoculation technique with AMA test with indirect inoculation technique without AMA test.
Methods: A number of 210 urine specimens were collected in Clinical Microbiology Laboratory at Sanglah General Hospital within a time period between 16 June until 16 July 2009.
Results: Antibiotics were detected in 40% of the urinary specimens; whereas 48.1% showed no evidence of UTI, that is negative AMA test and sterile urinary culture or colony growth < 10^5 CFU/ml. Only 11.9% of the specimens indicates urinary tract infections. The examination can be completed within 2-3 days which is shorter than indirect inoculation test which require 5-7 days. Direct inoculation technique can reduce the cost of production three-fold the costs require for an indirect inoculation test.
Conclusions: Application of AMA test and direct inoculation technique can give results more rapidly, reliable and useful for clinicians. This also decrease the laboratory's cost of production.

Key words: AMA test, urine culture, direct inoculation technique

INTRODUCTION

The Clinical Microbiology Laboratory at Sanglah General Hospital until today is still using indirect inoculation technique for culturing the microbial agents. This technique requires all the specimens be inoculated into enriched media before planted in agar media.[1] This process takes about 5 days so that clinicians will have difficulties in choosing the right antibiotics to replace the empirical ones.[2]

Urinary tract infections are the most common bacterial infections. Nosocomial urinary tract infections count almost 40% of all the nosocomial infections and cause 17% of secondary nosocomial sepsis.[3] In 2008, the Clinical Microbiology Laboratory at Sanglah Hospital had processed 2700 urine specimens or about 33% of all examined specimens.

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Ideally, in examination of urinary specimens, the result of examination should explain why the urine culture is sterile, or whether the bacteria isolated are significant as the cause of infections, or whether they are only contaminants during the process or maybe a normal regional flora in the human body that does not need any therapeutic antibiotics.[4]

If the isolated contaminant bacteria are mistakenly recognized as the causative agent and tested for antibiotics sensitivity, clinicians may likely give inappropriate antibiotics. This can be harmful not only for the patient but also for the hospital environment. Other disadvantages are longer inpatient hospitalization period caused by slower rate of recovery, higher hospital cost, and higher risk of nosocomial infections for the patient.[5]

Direct inoculation technique by putting the specimens directly into blood or MacConkey media will cut 1 day off the process. Nowadays, inoculation of specimens into enriched media prior to inoculation into agar media is not recommended anymore unless for certain purposes such as screening for specific disease, because inoculation into enriched media increases the risk of

contamination that leads to suppression of pathogenic bacterial growth[4]. Anti Microbial Activity (AMA) test is a testing procedure to detect the existence of antibiotics in the urine specimen. AMA test will help to differentiate whether the urine specimens are sterile because of antibiotics that have been taken by the patients or the urine specimens are really not containing any bacteria[4,6]. Based on the description above, since June 16, 2009, the Clinical Microbiology Laboratory at Sanglah Hospital has applied combined direct inoculation technique and AMA test. This aims to shorten the examination period, decrease the production cost of examination materials, and enrich the result of examination in order to help clinicians to choose the appropriate therapy.

METHOD

Every urine specimen underwent the following examinations:[6]

Examination Day-1

1. Microscopic examination: 10 mL of urine was centrifuged 300 rpm for 15 minutes. Two direct preparations were made, one for wet preparation and the other for gram staining. With 40x magnification, the leucocytes were counted on wet preparation. Leucocytes > 5 cells/field were reported as pyuria. On the Gram staining, with 100x magnification, leucocytes, epithels, and bacterial count were reported.
2. AMA test: 2-3 standard colonies (*Bacillus subtilis var globigii NCTC 10073)* were deluted until the solution reached 0.5-1 McFarland and then incubated into Muller Hinton media. One drop of urine specimen was put into the surface of MH media and incubated at 37^0C for 24 hours.
3. Colony count: 1 µl of urine was inoculated onto MH medium, incubated at 37^0C for 24 hours.
4. Urine culture: 1 µl of urine was dropped onto blood and MacConkey media agar.

Examination Day-2

1. The AMA test result: hemolytic zone on media considered as positive result indicating antibiotics in urine specimens. No hemolytic zone on media considered as negative.
2. The colony count result: colony count < 10 equal to < 10^4/ml, colony count 10-100 equal to 10^4/ml – 10^5/ml and colony count > 1000 equal to > 10^5/ml.
3. Evaluation of bacterial growth on media agar: if any growth detected, then Gram staining, identification, and sensitivity test were done on the colony.

Examination Day 3

1. The results of bacterial identification and sensitivity test were recorded.
2. The reported final results consisted of: leucocytes count on Gram and wet preparation, AMA test result, colony count, culture and sensitivity test result with expertise recommendations from the in charge clinical microbiologist.

RESULTS

During the period of June 16 – July 16, 2009 the laboratory had received and processed 210 urine specimens. The AMA test detected antibiotics in 40% of all the urine specimens. About 48.1% of the urine examinations resulted in no evidence of urinary tract infection, as indicated by negative AMA test with sterile culture result or colony count less than 10^5 cfu/ml. The examination result that indicated a process of urinary tract infection was found only in 11.9% of all the urine specimens (Tabel l).

Table 1
The Results of Urine Examinations by AMA Test (n=210)

AMA Test	Frequency (%)
Positive AMA test:	
Sterile	53 (25.40%)
Positive gram cocci	6 (2.90%)
Negative gram bacilli	18 (8.60%)
Yeast	5 (2.40%)
Mixture colony (2-3 colony types)	2 (0.950%)
Negative AMA test:	
Sterile	54 (25.70%)
Colony count < 10^5 cfu/ml	47 (22.40%)
Colony count > 10^5 cfu/ml	4 (1.90%)
Positive gram cocci	18 (8.60%)
Negative gram bacilli	3 (1.40%)
Mixture colony (2-3 colony types)	
Total	210 (100%)

Table 2 shows the comparison between the results of direct and indirect inoculation. For indirect inoculation, we used the data from February 2009 considering the number of examined specimens that were almost equal with the number of specimens used in this research. Indirect inoculation technique required 3-5 days whereas direct technique required only 2-3 days which allowed the patients to receive the definitive treatment faster. The cost of examination used per specimens did not show any significant difference between direct and indirect technique. But there was a big difference in production cost of the laboratory between these two techniques. Over the years the operational expenses of Clinical Microbiology Laboratory at Sanglah Hospital were

very high. For example, if we count the production cost of February 2009 that used indirect technique, the cost was higher than USD 2,321.00, whereas the cost of direct technique was only USD 772.00.

Table 2
The Comparison between Direct and Indirect Inoculation Technique on Urine Specimens Examination

Compared Variables	Direct	Indirect
Examination period (days)		
Sterile	2	3
Isolated gram positive cocci	3	5
Isolated gram Negative bacilli	3	5
Mixed colony	2	5
Examination cost per specimen ($US)		
Sterile	2.89	3.22
Isolated gram positive cocci	6.67	8.89
Isolated gram negative bacilli	11.11	11.11
Mix colony	2.89	16.67
Amount of specimen and isolated bacteria*		
Sterile	52.70% (106/210)	17.5% (44/251)
Isolated gram positive cocci	4.70% (10/210)	15.5% (39/251)
Isolated gram negative bacilli	17.10%(36/210)	65.7% (165/251)
Estimation of production cost	$US 772.8	$US 2321.78

DISCUSSION

Almost half of the spesimen were detected contain antibiotic. As already known, specimen collection for microbial culture should be done before antibiotics treatment was started.[3,4,7,8] This result also indicated the excess use of empirical antibiotics. Patient with no evidence of urinary tract infection needs no antibiotic therapy.[9] This result raised the question about the indication of urine specimens examination and showed the lack of sharp analysis by clinicians in determining the diagnosis.

In indirect technique, more bacteria were isolated because this technique required urine to be inoculated first in enriched BHI media before cultured into blood and MacConkey agar media. This technique not only allowed the causative agent to be grown excessively but also for normal regional flora or contaminant bacteria. This condition would suppress the real causative bacteria, so the subculture from enriched media would contain only the contaminant/normal flora bacteria. This was shown by the result of indirect technique in February 2009 in which the gram negative bacilli were highly isolated (65.70%) and if we look closely the species of isolated bacteria, it proved to be mainly of the types relevant to hospital contaminant bacteria. This result did not correspond to the real patient situation, thus the therapy given by clinician would errantly intended to treat the contaminant bacteria that may lead to the emergence of bacterial resistance.

This research is related with one of the hospital programs concerning in the rationale of antibiotics therapy in the hospital. The results of this research are used by the hospital as a foundation to continue the application of AMA test and direct inoculation technique at Sanglah Hospital. At the beginning, many clinicians denied the result of this new technique. To overcome this situation, the Clinical Microbiology Laboratory in cooperation with the nosocomial infections team have socialized and made a personal approach to the clinicians. Now every examination result sheet from the laboratory always contains an expertise suggestion regarding the interpretation of the culture result and choice of appropriate antibiotic. Today the clinicians have started to understand and accept the new format of the urine culture reports.

CONCLUSION

The examination of urine culture specimens using direct inoculation technique and AMA test have given advantages for: 1) the hospital management, by suppressing the expenses, preventing irrational use of antibiotics, and creating more effective work of laboratory staff; 2) the patients, by giving adequate and accurate therapy that would increase the recovery rate; and 3) hospital environment, by reducing the emergence of multi resistance bacteria.

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