

Evaluation of Etest 2.0 McFarland Method Compare with Population Analysis Profiles Test for Detection of Heterogeneous Vancomycin-intermediate *Staphylococcus aureus*

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are the major health problem worldwide including Thailand. An increasing use of glycopeptides especially vancomycin makes the organisms become more virulent and resistant. Further studies regarding the prevalence of infections or colonization caused by this organism, must depend on reliable laboratory tests for detection of this organism. Heterogeneous vancomycin-intermediate *S. aureus* (h-VISA) cannot be detected by a routine test for determination of the minimum inhibitory concentration (MIC) of vancomycin. The gold standard method for detection of this resistant strain is the population analysis profiles (PAP) method, but it is complicated, time-consuming, expensive, and needs well-trained experts. Using a laboratory method with comparable sensitivity and specificity but more convenient, simpler, and cheaper would be an alternative to the PAP method.

Objective: To evaluate of Etest 2.0 McFarland method, compared with PAP for detection of h-VISA.

Methods: All strains of MRSA, from all clinical specimens from King Chulalongkorn Memorial Hospital

and some specimens from Siriraj Hospital, were evaluated for h-VISA by two methods including the Etest 2.0 McFarland and the gold standard method (PAP).

Results: 4 total of 119 clinical specimens (103 from King Chulalongkorn Memorial Hospital and 16 from Siriraj Hospital) growing MRSA were enrolled. The PAP method can identify 6 h-VISA strains (5 from blood cultures and 1 from pus culture) from 4 patients of King Chulalongkorn Memorial Hospital. Thus, the prevalence of h-VISA strains was 6.35. Etest 2.0 McFarland was performed, and showed the sensitivity of 16.6 percent and specificity of 100 percent for detection h-VISA.

Conclusions: Etest 2.0 McFarland gives low sensitivity but 100 percent specificity for the detection of h-VISA. However, the Etest 2.0 McFarland is more convenient, less expensive, simpler, and does not need well trained experts. We suggest that if the result from the screening test by the one-point population analysis was positive for h-VISA, the Etest 2.0 McFarland should be performed first for a confirmation of h-VISA. If the result is positive, there is no need to perform the PAP method. But if the result is positive, the PAP method should be performed to determine h-VISA.

Detection of Dengue Virus in Bone Marrow by Reverse Transcription-Polymerase Chain Reaction

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Background: Our country is considered endemic for dengue virus infection. Serosurveillance indicates that almost all adults have been infected, mostly asymptotically. A long-held mechanism for clinical severity involves sequential infections by different serotypes. Even though some of its peer flaviviruses are known to reside persistently within the host and contribute to host illnesses, dengue virus has not been shown to behave in a similar fashion. As dengue is a hematotropic virus, we sought to find evidence of its persistence in the bone marrow of previously-infected persons.

Methods: We studied patients clinically suspected of hematologic malignancies and indicated to have diagnostic bone marrow studies. A fraction of cellular marrow was employed for RNA extraction for reverse transcription-polymerase chain reaction (RT-PCR) by dengue-specific primers. Serologic assessment by hemagglutination inhibition test (HI) and enzyme-linked immunosorbent assay (ELISA) was performed to minimize a chance of including patients with recent dengue infection. Demographic data of all patients were analyzed, especially for the history of prior recent febrile illness and diagnosis of dengue infection.

Results: Of 73 enrolled patients, dengue genome was detected in cellular marrow of three cases. These patients had had no history of febrile illness prior to the bone marrow study, and HI and ELISA results of single or paired sera of from these patients were, similar to those of the rest, compatible with either remote or remote/no infection by flaviviruses. An indication for bone marrow examination in these patients was a follow-up examination, and pathological results also confirmed they were in the stage of remission.

Conclusions: Dengue virus genome could be detected in the bone marrow of asymptomatic haematologic patients by using RT-PCR. Sequential infections by different serotypes seem to be a key in severe dengue pathogenesis. Its peer flaviviruses, however, have been shown both *in vitro* and *in vivo* to do so. The persistent first-serotype virus, defective or complete, could possibly confer a biological influence when co-infected with a second serotype later on in their life. As our understanding of dengue pathogenesis is far from perfect, this finding obviously opens up a door to a new arena of dengue research.

Predicting Factors for Successful Immune Response of Hepatitis B Vaccination in HIV-1 Infected Patients

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ABSTRACT

Background: Co-infection of hepatitis B virus (HBV) among HIV-1 infected patients has a great impact on morbidities and mortalities of the patients. A successful immunization of hepatitis B vaccination can prevent untoward consequences, while a suboptimal response was reported in this population. This study aimed to determine the predicting factors for successful hepatitis B vaccination among HIV-1 infected patients.

Methods: A prospective study was conducted among HIV-1 infected patients who had negative serologies for anti-HBs antibody, anti-HBc antibody, and HBs antigen. Anti-HBs antibody was evaluated one month after complete administration of 3-injection (0, 1, 6 months) course of hepatitis B vaccine. Patients who had anti-HBs antibody level of >10 mIU/mL were defined as responders.

Results: There were 65 patients with a mean age of 39±8.5 years, and 68 percent were females. Fifty-

seven (88%) patients had received antiretroviral therapy at a median (interquartile range) duration of 23 (9-40) months, and 75 percent of these had HIV-1 RNA of <50 copies/mL. The mean (SD) CD4 cell count and the percentage at the time. The mean of vaccination was 345 (194) cells/mm³ and 16 (7%), respectively. Of all, 30 (46%) patients were in the responder group. When compared to the non-responder group, the responder group had a higher mean CD4 cell count (p=0.047) and a trend towards younger age (p=0.052). Based on the multivariate analysis, younger age (p=0.049) and higher number of CD4 cell count (p=0.048) were predictors for successful response of hepatitis B vaccination.

Conclusion: Predicting factors for successful immunity of hepatitis B vaccination in HIV-1 infected patients were younger age and higher number of CD4 cell count. Test of antibody responses after vaccination is necessary to confirm the response.

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Received for publication: June 22, 2007.

Serum *Aspergillus* Galactomannan Antigen for the Diagnosis of Invasive Aspergillosis in Hematologic Malignancy and Hematopoietic Stem Cell Transplantation in Phramongkutklao Hospital

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ABSTRACT

Background: Invasive aspergillosis (IA) is the leading cause of infection-related mortality among recipients of allogenic hematopoietic stem cell transplantation (HSCT). IA is also a major cause of morbidity and mortality in patients with hematologic malignancies with neutropenia. With early diagnosis and treatment of this disease, the good outcomes have been proven. However, there are many obstacles in order to make a definitive diagnosis of IA in clinical practice. Therefore, the early diagnosis of this invasive disease is often difficult to establish.

Method: We performed a retrospective chart review on patients with hematologic malignancies and recipients of HSCT who were admitted to the Department of Medicine at Phramongkutklao Hospital between August 2005 and February 2007 with the diagnosis of IA, and testing for serum *Aspergillus*

galactomannan antigen was performed.

Results: A total of 19 patients were identified. All of the patients had pulmonary infection. Among them, 3 patients had associated sinus infection, and 1 patient had associated sinus and cerebral infections. Serum *Aspergillus* galactomannan antigen test was positive in 14 patients. The time between the onset of suspected symptoms or signs of IA and the first positive serum *Aspergillus* galactomannan antigen test was 9-155 days (average of 81.6 days). Sixteen patients (84.2%) died before the end of study.

Conclusions: The mortality due to IA is extremely high. We found that serum *Aspergillus* galactomannan antigen testing does not aid in the early diagnosis of IA in our patients, nevertheless, serum *Aspergillus* galactomannan antigen testing remains another useful tool in diagnosing IA.

A Bacteriologic Study of Ceftriaxone Treatment in Acute Pyelonephritis in Female Patients Caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* or *Proteus mirabilis* With or Without Extended-Spectrum- β -Lactamase Production

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Background: Extended-spectrum-beta-lactamase (ESBL)-producing *Escherichia coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis* have become recognized as a worldwide problem. Much controversy exists as to whether cephalosporin treatment is appropriate for infections caused by ESBL-producing organisms because no randomized studies have been performed to evaluate the microbiological outcome.

Objective: This study aimed to evaluate the microbiological outcome of ceftriaxone treatment of acute pyelonephritis in female patients caused by ESBL-producing *E. coli*, *K. pneumoniae*, *K. oxytoca*, or *P. mirabilis*, and to determine the prevalence of acute pyelonephritis caused by ESBL-producing organisms.

Results: We performed a prospective study of hospitalized female patients with acute pyelonephritis caused by *E. coli*, *K. pneumoniae*, *K. oxytoca*, or *P. mirabilis* with or without ESBL production between 2006 and 2007. The microbiological outcomes were assessed at 72 hours after ceftriaxone therapy. There

were seventy-three patients (the mean age of 66.15 ± 20.69 years). The prevalence of ESBLs was 33.7 percent. Independent risk factor for ESBL-producing strains, analyzed by multivariate analysis, was underlying cerebrovascular disease or a recent history of antibiotic use within 1 months. The microbiological outcome at 72 hours (the response rate were 67.9% and 100%, $p=0.001$) and 14 days (the response rate were 40% and 100%, $p=0.015$) after therapy in the ESBL-producing group was poorer than the non-ESBL producing group, respectively. However, the clinical outcome at 72 hours and 14 days was not significantly different between the ESBL-producing and non-ESBL producing group, respectively.

Conclusion: There is a different microbiological outcome after ceftriaxone treatment of acute pyelonephritis in female caused by ESBL-producing *E. coli* or *K. pneumoniae*, or *P. mirabilis*, in comparison with ESBL-nonproducing strains. We do not recommend ceftriaxone in the treatment of acute pyelonephritis in female patients caused by ESBL-producing organisms.