HEALTH SCIENCES DIVISION

HSD-1

CLIMATIC FACTOR CORRELATES WITH DENGUE INCIDENCE IN METRO MANILA, PHILIPPINES

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Dengue is a serious public health problem in the tropical regions particularly in Metro Manila, Philippines. Dengue infections are caused by a virus categorized in the family of *Flaviviridae* and is transmitted by the *Aedes aegypti* mosquito. The concern over today's increasing dengue incidence has been attributed to climate change. Contradicting reports indicate that climatic factors such as temperature and rainfall considerably increase the toll of infectious diseases like dengue. Such reports show that the relationship of dengue with the climatic factors remains inconclusive. To date, little research has been conducted on how climatic factors influence the burden of ill health particularly on dengue in Metro Manila, Philippines. This study investigates the climatic factors temperature and rainfall influence on the dengue incidence in Metro Manila for the 10-year period of 1996-2005. The information obtained could help bridge the gap on understanding the complex link of the environment and human health. Monthly dengue incidence data for Metro Manila were collected over a 10-year period from the epidemiologic reports of the National Epidemiology Sentinel Surveillance System (NESSS), Department of Health. Monthly climatic factors for Metro Manila were collected at the same period from the Philippine Atmospheric, Geophysical and Astronomical Services (PAGASA). Climatic factors temperature and rainfall were linked with dengue incidence through a multiple linear regression analysis. Results showed that the predictive model equation plots dengue incidence *(Y)* versus rainfall (X), which suggests that climatic factor rainfall is significantly correlated to dengue incidence ($r = 0.337$, $p<0.05$). No significant correlation on the relationship of temperature and dengue incidence was established ($p > 0.05$). Considerable evidence shows dengue incidence in Metro Manila is likely to increase in changing rainfall patterns.

Keywords: climate, dengue, rainfall, temperature, Philippines

HSD-2

MOLECULAR INITIATIVES AND BIOINFORMATICS IN THE STUDY OF DENGUE VIRUS IN THE PHILIPPINES

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Research on Dengue hemorrhagic fever in the Philippines has been limited mainly to clinical research, incidence and occurrence. This work aims to show the molecular initiatives and bioinformatics used in our 10-year study of the dengue virus.

From 1995 up to present, we have accumulated a total of 9,062 serum samples coming from different regions in the Philippines now stored in the St. Luke's Dengue Serum Bank. Corresponding patient data, demographics and research results have been encoded in a Dengue Database. Dengue virus was first detected then isolated and cultured from serum using *Aedes albopictus* C6/36 cell lines. Serotypes of positive isolates were identified by reverse transcriptase polymerase chain reaction (RT-PCR). Molecular genetic markers in the virus are currently being probed to associate virulence of the pathogen with severity of the disease. In phylogenetic analysis, our Philippine DEN 1 isolates clustered in a unique group while DEN 2 formed two clusters, where more recent isolates clustered distinctly and separately from earlier isolates. Generation of a homologous 3-D protein structure derived from the entire envelope gene permitted the localization of potential antigenic sites. Sequence alignment allowed the identification of a consensus region of DEN 3 NS1, where LUX-based primers were designed for more efficient detection and quantitation by real-time PCR.

Conventional ways of detecting dengue infection include IgM-capture ELISA, Focus Formation Assay (FFA) and Immunofluorescence Assay (IFA). Virus overlay protein-binding assay (VOPBA) is used to look for putative dengue-binding proteins on the host cell surface. Monoclonal antibodies derived by hybridoma technology, single-chain variable antibody fragments (ScFv) prepared by phage technology, and diagnostic antigens are being tested using flow cytometry and animal immunogenicity test.

These thrusts are valuable components in any research on pathogen genomics as shown by the Dengue Research Group at St. Luke's Medical Center.

Keywords: dengue, bioinformatics, database, molecular biology, genomics

HSD-3

MONOCYTES (CD14+) AND B CELLS (CD 19+) ARE THE PRINCIPAL TARGET CELLS OF DENGUE VIRUS

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Laboratory diagnosis of dengue virus infection involves techniques such as cell culture, immunofluorescence, immunochromatography, enzyme-linked immunosorbent assay, and polymerase chain reaction. To date, monoclonal antibodies and cell fixation-permeabilization techniques in flow cytometry have been reported for the rapid detection of dengue virus infected cells. In this study, phycoerythrin (PE) labeled anti-CD3, CD14, CD16, CD19 and 6B6C anti-flavirus antibodies were used in conjunction with fixation-permeabilization techniques to examine their possible application in the detection of dengue virus in peripheral blood mononuclear cells by flow cytometry. Cells were collected from suspected dengue-patients and analyzed on a BD FACSCaliburÔ Flow Cytometer. Dimly to brightly positive expression was noted in monocytes and B cells in the blood samples tested. Results suggest that fluorescent activated cell sorter (FACS) can be a powerful tool to detect dengue virus infected cells by flow cytometry.

Keywords: Dengue virus, Fixation, Permeabilization, Phycoerythrin, 6B6C, T cells, Monocytes, NK cells, B cells, Flow Cytometry

HSD-4

LUX™-BASED FLUOROGENIC PRIMERS FOR REAL-TIME PCR DETECTION AND QUANTITATION OF DENGUE VIRUS

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Real-time Polymerase Chain Reaction (PCR) using fluorogenic probes or primers presents many advantages over conventional PCR methods, including sensitivity, speed, and the ability to accurately quantify the amount of template nucleic acid present in a sample. In this study, we developed a real-time PCR assay to detect and quantify dengue virus type 3 (DEN 3) using the novel LUX™ (Light Upon eXtension) fluorogenic technology. Briefly, this platform utilizes a hairpin conformation at the fluorophore-labeled oligonucleotide primer to quench the fluorescence signal. Upon annealing and extension, the primer linearizes resulting in release of fluorescence. The LUX™ designer software from Invitrogen was used to design a FAM-labeled forward primer and a standard reverse primer. This primer pair targets a conserved region within the non-structural 1 (NS1) gene of DEN3 strains. Using the Rotorgene 3000 (Corbett) real-time PCR thermocycler, DEN3 was detected in cDNAs generated from total RNA isolates of DEN3-infected culture fluids. Serially diluted plasmid DNAs containing the DEN3 NS1 gene were used as quantitation standards. The standard curve generated was used for extrapolating quantitative information for targets of unknown concentration. The detection limit of the assay was observed to reach as low as 100 copies/ml. This assay protocol is used to detect and quantify dengue 3 virus in infected culture fluids from C6/36 *Aedes albopictus* cells and Human Pulmonary Arterial Endothelial cells (PAE), and from clinical serum samples of dengue-suspected patients. Our results demonstrate that the LUX-based real-time PCR assay may be utilized as a rapid, convenient, and sensitive screening tool for dengue virus infections.

Keywords: dengue, real-time PCR, quantitation, LUX, fluorescence

HSD-5

IDENTIFICATION OF HBV GENOTYPES IN THE FILIPINO POPULATION BY RESTRICTION FRAGMENT LENGTH POLYMORPHISM

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Hepatitis B virus (HBV) is a DNA virus of the family *Hepadnaviridae*. It contains a small, circular, partially double stranded segment of DNA of approximately 3.2 kb in size. HBV infection is a global public health problem that infects approximately 300 million carriers from which one million die annually from HBV related liver diseases. At least 8 major genotypes have been documented and a significant number of data indicates that HBV genotypes influence HBesAg seroconversion rates, severity of liver disease, development of antiviral drug-resistance and response to treatment. This study aims to identify existing genotypes in the Filipino population by restriction endonuclease cleavage of the PCR-amplified S gene region of the HBV genome. Samples found positive for HBV DNA either by conventional in-house PCR detection or commercially available PCR assays (Amplicor HBV Monitor Test, Roche Molecular Systems, NJ, USA) at the Research and Biotechnology Division, St. Luke's Medical Center were included in the study. DNA from serum or plasma was extracted and PCR was performed in a single tube assay using nested primers based on the conserved nucleotide sequences in the regions of the pre-S1 through S genes of the HBV genome. Restriction digests using (a) *Bsr*I (b) *Sty*I (c) *Hpa*II (d) *Eae*I and (e) *Dpn*I enzymes were carried out on the final PCR products. After electrophoresis through a 10% polyacrylamide gel, restriction patterns were determined by comparing digestion products with that of established genotypes.

Out of 226 HBV DNA samples that were positive by conventional HBV PCR detection or Amplicor HBV Monitor Test, 110 (48.7%) were identified as genotype A, 49 (21.7%) as genotype B and 67 (29.6%) as genotype C. Our ongoing work is designed to correlate the identified HBV genotypes with viral DNA level, serological and biochemical markers.

Keywords: Hepatitis B Virus, HBV genotypes, restriction endonuclease cleavage, nested PCR, restriction fragment length polymorphism, Amplicor HBV Monitor

HSD-6

DETERMINATION OF *Helicobacter Pylori VacA* GENOTYPES AND *CagA* GENE FROM GASTRIC BIOPSY SAMPLES IN A SPECIAL *Pylori* TEST PAPER

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Helicobacter pylori, attributed to be the major cause of peptic ulcer disease is difficult to detect. Tests to confirm presence of *H. pylori* are mostly invasive in nature, requiring endoscopy.

The special *Pylori* test paper is easy to use and has been found to be reliable. It consists of an agar gel containing urea, phenol red, buffers and bacteriostatic agent in sealed plastic slide. If the urease enzyme of *H. pylori* is present, the resulting breakdown of urea causes the pH to rise and the gel turns from yellow to bright magenta. In this study, gastric biopsy samples were used to determine the *vacA* genotypes and *cagA* gene status in a special *Pylori* test paper. DNA was isolated from gastric biopsies whether positive or negative for the rapid urease test. To confirm presence of *H. pylori*, primers for the *H. pylori* (Hp-specific) *16s rRNA* and *glm* were used and *vacA* genotypes and presence of *cagA* were also determined.

Seventy-two gastric biopsies placed in special *Pylori* test paper were used. Of the 72 gastric biopsies tested, 69 were positive for *H. pylori* using Hp specific *16s rRNA* and 26 were positive for *glm*. Thirty-eight samples were positive for *vacA*: 4 *s1a/m1*; 8 *s1b/m1*; 2 *s1b/m2*; 3 *s1c/m2*; 1 *s2/m2*; 2 *s1a,s1b/m1*; 1 *s1a,s1c/m2*, 5 with *s1b* only and 12 with only *m1*. Thirty-four samples were negative for *vacA* and twenty-four samples were positive for *cagA*.

This study shows that gastric biopsy samples used for the Rapid Urease Tests can be used to determine *vacA* genotypes and presence of *cagA*. This would greatly facilitate genetic studies of *H. pylori* as it does not require additional biopsy samples.

Keywords: *Helicobacter pylori*, *vacA*, *cagA*, *16s rRNA*, Special Pylori Test Paper

HSD-7

DECREASED INVASION OF RESPIRATORY PATHOGENS IN HUMAN PHARYNGEAL CELLS DUE TO SUBINHIBITORY CONCENTRATIONS OF CARBOXYMETHYLCYSTEINE

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Adherence of pathogens to cell surface membrane is an essential first step in invasion and successful infection. Pathogens attach to the respiratory epithelial cells avoiding elimination by mucociliary action. Interfering with bacterial adhesion is a novel approach in the treatment of respiratory infections overcoming problems associated with antibiotic resistance. Inhibition of bacterial adhesion is an appropriate early strategy in preventing respiratory infections. The mucolytic agent S-carboxymethylcysteine (S-CMC) is used in the treatment of various respiratory illnesses characterized by abnormal mucous secretions. S-CMC inhibits the attachment of *Moraxella catarrhalis*, *Haemophilus influenzae* and *Streptococcus pneumoniae* to epithelial cells. In this study, the effect on the attachment of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* to human pharyngeal cells was investigated. Briefly, bacteria at a cell density of 1×10^6 cfu/ml were incubated with various concentrations of S-CMC alone, S-CMC with placebo and placebo alone. Bacteria treated with culture medium but without any drug represented the control sample. Treated bacteria were added to the pharyngeal cells and incubated for 2 hours at 37°C with 5% CO_2. The unattached bacteria were washed off using sterile PBS. Bacteria remaining in the extracellular environment were killed with antibiotic treatment. The cells were washed and lysed to release intracellularly located bacteria. The lysate was plated in nutrient agar and incubated at 37°C. Thereafter, bacterial colonies were counted and expressed as colony forming units. A significant decrease in the number of bacteria colonies was observed after 1 hr incubation of carbocisteine. Increasing amounts of carbocisteine further decreased the number of colonies. The placebo alone did not decrease the number of colonies. However, co-incubation of carbocysteine and placebo still resulted to a decrease in the number of colonies observed suggesting specificity of binding inhibition.

Keywords: bacterial adhesion, carbocysteine, pharyngeal cells

HSD-8

PCR-BASED DETECTION OF MUTATIONS IN THE *katG*, *rpoB*, AND *embB* GENE OF *Mycobacterium tuberculosis*

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Tuberculosis is a leading cause of death by an infectious microorganism. Rapid detection and early diagnosis specifically of multi-drug resistance are essential for effective treatment and control of *M. tuberculosis*. Current assays for determining antibiotic susceptibility of *M. tuberculosis* require weeks to perform due to the slow growth of the bacilli. Alternatively, PCR-based methods are used in the detection of *M. tuberculosis* and identification of mutations responsible for resistance. In this study, specific mutations in the rpoB, katG and embB codons were detected by Amplification Refractory Mutation System (ARMS) and Multiple allele Specific (MAS) PCR assay. Briefly, genomic DNA was extracted from TB bacilli identified and phenotypically characterized at the Quezon Institute Hospital. Specific primers were used to amplify regions in the rpoB, katG and embB gene. Mutations were identified based on the presence or absence of specific amplicons. Mutations in the katG and embB were further confirmed using PCR-RFLP. 61% were found to be resistant to isoniazid (INH) using culture sensitivity assay. Only 45% of these resistant isolates have mutations in the katG codon 315. 39% were observed to be sensitive to INH by culture. However, mutations in the katG codon were detected in 8 culture sensitive isolates. 28% were observed to be resistant to ethambutol by way of culture. Only 50% of the ethambutol resistant strains have mutations in the embB codon 306 gene. While 72% were observed to be sensitive to ethambutol, mutations in the embB codon 306 were detected in 9 out of the 62 ethambutol sensitive isolates. 39% were found to be resistant to Rifampin by culture. Only 24 out of the 31 resistant isolates have mutations in the rpoB codon. 61% were observed to be sensitive to rifampin. However, 23 out of the 49 culture sensitive isolates have mutations in the rpoB codon. The above results indicate that while mutations in the rpoB, katG and embB are present and can be detected by gene amplification techniques, there are other factors that further contribute to the emergence of drug resistance.

Keywords: PCR, mutations, *Mycobacterium tuberculosis*, multi-drug resistance

HSD-9

ASSOCIATION OF *CETP Taq1B* SINGLE NUCLEOTIDE POLYMORPHISM AMONG DYSLIPIDEMIC FILIPINOS WITH THEIR RESPONSE TO SIMVASTATIN TREATMENT

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Lowering of cholesterol levels with statins among middle aged-dyslipidemic patients reduces the incidence of coronary heart disease and lowers the risk of death. However, it was observed that a substantial percentage of patients were not protected from myocardial infarction. This variation in response to statin treatment was attributed to genetic factors. An association of the variation in response to cholesterol-lowering strategies and *cholesteryl ester transfer protein (CETP)* polymorphism was reported by a previous study among Dutch patients. *CETP Taq1B* single nucleotide polymorphism (SNP) includes two different variants, *B1* and *B2* alleles characterized by the presence and loss, respectively, of a restriction site for the enzyme *Taq1* in intron 1. The present study determined the *CETP Taq1B* gene polymorphism using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 130 healthy Filipino volunteers. An association study of *CETP Taq1B* SNP with the response to simvastatin treatment of 24 middle-aged dyslipidemic patients for 8 weeks was also carried out. The allele frequencies of *B1* and *B2* alleles were 0.625 and 0.375, respectively. The genotype frequencies were in Hardy-Weinberg equilibrium. The rapid increase in high density lipoprotein (HDL) after 2 weeks and continuous increase in HDL during the 8th week simvastatin treatment were commonly observed in individuals with *CETP B1B1* genotype. The expected HDL elevation among individuals with *CETP B1B2* genotype was observed only after the 8th week treatment. For patients with *CETP B1B1* genotype, twenty (20) mg of simvastatin was adequate to cause an early response and continuous increase in HDL-C level during the 8-week treatment period.

Keywords: *CETP Taq1B*, single nucleotide polymorphism, Simvastatin, cholesterol

HSD-10

DETECTION AND ISOLATION OF *Acanthamoeba* sp. FROM THE NASAL CAVITY OF SELECTED FILIPINO POPULATIONS

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Free-living potentially pathogenic and opportunistic amebae especially those belonging to the genera *Acanthamoeba* and *Naegleria* are ubiquitous unicellular organisms. They have been isolated from soil, dust, air, water, sewers, eyewash stations, air-conditioning units, contact lenses and many other common areas. Some *Acanthamoeba* species cause fatal granulomatous amebic encephalitis (GAE), amebic keratitis as well as cutaneous lesions and sinusitis in humans. They may also harbor in their cytoplasm pathogenic bacteria such as *Legionella sp.* In this study, nasal swabs were obtained from volunteers of four different sample populations of diverse occupations to associate the presence of the ubiquitous free-living amebae with occupational risk. The populations were composed of 50 y high-rise office workers, 50 factory workers, and 50 public utility jeepney (PUJ) drivers. An additional 57 street children volunteers were included in the study. The nasal swabs were immediately placed in tubes containing 300 ml of sterile Page's ameba saline and brought to the research laboratory. The tubes were mixed thoroughly and the contents including the cotton swab were placed on the surface of non-nutrient agar plates containing heat-killed *Escherichia coli.* All plates were incubated at 37°C and were observed daily for 2 weeks. No amebae were observed in the plates containing nasal swabs from office and factory workers as well as the street children. However, trophozoites and cysts were observed in the plates inoculated with nasal swabs from 2 PUJ drivers after 2 weeks of incubation. The isolated amebae were further propagated in non-nutrient agar containing heat-killed *E. coli.* The cysts were round to oval and double-walled. The outer and inner walls meet to form the edges of the cyst pore. Trophozoite and cyst morphology suggest they belong to the genus *Acanthamoeba.* This is the first report on the detection and isolation of potentially pathogenic amebae which may be associated with occupational hazard.

Keywords: *Acanthamoea*, *Naegleria,* trophozoite, ameba cyst, nasal cavity

HSD-11

MULTIDRUG RESISTANT COMMENSAL *Staphylococcus aureus* AMONG NON-INSTITUTIONALIZED POPULACE

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Detection and recovery of non-hospital strains of multiple drug resistant *Staphyloccocus aureus* are increasingly being reported from various regions of the world. The former exclusive nosocomial pathogen has been isolated from both children and adults with no obvious traditional risk factors. The emergence and spread of this multi-resistant strain in the community poses a serious health threat leaving only limited therapeutic options.

Clinical specimens were collected from non-institutionalized children and adult women (114 and 148 individuals respectively) of Iligan City. Seventy-one percent of the study group (70 children and 116 women) were found to be colonized with *S. aureus*. The presumptively identified *S. aureus* were further screened for antibiotic *in vitro* susceptibility profiles. Less than half of the strains recovered from both pediatric and adult carriers (41% and 40%) exhibited multidrug resistance. Four percent of the total 75 heteroresistant strains were found to be no longer susceptible to all five antibiotic agents and an alarming 36% and 96% were resistant to four and three antibiotics, respectively. Vancomycin remained as the antibiotic of choice with the least number of resistant strains (17%). Erythromycin and clindamycin, which are the most commonly prescribed antibiotics showed the least efficacy. The lowered susceptibility to these agents may be attributed to misuse due to self-medication, easy availability and inducible clindamycin resistance by erythromycin resistant strains.

Prevalence of MDRSA was observed to be higher among antibiotic users and those who had current illnesses due to compromised immune systems although there was no significant correlation between colonization of MDRSA and the stated variables.

Keywords: multi-drug resistant *Staphylococcus aureus* (MDRSA), heteroresistant strains

HSD-12

HPLC ANALYSIS OF COTININE FROM URINE AMPLES OF ACTIVE AND PASSIVE SMOKERS

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Secondhand smoke or environmental tobacco smoke (ETS) is a combination of the smoke from a burning cigarette and the smoke exhaled by the smoker. Inhalation of this dangerous ETS is called passive smoking. ETS exposure can lead to respiratory problems and even lung cancer in non-smokers.

ETS exposure can be detected by analysis of cotinine, a metabolite of nicotine and a better biological marker than nicotine.

Analysis of cotinine was carried out using HPLC with UV detection at 254 nm; a C-18 column and MeOH: 0.1 M KH_2PO_4 pH 3.4 (50:50 v/v) buffer. The retention time of cotinine was around 6.3 minutes. Linear response was obtained over a concentration range of 25 ppb to 3.0 ppm while the limit of detection was 5.0 ppb. Solid phase extraction (SPE) with addition of trichloroacetic acid (TCA) was found effective and utilized for pretreatment of urine samples.

The method was tested by analysis of urine samples from three active smokers and three passive smokers. The cotinine levels of active smokers ranged from 683-839 ppb while that of passive smokers were 353-484 ppb.

Keywords: cotinine, secondhand smoke or environmental tobacco smoke (ETS), high performance liquid chromatography, solid phase extraction (SPE), passive smoking

HSD-13

ANODIC STRIPPING VOLTAMMETRY: A SIMPLE AND INEXPENSIVE TECHNIQUE FOR ANALYSIS OF MERCURY IN HUMAN BLOOD

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Mercury is a toxic heavy metal that damages the central nervous system, endocrine system, kidneys and other organs. Exposure to hazardous mercury levels can result in brain damage and ultimately death. Currently, the most commonly utilized technique to analyze mercury in blood is the coldvapor absorption technique.

In this study, a differential pulse anodic stripping voltammetry (DPASV) is applied for the analysis of mercury. In this simple and inexpensive electrochemical method, the three-electrode configuration consisted of a gold electrode as working electrode , a glassy carbon electrode as the auxiliary electrode and Ag/AgCl electrode as the reference electrode in 0.1 M Nitric acid supporting electrolyte.The stripping peak potential of Hg occurred at +0.60 V. Linear and reproducible response was obtained in concentration range from 5 ppb to 50 ppb.The limit of detection was 0.08 ppb. Mercury spiked in human blood was analyzed using the standard addition method. Using 4 samples, the % recovery obtained ranged from 84.6 % to 99.9 %.

Keywords: mercury, differential pulse anodic stripping voltammetry (DPASV), blood, gold electrode, electrochemical method

HSD-14

MORPHOMETRIC GEOMETRY OF THE EDEMATOUS FACE IN DIABETIC PATIENTS

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This study is an attempt to explore morphological variations in the face of people with diabetes which may be consequences of facial edema brought about by diabetes mellitus. For data acquisition, digital images of the faces of each patient were taken. For standardization, only pictures of patients with no moustache, no beard, and no eye glasses and with neutral face expression were considered. A total of 43 manually positioned anthropomorphic landmarks were collected, the Cartesian coordinates of which were extracted using an image analysis and processing software, SCIONIMAGE. The faces were then aligned using Procrustes alignment of the Cartesian coordinates to eliminate size differences and rotational translation. The size residuals left after the alignment were then used to reconstruct the face truss network using thin-plate spline grids. Variations in facial morphology were then explored using the methods of relative warps analysis and partial warps analysis supplemented with various multivariate statistical analyses. Results showed drooping of the brow ridge portion of the face in most of the patients, drooping of the chin and bulging of the cheek surface among the affected individuals. Also, facial asymmetry seems to be a common feature among the individuals surveyed as shown by the direction of shape change indicated by the first partial warp. Ordination of the samples based on the shape residuals showed similar shape changes for both sexes as shown by the overlapping of the convex hulls. The results indicate that similar manifestations can be seen in the two sexes as a result of the accumulation of fluid in the underlying tissues of the face.

Keywords: morphological variation, edematous face, diabetic patients

HSD-15

CYTOGENETIC FINDINGS IN TWO FILIPINO CHILDREN WITH AMBIGUOUS GENITALIA

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When babies are born and their outer genitals do not conform to the typical appearance of either sex, parents become emotionally troubled. This is because of the unconscious emotional significance we give to these reproductive structures and probably the consequent impact of deformities on future generations. The presence of the Y chromosome dictates the undifferentiated gonad to develop into a testis at about the 8th month of fetal life. At the molecular level, this is the job of the testis-determining factor (TDF), a 35-kilobase pair (kbp) sequence on the 11.3 subband of the Y chromosome, an area termed the sex-determining region of the Y chromosome (SRY). When this region is absent or altered, the gonad differentiates into an ovary. However, reported cases of XX sex-reversed males who have testicular tissue in the absence of an obvious Y chromosome or SRY genetic material clearly require other genetic explanations. Various studies claim that autosomal genes play a role in sexual differentiation. We report two cases of Filipino children with ambiguous genitalia. Chromosomes cultured from peripheral blood of these patients were analyzed using routine G (Giemsa)-banding technique. In both cases abnormalities in chromosome 6 were noted. The first case is a newborn female with very prominent labia majora. Cytogenetic analysis showed a reciprocal translocation between the entire short arm of chromosome 6 and the terminal region of the long arm of chromosome 15. This finding was confirmed by fluorescence *in situ* hybridization (FISH). The second case is a 10-month old baby with immature male external genitalia with big, deformed penile head and cleft palate. Karyotype revealed an additional material of unknown origin attached to the short arm of the chromosome 6. These novel chromosomal mutations may point to the involvement of other autosomal genes in sexual differentiation.

Keywords: ambiguous genitalia, Y chromosome, testis-determining factor (TDF), Sex determining region of the Y chromosome (SRY), autosomal genes

HSD-16

ANTHOCYANIN-RICH TAPUY: A NEW AND HEALTHIER WAY TO ENJOY RICE WINE

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Dietary antioxidants have attracted considerable interest due to their beneficial effects to human health especially disease prevention. Among various phytochemicals, anthocyanins have been shown to exhibit high antioxidant levels. Substantial amount of anthocyanin is found in pigmented rice. In this study, purple rice from various sources were utilized in the production of rice wine, locally known as tapuy. Four glutinous rice samples prepared in unpolished (U) and polished (P) forms were evaluated namely, *Ballatinao* from Baguio (UBB & PBB), purple rice from Palawan (UPP & PPP), purple rice from PhilRice (UPI & PPI), and NSIC Rc19 (UNI & PNI) also from PhilRice. Physicochemical analysis revealed that among the four samples tested, UBB and PPI had the highest wine yield and alcohol content. The sweetest wine came from UNI and PPP based on physicochemical test and sensory evaluation. In terms of over-all acceptability, wines produced from UPP and PNP were considered the best by the panelists.

UPP was found to have significantly highest anthocyanin content (1512.60 mg/kg) while UNP and PNP significantly produced the lowest with only 11.14 mg/kg and 11.02 mg/kg respectively. The result also showed that UPP and PPP produced the wine with the highest anthocyanin content, 19.95 mg/L and 0.76 mg/L, respectively. These were comparable to cyanidin-3-glucoside content of other types of pigmented wines (red wine, blueberry wine, etc.). Thus, the utilization of purple rice of Palawan especially in its unpolished form has a great potential in the development of anthocyanin-rich wine of high over-all acceptability, making it an excellent source of dietary antioxidants.

Abbreviations: NSIC – National Seed Industry Council, PhilRice – Philippine Rice Research Institute

Keywords: anthocyanin, brown rice, cyanindin-3-glucoside, physicochemical analysis, pigmented rice, polished rice, rice wine, sensory evaluation, unpolished rice

HSD-17

THE *in vitro* ANTI-TUBERCULOSIS ACTIVITY OF VIRGIN COCONUT OIL

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Many testimonials are continuously being heard about the many health benefits of virgin coconut oil (VCO), but none so far has indicated activity against tuberculosis (TB). Since VCO is a safe product readily available in the market for human consumption this study was conceived to know if TB patients would benefit from it. Using the Microplate Alamar Blue Assay (MABA) two commercial samples of virgin coconut oil (VCO-HF and VCO-PM) and two coconut oil derivatives (monolaurin, ML, and ß-monolaurin, BML) were found to exert activity against *Mycobacterium tuberculosis* H37Rv and two clinical isolates (Mtb 22 and Mtb 78) identified as *Mycobacterium tuberculosis* at an MIC (minimum inhibitory concentration) range of 39-1250 µg/ml. Results further indicate that BML had a slightly better activity than ML. If the study can be expanded, duplicated, and VCO passes clinical trials, virgin coconut oil will prove to be a breakthrough pharmacy in a bottle. The product is safe, affordable, and easily available to TB patients, most of whom are in the lower economy class. Problems of non-compliance will be solved, and in the end, tuberculosis might be eradicated.

Keywords: tuberculosis, *Mycobacterium tuberculosis*, virgin coconut oil, monolaurin, ß-monolaurin

HSD-18

DETERMINATION OF THE ANTI-FUNGAL ACTIVITY OF FIVE MEDICINAL PLANTS AGAINST SOME MEDICALLY IMPORTANT FUNGI

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The activity of five medicinal plants (*akapulko*, basil, caballero, guava and garlic) against selected medically important fungi was determined. Two types of plant preparations, extract and decoction, were tested against 24 cultures which included clinical and non-clinical strains of *Candida albicans, C. parapsilosis, C. guillermondii, C. lusitaniae, C. krusei, C. stellatoidea* and *C. tropicalis*, and the two molds *Aspergillus niger* and *Rhizopus stolonifer.*

Anti-fungal activity was determined using the Disk Diffusion Assay (Kirby-Bauer Method). The initial viable counts of the fungal suspensions prepared were 5.82×10^7 colony forming units (CFU)/ml for the *Candida* strains and 7.30×10^6 CFU/ml for the molds. Mycostatin (10,000 units/ml) and sterile distilled water were used as positive and negative controls, respectively.

Of the medicinal plants and preparations evaluated, only the garlic extract exhibited anti-fungal activity and this was against all the organisms tested. The average diameter of the zones of inhibition (ZOI) exhibited by the garlic extract against the 22 strains of *Candida* was 14.7 mm while that for mycostatin was 17.7 mm. A smaller average ZOI diameter of 14.2 mm was noted for the clinical strains of *Candida* compared to 17.4 mm for the non-clinical strains. With reference to the molds, the garlic extract (with an average ZOI diameter of 25 mm) was more inhibitory than mycostatin (with 18.8 mm-average ZOI).

This study found garlic extract to be inhibitory to the species of *Candida, Aspergillus and Rhizopus* tested. It was more inhibitory to the molds than to the *Candida* species, and to the non-clinical strains of *Candida* compared to the clinical isolates. No anti-fungal activity was observed from the garlic decoction probably due to its being more diluted and/or due to the boiling step which could have destroyed its inhibitory properties. The potential active ingredients may have also not been extracted.

Keywords: anti-fungal, disk diffusion, medicinal plants, medically important fungi, *Candida, Aspergillus, Rhizopus*

HSD-19

THE INHIBITORY ACTIVITY OF SELECTED PLANT EXTRACTS AND THEIR INTERACTION AGAINST COMMON RESISTANT PATHOGENS BY DOUBLE-DISK SYNERGY TECHNIQUE

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Effective drug combinations using synthetic drugs have been experimented to come up with remedy to battle the outbreak of the multi-resistant pathogens. With this technology, a new approach in the use of herbal plants was tried and experimented in an attempt to come up with innovative technique and new knowledge in natural products.A two-plant extract combination from *Daucus carota Linn, Imperata cylindrical, Hibiscus rosasinensis and Anona muricata* was prepared and used to determine possible synergistic activity using the Double-Disk Synergy Test (DDST).The inhibitory effects of each plant extract alone and in combinations were observed against the Extended-Spectrum Beta-Lactamase (ESBL) *Escherichia coli and Klebsiella pneumoniae* and Oxacillin-Resistant *Staphylococcus aureus* (ORSA). Phytochemical screening revealed the presence of the essential biological constituents such as the flavonoids, tannins, saponins, anthraquinones, higher alcohols, steroids and essential oils. Disk diffusion assay of the plant extracts showed that *Hibiscus rosasinensis*, had the greatest inhibitory activity with 25 mm and 26 mm zones of inhibitions against ESBL-producing *E. coli* and *K. pneumoniae* respectively, with the oxacillin-resistant *S. aureus* as the most susceptible with 35 mm zone of inhibition. *Anona muricata* yielded a zone of inhibition of 31 mm against oxacillin resistant *Staphylococcus aureus. Imperata cylindrica* yielded the highest zone of inhibition of 21 mm against Oxacillin resistant *Staphylococcus aureus.*. Finally, Double-Disk Synergy Test(DDST) on the different plant extract-combinations against the common resistant pathogens yielded excellent results revealing the synergistic activities of the plant extracts by revealing an enhancement or bridging at or near the junction of the two zones of inhibition.

Keywords: *Anona muricata,* anthraquinones, *Daucus carota Linn,* DDST, ESBL, flavonoids, *Hibiscus rosasinensis, Imperata cylindrical,* higher alcohols ,ORSA, saponins, steroids, tannins.

HSD-20

ANTI-BREAST CANCER ACTIVITY OF LOCALLY GROWN *Annona muricata* L. LEAVES

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This study explored the potential anti-cancer activity of the leaf crude extract from the locally grown *Annona muricata* using human breast cancer cell line (ATCC MCF-7), along with a normal cell line from Chinese hamster ovarian cells (ATCC CHO-AA8) for comparison. Breast cancer has been the leading cancer among Filipino women today, and there have been studies showing that the Philippines has the highest incidence rate of breast cancer in Asia and has the ninth highest incidence rate in the whole world. Numerous plants have been valued for their medicinal importance. It is very apparent that in the midst of the advent in science and technology, the natural curative power of medicinal plants still appears to be the best remedy for some illnesses. One family of plants, the *Annonaceae* has been reported to have a potential anti-cancer activity. Culture cells were treated with the test sample at two concentrations: 5 µg/mL and 50 µg/mL. Determination of the cell survival was determined by MTT assay. Results obtained showed that the crude extract has no toxic activity towards normal mammalian cells at both concentrations used. Cytotoxic activity against cancer cells was determined at the 50ug/mL dose, resulting to a relatively small fractional survival value.

Keywords: ATCC MCF-7, ATCC CHO-AA8, *Annonaceae*, MTT

HSD-21

CYTOTOXICITY OF $[Bu_3Sn_3(P_2W_{15}O_{59})]^{9-}$ ON ADRIAMYCIN-RESISTANT AND WILD TYPE BREAST CANCER CELLS *IN VITRO*

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Breast cancer cell proliferation experiments using $[Bu_3Sn_3(P_2W_{15}O_{59})]^{9-}$ showed that the cell interaction of this heteropolyanion differed with the type of breast cancer cells used and whether the heteropolyanion was dissolved in aqueous solution or encapsulated in phospholipid vesicles.

In this study, it was found that when wild type breast cancer cells were incubated with vesicle-encapsulated $[Bu_3Sn_3(P_2W_{15}O_{59})]^{9-}$, the survival rate of the breast cancer cells was only 11% at a concentration of 0.2 μM. On the other hand, incubating breast cancer cells with the same concentration of $[Bu_3Sn_3(P_2W_{15}O_{59})]^{9-}$ but was not vesicle-encapsulated had a survival rate of 91%. These differences demonstrated that encapsulation of heteropolyanions in phospholipids vesicles alter the interaction of polyoxometalates with breast cancer cells.

Keywords: heteropolyanion, breast cancer, adriamycin, liposomes, cytotoxicity

HSD-22

In vitro CYTOTOXIC ACTIVITY OF DIFFERENT CHEMOTHERAPEUTIC DRUGS AGAINST DIFFERENT HUMAN TUMOR CELL LINES AS A MEANS TO EVALUATE POTENCY

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Chemotherapy using cytotoxic drugs is the main treatment modality for cancer. Storage and temperature requirements must be strictly monitored and adhered to when transporting these chemotherapeutic drugs from one facility to another. Deviations from the prescribed storage and transport conditions may

impact on the activity of the drug. Chemotherapeutic drugs must be potent to successfully treat tumor tissues or cells. Potency is an expression of activity of a drug in terms of concentrations required to achieve a desired effect. In this study the cytotoxic potency of seven (7) anti-cancer drugs was determined *in vitro* by a high-throughput cytotoxic assay using five different human tumor cell lines. Half-maximal inhibitory concentration (IC_{50}) of the different chemotherapeutic agents was obtained from an experimentally derived dose-response curve. Potency of the different chemotherapeutic drugs was statistically calculated using Parallel Line Assay. Tamoxifen and Mitoxantrone were found to be the most cytotoxic having the lowest IC_{50}. Different human cancer cell lines showed similar cytotoxic responses to the individual drugs used. *In vitro* cell-based cytotoxicity assay promises to be a useful and reliable method for evaluating potency of chemotherapeutic drugs under storage or being transported from one site to another.

Keywords: in vitro cytotoxic assay, chemotherapeutic drugs, potency, tamoxifen, mitoxantrone