HEALTH SCIENCES

HS-01

MOLECULAR IDENTIFICATION OF T4 AND T5 GENOTYPES OF *Acanthamoeba* ISOLATES IN THE PHILIPPINES

Corazon C. Buerano[1,3], Abigail D. Trinidad[1], Lindsay Sydney N. Fajardo[3], Irwin Y. Cua[2], **Michael O. Baclig**[3], Filipinas F. Natividad[3]
[1]University of the Philippines Diliman, [2]International Eye Institute, and [3]Research and Biotechnology Division, St. Luke's Medical Center;
mobaclig@stluke.com.ph

Acanthamoeba species are ubiquitous free-living single-celled, opportunistic pathogens, which can be isolated from water, soil, dust in the air, and from other organisms. It can cause sight threatening *Acanthamoeba* keratitis as well as the rare but fatal encephalitis in humans. Traditionally, laboratory procedures to detect this organism include culture on non-nutrient agar with heat-killed *E. coli* (NNE) and microscopic examination. Identification can easily be done at the genus level but not at the species level. A recent way of detecting and identifying the organism propagated on NNE is through molecular means. This technique is based on the presence of ASA.S1, a partial 18S ribosomal DNA (Rns) gene unique to the genus. Subgeneric level of identification can be achieved by genotyping. Here we report on the genotyping of *Acanthamoeba* species in corneal scrapings from three keratitis patients and in nasal swabs from six unrelated healthy volunteers. A 461-bp amplicon was amplified using genus specific JDP1 and JDP2 primers. DNA sequencing of the PCR product was carried out using conserved 892 and 892C primers to determine the sequence of diagnostic fragment (DF3) of Rns. Phylogenetic tree was constructed using MEGA5. Results showed that isolates from all three corneal scrapings and from five out of six nasal swabs belonged to genotypeT4, whereas one nasal swab was of the genotype T5. Phylogenetic analysis showed that these isolates clustered with the reference sequences most similar to them. T4 (89%) is the predominant genotype found among nine isolates analyzed in this study. Molecular-based technique is a useful tool for the identification of genotypes of *Acanthamoeba* from other free-living amoebas. Genotyping helps in decision-making for clinical management of *Acanthamoeba* infection, in tracking the source of infection, as well as in epidemiological and environmental studies.

Keywords: *Acanthamoeba*, genotyping, keratitis, 18S ribosomal DNA

HS-02

ASSESSMENT OF DISTAL GUT MICROBIAL DIVERSITY AMONG FILIPINO CHILDREN OF DIFFERENT NUTRITIONAL STATUS THROUGH THE rRNA GENE

Leslie Michelle M. Dalmacio[1], Raul V. Destura[2] and Evelyn Mae Tecson-Mendoza[3]
[1]College of Medicine, [2]National Institutes of Health, UP Manila and
[3]Institute of Plant Breeding, UP Los Baños; lesmdmc@gmail.com

Acanthamoeba species are ubiquitous free-living single-celled, opportunistic pathogens, which can be isolated from water, soil, dust in the air, and from other organisms. It can cause sight threatening *Acanthamoeba* keratitis as well as the rare but fatal encephalitis in humans. Traditionally, laboratory procedures to detect this organism include culture on non-nutrient agar with heat-killed *E. coli* (NNE) and microscopic examination. Identification can easily be done at the genus level but not at the species level. A recent way of detecting and identifying the organism propagated on NNE is through molecular means. This technique is based on the presence of ASA.S1, a partial 18S ribosomal DNA (Rns) gene unique to the genus. Subgeneric level of identification can be achieved by genotyping. Here we report on the genotyping of *Acanthamoeba* species in corneal scrapings from three keratitis patients and in nasal swabs from six unrelated healthy volunteers. A 461-bp amplicon was amplified using genus specific JDP1 and JDP2 primers. DNA sequencing of the PCR product was carried out using conserved 892 and 892C primers to determine the sequence of diagnostic fragment (DF3) of Rns. Phylogenetic tree was constructed using MEGA5. Results showed that isolates from all three corneal scrapings and from five out of six nasal swabs belonged to genotypeT4, whereas one nasal swab was of the genotype T5. Phylogenetic analysis showed that these isolates clustered with the reference sequences most similar to them. T4 (89%) is the predominant genotype found among nine isolates analyzed in this study. Molecular-based technique is a useful tool for the identification of genotypes of *Acanthamoeba* from other free-living amoebas. Genotyping helps in decision-making for clinical management of *Acanthamoeba* infection, in tracking the source of infection, as well as in epidemiological and environmental studies.

Keywords: *Acanthamoeba*, genotyping, keratitis, 18S ribosomal DNA

HS-03

DIAGNOSIS AND MOLECULAR CHARACTERIZATION OF *Trichomonas vaginalis* IN SEX WORKERS IN THE PHILIPPINES

Macario Ireneo P. Queza[1] and **Windell L. Rivera**[1,2]
[1]Institute of Biology, College of Science, [2]Molecular Protozoology Laboratory, Natural Sciences Research Institute, University of the Philippines Diliman; wlrivera@science.upd.edu.ph

Trichomonas vaginalis is a pathogenic protozoan which causes the sexually transmitted infection, trichomoniasis. The absence or non-specificity of symptoms often leads to misdiagnosis of the infection. In this study, 969 samples consisting of vaginal swabs and urine were collected and screened from social hygiene clinics across the Philippines. Of the 969 samples, 216 were used for the comparative analysis of diagnostic tools such as wet mount microscopy, culture and PCR utilizing universal trichomonad primers, TFR1/2 and species-specific primers, TVK3/7 and TV1/2. PCR demonstrated higher sensitivity of 100% compared to 76.92% of the wet mount. PCR primer set TVK3/7 and culture had the same and the best expected average performance (ROC, 0.9848). Prevalence of infection in the sample population was 6.81%. Restriction fragment length polymorphism (RFLP) and phylogenetic analyses of the 18S rRNA gene and ITS1-5.8S-ITS2 region revealed that majority of the *T. vaginalis* isolates belonged to one main group. This study could serve as a trigger in enhancing cooperation among health institutions including local government units, health departments, non-government organizations, research and the academe to improve the prevention of the increasing cases of STI/STDs in the country.

Keywords: *Trichomonas vaginalis*, diagnosis, PCR, microscopy, culture, phylogenetic analysis

HS-04

Trichomonas vaginalis INDUCES APOPTOSIS IN HUMAN LUNG ALVEOLAR BASAL CARCINOMA EPITHELIAL CELL LINE A549

Daile Meek C. Salvador[1], Sonia D. Jacinto[1] and Windell L. Rivera[1,2]
[1]Institute of Biology, College of Science, [2]Molecular Protozoology Laboratory, Natural Science Research Institute, University of the Philippines Diliman;
dmcsalvador@gmail.com

Trichomonas vaginalis, a known inhabitant of the genitourinary tract has been identified in the respiratory tract of neonates and adults. The unusual presence of *T. vaginalis* in this site is associated with respiratory infections. However, the medical significance of this occurrence is unclear. In this study, the pathogenic potential of *T. vaginalis* in human lung alveolar basal carcinoma epithelial cell line A549 was investigated. It was shown that *T. vaginalis* can induce apoptosis in A549 cells as determined by TUNEL assay and transmission electron microscopy. After six hours of incubation with *T. vaginalis* there were about 20% TUNEL-positive A549 cells indicating apoptotic cells. Electron microscopic observations of infected A549 cells with trichomonads demonstrated apoptotic morphological features such as nuclear membrane disintegration, intense vacuolarization in the cytoplasm and chromatin condensation in the nucleus. Results from this study suggest the possible pathogenic effect of *T. vaginalis* to lung cells. To our knowledge, this is the first study to document the apoptotic potential of *T. vaginalis* in A549 cells. Continued researches are recommended to establish the clinical presentation of *T. vaginalis* in lung cells.

Keywords: *Trichomonas vaginalis*, A549 cells, apoptosis, host-parasite interactions, human lung cells

HS-05

IMMUNOMODULATORY EFFECT OF *Tinospora rumphii* Boerl LOTION IN *Sarcoptes scabiei* var *hominis*-INFECTED PATIENTS AND ITS PREDICTED SHELF LIFE: A PILOT STUDY

Agnes L. Castillo[1,2], Marina O. Osi[2] , John Donnie A. Ramos[2], Jean L. De Francia[3], Marylaine U. Dyjunco[4] and Peter F. Quilala[1,5]
[1]Faculty of Pharmacy [2]The Graduate School, University of Santo Tomas, [3]Dept of Pediatrics, [4]Dept of Internal Medicine, [5]Dept of Emergency Medicine, St. Luke's Medical Center; agnes_412@yahoo.com

Scabies is a major public health problem affecting 10% of the general population. It is caused by the *Sarcoptes scabiei* mite that has the ability to modulate the host's inflammatory and immune responses. A randomized, controlled, double-blind, pilot clinical study was performed to investigate the immunomodulatory effect and clinical efficacy of the *Tinospora* lotion in 66 scabies-infected patients through Enzyme-linked Immunosorbent Assay (ELISA) for Interleukin-1, Interleukin-6, Interleukin-8 and Monocyte Chemoattractant Protein-1 (MCP-1) in the serum samples. The pediatric patients were treated with *Tinospora* and Permethrin lotions for three consecutive days for two weeks and blood extraction was performed before treatment, during and after treatment. Clinical assessment of each patient was performed every week for five weeks. *Tinospora* lotion is comparable with Permethrin as anti-scabies agent ($p=0.315$) with significant reduction in the mean global evaluation score from baseline (7.20 ± 0.48 vs 7.264 ± 0.44) to day 28 (0.933 ± 0.35 vs 0.95 ± 0.25). No significant difference in the clinical improvement of the patients treated by both lotions ($p=0.9123$) and at different periods of observation ($p=0.4747$). The mean clearance time is 23, 20.47 to 25.53; and 21, 17.39 to 23.67; $p=0.226$ for *Tinospora* and Permethrin lotions, respectively. *Tinospora* lotion significantly reduced the IL-1, IL-6, lL-8 levels from Day 14 to Day 28 ($p=0.0002$, $p=0.0002$, $p=0.0065$) which is comparable to Permethrin lotion ($p<0.050$) with the exception of MCP ($p=0.3497$). Its predicted shelf life is 6 months. *Tinospora* lotion exhibits significant antiscabies activity through down-regulation of IL-1, IL-6 and IL-8 levels. Its incorporation as therapeutic reagent in Sarcoptes scabiei infections is highly recommended.

Keywords: *Tinospora*, scabicide, immunomodulatory,interleukin, MCP-1

HS-06

A DOUBLE BLIND RANDOMIZED CONTROLLED TRIAL ON THE EFFECTIVENESS OF 10% LEMONGRASS OIL (*Cymbopogon citratus*) VS. 1% CLOTRIMAZOLE SOLUTION IN TREATING *TINEA CORPORIS* AND *TINEA CRURIS*

Pamela Christine C. Cue[1], Rosalinda C. Torres[2] and Arnelfa C. Paliza[1]
[1]Department of Dermatology, University of Santo Tomas Hospital, [2]Chemicals and Energy Division, Industrial Technology Development Institute, Department of Science and Technology; pamelacue@yahoo.com

Superficial fungal infection is among the most common reasons for dermatologic consultation. This superficial infection is usually treated with topical antifungal agents such as the azoles & allylamines, sold usually as topical creams but not in solution forms. The essential oil of *Cymbopogon citratus* (lemongrass) exhibits antifungal activity. This project therefore aims to compare the efficacy of 10% lemongrass oil with 1% clotrimazole solution in treating *tinea corporis* and *tinea cruris* in terms of complete cure and adverse events. Ninety-six patients clinically and mycologically diagnosed with *tinea corporis* and/or *tinea cruris* were assigned randomly to apply either 10% lemongrass oil or 1% clotrimazole solution twice daily for 4 weeks. Clinical and mycological evaluations were conducted at baseline, and weekly up to 2 weeks post-therapy. Complete cure was achieved if there was clinical and mycological cure at 4 weeks. There was no statistically significant difference in terms of complete cure at four weeks between the two groups ($p = 1.0$, Fisher's exact test). There was no recurrence 2 weeks post-treatment in both groups. Erythema and burning sensation from the application of lemongrass were observed in two patients. This randomized controlled trial showed that 10% lemongrass oil was as effective as 1% clotrimazole solution in treating *tinea corporis* and *tinea cruris* based on clinical, mycological and complete cure assessments.

Keywords: lemongrass, tanglad, clotrimazole, *tinea corporis*, *tinea cruris*

HS-07

CELLULAR RESPONSE TO *Aglaia loheri* Blanco ACTIVE PRINCIPLE, MALDI 531.2[M+H]$^+$ IS PREDICTED BY GENES

Else F. Galutan[1], Sonia D. Jacinto[1] and Thomas Efferth[2,3]
[1]Institute of Biology, University of the Philippines Diliman, [2]University of Mainz, Germany, [3]German Cancer Research Center, Heidelberg, Germany;
akosielse@gmail.com

The expression of genes can be influenced by the presence of drugs or chemicals in cellular environment. The newly isolated *Aglaia loheri* active principle, Maldi 531.2[M+H]$^+$ was investigated for its *in vitro* cytotoxicity against human leukemia cell lines, CCRF-CEM and their multidrug resistant (MDR) type, ADR5000-CEM. Changes in the regulation of genes of two human leukemic cell lines were also evaluated after treatment with the active principle. XTT tetrazolium hydroxide for the non-radioactive quantification of cell proliferation and viability was used for cytotoxic test, and human illumina chip ID 6247215020 for DNA microarray analysis. Maldi 531.2[M+H]$^+$ showed potent anticancer activity against both CCRF-CEM and ADR5000-CEM cells with IC50 of 0.02 and 0.03µM respectively. The active principle further caused down-regulation of genes associated with cell survival: *ALDH1A2* and *AKR1C3,* including genes which play a role in maintaining mitochondrial DNA, NIPSNAP1. The data indicate that cytotoxic principles derived from *A. loheri* maybe a valuable source for the development of novel treatment options for cancer as it is seen that cellular response to Maldi 531.2[M+H]$^+$ is predictable by genes.

Keywords: *Aglaia loheri*, cytotoxicity, expression analysis, illumina sequencing, multidrug resistance, leukemia

HS-08

HAIR LEAD BIO-MONITORING AMONG SCHOOL CHILDREN IN THE PROVINCE OF CAVITE, PHILIPPINES

Janet P. Macawile[1] and Glenn L. Sia Su[2]
[1]De La Salle University Dasmariñas, Cavite
[2]University of the Philippines Manila; siasug@gmail.com

Airborne lead is one of the pressing environmental problems that cause neuropsychological impairments to people who are exposed to it. In assessing people's exposure to airborne lead, bio-monitoring techniques has been used as an indicator of chemical exposure. This study aims to determine the hair lead concentrations among school children in the Province of Cavite and compare the hair lead concentrations of the school children living and studying in the urban and rural areas of the Province of Cavite, Philippines. Consenting public and private school children of the municipalities of Bacoor and Alfonso were involved in the study. Hair strands were obtained from each student and analyzed for lead concentrations. Results of hair lead concentrations were compared for significant differences between the public and private schools in both areas using the t test under the $P < 0.05$ level of significance. A total of 922 consenting school children participated in this study. The mean $\pm$ SD hair lead concentrations of all school children surveyed was 0.2814 ± 0.1245 ppm. Hair lead concentrations of children studying in public schools (0.3044 ± 0.1081 ppm) were higher relative to those in private schools (0.2259 ± 0.1428 ppm). School children residing in the urban areas (0.3079 ± 0.1442 ppm) had a higher hair lead concentrations compared to those residing in the rural areas (0.2499 ± 0.0863 ppm). Hair lead concentrations of school children living in urban and rural areas and those studying in private and public schools in Bacoor and Alfonso were found to be significantly different ($t = 9.096$ and $t = 6.867$, respectively, $P < 0.05$). Findings indicate that school children are exposed to airborne lead. Higher hair lead concentrations were evident among school children who were residing in urban areas and studying in public schools.

Keywords: Airborne Lead, School children, Bio-monitoring, Cavite, Chemical Exposure

HS-09

HPLC ANALYSIS OF CORTISOL AND CORTISONE IN HUMAN URINE

Ma. Cristina B. Portilla[1], Jessa Karyl R. Apa[2],
Jason Paul C. Monlinong[1] and **Cherrie B. Pascual**[1,2]
[1]Research and Biotechnology Division, St. Luke's Medical Center,
[2]Institute of Chemistry, University of the Philippines Diliman;
mcbportilla@yahoo.com

Cortisol is a steroid hormone which increases blood sugar, suppresses the immune system and aids in fat, protein and carbohydrate metabolism. It is clinically important to measure urinary free cortisol and its metabolite, cortisone, to diagnose and treat adrenal dysfunctions like Cushing's and Addison's syndrome. A reversed-phase HPLC method was developed for the determination of free cortisol and cortisone in human urine, using 6α-methylprednisolone as internal standard. The steroids were separated on a Lichrosphere C18 column using mobile phase of 40:60(v/v) acetonitrile:water mixture with UV detection set at 248 nm. The average retention times were 7.9 minutes for cortisol, 8.5 minutes for cortisone and 10.0 minutes for 6α-methylprednisolone. Linear response for cortisol and cortisone dissolved in mobile phase and spiked in urine was within the range 0.50-10.00 μg/mL. The limit of detection (LOD) for cortisol and cortisone was 0.002 μg/mL and 0.001 μg/mL respectively, while the the limit of quantification (LOQ) was 0.007 μg/mL and 0.003 μg/L respectively. Intra-batch and inter-batch CV were all less than 13%. Prior to chromatography, samples were extracted with solid-phase extraction (SPE) column. Recoveries after SPE ranged from 90.3-115.3% for cortisol and 93.0-107.1% for cortisone. Human urine samples were analyzed and cortisol concentration ranged from 0.06-0.09 μg/mL which was within the normal cortisol concentration range of 0.05-0.16 μg/mL. The method described here may be used to quantify cortisol and cortisone in human urine.

Keywords: HPLC, cortisol, cortisone, chromatography, SPE

HS-10

DETECTION OF DENGUE VIRUS USING A QUARTZ CRYSTAL MICROBALANCE (QCM)-BASED IMMUNOSENSOR

Jason Paul C. Monlinong[1], **Olga Corales**[2], Roland Jay Miguel[3], Luis Sison[3], Cherrie Pascual[1,2] and Ronald Matias[1]
[1]Research and Biotechnology Division, St. Luke's Medical Center,
[2]Institute of Chemistry and [3]College of Engineering,
University of the Philippines Diliman; jpcmonlinong@yahoo.com

Continuing efforts to develop fast and reliable methods for the early detection of dengue virus in human blood samples prompted us to develop a Quartz Crystal Microbalance (QCM)-based immunosensor. Following surface functionalization on the gold electrode surface of the quartz crystal, the immunosensor was used to detect dengue viral antigen using a laboratory-fabricated QCM set-up. Dengue monoclonal antibody (mAb) was immobilized on the gold electrode surface of the 5-MHz crystal using Protein A. C6/36 cells were then infected with dengue 2 viruses and propagated. Harvested infected culture fluid was utilized to determine the sensitivity of the QCM-immunosensor. Binding of the dengue virus antigen to the immobilized dengue monoclonal antibody induced detectable changes in the oscillation frequency of the quartz crystal. Baseline oscillation frequencies (finitial) were measured and compared with the oscillation frequency at the time of binding of the dengue antigen to the dengue mAb (f_{final}). Quantification of the frequency shifts ($\Delta f = f_{final}-f_{initial}$) yielded a reliable signal for the detection of the dengue virus. Parameters that were optimized for the QCM-immunosensor include dengue mAb concentration, Protein A concentration and incubation time. Optimum parameters used in the fabrication of the immunosensor were the following: 120 min of Protein A incubation using 10.0 mg/mL Protein A concentration and 180 min of dengue mAb incubation using 0.1 mg/mL dengue mAb. The QCM-immunosensor shows promise as a reliable diagnostic method for the detection of dengue. Using this technology, clinical samples will be tested parallel to IgM Capture ELISA and real-time PCR methods which are currently used to diagnose dengue virus infection.

Keywords: dengue viral antigen, Quartz Crystal Microbalance (QCM), immunosensor, Protein A, monoclonal antibody (mAb)