Laboratory Studies

Chair: W McLaughlin and L Young-Martin

(O – 14)

Assessing potential for drug-herb interactions: evaluation of the impact of a *Guazuma ulmifolia* extract on the activities of cytochrome P450 enzymes

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Objective: To determine the potency of a *Guazuma ulmifolia* tea on the *in vitro* activities of cytochrome P450 (CYP) enzymes known to play central roles in phase I drug metabolism.
Method: Evaluations were conducted using a fluorescence based assay in a 96-well plate, from which IC50 values (that is, the inhibitor concentration that causes a 50% reduction in enzyme activity) were determined. Heterologously expressed human CYP enzymes (CYPs 1A2, 2C19 and 2D6) and human liver microsomes (CYP2C19 and CYP3A4) were used to assess the impact of varying test inhibitor concentration in the presence of fluorogenic substrates. IC50 values were obtained using Sigma Plot Version 10.
Results: *Guazuma ulmifolia* (traditionally used for the treatment of diarrhoea, urinary infections, skin injuries and skin conditions) was found to be at least moderately potent against the drug metabolizing enzymes, with all IC50 values less than 100 µg/mL. This, therefore, warrants clinical evaluation into potential adverse reactions from the co-administration of this tea with pharmaceutical drugs metabolized by CYPs3A4, 2D6, 2C19 and 1A2. It was found to be especially potent against CYP1A2 activity *in vitro*, with an IC50 value of 5.18 µg/mL. Potency against these enzyme activities by *Guazuma ulmifolia* validates further definitive assessment into its potential for drug-herb interactions that may lead to adverse reactions.
Conclusion: Moderate to strong potency was observed by *Guazuma ulmifolia* against the drug metabolizing enzymes. With its documented traditional usage, it is advised that *Guazuma ulmifolia* undergo clinical investigations for definitive evaluation into the safety of its usage with concomitant administration of clinical drugs, especially those metabolized by CYP1A2, such as tamoxifen, phenacetin and warfarin.

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Distribution of uropathogenic virulence factors among antimicrobial-resistant and -susceptible *Escherichia coli* implicated in urinary tract infections

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Objective: The uropathogenic proficiency of *Escherichia coli* is dependent on the correlation of virulence factor expression with antimicrobial resistance, which influences the severity of infection. To obtain a more comprehensive outlook on the potential hazard presented by antimicrobial resistant uropathogens, we assessed distribution and diversity of virulence genes among uropathogenic *E coli* strains susceptible and resistant to nalidixic acid, ciprofloxacin and trimethoprim-sulfamethoxazole.
Methods: A total of 174 uropathogenic *E coli* isolates recovered from cases of non-complicated urinary tract infections in Jamaica were screened by polymerase chain reaction (PCR) for six virulence factors encoding the adhesins (afimbrial, Type 1 fimbriae, pyelonephritis associated pili, S fimbriae) and toxins (cytotoxic necrotizing factor, haemolysin). Susceptibility to nalidixic acid, ciprofloxacin and trimethoprim-sulfamethoxazole was determined using the disc diffusion assay.
Results: Nalidixic acid, ciprofloxacin and trimethoprim-sulfamethoxazole resistance frequencies were high: 82%, 78% and 59%, respectively. Twelve distinct patterns were identified for the carriage of the virulence determinants *afaBC*, *cnfI*, *fimH*, *hylA*, *papEF* and *sfaDE*. The toxin gene, *cnfI* (75%), was the second most prevalent marker to the adhesin, *fimH* (97%). The significant association of *sfa-hyl* loci ($p < 0.01$) with susceptible strains was also observed, notwithstanding an overall higher occurrence of virulence factors.
Conclusion: Virulence factors among uropathogenic *E coli* implicated in urinary tract infections are diverse. As the first report highlighting virulence and antimicrobial resistance in Jamaica, these findings emphasize the need for effective strategies for monitoring uropathogenic *E coli* and the rational use of antimicrobial agents.

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Lack of association of quorum sensing genes with pathogenicity in *Staphylococcus aureus* using a *Caenorhabditis elegans* model

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Objective: *Staphylococcus aureus* is a significant human pathogen that employs the *agr* quorum sensing system to regulate virulence gene expression. This study compared pathogenicity in methicillin-sensitive and methicillin-resistant *S aureus* and investigated the association with the *agr* quorum sensing locus using a *Caenorhabditis elegans* pathogenicity model.
Method: Antibiotic resistance phenotypes of 102 *S aureus* to 12 antibiotics were determined using the disk diffusion method. Multiplex polymerase chain reaction (PCR) was used to identify the presence of *agr* alleles while *C elegans* infectivity and lifespan assays were used to determine pathogenicity of the isolates. Significant differences in antibiotic resistance frequencies were assessed using the Chi-squared test while Kaplan-Meier survival estimates were used to analyse survival curves. Any significance in the observed survival rates was evaluated using the log rank test.
Results: Antibiotic resistance phenotypes for oxacillin, clindamycin, tetracycline and trimethoprim/sulfamethoxazole were significantly associated with MRSA ($p < 0.05$). Survival assays showed a significant difference in the killing time between MRSA and MSSA ($p < 0.001$), with MSSA having a lower median lifespan (three days) than MRSA (six days). There was no association of pathogenicity with *agr* positivity or negativity ($p > 0.05$).
Conclusion: The quicker killing time of MSSA when compared to MRSA, coupled with the significant association of antibiotic resistance in MRSA suggests that the carriage of antibiotic resistance genes may influence pathogenicity. Further, the lack of association between pathogenicity and the *agr* locus may indicate that *agr*-negative strains are otherwise adapted for pathogenicity *via* other quorum mediators.

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Migration of bone marrow-derived cells to the vasculature of organs is enhanced by moderate intake of alcohol

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Objective: To examine the contribution of bone marrow (BM)-derived cells to adult angiogenesis and the influence of moderate intake of alcohol in this phenomenon. Endothelial turnover in adult animals is slow and ten months after bone marrow transplantation, around 3–4% of luminal cells are BM-derived.
Methods: Using BM-directed gene transfer and permanent transduction *via* recombinant SV40-derived vectors, we previously reported that BM-derived cells may be progenitors of central nervous system (CNS) cells, such as hippocampal neurons in normal adult animals. We demonstrated by the same method that, under physiologic conditions and in the absence of CNS or vascular injury, marrow-resident precursors can migrate to, and form an endothelial lining for, brain blood vessels of the striatum. We asked here if moderate ethanol consumption can increase the migration of BM-derived cells to the vasculature of different organs (brain, heart and skeletal muscle) and their incorporation into vascular structures. We used BM-directed gene transfer and permanent transduction *via* SV40-derived gene delivery vector, carrying a marker epitope (FLAG), appended to carrier protein, in one month-old paired ethanol fed and control rats.
Results: Forty-eight weeks after intramarrow injection of the vector, SV(Nef-FLAG), transgene expression was examined in the vasculature of different organs. The numbers of FLAG-expressing cells *per* vascular structures were significantly higher in the ethanol fed animals. No FLAG-positive cells were seen after intramarrow injection of SV(BUGT), a control vector.
Conclusion: These results show that moderate ethanol consumption increased the migration and the incorporation of BM-resident cells toward the vasculature of different organs.

(O–18)

Evaluation of the Dengue Duo® rapid diagnostic kit in Jamaica

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Objective: To evaluate the diagnostic performance of the Dengue Duo® rapid diagnostic kit and to determine if the diagnostic sensitivity was improved by using a combined testing strategy.
Methods: The Dengue Duo rapid immunochromatographic kits were evaluated against a panel of 176 acute dengue and 163 non-dengue serum samples which were characterized by a reference enzyme-linked immunosorbent assay (ELISA). The 339 consecutive archived serum samples were selected using a retrospective cross-sectional study design over a three-month period (October–December 2012).
Results: The overall diagnostic sensitivities of the Dengue Duo rapid test for IgM, IgG and NS1 were 49.3% (95% CI: 41.3, 57.4), 39.1% (95% CI: 33.3, 45.2) and 90% (95% CI: 82.1, 94.7), respectively. The IgM and IgG detection rates were significantly lower than that of the NS1 antigen ($p < 0.001$). However, the combination of the anti-IgM detection with either anti-IgG or NS1 antigen detection or both resulted in a significant ($p < 0.001$) increase in sensitivity to 80.2% (95% CI: 67.4, 81.9), 97.5% (95% CI: 92.9, 99.2) and 98.9% (95% CI: 96.0, 99.7), respectively. These higher sensitivities were achieved without any decrease in specificities.
Conclusion: This study reveals that combining two or more parameters of the Dengue Duo rapid kit significantly improved the sensitivity of diagnosis of acute dengue virus infection and supports its usefulness in Jamaica.

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The induction of pro-inflammatory cytokine by *Stenotrophomonas maltophilia*

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Objectives: (i) To investigate the secretion of tumour necrosis factor-alpha (TNF-a) induced by *S maltophilia* and (ii) to determine the main pathogen associated molecular pattern (PAMP) and pattern recognition receptor (PRR) involved.
Method: Peripheral blood mononuclear cells (PBMC) obtained from healthy donors were stimulated *in vitro* with whole *S maltophilia* bacteria and fractionated sub-cellular components. Tumour necrosis factor-alpha production was measured by an EPICS ALTRA flow cytometer (Beckman Coulter).
Results: Significant amounts (96%) of TNF-a were produced predominantly from monocytes. The removal of the lipopolysaccharide (LPS) component of *S maltophilia* by polymyxin B adsorption revealed that LPS was the major PAMP that induces the production of TNF-a ($p = 0.009$). Significant reduction of TNF-a production was observed when anti-TLR4 blocking antibodies were used ($p = 0.009$). In contrast, no significant reduction was observed with the use of anti-TLR2 blocking antibodies. This showed that *S maltophilia* induced TNF-a production in PBMC through a TLR4 dependent pathway.
Conclusion: The LPS component (PAMP) of *S maltophilia* is a potent stimulus for the production of the pro-inflammatory cytokine TNF-a and the mechanism is through a TLR4-dependent (PRR) pathway. The ability to induce pro-inflammatory cytokine may help to explain the immunopathogenesis of infections with *S maltophilia.*

(PO – 02)

A five-year review of acute accidental poisoning in children at the Bustamante Hospital for Children: 2007–2011

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Objective: To review the epidemiology, management and outcome of children seen at the Bustamante Hospital for Children (BHC) with a diagnosis of acute accidental poisoning during the period January 2007–December 2011.
Methods: This was a descriptive retrospective study. Patients were identified using admission books. Data were collected, using a data extraction sheet, from the dockets of all patients with acute poisoning seen at BHC during the study period.
Results: There were 196 males and 195 females with a mean age of 29.4 months. Fifty-four per cent were 13–36 months old. Seventy-four per cent of children were from Kingston while 21% were from St Catherine. The mother was the supervisor in 51% of the cases. Eighty-nine per cent of the ingestions occurred in the home. Bleach (25%) was the most commonly ingested toxin. There were 62% and 34% of patients admitted to the medical and surgical wards, respectively. Vomiting occurred in 71% of cases of bleach ingestion and dystonia in 88% of ingestion of antipsychotic medication. Sixty per cent of patients were discharged within two days of admission. All patients admitted to the surgical ward received oesophagoscopy.

Complications were recorded in 6% of cases. The social work department was involved in the management of 92% of only 152 cases referred. Only 45% of patients and caregivers were educated regarding the importance of adequate supervision and storage of toxins while 9% of patients were given medications upon discharge.

Conclusion: Bleach was the most commonly ingested toxin. Children aged 2–3 years old were most commonly affected.