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Scientific Tracks & Abstracts

Day 1

Mardiastuti, Clin Microbiol 2018, Volume 7
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Real-time polymerase chain reaction assay for the detection of *H. pylori* in patients with dyspepsia: Comparison with histopathology examination

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Helicobacter pylori infection is still a health problem in Indonesia and may lead to malignancy. Early detection may increase the effectiveness of treatments and prevent complications. This study was aimed to determine the accuracy of real-time Polymerase Chain Reaction (rPCR) compare to histopathology examinations. Endoscopies of antral and corporal biopsies were performed in 34 consecutive patients with dyspepsia who did not take antibiotics and proton pump inhibitor for two weeks (February-October 2017). The tissue biopsies were stained with HE and the rPCR was conducted using primers previously. The thermal cycle of rPCR was 95 oC, 3'; 45 cycles of denaturation 95 oC, 15"; annealing temperature was 64 ^{O}C, 1'. The primer and probe concentration were 0.8 µM and 0.6 µM, respectively. Some of the positive specimens were sequenced to confirm the presence of H. pylori. The minimal DNA concentration detected was 3.8^{O}10-11 ng/ul. No other microbes showed positive result. Real-time PCR revealed a higher positivity rate 32.35% (11/34) compared to histopathology examinations 20.59% (7/34). The positivity rate of rPCR from the antral was higher than from the corporal specimens. There was only one discordance result in which histopathology showed a positive result, while the rPCR was negative. All of specimens sequenced (7/34) were confirmed as H. pylori. Real time PCR is able to enhance the positivity rate in detecting *H. pylori* directly from the specimen. Furthermore, rPCR was specific, sensitive, less time consuming and more cost effective than histopathology examinations.

Biography

Mardiastuti has completed his MSc in Microbiology from Western Illinois University in 1991 and PhD in Medical Education from Gadjah Mada University in 2013. She is the Director of Postgraduate training program of Clinical Microbiology, Faculty of Medicine, University of Indonesia. She is also the Research Coordinator at Department of Medical Education at the same institution.

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Modified rapid urease test for the detection of *Helicobacter pylori* from gastric biopsies in patients with dyspepsia

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Up to now, rapid detection of *Helicobacter pylori* is still a problem due to its difficulty in culturing and having accurate histopathology result. In clinical setting, internists usually perform urea breath test. In laboratory setting, urease test, MIU test, culture, PCR and histopathology examination are utilized for detecting *Helicobacter pylori*. The objective of this study is to detect *H. pylori* in gastric biopsies using a modified rapid urease test. This is a cross sectional study. We obtained gastric tissue biopsy specimens (antrum and corpus) from untreated dyspepsia patients who come to Gastroenterohepatology Division, Department of Internal Medicine, dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia. One set of specimen was fixated in 10% formalin and was sent to Anatomy Pathology Laboratory. The other was sent directly to Microbiology Laboratory without fixation. Rapid Urease Test medium was prepared by modifying Christensen Urea and Motility Indole Urease Test. Urea concentration, pH indicator concentration (phenol red) and incubation temperature were optimized. The optimum concentration of urea was 40%, while the optimum concentration of phenol red was 0.5%. Of 34 samples, 12 (35.3%) were positive by rapid urease test (RUT) (either antrum and/or corpus). There were 2 (5.8%) samples which only positive by histopathology examination, 7 (20.6%) samples were only positive by RUT and 5 (14.7%) samples were positive by histopathology and RUT (14.7%). We assumed that *Helicobacter pylori* detection should not only based on histopathology examination, but also in combination with RUT.

Biography

Angky Budianti has completed her Post-graduate training program in Clinical Microbiology from University of Indonesia. She is an Academic Staff at the Department of Microbiology, Faculty of Medicine, University of Indonesia, since 2010. She also works as a Clinical Microbiologist in Clinical Microbiology Laboratory in several hospitals in Tangerang, Indonesia. She is currently a candidate of PhD program in Medical Sciences at Faculty of Medicine, University of Indonesia.

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John James Stewart Cadwell, Clin Microbiol 2018, Volume 7
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The hollow fiber infection model: Principles and practice

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Emerging antibiotic resistance presents a serious global health threat. 2 million people in the United States were infected with antibiotic resistant bacteria in 2014 and more than 20,000 died as a direct result of these infections, many more from complications. Antimicrobial resistance has been identified as one of the three greatest threats to human health. Antibiotic discovery and development require static susceptibility testing to screen compounds, *in vitro* Pharmacodynamics/ Pharmacokinetic (PK/PD) studies to model drug dynamics and efficacy and testing in animal models to provide critical information prior to the clinical evaluation of new antibiotics. The one compartment PK/PD model typically consists of an open central reservoir containing the organism of interest, a source of diluent and a waste reservoir: (1) Open system, not bio safe, (2) bacteria numbers change over time, (3) large volume requires large amount of drug and diluent and (4) rapid changes in drug concentration not possible, cannot model short half-lives. Animal models have many shortcomings though they have served as a primary development tool for many years: (1) PK/PD may not match human values, (2) cannot sample same animal over time, (3) difficult to study large numbers of bacteria to reveal resistance and (4) many infections cannot be modeled in a mouse or other animal. To address these shortcomings the two-compartment *in vitro* pharmacokinetic model utilizing hollow ber bioreactors was developed, the Hollow Fiber Infection Model (HFIM). The advantages of the HFIM are as follows: (1) Closed, bio-safe system, (2) large number of organism can be tested, revealing resistance, (3) Precisely simulates human PK/ PD, (4) repetitive sampling over time, both drug and organism, (5) total kill can be determined, (6) single use, disposable, reproducible, (7) two drug models can be tested, (8) can model both dosing curve and elimination curve and (9) can look at bacteria in different growth phases and in combination with cells. The clinical utility of the HFIM has been demonstrated and is now endorsed by the EMA. An overview of historic PK/PD models is presented and the utility of the system as it relates to antibiotics and other drugs are discussed.

Biography:

John James Stewart Cadwell has received his degree in Pharmacology from the University of Miami in 1981. He spent additional time studying at the University of Nottingham and the National Institute of Medical Research at Mill Hill, UK. In 2000 he founded FiberCell Systems Inc., a company specializing in the research and supply of hollow fiber bioreactors. He has over 10 publications in the field and three patents relating to hollow fiber systems and is considered a world expert in the field.

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Day 2

Baveja C P et al., Clin Microbiol 2018, Volume 7
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Diagnostic solutions for musculoskeletal tuberculosis in disease endemic regions

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Musculoskeletal tuberculosis most often involves the spine, followed by tuberculous arthritis in weight-bearing joints and extra spinal tuberculous osteomyelitis. Microbiological diagnosis of osteoarticular tuberculosis is difficult since it is mostly a paucibacillary disease. This study had been designed to evaluate the results of the Mycobacteria Growth Indicator Tube (MGIT) over conventional methods for diagnosis of musculoskeletal tuberculosis. Clinically suspected cases of *musculoskeletal tuberculosis* having an abscess were enrolled in the study. A total of 30 aspirate samples were collected and processed as follows: (1) Direct microscopy by Ziehl-Neelsen staining, (2) Culture on Lowenstein-Jensen (LJ) medium and (3) Culture in mycobacteria growth indicator tube. The samples that yielded a positive culture for Mycobacterium tuberculosis on LJ medium and MGIT were further subjected to drug susceptibility testing using proportion method and MGIT SIRE kit, respectively. Out of the 30 samples tested, 10 (33.33%) showed growth of *Mycobacterium tuberculosis* in MGIT, 7 (23.33%) showed growth of MTB on Lowenstein-Jensen medium and only 4 (13.33%) showed presence of Acid Fast bacilli on Ziehl-Neelsen staining. The mean duration for detecting MTB by MGIT was 15.09 days whereas on LJ culture it was 29.33 days. Of the 10 samples that were positive for MTB using MGIT, 1 (1.00%) sample was detected as Rifampicin and Isoniazid resistant. No drug resistant MTB was detected using proportion method. The key advantage of MGIT was that it detected MTB in 3 cases that showed no growth on LJ culture. The MGIT results were available in about half the time. MGIT also yielded one resistant case which was missed by LJ culture. Thus, MGIT offers a faster and improved method of detecting musculoskeletal tuberculosis in disease endemic regions.

Biography:

Baveja C P is the Director, Professor and Head of Microbiology at Maulana Azad Medical College, New Delhi. He was awarded an International Fellowship at Royal Postgraduate Medical School and Hammersmith Hospital, London, UK. He has also conducted research work on Polymerase Chain Reaction at London School of Hygiene and Tropical Medicine. He has been teaching microbiology to medical undergraduates for past three decades. He was honored with the Best Medical Educationist award in 2000. He has been mentoring postgraduates for the last 25 years. He has been involved in research work with particular emphasis on diagnosis of tuberculosis. He has supervised a number of PhD students with research work on tuberculosis. He is also the Nodal Officer and In-Charge for State Reference Laboratory for HIV testing.

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Jaskanwal Kaur, Clin Microbiol 2018, Volume 7
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Resistant fungal infection in Indian subcontinent

Jaskanwal Kaur
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Fungi are ubiquitous heterotrophs and exist as saprophytes or parasites. Because of the increase in worldwide travel mycoses has crossed all geographical borders. Dermatomycosis is nowadays becoming recalcitrant and recurrent especially in the tropical climates of India. Dermatophytes which infect skin, hair and nails are limited to dead keratinous tissue but the immune response occurs in dermis. Antifungal drug resistance is increasing at an alarming rate and patients are doing the rounds of OPDS under distress. Dermatophytosis caused by *Epidermophyton, Trichophyton* and *Microsporum* species is flourishing due to decreasing effect of drugs like terbinafine and griseofulvin and itraconazole which are the preferred drugs. 20% to 30% of daily OPD turns out to be patients of fungal infection not responding to treatment appropriately. Most commonly patients come with self-treatment with topical steroid ointments due to its OTC availability. There is also rampant use of systemic steroids which flairs the infection. There is large volume of cases of tinea cruris especially in adult males due to usage of tight fitting undergarments and denims which adds to moist condition in already hot and humid weather. Diabetic, atopic and immune-compromised patients are most difficult to treat. It is need of the hour to curtail this growing fungal resistance in collaboration with microbiologists and conducting studies for other causative factors and further lay down guidelines for management of such patients so that it does not become a global problem.

Biography:

Jaskanwal Kaur has served as a Physician for almost 20 years. She is in the field of dermatology for the last 8 years and has presented papers at the national and international level and obtained scholarship at Vancouver by International League of Dermatological Society for research work in alopecia areata.

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Wondimu Ashagre et al., Clin Microbiol 2018, Volume 7
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In vitro starvation model for assessing phenotypic drug tolerance on *Mycobacterium tuberculosis* lineages in Ethiopia

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Background: *Mycobacterium tuberculosis* persist in the human host for decades and reactivation can occur at any point. Becomes dormant and phenotypically drug tolerant when exposed adverse conditions. Understanding of the signals and processes which allow the bacteria to achieve this feat could potentially be used as a baseline to design new types of drugs or modify old drug regimens for improved cure and avert development of drug resistance.

Objective: To use *in vitro* starvation model in assessing if nutrient deprivation affects phenotypic drug tolerance in *Mycobacterium tuberculosis* lineages circulating in Ethiopia.

Methods: Three MTB lineages and one standard susceptible reference strain (H37Rv) were tested by different test methods at different time point from March to September 2017. All lineages tested to be sensitive to first line anti Tb drugs. Log phase (highest colony count on week 3-4) culture from Lowenstein Jenson medium was sub cultured to Middle-brook7H9 with 10% oleic acid albumin dextrose catalase as a normal, Phosphate Buffer Solution (PBS) (pH 7.2) and Sterile Distilled Water (SDW) as starvation media were used. Each week we performed culture growth reading, acid fast stain (AFS) by Ziehl Neelson (ZN), Lipid Bodies (LB) by Sudan black stain and viability by Fluorecin Diacetate (FDA) staining. On week 0, 3 and 6 drug susceptibility test was done by colorimetric MTT assay. Graph pad prism 6 and SPSS V20 used for data analysis.

Results: A total of 576 experiments were performed using4 strains of *Mycobacterium Tuberculosis* sub-cultured on SDW, PBS and 7H9. Of these, 324 microscopic tests using (108 (ZN) acid fastness, 108 (FDA) viability and 108 (Sudan black stain) lipid bodies), 108 culture growth reading done. After week 6 acid fastness, viability and culture growth decreased. 144 phenotypic DST done using MTT assay. A higher inhibitory drug concentration was required at the 6th week compared to the baseline and C50 (RMP=0.5; INH=0.1; STM=2.0 and for EMB=4.0), yet the proportion of lipid body containing bacilli increased continuously in all lineages.

Conclusion: Our study showed that the mycobacteria lineages behaved similarly in all media systems and reached stationary phase at similar time. The increased drug concentration observed at the 6th week coincided with the decline in viable *Bacilli* in all media systems, thus attributing this phenomena to lipid body accumulation alone was difficult.

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