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May 3-5, 2012, Vancouver, British Columbia

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ORAL PRESENTATIONS
THURSDAY May 3, 2012
Room: Junior Ballroom A
Session A

A1
THE IMPACT OF CHANGING FROM AN ENZYME IMMUNE ASSAY (EIA) TO A POLYMERASE CHAIN REACTION (PCR) METHOD FOR DETECTING *CLOSTRIDIUM DIFFICILE*

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OBJECTIVE: PCR assays for detection of *C. difficile* infection (CDI) in symptomatic patients have been shown to have higher sensitivities than EIAs. The objective of this study was to assess the impact of changing from an EIA to a PCR-based method for *C. difficile* toxin B gene detection on healthcare-associated CDI (HA-CDI) rates, patient outcomes and CDI-related isolation days in hospital.

METHODS: An observational study was carried out on patients positive for *C. difficile* toxin by EIA (Oct 2008-Sept 2009) and PCR (Oct 2009-Sept 2011). All patients with a positive test were reviewed to confirm adherence to a case definition for CDI. Patient data collected included demographics, complications, 30-day outcomes, and length of stay (LOS). The number of isolation days for all cases of suspected and confirmed CDI were tracked. HA-CDI rates were reported per 10,000 patient-days. Cases detected by the two methods were compared by t-test and χ^2 analysis.

RESULTS: The mean incidence of HA- CDI did not differ between the EIA and PCR test periods (6.4 vs. 6.6/10,000 patient-days; p=0.79). A significant decrease was seen in the mean LOS overall (51 vs 32 days; p=0.007), and after confirmation of infection (32 vs 20 days; p=0.011); the mean number of isolation days per patient decreased from 7.7 to 6.3 days (p<0.001) after the introduction of the PCR assay. No significant difference in patient outcomes (ICU admission, colectomy or death at 30 days) was observed.

CONCLUSION: The introduction of PCR testing did not affect HA-CDI rates, but was associated with reduced isolation days and hospital LOS, thereby improving patient care and safety.

A2
COMPARATIVE EVALUATION OF TECHLAB C. DIFF QUIK CHEK COMPLETE® MEMBRANE IMMUNOASSAY WITH TOX A/B II ELISA™ AND TOXIGENIC CULTURE FOR THE DETECTION AND IDENTIFICATION OF *CLOSTRIDIUM DIFFICILE* STRAINS

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OBJECTIVE: Timely detection of *Clostridium difficile* infection (CDI) is essential to initiate treatment and isolation and to control outbreaks. To identify sensitivity and specificity of C. DIFF QUIK CHEK COMPLETE (CDCC, membrane EIA), which takes 30 min to perform and does not need any instrumentation, we compared it to another EIA-based test, Techlab Tox A/B II™ (TTAB) and toxigenic culture (TC).

METHODS: 200 specimens (80 positive and 120 negative by TTAB) were tested with CDCC and TC. For TC (gold standard), colonies grown on CDMN plates were checked for fluorescence and DNA extracted from isolates (4 colonies each) was tested for toxins genes, typed by MLVA to identify strains diversity and ribotyped.

RESULTS: Among 120 specimens negative by TTAB 11% (n=13) were positive by CDCC (GDH band, toxins band was negative) and 50% (6/12) of them were toxigenic, 1 with 027 ribotype. 3 specimens positive by TTAB were negative by CDCC and TC. 12/13 (92%) specimens positive by CDCC were also positive by TC. 24% of CT-positive specimens (21/87) were of ribotype 027.

CONCLUSION: The Techlab CDCC test is more sensitive than TTAB for detection of CDI. It identified CD in 13/120 additional specimens, half of them toxigenic. There was an excellent concordance of CDCC with toxigenic culture. Specimens negative for toxins with CDCC can be retested after culture enrichment or with another method (TTAB or PCR). Because of its user-friendly format and short time required to get results it can be used in any settings to identify cases of CDI to manage patients and rapidly identify outbreaks.

A3
COMPARISON OF TWO EIA TESTING METHODOLOGIES AND LOOP-MEDIATED ISOTHERMAL DNA AMPLIFICATION FOR THE DETECTION OF *CLOSTRIDIUM DIFFICILE*

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Screening tests for *C. difficile* currently rely on EIAs that detect glutamate dehydrogenase antigen (GDH) which is highly sensitive but requires confirmatory testing by EIA ,cytotoxin neutralization assay (CTN) or loop-mediated isothermal DNA amplification (LAMP).The purpose of the study was to validate and compare the illumigene® LAMP assay against: 1) C Diff QUIK CHEK® antigen (QC-Ag), 2) C DIFF QUIK CHEK® toxin A/B (QC-TOX A/B) and 3) C DIFF QUIK CHEK® COMPLETE (QC-COMP). 346 clinical diarrheal stool samples were tested using 1) QC-Ag and QC-TOX A/B (if QC-Ag positive) and 2) the illumigene® LAMP platform. For comparison of EIAs, a subset of samples (135) was also tested by the QC-COMP. Uninterpretable results were resolved by CTN.

Of 346 samples, 207 were negative and 48 were positive for both QC-Ag and QC-TOX A/B. All GDH positive samples were also positive by LAMP and an additional 27 and 48 of 91 EIA-uninterpretable samples were positive by CTN and LAMP, respectively. Both EIA methods showed complete agreement for GDH detection with 17 positives, and confirmed by LAMP. 6 additional samples were toxin positive by QC-COMP, confirmed by CTN and LAMP. There is no difference in antigen detection between the one and two-step EIA methods. The QC-COMP is as sensitive for GDH detection as LAMP but is less sensitive for detection of toxin. Because LAMP detects the presence of the toxin gene, rather than its expression, carriers of toxigenic *C. difficile* with diarrhea from other causes will be detected by LAMP, requiring clinical review of these cases. The QC-COMP is a quick and cost-effective assay for the majority of samples and LAMP provides rapid results on uninterpretable results.

A4
MOLECULAR DIAGNOSTIC ASSAYS FOR THE DETECTION OF TOXIGENIC *CLOSTRIDIUM DIFFICILE* INFECTION

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BACKGROUND: Toxin-producing *Clostridium difficile* (CDT) is the causative agent for a spectrum of clinical manifestations that range from mild diarrhea to life-threatening complications such as pseudomembranous colitis. Thus, rapid and accurate detection is imperative for disease management. We sought to compare the performance of the BD GeneOhm CDiff and the Meridian Illumigene *C. difficile* assays against the Remel ProSpect enzyme immunoassay (EIA) for detection of CDT from stool samples.

METHODS: 95 liquid or semi-formed stool specimens were evaluated. EIA was performed within 24 h of receipt. The stools were subjected to one freeze-thaw cycle and the GeneOhm and Illumigene assays were performed simultaneously. The gold standard toxigenic culture assay was also performed on all specimens.

RESULTS: Toxigenic culture yielded 26 (27.4%) positives and 69 (72.6%) negatives. Both the GeneOhm and Illumigene assays detected 25 positives; the one discrepant sample grew low number on CCFA. In contrast, EIA detected 20 true positives and 2 false positives. Both molecular methods

displayed a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 96%, 100%, 100%, and 99%, respectively. In contrast, EIA demonstrated 77% sensitivity, 97% specificity, 91% PPV and 92% NPV. Of the 95 patient stools tested, the GeneOhm and Illumigene assays were able to detect 5 additional CDT isolates (5.3%).
CONCLUSION: The GeneOhm and Illumigene assays were significantly more sensitive than EIA and would be an appropriate option for laboratory diagnosis of *C. difficile* infections.

A5
DETERMINATION OF THE OPTIMAL STORAGE DURATION AND CONDITIONS FOR FAECAL TRANSPLANTATION (FTX) SAMPLES

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OBJECTIVE: Disruption of normal bowel flora is a precondition to *Clostridium difficile* infection (CDI). FTX has been used for recurrent CDI as a means to restore normal flora. Optimal conditions for stool (STL) and STL filtrate (SF) storage prior to TX are not known. We examined the impact of different storage conditions on semi-quantitative (SQ) growth of fecal flora from freshly donated SF.
METHODS: Fresh STL from 5 donors were processed. 2 aliquots of each were kept at room temp (RT) and 2 at 2-5°C. At 0, 4, 24, 48, and 72 h, 5 g of each was processed into SF using 100 mL of sterile saline. 50 µL of SF was plated at 0, 1, 2, and 3 h using selective and non-selective aerobic and anaerobic agars, incubated for 24 and 72 h respectively. SQ growth was determined by three readers. The time at which there was a loss of fecal flora (LFF) (defined as ↓ growth by ≥1 SQ quadrant on >1 plate or ≥2 quadrants in any plate vs. baseline) was determined. Results were collated and the optimal condition and storage times were determined.
RESULTS: For SF prepared from STL stored at RT: 7%, 26%, and 53% had a LFF after 24, 48, and 72 h of STL storage respectively. When SF from STL stored at RT was stored at RT, LFF was noted as early as 1h. For SF prepared from STL stored at 2-5°C: 0%, 8%, and 33% had a LFF after 24, 48, and 72 h of STL storage respectively. When SF from STL stored at 2-5°C was stored at RT, LFF was not noted except for STL stored for 72 h. The cumulative % of SF with LFF was higher at all time points when STL was stored at RT vs. 2-5°C ($p=0.02$ χ^2 $test_{Trend}$).
CONCLUSIONS: FTX programs should advise donors to store STL at 2-5°C and deliver it for processing within 24 h of donation. Once prepared as SF, there is at least a 3 h window to administer it to the patient before LFF may occur.

Room: Junior Ballroom B
Session B

B1
OPTIMAL NUMBER OF SPECIMENS TO TEST DURING INSTITUTIONAL RESPIRATORY INFECTION OUTBREAKS

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OBJECTIVES: Laboratory testing is crucial in the identification of institutional respiratory outbreaks. Determining the number of specimens to be tested during an outbreak is challenging as outbreak needs and testing costs must be balanced. The objective of this study was to determine the optimal number of specimens to be tested during an outbreak.
METHODS: We reviewed respiratory specimens from outbreaks submitted for testing from September 1, 2009 to August 31, 2011. Specimens were tested by Multiplex Respiratory Virus PCR (MRVP). Outbreaks were compared based on the chronological specimen that yielded the first detected organism.
RESULTS: A total of 5,760 specimens from 1,707 respiratory outbreaks were tested for viruses. Of all outbreaks, 1,454 (85.2%) had at least one virus detected. Of those, 1,142 (78.6 %) had a virus detected on the 1st specimen, 229 (15.7 %) on the 2nd specimen, 48 (3.3 %) on the 3rd specimen, and 23 (1.6 %) on the 4th specimen. Only 12 (0.8%) of the remaining outbreaks had the 1st virus detected on the 5th or subsequent specimen. Eighty (5.5%) of positive outbreaks had a new 2nd, 3rd or 4th virus identified on the 5th or subsequent specimen and in 8 (10%) of these the new virus was influenza.
CONCLUSIONS: This study suggests that initial testing of 4 specimens is sufficient to detect viral causes of respiratory outbreaks. Further viral testing should be reserved for a minority of outbreaks where a pathogen is not found and the outbreak is continuing to evolve.

B2
COMPARISON OF RAPID TESTS AND REAL-TIME RT-PCR FOR INFLUENZA DIAGNOSTICS

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OBJECTIVE: Influenza is a highly contagious acute viral respiratory disease. The Saskatchewan Disease Control Laboratory uses real-time RT-PCR as well as rapid antigen tests to aid in the diagnosis of influenza. The objective of our study was to compare influenza A RT-PCR cycle threshold (Ct) values with the results obtained from commercially available rapid tests.
METHODS: 25 nasopharyngeal swabs that were positive for influenza A by RT-PCR were subsequently tested by three different enzyme and microparticle-based immunoassays: Meridian Tru Flu Influenza A & B (Somagen Diagnostics, Edmonton, AB); Binax NOW Influenza A & B (Alere, Inc., Ottawa, ON) and the Quidel QuickVue Influenza A & B (Quidel, San Diego, CA).
RESULTS: Compared to real-time PCR for influenza A, the sensitivity of the Meridian Tru Flu Influenza A & B Test and the Binax NOW Influenza A & B Test was 48%, and the sensitivity of Quidel QuickVue Influenza A & B Test was 56%. There was a strong correlation between PCR Ct values and antigen test results. No rapid tests were positive on specimens with PCR Ct values higher than 23.68.
CONCLUSION: Rapid influenza detection tests facilitate the timely initiation of antiviral therapy and reduction in both antibiotic use and the cost of hospital care. However, rapid tests are dependent upon high virus titres and specimens collected at the wrong time, or improperly collected or transported, may have a lower viral load, which in turn could lead to false negative results. Negative results obtained using these rapid influenza detection tests do not rule out influenza virus infection and should not be used as the sole basis for treatment or other clinical management decisions.

B3
COST ANALYSIS OF COMMERCIAL MULTIPLEX NAT TESTING OF RESPIRATORY SAMPLES VERSUS AN IN-HOUSE RT-PCR SCREENING FOR INFLUENZA AND RSV FOLLOWED BY COMMERCIAL MULTIPLEX NAT TESTING OF SELECTED SAMPLES

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OBJECTIVE: To determine the relative cost of using a commercial multiplex NAT assay only versus pre-screening samples first with an in-house developed multiplex real-time RT-PCR that detects Influenza A/B and RSV followed by secondary testing by a commercial multiplex NAT assay for a subset of samples.
METHODS: We analyzed, in parallel, 1,426 respiratory samples using a 4plex in-house real-time RT-PCR assay that detects Influenza A/B, and RSV and compared it to testing with a multiplex NAT assay detecting 15 respiratory viruses to determine the cost per reportable result.
OUTCOMES: We received an average 28.5 respiratory samples per day. By our testing criteria 70.1% of the samples would require secondary testing if Influenza or RSV were not detected. On average 14% of the samples

had Influenza/RSV detected with 17 samples per day requiring secondary multiplex testing. Cost analysis was based on a cost of x$ per reportable for our in-house assay vs. 19x$ per reportable for the commercial assay. We determined that the average cost per reportable test for all samples was approx. $37.54 (range $13.05 to $101.40).
CONCLUSIONS: We estimated a potential cost reduction of approx. $26,000 for testing the 1,426 samples using a two stage testing algorithm. However, savings realized by a two stage approach will vary based on the sample number, the Influenza/RSV positivity rate and the number of samples requiring secondary testing. The impact on laboratory staff in terms of extra labour, potential errors due to secondary testing and turnaround time delays to clients although not easy to calculate, is being investigated and may overshadow the savings created by a two stage testing algorithm.

B4

RISK FACTORS FOR HUMAN METAPNEUMOVIRUS DISEASE SEVERITY IN CHILDREN AGED <3 YEARS

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OBJECTIVES: Human metapneumovirus (hMPV) is a major cause of respiratory tract infections (RTI) in children. However, risk factors for severe disease requiring hospitalization remain unknown. Thus, we aimed to identify environmental, host and viral determinants for severe hMPV infection in children <3 years.
METHODS: We studied a prospective cohort of >1000 children aged <3 years hospitalized or presenting to a pediatric clinic for acute lower RTI during 2006-10. We collected clinical data at enrolment and 1-month follow-up, and tested nasopharyngeal secretions for respiratory viruses by multiplex PCR/DNA hybridization assay. Disease severity was defined as hospitalization, and also assessed with a severity score (1 point/variable) including: FiO2 ≥30%, hospitalization >5 days, and PICU admission.
RESULTS: hMPV was identified in 58/305 (19.0%) outpatients and 69/734 (9.4%) hospitalized children, second only to respiratory syncytial virus (RSV) in both settings (48.2% and 63.6%, respectively). In multivariate regression analysis of hMPV cases, age <6 months (OR 2.66, 95% CI 1.04-6.81), and household crowding (OR 2.86 [95% CI 1.17-6.98]) were associated with hospitalization. Among hospitalized patients, female sex (OR 4.32 [95% CI 1.3-14.9]), prematurity (OR 13.97 [95% CI 1.50-130.0]) and hMPV genotype B (OR 4.34 [95% CI 1.26-14.9]) were associated with severe hMPV disease (severity score ≥1).
CONCLUSIONS: Young age, prematurity, female sex, household crowding, and viral genotype were identified as independent risk factors for severe hMPV outcomes. These findings will inform hMPV treatment and prevention strategies currently under development.

B5

SURVEILLANCE OF MEASLES, MUMPS AND RUBELLA IN CANADA IN 2011

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OBJECTIVE: Molecular epidemiology surveillance for measles, mumps and rubella aids in linking cases, documenting importation, mapping global activity, and documenting elimination of measles and rubella in Canada.
METHODS: Clinical specimens submitted by provincial laboratories were screened for target genes by real time RT-PCR. Positive specimens were amplified by conventional RT-PCR.WHO standardized windows were sequenced and compared to WHO reference strains to determine genotype.
RESULTS: Measles: Five genotypes were identified from 157 cases: A (vaccine), B3, D4, D8 and D9. D8 was found as 4 unique strains in AB, BC and ON. D9 was associated with 2 unique clusters in SK and 2 isolated cases in ON. Most D4 strains had the identical sequence in QC and BC. Two additional D4 strains were identified in QC and 1 in AB. Another D4 strain was associated with a cluster of cases in ON. Mumps: Five genotypes were identified from 171 cases: D, F, G, H and New1. Genotypes D, F, H and New1 were associated with sporadic cases. The majority of the G strains (detected in BC, ON and QC) were identical and is the same strain that has been circulating since 2007. The remaining G strains are variants of this predominant strain. Rubella: Three genotypes were identified from 3 cases: 1A (vaccine) in ON, 1J in BC, and 2B in MB.
CONCLUSION: Mumps surveillance over the years indicates that we may have an endemic strain. A variety of measles and rubella genotypes were found in Canada in 2011, reflecting separate importation events and supporting elimination. The identical measles genotype D4 was identified globally (Europe and the Americas), posing a challenge to distinguish local transmission from repeat importation events.

Room: Port McNeil
Session C

C1

A CANADIAN MULTI-SITE COMPARISON OF BRUKER BIOTYPER, BIOMERIEUX VITEK MS AND STANDARD METHODS FOR IDENTIFICATION OF BACTERIA AND YEASTS

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OBJECTIVE: To compare the accuracy and speed of bacterial and fungal identification by the two newly available MALDI-TOF mass spectrometry platforms with that of standard methods.
METHODS: The mass spectrometers were installed sequentially at each laboratory, with the exception of C&W, which performed Vitek MS evaluations at SPH. Each site assembled a retrospective panel of organisms considered representative of their clinical service and organisms were enrolled prospectively for a total of >3000 isolates. All isolates were tested by both systems. Spectra were analysed using the Bruker Biotyper 3.0 database and the Vitek MS 2.0 database, available at the time of the study (Spring 2011). The Saramis research database was not evaluated. For a subset of 75 prospective organisms, detailed information on turnaround time and standard test utilization was collected. Organisms yielding discordant results were forwarded to BCCDC for biochemical or sequence-based identification.
RESULTS: Of the first 1487 organisms analysed, >87% of isolates were correctly identified to species by MALDI-TOF. Incorrect identifications were uncommon (3-4%), and most were due to *Shigella spp*. or *Streptococcus mitis* group (Bruker). *Streptococcus pneumoniae* isolates were all correctly identified. Partial identifications to genus were more common for Bruker (5%), and "No Identifications" were more common for Vitek MS (4%). Extraction was required for about 5% (Vitek) and 15% (Bruker). Aerobic Gram-positive bacilli were the most troublesome with only 64-72% identified to species. Processes and turnaround time varied by site, but on average, final identification was available in under 2 hours for MALDI-TOF and 36 hours for standard methods.
CONCLUSIONS: Both mass spectrometry systems are accurate for Canadian isolates and were at least equivalent to standard methods plus reference sequencing. Some additional testing is required for a small number of well-described limitations. Turnaround times were dramatically shorter, and improvements in patient outcomes are anticipated. Widespread implementation of MALDI-TOF mass spectrometry is strongly recommended for Canadian laboratories.

C2

COMPARISON OF BRUKER BIOTYPER™ (BRBT) AND BIOMÉRIEUX VITEK MS (VTMS) FOR RAPID IDENTIFICATION (ID) OF *CANDIDA* SPECIES USING MATRIX-ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT (MALDI-TOF)

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OBJECTIVES: Reporting sterile site yeast ID at the time of isolation will improve patient safety and reduce costs by avoiding use of inappropriate anti-fungal therapy. This study assessed MALDI-TOF ID accuracy using retrospective, previously characterized, clinically significant strains from ProvLab Alberta.

METHODS: IDs of 169 *Candida* were confirmed by Vitek II YS01 and API20C on receipt in Toronto. To evaluate the BRBT and VTMS, yeasts were grown on 5% sheep blood (BA) and Sabouraud (SAB) agars for blinded, parallel testing (SAB after 16hx37ºC; BA: after 22h and 36hx37ºC). As instructed, colonies were applied to reusable BRBT target via wooden toothpick, and to VTMS slide via plastic 1µl loop. Formic acid extractions were completed directly on the target/slide for both systems. If a "No reliable ID" was obtained, the test was repeated.

RESULTS: ID by BRBT and VTMS was 100% from SAB and BA (67 *C. albicans*, 1 *C dublinensis*, 49 *C. glabrata*, 11 *C. kefyr*, 16 *C. krusei*, 2 *C. lusitaniae*, 11 *C parapsilosis*, and 12 *C tropicalis*). 16h growth was generally good on SAB: only 2 (1.2%) and 5 (2.9%) required re-testing on BRBT and VTMS, respectively. On BA at 22h/36h, *C. glabrata* growth was often poor: 34 (20.1%)/27(16%) yeasts were re-tested by BRBT vs. 4 (2.3%)/3 (1.8%) by VTMS. This difference appeared due to the application method (toothpick vs. loop) rather than to an ID deficiency, since reliable ID was achieved if loops were used on yeasts with poor growth for BRBT.

CONCLUSIONS: Both the BRBT and VTMS were equally able to accurately ID all *Candida* using MALDI-TOF. For BRBT, ID was more easily achieved from SAB than BA given the propensity for *C. glabrata* to grow poorly on BA. However, use of a plastic loop improved the ability of BRBT to ID *C. glabrata* from poor growth.

C3

RAPID IDENTIFICATION OF BACTERIA AND YEAST BY MALDI-TOF MS (VITEK MS, BIOMERIEUX) IN A ROUTINE LABORATORY SETTING

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OBJECTIVE: Currently in our laboratory, identification of bacteria and yeast is performed by semi-automated identification systems or by traditional methods which are slow, laborious and costly. MALDI-TOF mass spectrometry offers a quick and inexpensive method for identification. We evaluated the Vitek MS method of identification with a variety of routine clinical isolates.

METHODS: 662 clinical isolates from the routine laboratory were identified using both conventional methods and the Vitek MS according to the manufacturer's directions or laboratory protocols. These isolates included gram positive and negative aerobic bacteria, anaerobes and yeast. Congruent identification results of genus and species were considered to be a correct identification. Discrepant results were repeated and then analyzed by 16S sequencing.

RESULTS: Eighty five percent of isolates were correctly identified and an additional 5% were one of the correct choices given. Two percent of isolates produced no identification result and 8% were misidentified. The highest percentage of misidentification or no identification occurred with glucose non-fermenting gram negative bacilli and anaerobes. Results were available from the Vitek MS in approximately 10 minutes while conventional method identifications took from 18 hours to several days.

CONCLUSIONS: Vitek MS provides an accurate, cost effective and rapid identification method for common, routine clinical bacteria and yeast isolates from a clinical laboratory. Enhancement of the database to include other groups of organisms will further improve its performance in a clinical laboratory.

C4

IMPACT OF THE BRUKER MALDI BIOTYPER ON THE ANAEROBIC BENCH PROTOCOL IN DIAGNOSTIC MICROBIOLOGY

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OBJECTIVES: Workup of anaerobic isolates from appropriate diagnostic samples is very time consuming in the Clinical Microbiology diagnostic lab. The first step is the aerotolerance test used to determine if the isolate growing on anaerobic media is an anaerobe. The objective of this evaluation was to compare the efficacy and turn-around-time of the MALDI BioTYper (MBT) identification of anaerobes to traditional aerotolerance and biochemical identification methods.

METHODS: Consecutive clinical isolates from appropriate specimens that were requested to have anaerobic culture were assessed. All isolates growing on the direct anaerobic media were evaluated by aerotolerance testing using traditional methods as well as by MBT. Extraction was used for the MBT method if the first attempt at identification did not provide an acceptable identification. The turn-around time (TAT) was tracked for both approaches. The anaerobic isolates detected were further assessed to compare the reliability of identification from the MBT compared to traditional biochemical and high potency disk identification algorithms currently being used.

RESULTS: A total of 180 bacterial isolates were evaluated. Of these 100 were confirmed to be strict anaerobes that required identification (80 were facultative anaerobes). Using the MBT approach 83.8% of aerotolerance testing could be eliminated and 87% of the anaerobes were directly identified without needing additional biochemical testing. The remaining 13% of isolates required other methods of identification. The average TAT for the MALDI was 38.9 hours compared to 66 hours for the conventional approach. Data base limitations account for some of the 13% of anaerobes that could not be identified by MBT.

CONCLUSIONS: MALDI-TOF MS is a rapid and highly accurate method for direct identification of anaerobic pathogens from clinical isolates. The MALDI-TOF technology dramatically reduced the amount of time to definitive identification and essentially replaced aerotolerance testing for the majority of suspect anaerobic isolates. This improvement in rapid reporting of anaerobes should facilitate improved patient care.

C5

INTEGRATION OF THE BRUKER MALDI BIOTYPER AS A RAPID METHOD FOR IDENTIFICATION OF UROPATHOGENS DETECTED ON THE ROUTINE URINE BENCH

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OBJECTIVES: The use of chromogenic media for urine cultures has helped streamline the workflow on the urine bench in diagnostic Microbiology labs. The objective of this evaluation was to compare the efficacy and turn-around-time of the MALDI BioTyper (MBT) identification of significant uropathogens to traditional reporting based on colony color and rapid biochemical identification methods.

METHODS: Consecutive clinical isolates from all types of urine specimens that were considered to require identification (>10^7 cfu/L) by the existing algorithm were included in this study. All isolates were identified using traditional methods (ie, color and colony characteristics on Orientation chromogenic media, spot tests & Vitek ID where needed) as well as by MBT. The turn-around time (TAT) was tracked for both approaches.

RESULTS: A total of 418 bacterial isolates were considered significant uropathogens and were evaluated. Of these 400 isolates (95.7%) were a "green" level of identification by MBT, and 18 (4.3%) were a "yellow" level identification. There were no "red" (ie, unacceptable level) identifications. Overall the MBT method demonstrated 100% accuracy for the 418 uropathogens evaluated. The combination of direct identification from Orientation media along with the MBT provided significant improvement to the TAT for identification of uropathogens. There was a 9 hour reduction in overall TAT for *E. coli* and Enterococcus isolates (most of these isolates can be reported directly on colony colour and morphology from the Orientation chromogenic media). For isolates that required a Vitek identification card there was a 20.8 hour reduction in the TAT using MBT.
CONCLUSIONS: MALDI-TOF MS is a rapid and highly accurate method for direct identification of uropathogens from urine samples. The MALDI-TOF technology dramatically reduced the amount of time to definitive identification and when combined with Orientation chromogenic media there were significant reductions in TAT. This improvement in rapid reporting of uropathogens should facilitate improved patient care.

ROOM: Port Alberni
Session D

D1
MACROLIDE-BASED REGIMENS AND MORTALITY IN HOSPITALIZED PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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OBJECTIVES: Macrolides may have a favourable effect on pneumonia outcomes, despite increasing antimicrobial resistance, due to their immunomodulatory properties. We systematically reviewed all studies of macrolide use and mortality among patients hospitalized with community-acquired pneumonia (CAP).
METHODS: All RCTs and observational studies comparing macrolides to non-macrolide regimens in adults hospitalized with CAP were identified through electronic databases and grey literature searches. Primary analysis examined any macrolide use and mortality; secondary analysis compared only IDSA/ATS guideline-concordant regimens. Risk of bias was assessed and random effects models used to generate pooled risk ratios (RR) and evaluate heterogeneity (I^2).
RESULTS: We included 23 studies (5 RCTs, 18 observational) and 137,574 patients. Overall, macrolide use was associated with a statistically significant mortality reduction compared with non-macrolide groups (3.7% vs. 6.5%; RR 0.78; 95%CI 0.64–0.95; p=0.01; I^2=85%). There was no survival advantage and heterogeneity was reduced when analyses were restricted to RCTs (4.6% vs. 4.1%; RR 1.13; 95%CI 0.65-1.98); p=0.66; I^2=0%) or to patients treated only with guideline-concordant antibiotics (macrolide/beta-lactam combinations 5.3% vs. respiratory fluoroquinolones 5.8%; RR 1.17; 95%CI 0.91–1.50; p=0.22; I^2=43%).
CONCLUSIONS: In hospitalized CAP patients, macrolide-based regimens were associated with a significant 22% reduction in mortality compared to non-macrolides; however, this benefit did not extend to patients in RCTs or those that received guideline-concordant antibiotics. Our findings suggest guideline- concordance is more important than antibiotic choice when treating CAP.

D2
ANTIMICROBIAL CONSUMPTION IN A PAEDIATRIC HOSPITAL; AN ALTERNATIVE TO DAYS OF THERAPY (DOTS)

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BACKGROUND: Monitoring antimicrobial consumption is recognized for its importance in antimicrobial stewardship programs. Defined daily doses (DDD) are the standard unit of measure but are a poor metric for assessment of populations such as paediatrics because of considerable variation from standard adult dosing. Days of therapy (DOTs) is a metric that avoids this problem since it is not related to dose. DOTs are difficult to calculate with most hospital pharmacy software systems in Canada.
PURPOSE: To determine if antimicrobial expenditures are an acceptable surrogate measure of DOTs of antibacterial consumption in a paediatric hospital.
METHOD: Hospital level, inpatient monthly antibacterial expenditures and DOTs were determined for each antibacterial class retrospectively from 2008-2011 in a large paediatric acute care centre. DOTs were determined by counting the start date, stop date and each day in between as equal to one DOT for all systemic antibacterial orders. Expenditures were obtained from hospital pharmacy reports and regressed against DOTs by linear regression.
RESULTS: Forty-eight months of data of 8 antibacterial classes were assessed. The median monthly antibacterial use was 106.05 DOTs/100 patient days (IQR 102.5-110.7). Median antibacterial expenditures were $31,416 (IQR $28,298-$34,252). *Pearson* R-square for correlation of DOTs versus expenditures was 0.74 (p<0.01), suggesting good correlation in this data set.
CONCLUSION: Antimicrobial expenditures may be an acceptable surrogate for DOTs, which may help reduce the labour burden of monitoring antimicrobial utilization. Replication of results in other centres would further validate the method.

D3
ANTIMICROBIAL RESISTANCE: CAN COLLABORATIVE WORK AND PARTNERSHIPS HELP SOLVE WICKED PROBLEMS?

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National Collaborating Centre for Infectious Diseases, Winnipeg, MB

OBJECTIVE: In February 2010, the National Collaborating Centre for Infectious Diseases (NCCID) hosted a consultation to identify priorities for addressing AMR.
METHODS: The consultation included 50 experts in human, animal, and environmental antimicrobial resistance (AMR). Participants created an action plan outlining key activities and next steps in four areas: leadership and governance, education and awareness, surveillance, and research. The action plan further elaborated the activities that could be supported by NCCID that would have the greatest impact on AMR.
RESULTS: NCCID has focused on the first 3 key areas identified at the consultation:
LEADERSHIP AND GOVERNANCE: A working group was established with PHAC to coordinate AMR activities and improve communication. Two additional working groups were created to provide expert advice and direction and to coordinate and deliver professional education campaigns.
EDUCATION AND AWARENESS: Consisting of 11 Canadian and 3 international health organizations, the Communications and Education Task Group hosted three events for professionals. Planning is underway for another Antibiotic Awareness Week this November.
SURVEILLANCE: Work is underway on a comprehensive report that summarizes current FPT AMR surveillance and antimicrobial use monitoring programs. The final report, due in 2013, will also provide recommendations for addressing barriers and for creating optimal AMR surveillance programs in Canada.
CONCLUSION: NCCID is supporting AMR activities in 3 key areas and will continue to do so over the upcoming years. All activities will be evaluated as appropriate.

D4

CATEGORY I ANTIMICROBIAL RESISTANCE IN BACTERIA RECOVERED FROM RETAIL MEATS IN CANADA

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INTRODUCTION: The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) tracks temporal and regional trends in antimicrobial use, and antimicrobial resistance in selected species of enteric bacteria obtained at different points along the food chain and from humans. Routinely sampled commodities are raw meat and poultry commonly consumed by Canadians including: chicken, pork and beef; turkey was added in early 2011. *Campylobacter*, *Salmonella*, and generic *E. coli* from chicken and turkey are tested routinely; only generic *E. coli* is routinely tested in pork and beef. *Campylobacter* and *Salmonella* are the most common causes of bacterial foodborne gastroenteritis in Canadians. Notable Category I antimicrobials (of very high importance in human medicine) routinely tested include third generation cephalosporins and fluoroquinolones.

RESULTS: Sampling involves regular sample collection from randomly selected census divisions, weighted by population, in each participating province/region (the maritime provinces, Québec, Ontario, Saskatchewan and British Columbia). Temporal (2003-2010) resistance data for Category I antimicrobials, stratified by region, will be presented for the commodity-bacteria pairs. Notable findings include an increase in the prevalence of ciprofloxacin-resistant *Campylobacter* isolated from chicken in western Canada since 2007 and re-emerging ceftiofur resistance in *Salmonella* from chicken in Ontario and Québec.

CONCLUSIONS: The regional increases in ciprofloxacin-resistant *Campylobacter* and ceftiofur-resistant *Salmonella* from retail chicken suggest that Category I antimicrobials are being used in poultry. Unfortunately, antimicrobial use data from the poultry sector are not currently available.

ORAL PRESENTATIONS
Friday May 4, 2012

ROOM: Junior Ballroom A
Session E

E1

SHORT-TERM STABILITY OF NUCLEIC ACIDS IN A DIAGNOSTIC MICROBIOLOGY LABORATORY

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OBJECTIVE: Preservation of the quality and quantity of amplification targets through proper storage of clinical specimens could be critical for the diagnostic accuracy of molecular assays. However, limited data is available on the effect of short-term storage conditions on the detection of pathogen specific nucleic acids. The objective of this study was to assess the stability of DNA or RNA amplification targets in crude clinical specimens and in purified nucleic acid extracts stored at various temperatures.

METHODS: Fresh, never previously frozen serum, CSF and EDTA-blood samples (1 ml) were spiked with a CMV patient isolate, an Enterovirus 70 strain (ATCC VR-836) and a *Neisseria meningitidis* strain (ATCC 13090), respectively. 50 µl of each sample was used for nucleic acid extraction followed by detection of the respective pathogen by Taqman real time PCR, immediately after spiking or after incubation at room temperature (RT), 4°C and −80°C, respectively, for 1, 2, 4, 7 and 16 days. Similar experiments were carried out to analyze DNA/RNA stability in purified samples using nucleic acid extracts from EBV positive serum and Influenza A positive nasopharyngeal wash (NPW) samples.

RESULTS: Kinetic data based on C_T values indicate that the half-life of CMV DNA in serum at RT, 4°C and −80°C were <1, 2 and 3 days, respectively. The half life of enterovirus RNA in CSF at RT was 9 days, but was stable at 4°C and −80°C over two weeks. *N meningitidis* DNA in EDTA-blood remained stable for 2 weeks at all temperatures tested. After two weeks of incubation, the average loss of CMV DNA at RT, 4°C and −80°C was 79.7%, 50% and 36.9% ($p<0.001$; $n=10$), respectively, and at RT only, enterovirus RNA quantity decreased to 17.9% ($p<0.001$; $n=5$). Consistent with kinetic data, there was no significant ($p>0.05$; $n=10$) decrease of spiked *N meningitidis* DNA in random blood samples. In contrast to crude clinical specimens, EBV specific DNA and Influenza A specific RNA were remarkably and reproducibly stable in purified nucleic acid extracts over a 1 month period.

CONCLUSIONS: Our results indicate that the levels of viral DNA/RNA or bacterial DNA do not abruptly change during incubation at RT or 4°C over a few days. However, the suitability of serum samples for quantitative viral load analysis needs to be carefully assessed.

E2

MONITORING FOR PCR INHIBITION IN FOLLOWING HOMOGENIZATION AND HEAT TREATMENT

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OBJECTIVES: Most laboratories use a nucleic acid extraction to remove PCR inhibitors. Homogenization followed by heat treatment was recently shown to be a rapid and cost effective method to recover DNA from swabs submitted for herpes simplex virus (HSV) real-time PCR. Homogenization uses multidirectional motion to disrupt cells through contact with silica beads. Despite a subsequent heat treatment, this crude method to recover DNA may not inactivate PCR inhibitors. To assure its optimal performance after introduction, this study established a quality control system and interpretive criteria to a monitor for PCR inhibition using an exogenous internal control (IC).

METHODS: Following 48 consecutive experiments, crossing point (Cp) values of the IC in 750 HSV-negative specimens were analyzed to assess: 1) the distribution within and between experiments; 2) the deviation from the control in each experiment; and 3) the deviation from the average obtained for all HSV-negative specimens in each experiment (intra-experimental average).

RESULTS: Cp values were affected by intra- and inter-experimental variation; however, when normalized to the control or to the intra-experimental average, the deviations displayed a normal Gaussian distribution. Using a cut off of Cp ± 2 standard deviations, normalization to the control identified potential inhibition in a single specimen whereas data normalized to the intra-experimental average, an additional 15 specimens were identified with possible PCR inhibition.

CONCLUSIONS: This study describes a rigorous method to monitor for potential PCR inhibition after sample homogenization and heat treatment that would have otherwise been masked by the intra- and inter-experimental variation.

E3

MOLECULAR TESTING FOR ENTERIC PATHOGENS USING STOOL SPECIMENS SUBMITTED FOR BACTERIAL CULTURE

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OBJECTIVE: Investigation of gastrointestinal infections often requires examination of multiple stool samples using a range of culture media and techniques. Molecular screening for viral and bacterial pathogens using a multiplex PCR system was applied to stools submitted for culture in Cary-Blair transport medium (CB).

METHODS: Consecutive stool specimens submitted in CB were cultured and then total nucleic acids were extracted and stored at −70°C. The Seegene SeePlex Diarrhea ACE Detection assay was used on all extractions, and products were detected on a LabChip instrument. The SeePlex assay detects 4 viruses and 10 bacteria: rotavirus, norovirus, adenovirus,

astrovirus, *Salmonella*, *Shigella*, *Vibrio*, *Campylobacter*, *Clostridium difficile*, *C. perfringens*, *Y. enterocolitica*, *Aeromonas*, *E. coli* O157:H7 and verotoxigenic *E. coli*. Real-time PCR were used for correlation to SeeGene results for norovirus and enteropathogenic *E. coli* and Shiga toxin-producing *E. coli*.
RESULTS: 499 stools yielded 11 culture positive results. The SeeGene assay failed to detect 3/5 culture positives for *Salmonella* and *Campylobacter*. The SeeGene assay identified positive targets in 164/488 culture negative specimens. Real-time PCR confirmed 36/40 positive norovirus specimens by the SeeGene assay.
CONCLUSION: A multiplex PCR assay can be applied to stool specimens in CB for the detection of viral and bacterial targets. This approach allowed the detection of viral pathogens in patients who were not investigated for viral diarrhea. However, the SeePlex assay did not detect all bacterial pathogens isolated from the specimens studied. Appropriate and alternative PCR targets with established limits of detection are necessary before molecular testing can supplant stool culture.

E4

COMPARISON OF THE BD VIPER™ SYSTEM WITH XTR™ TECHNOLOGY TO THE GEN-PROBE APTIMA COMBO 2® ASSAY USING THE TIGRIS® DTS® SYSTEM FOR THE DETECTION OF *CHLAMYDIA TRACHOMATIS* AND *NEISSERIA GONORRHOEAE* IN URINE SPECIMENS

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OBJECTIVE: The performance of the BD ProbeTec™ CT/GC Q^x Amplified DNA Assay reagents on a BD Viper System with XTR Technology (BD Diagnostics, Sparks, MD) and APTIMA COMBO 2 Assay reagents on a TIGRIS DTS platform (Gen-Probe, Inc., San Diego, CA), for detection of both *C. trachomatis* and *N. gonorrhoeae* was compared.
METHODS: A total of 1,018 first-void urine specimens were tested for the presence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA using the two assays.
RESULTS: *C. trachomatis* was detected in 143 specimens (14%). Eight specimens exhibited discordant results and they were divided equally between the two assays. The overall agreement was 99.2% (positive agreement was 97.1% and negative agreement was 99.5%). Cohen's Kappa was 0.967. *N. gonorrhoeae* was detected in 27 specimens (2.6%). Two specimens exhibited discordant results and they were divided equally between the two assays. The overall agreement was 99.8% (positive agreement was 96.2% and negative agreement was 99.9%). Cohen's Kappa was 0.961. Nine of ten discordant specimens re-tested with a different target (Gen-Probe) gave the same result, while only one of the ten discordant specimens gave the same result when repeated with the same target (BD). The combined rate of false-positives and false-negatives was 0.049% (Gen-Probe) and 0.44% (BD).
CONCLUSION: There was a high level of agreement between the systems for both *C. trachomatis* and *N. gonorrhoeae* detection. Selection of a NAAT platform for detection of *C. trachomatis* and *N. gonorrhoeae* should be made after consideration of factors such as daily specimen throughput, the ease of use, potential efficiency gains in the laboratory, and the environmental footprint.

E5

DETECTION OF *NEISSERIA GONORRHOEA* USING VIPER XTR; CONFIRMATION IS NECESSARY TO AVOID FALSE POSITIVE RESULTS

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OBJECTIVES: Molecular methods for the diagnosis of *Neisseria gonorrhoea* (NG) have the potential for detection of non-NG *Neisseria* species, a problem that leads to false positive results. Here we describe our experience with false-positive tests using the Viper XTR.
METHODS: 1) Genital and throat specimens collected over a 10-week period were tested using the Viper XTR. Positive specimens were confirmed using the EraGen MultiCode®-RTx assay directed at conserved regions of the NG 16s rDNA. 2) To assess cross reactivity, a 10-fold dilution series of 6 different *Neisseria* species was tested with the Viper XTR. NG dilutions were tested in parallel with the EraGen kit.
RESULTS: 1) Of the 6694 specimens tested, 13 tested positive for NG. Three of the 10 genital specimens positive for NG on the Viper XTR failed to confirm using the EraGen PCR. All 3 throat specimens positive for NG failed to confirm. 2) The LOD of the EraGen and Viper XTR assays are equivalent at 500 copies/ml. The Viper XTR NG assay cross reacted with *N. cinerea*, *N. lactamica*, and *N. meningiditis* but did not cross react with *N. sicca* or *N. subflava*. No cross reactivity was observed with the EraGen assay.
CONCLUSION: Cross-reactivity with non-NG Neisseria species does occur with the Viper XTR. Confirmation with a second molecular assay is necessary and particularly important for all non-genital specimens.

E6

COMPARISON OF MULTIPLE APPROACHES TO THE IDENTIFICATION OF PHENOTYPICALLY ATYPICAL NON-FERMENTING GRAM NEGATIVE BACILLI ISOLATED FROM PATIENTS WITH CYSTIC FIBROSIS

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OBJECTIVE: The accurate identification of non-fermenting gram negative bacilli can be challenging especially when isolates are recovered from patients with cystic fibrosis. Often phenotypic characteristics are unusual. When used in this setting conventional biochemicals and commercial instrument systems may be inaccurate
METHODS: We identified atypical non-fermenting gram-negative bacilli from cystic fibrosis patients which grew on oxidation/fermentation-polymyxin-bacitracin-lactose agar and for which an abbreviated set of conventional biochemicals produced questionable results. Included were atypical strains of *S. maltophilia* (5), *B. cenocepacia* (2), Achromobacter, sp.(3), Chrysobacterium sp (1), Acinetobacter sp.(2) and *Pseudomonas* sp.(8). We compared the ability of the Vitek 2 GN card, API NE, Vitek MALDITOF-MS (using the Myla™ software application) (bioMérieux, QC, Canada) and 16S PCR to speciate each stain. Testing was done in a blinded fashion.
RESULTS: Using 16s sequence analysis 20/21 were identified to the species level. In one case sequencing was unable to differentiate between Alcaligenes sp and Achromobacter sp. Using the 16S sequence as the gold standard, the Vitek MS identified 20 of 21 correctly to the genus level, one Acinetobacter sp. was not identifiable. One strain of B. cenocepacia could not be differentiated from *B. vetnamiensis*, The strain which 16S could not differentiate Alcaligenes sp. from Achromobacter sp. was called an Achromobacter sp. by the Vitek MS. The Vitek 2 correctly identified only 8 of 21 isolates. The API 20 NE correctly identified only one strain.
CONCLUSION: The Vitek MALDITOF-MS produced results equivalent to that of 16S PCR based sequencing and proved surprisingly accurate in the identification of this very challenging collection of isolates. The API NE system was unable it identify most stains and should not be used in this setting.

E7

DUAL-TARGET QUANTITATIVE PCR ASSAY FOR RAPID DETECTION OF *MYCOBACTERIUM TUBERCULOSIS*

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BACKGROUND: Traditional methods for the detection of *Mycobacterium tuberculosis* (MTB) in clinical specimens, such as microscopy and culture, either have poor sensitivity and specificity or slow turn-around times. Molecular assays fill this niche by providing rapid and reliable detection of MTB.
OBJECTIVE: We developed a dual-target quantitative PCR assay for MTB complex that is compatible with a wide range of specimen types. The

performance of the assay was compared against acid-fast bacilli (AFB) smears, mycobacterial culture and the Gen-Probe Amplified *M. tuberculosis* direct (AMTD) assay.

RESULTS: The analytical sensitivity of our TB PCR was 10 MTB genome copies. Compared to mycobacterial culture, our TB PCR had 97% sensitivity (100% upon repeat testing of indeterminate results). The specificity was 81% suggesting that the assay also detected MTB that did not grow in culture. There was a correlation between the Ct value and the AFB smear result with lower sensitivities for samples that were below 1+ AFB. Our TB PCR assay also had comparable performance against the Gen-Probe AMTD with an inter-assay concordance of over 98% on respiratory specimens.

CONCLUSION: We have developed, validated and implemented an in-house TB PCR assay that is less expensive than commercial assays while providing comparable performance on a wider range of specimen types with higher throughput and faster turn-around times. The assay complements traditional MTB tests by providing rapid MTB detection of AFB smear-positive specimens. It also allows for quality control of AFB smear results as well as having the potential to eventually replace the use of AFB smears for TB infection control.

E8

GENETIC CHARACTERIZATION OF CATEGORY A/B BACTERIAL PATHOGENS IN CANADA: RECENT DEVELOPMENTS

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OBJECTIVES: Historically, DNA fingerprinting has provided insights into the spread of several etiological agents of disease, the linkage between infections of humans and animal reservoirs, and the emergence of drug resistance. More recently, the ease of whole genomic sequencing (WGS) and resultant data acquisition has pushed the notion of "subtyping" into a new era. As part of a CRTI project aimed at Microbial Forensics, the National Microbiology Laboratory has integrated both classical subtyping and WGS capabilities for the Category A/B agents *B. anthracis*, *F. tularensis*, *Y. pestis* and *Brucella* sp. into their diagnostic capacity. Here we discuss our new capabilities and results to date.

METHODS: Multiple locus variable number tandem repeat analysis (MLVA), single nucleotide polymorphism (SNP) assays and WGS capabilities via 454 and Illumina technologies have been adapted and adopted as part of the Bioforensics Assay Development and Diagnostics group (NML). The majority of testing has been validated to ISO17025 standards or is in process. A variety of bioinformatic software has been applied for analysis, including Bionumerics, GeneMarker, CLC Workbench and in-house developed WGS tools. A Bionumerics database has been constructed to house all subtyping data.

RESULTS: Our in-house genotyping database has MLVA patterns for 673 reference and clinical strains of *B. anthracis* and *F. tularensis* available for comparison. Canonical SNP data is available for *B. anthracis*, *F. tularensis* and *Brucella* sp, which identifies lineages and/or subtypes accurately and can have a direct impact on control measures. Whole genomic sequence data is also available for most strains of the Category A agents in our collection. Pros and cons of relying on one or the other type of data is outlined.

CONCLUSIONS: Although WGS provides greater resolution and is ideal for population genetics, MLVA has proven invaluable for epidemiologists and prevention/control authorities and is now available for high consequence pathogens in Canada. Having well-described population of strains serves a variety of purposes, including the unique prospect of determining the diversity of these agents in Canada. Here we present new capabilities offered to provincial/territorial partners and an opportunity for collaborative activities.

ROOM: Junior Ballroom B
Session F

F1

THE VALUE OF THE ANAEROBIC BLOOD CULTURE BOTTLE

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OBJECTIVE: Anaerobic blood culture media are not universally used in blood culture sets to detect blood borne pathogens. Recent publications suggest an anaerobic(AN) and aerobic bottle have increased bacterial recovery over 2 aerobic bottles per blood culture set. Detection of strict anaerobes is uncommon, but the utility of the AN bottle may be to aid in detection of aerobic organisms.

METHODS: Over an 8 month period in a 477 bed urban, level I trauma centre, all blood cultures drawn as an aerobic/AN set (BACTEC FX, Becton Dickinson) were reviewed. Sets with growth only in the AN bottle were included. Data collected included: overall bacterial yield, species identification, pathogen/contaminant, and anaerobe/aerobe classification. Septic events, defined as the recovery of a presumed pathogen from patient within a 7 day period were counted. Missed septic events were calculated by identifying which patients had another positive culture with the same organism in an aerobic bottle within 48 hrs of the original collection.

RESULTS: Overall 9,395 cultures were collected hospital wide, with 1,265 positive cultures (13.5%). Growth in AN bottles only occurred in 203 cases, representing 191 patients, and 16% of the total positive cultures. Of these 203, 83(41%) grew aerobic pathogens, 16(7%) grew AN pathogens, 77(38%) aerobic contaminants, 23(11%) AN contaminants, and 4(2%) grew organisms of indeterminate clinical significance. 92 septic events were identified, representing 25 distinct organisms. Of the 77 patients growing only aerobic pathogens, 24 (31%) septic events would have been identified within 48 hours by another aerobic culture. However, in 53/77 (69%) cases, no other cultures were positive within 48 hrs of collection. One patient had 3 serial cultures positive for *E. faecalis* only in the AN bottle. Organisms identified included *Staphylococcus aureus*, *E. coli*, and *E. faecalis*, in 28, 19, and 6 cultures respectively.

CONCLUSIONS: The importance of the AN bottle for aerobic recovery is clear, as 83/203 (41%) of the AN bottles grew aerobic pathogens. Furthermore, 53/77(69%) of septic events identified only by the AN bottle grew aerobic pathogens. Had an anaerobic bottle not been drawn, these events would have gone unrecognized.

F2

DIFFERENCE IN TIME TO REPORT POSITIVE BLOOD CULTURES BETWEEN THE CENTRAL HOSPITAL AND COMMUNITY HOSPITALS IN A CONSOLIDATED MICROBIOLOGY LABORATORY

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OBJECTIVE: Consolidation of microbiology laboratories is being pursued with a goal of achieving cost savings, but with little data on the potential clinical impact. Following consolidation of community hospital microbiology laboratories to a central hospital laboratory, an analysis of the reporting time of blood cultures positive for *Enterobacteriaceae* was performed.

METHODS: Data were compiled on positive blood cultures collected at the central hospital and at three community hospitals that refer their microbiology specimens to the central laboratory. The following time points were recorded for blood cultures positive for coliforms: specimen collection, arrival at community hospital laboratory, arrival at central laboratory, detection of positive culture, and initial report of positive blood culture to the clinical ward. Mean and 90th percentile intervals between these time points were calculated.

RESULTS: From October 2009 to September 2010, 188 blood cultures that grew coliforms were collected at the central hospital and 166 at the community hospitals. At the 90th percentile, there was a nine-hour delay

in the time to report positive cultures from community hospitals, 37.3 h versus 28.3 h for central hospital collections. Community samples had longer intervals between collection and arrival at the central laboratory (mean 5.5 h; p<0.001); between collection and detection of growth (mean 2.5 h; p=0.019); and between collection and initial report to ward (mean 4.5 h; p= 0.003).

CONCLUSIONS: Consolidation of microbiology services from community hospitals results in a delay of detecting and reporting blood cultures positive for *Enterobacteriaceae*. This may have a negative impact on the clinical management of patients with significant bacteremia

F3

EVALUATION OF BIOMÉRIEUX BACT/ALERT® (BTA) POLYMERIC BEAD-BASED FA PLUS AND FN PLUS CULTURE BOTTLES FOR CULTURING STERILE BODY FLUIDS

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OBJECTIVE: Recovery of microorganisms in sterile body fluids using culture bottles was evaluated for (1) recovery rates, (2) time to detection (TTD in hours) and (3) ease of Gram stain by comparing new polymeric bead-based BTA FA Plus/FN Plus culture bottles with current charcoal-based BTA FA/FN culture bottles.

METHOD: Sterile body fluids (ie, CSF, pleural, dialysate, synovial, peritoneal) were cultured as per standard laboratory protocol. Excess specimen was inoculated in equal volumes (range of 0.1 to 10.0 mL) in random order to a bottle set (FA/FA Plus, or FN/FN Plus, or FA/FN/FA Plus, or FA/FN/FN Plus, or FA/FN/FA Plus/FN Plus) and placed into the BTA Microbial Detection System for up to 5 days incubation. Positive bottles were Gram stained and sub-cultured after detection of microbial growth, while negative bottles were terminally sub-cultured after 5 days.

RESULTS: 53/243 specimens (21.8%) were positive for clinically relevant microorganisms with the following bottle positivity rate: FA (17.7%), FN (15.0%), FA Plus (21.7%), FN Plus (18.4%). Polymeric bead-based FA Plus/FN Plus bottle Gram stains were easier to read, and the time to find and identify microorganisms was faster compared to the charcoal-based bottles.

	TTD (faster)	p-value	n
FN/FN Plus pair POS	1.2 (FN Plus)	<0.05	27
All bottles (FA/FN/FA Plus/FN Plus) POS	1.2 (Plus)	<0.05	19
One bottle of each (FA/FN+FA Plus/FN Plus) POS	1.7 (Plus)	<0.05	44

CONCLUSION: The new polymeric bead-based BTA FA Plus and FN Plus culture bottles provide improved recovery and time to detection, and provide a clearer Gram stain when compared to the charcoal-based culture bottles.

F4

CLINICAL VALIDATION OF MRSA*SELECT*™ CHROMOGENIC MEDIUM FOR EARLY DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM BLOOD CULTURES

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OBJECTIVE: MRSA bacteremia is a clinical entity which requires, a rapid turnaround time (TAT) for detection from positive blood cultures. While the initial gram stain result often suggests *Staphylococcus* by morphology, direct tests such as Thermostable DNAse (TDNAse) and tube coagulase offer rapid preliminary identification of *S. aureus*. Confirmatory identification of the isolate and antimicrobial susceptibility results take another ~48 hours using conventional methods. The objective of our study was to validate the use of the chromogenic medium MRSA*Select*™ for direct detection of MRSA from blood cultures showing growth of gram positive cocci in clusters to reduce the TAT.

METHODS: The study was carried out at three microbiology laboratories in Manitoba. Four hundred and ninety positive blood cultures from the three sites were included in the study. Samples from positive blood cultures growing gram positive cocci, were inoculated to MRSA*Select*™ medium (Bio-Rad) in addition to routine media. Identification and antimicrobial susceptibility testing were done as per laboratory protocol.

RESULTS: Of the 490 positive cultures, 53 grew MRSA. Fifty-two of the 53 were detected by MRSA*Select*™ upon direct subculture. The remaining 439 blood cultures grew, 223 methicillin susceptible *S.aureus*, (MSSA) 208 coagulase-negative staphylococci and 8 *Micrococcus* spp.

CONCLUSION: The sensitivity of direct inoculation on MRSA*Select*™ and the specificity was 100%. TAT to identification of MRSA was reduced to <18 hours. We recommend the use of this method for the rapid detection of MRSA from blood cultures.

F5

EVALUATION OF BIOMÉRIEUX CHROMID™ VRE AGAR FOR THE DETECTION OF VANCOMYCIN RESISTANT ENTEROCOCCI (VRE)

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OBJECTIVE: To compare the sensitivity, specificity, PPV, NPV and ease of use of bioMérieux chromID™ VRE and Oxoid® Brilliance VRE plates using a real-time PCR method.

METHOD: A total of 500 specimens from screening samples (rectal swab) and stool samples submitted for *C. difficile* toxin were analyzed. Specimens were suspended in saline, vortexed, cultured on both media using the bioMérieux PREVI™ Isola, then incubated and read at 1 and 2 days. Confirmatory real-time PCR for *vanA* and *vanB* genes, was performed on positive cultures using an in-house developed, internally controlled PCR reaction. In addition, identification by Vitek 2™ GP cards and vancomycin MIC susceptibility testing was performed on all positive cultures according to manufacturers' instructions.

RESULTS: 43/500 specimens were confirmed positive for VRE; 43/43 were confirmed by PCR as carrying *van A*.

	Sensitivity (%)		Specificity (%)		Breakthrough (# colonies)	
Day	1	2	1	2	1	2
chromID VRE	88.4	100	99.6	98.9	5	21
Brilliance VRE	88.4	100	99.6	98.1	6	92

Breakthrough: Non-chromogenic colonies (clear or white). Shaded boxes are the manufacturers' recommended incubation and reading time.

CONCLUSION: At 24 hours, bioMérieux chromID™ VRE Agar exhibited similar sensitivity and specificity, albeit with less breakthrough of non-chromogenic colonies (clear or white), compared to Oxoid® Brilliance VRE. For an optimized VRE recovery, an additional reading at 48 hours is recommended for all media.

F6

OPTIMIZATION OF GROUP B STREPTOCOCCUS RECOVERY IN STREPB CARROT BROTH

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BACKGROUND: Group B streptococcus (GBS) is the leading infectious cause of morbidity and mortality among newborns. In 2010, the Centers for Disease Control and Prevention (CDC) guidelines included GBS identification by StrepB Carrot Broth (CB). However, limited data are available to determine the optimal storage and transport medium (TM). We sought to compare recovery of GBS in CB when ESwab and Amies TM are stored for up to 144 h at 4°C, 21°C and 24°C.

METHODS: 50 isolates of GBS were evaluated. 100 µL aliquots of 10^3 CFU/mL suspensions were inoculated onto Amies and ESwab TM and

stored at 4°C, 21°C or 24°C for up to 144 h. At 24 h intervals, Amies and ESwab TM were inoculated into CB and incubated at 35°C for 24 h. Results were quantified as 0, 1+, 2+, 3+, and 4+. The difference in GBS survival were compared.

RESULTS: For Amies TM at 4°C, GBS recovery was 100% at 96 h and 84% at 144 h. Recovery of GBS at 144 h decreased significantly to 34% and 54% when Amies TM were stored at 21°C and 24°C, respectively (P=0.0001). For ESwab TM at 4°C, GBS recovery was 100% up to 120 h and declined to 88% at 144 h. In contrast, 100% of GBS in ESwab TM stored for 144 h were recovered at 21°C and 24°C. At 21°C and 24°C, 0/50 GBS were recovered at 3+/4+ using Amies TM whereas 43/50 was recovered at 3+/4+ using ESwab TM (P=0.0001).

CONCLUSION: Optimal specimen collection and TM are essential for identification of GBS. Results from this study support the use of CB to facilitate detection of GBS. Furthermore, optimal recovery is observed at 4°C for Amies TM and 21°C/24°C for ESwab TM. Overall, this study demonstrates that ESwab TM is superior to Amies TM for recovery of GBS, particularly when stored at 21°C and 24°C.

F7

EVALUATION OF A WHO RECOMMENDED RAPID SYPHILIS POINT OF CARE TEST (POC) IN A HARD TO REACH POPULATION OF BRITISH COLUMBIA, CANADA

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OBJECTIVES: The goal of the study was to evaluate the syphilis SD BIOLINE 3.0 Point of Care Test (POC) with an aim to use it for testing in hard to reach populations, including sex trade workers in British Columbia.

METHODS: Blood samples were collected from each patient in a serum separator tube used for both standard serological testing as well as POC tests in the BCCDC Public Health Microbiology and Reference Laboratory. Blood samples obtained from finger-prick were used in the Sexually Transmitted Infection Clinic at BCCDC to carry out POC tests as per the manufacturer's instructions. The POC test results were compared against the Health Canada approved syphilis antigen test, *Treponema pallidum* Particle Agglutination Assay (TPPA).

RESULTS: A total of 290 patients were enrolled in this study. Head to head comparison of the whole blood POC tests (done in clinic/field) were made with the TPPA test (performed in Laboratory) using serum on 275 patients. The sensitivity and specificity with whole blood were 85% (missed 7 positive cases) and 100% respectively. When the POC tests were done using serum in the laboratory and compared with TPPA, the sensitivity and specificity were 95% and 100%.

CONCLUSIONS: SD BIOLINE 3.0 kit demonstrated excellent specificity (100%), however, missed 7 positive cases (sensitivity 85%) in whole blood samples tested in the clinic. A handheld strip reading device may increase sensitivity by capturing weakly reactive (faint band) cases.

F8

LEGIONELLA ….OR NOT *LEGIONELLA*…..THAT IS THE QUESTION

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OBJECTIVE: The genus *Legionella* was only 'discovered' in the 1970s because these species could not be recovered *in vitro* on usual laboratory agar (eg sheep blood (SBA), chocolate (CHOC) or trypticase soy agars (TSA), due to requirements to grow only media enriched with cysteine and iron, preferably in the presence of charcoal. In this study, bacteria which had upon casual inspection legionella-like colonies after growth on legionella agar (Buffered Cysteine Yeast Extract agar enriched with cysteine, iron and pH 6.9 (BCYE)) but not on SBA, CHOC or TSA at 72h, were found to be identifiable as members of the genus *Tepidimonas*. Features which provided differentiation from true *Legionella* species, are reviewed.

METHODS: A polyphasic approach was used, using standard phenotypic, chemotaxonomic, 16S rRNA gene sequencing, AST and MALDI-TOF methods

RESULTS: NML 110652 & 110653 were recovered during study of environmental water flora from a legionella case site where a strain of *Legionella pneumophila* sg 10, ST 378 had been previously recovered. Both: 1) grew on BCYE with or without enrichment (*Legionella* grow only on enriched BCYE) 2) had cellular fatty composition profiles consistent with *Tepidimonas* spp., which is qualitatively very different from legionella 3) by 16S, were closest to (98.7% identity) *T. ignava* and not related to *Legionella* 4) lacked the *mip* gene (associated with pathogenic *Legionella spp.*) 5) biochemically were inert (like *Legionella*) and thermophilic.

CONCLUSION: Microbiology labs doing water enumeration studies should be aware that legionella-mimicking bacteria co-exist with *Legionella* in waters being tested as part of a case followup. Several approaches including molecular methods should be used to rapidly differentiate these phenotypically-similar taxa.

ROOM: Port McNeil
Session G

G1

INTERIM ANALYSIS OF A RANDOMIZED CONTROLLED TRIAL OF CHLORHEXIDINE GLUCONATE, INTRANASAL MUPIROCIN, RIFAMPIN AND DOXYCYCLINE VERSUS CHLORHEXIDINE GLUCONATE AND INTRANASAL MUPIROCIN ALONE FOR THE ERADICATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* COLONIZATION IN AN OUTPATIENT POPULATION

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OBJECTIVES: This study aims to compare a topical decolonization protocol for MRSA eradication with a systemic decolonization protocol in order to examine their sustained efficacy and adverse effects.

METHODS: Outpatients colonized with MRSA were randomized (3:1 allocation) to receive 7-day systemic or standard treatment. The standard protocol employed daily 4% chlorhexidine gluconate baths with twice daily 2% intranasal mupirocin. The systemic protocol included the addition of oral rifampin and doxycycline. Patients were assessed for successful initial decolonization and for sustained decolonization at 3, 6, and 12 months post-treatment.

RESULTS: Of the 79 patients enrolled in the study, 25 (31.6%) decolonized without treatment. Of the remaining 54 patients (41 systemic; 13 standard), 12.2% of systemic were unable to complete treatment due to side effects. Initial decolonization was achieved for 58.3% of patients receiving standard treatment versus 75.0% of those assigned to systemic. Three month follow-up showed sustained decolonization in 50% of standard treatment patients and 54.2% of systemic. Sustained decolonization among patients treated with standard therapy dropped to 40% and 22.2% at 6- and 12-month follow-up respectively. At the same intervals, systemic patients had sustained decolonization rates of 45.0% and 42.1% respectively. No difference in mupirocin resistance patterns has been noted thus far.

CONCLUSIONS: While it is early to draw definitive conclusions, systemic decolonization appears to be superior for sustained decolonization over standard decolonization therapy based on this interim analysis. However, side effects and an inability to complete therapy were more common in the systemic treatment arm.

G2

INJECTING INSULIN THROUGH CLOTHES? A CASE OF *M. IMMUNOGENUM* CUTANEOUS INFECTION AND REVIEW OF THE LITERATURE

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OBJECTIVE: We report a case of cutaneous *M. immunogenum* infection in a diabetic related to insulin injections through clothing and provide a literature review.

METHODS: A chart review was conducted to collect clinical data on this case. Pubmed was searched for similar cases reported in the English language literature.

RESULTS: A 37-year old female with longstanding type 1 diabetes mellitus presented with a 2-month history of fevers, night sweats, and a non-healing thigh nodule unresponsive to oral antibiotics. She denied recent foreign travel, but described subcutaneous insulin injections to her thighs through clothing. Physical exam confirmed a 5cm tender, erythematous nodule. Biopsy demonstrated non-specific inflammation with negative acid-fast bacilli (AFB) stains. Culture isolated *Mycobacteria spp.* after extended incubation of 62 days. 16S rRNA sequencing identified the organism as *M. immunogenum*. Antimicrobial susceptibility documented sensitivity only to clarithromycin and tigecycline. The patient underwent operative debridement followed by tigecycline and azithromycin for 6 weeks. She remains on azithromycin and is disease free at 3 months of follow-up. Literature review revealed 4 cases of cutaneous *M. immunogenum* infection and a case series associating non-tuberculous mycobacterial (NTM) infection with insulin injection through clothing.

CONCLUSION: This case adds to growing literature supporting the pathogenicity of the organism as well as the association of NTM infection with insulin injection through clothing questioning the safety of this practice. The prolonged incubation required for isolation questions whether the standard 49 days is adequate time to detect mycobacterial pathogens.

G3

CENTRAL LINE ASSOCIATED BLOOD STREAM INFECTIONS (CLABSIS) AT A TERTIARY CARE HOSPITAL 2006-2010 – A COMPARISON BETWEEN ICU AND NON-ICU HOSPITAL SETTINGS

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OBJECTIVES: To determine the rates of CLABSI in the ICU and ward settings of a tertiary care hospital and to describe any microbiologic or demographic differences between infections in these two settings.

METHODS: Data collected prospectively by infection control practioners between 1 Jan 2006 and 31 Dec 2010 was analyzed and reported per 1,000 line days. Line days outside of the ICU setting were extrapolated from line days collected on 4 representative nursing units over a 2 month period. CLABSI rates in and out of the ICU were compared as were the presence of other risk factors.

RESULTS: We identified 275 episodes of line infection among 257 patients. A striking difference by gender was observed (men accounted for 91% of ICU cases and 64.1% of ward cases). The greatest numbers of cases were observed in general systems ICU and hematology ward. Little difference was found in organisms cultured by setting. Substantially more PICCs and tunnelled catheter infections occurred on the ward. ICU rates were significantly higher than ward rates from 2006-2008 then dropped to match the ward rates in 2009-2010. The average rate over the 5-year period was 1.5/1000 line days.in the ICU and 1.3/1000 line days in the wards. The all cause mortality rate in this population was 17.2% regardless of setting.

CONCLUSIONS: More than half of all central line days occur outside of the ICU. The type of line differed with more PICC line infections outside of the ICU. The rates of infection and the microbiology in and out of the ICU are comparable. Literature would suggest that CLABSI rates outside of the ICU should be lower. This may be an area that requires more intensive infection prevention strategies.

G4

INVESTIGATION OF AN *ENTEROBACTER CLOACAE* OUTBREAK IN A NEONATAL INTENSIVE CARE UNIT

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OBJECTIVE: Isolates of ertapenem-resistant *Enterobacter cloacae* were identified in a neonatal intensive care unit [NICU]. An investigation was initiated with the following objectives: (1) to define the extent of the outbreak, (2) to determine a source, and (3) to implement appropriate control measures.

METHODS: An outbreak investigation was conducted in a 65-bed tertiary care Level II/III NICU. Infants colonized or infected with *E. cloacae* from April to May 2011 were included. Infection prevention and control [IPC] and environmental assessments, pulsed-field gel electrophoresis [PFGE] for strain characterization, and polymerase chain reaction-based testing for carbapenemases were performed.

RESULTS: There were 8 patients from whom *E. cloacae* was isolated. Six isolates were the same strain based on PFGE; five were considered to be infections. There were varying susceptibilities to third-generation cephalosporins and ertapenem. Four of the six isolates had a derepressed AmpC gene. No carbapenemases were present. Plasmid typing and porin-specific characterization were not done. IPC and environmental assessments did not identify any obvious source, but it was noted that staff did not routinely use gloves for diaper changes. IPC measures included institution of contact precautions, enhanced environmental cleaning and cohorting of patients and staff.

CONCLUSION: We hypothesize that lack of hand hygiene and ensuing environmental contamination contributed to transmission. The complexities of laboratory identification of resistant Gram-negative bacilli are highlighted. Different genotypic and phenotypic characteristics within a single species create challenges for IPC with respect to interpretation of significance and an appropriate response.

G5

CONTAMINATED ICU SINKS AS THE SOURCE OF AN EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) PRODUCING *KLEBSIELLA OXYTOCA* OUTBREAK

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BACKGROUND: *Klebsiella* spp. may be transmitted person to person, but also from environmental sources in hospitals. We describe a prolonged outbreak of ESBL-producing *K. oxytoca* (EKO) associated with sink colonization in a tertiary care hospital.

METHOD/RESULTS: From 10/2006 to 1/2007, 5 infections due to two related PFGE clones of EKO were identified in our 16 bed medical/surgical ICU. Initial investigation identified 4 additional patients colonized. Despite routine rectal screening (MacConkey agar with cefpodoxime [2µg/mL]) for ICU admissions and contact precautions for colonized and infected patients, there were 5 clinical EKO isolates in 2007 and 9 in 2008. EKO with the same PFGE pattern was cultured from 15 of 16 free-standing porcelain handwashing sinks in ICU rooms, primarily in drain cultures (43/136, 31.6%). Thrice daily disinfection (Q4 2008) reduced the positivity rate of sink cultures from 20.5% (9/2008) to 3.9% (10-12/2008), but was unable to eradicate EKO from sinks. In Q1 2009, an antimicrobial stewardship program was introduced. After 7 months without clinical isolates, 4 were identified in Q3/4 2009 prompting the removal of sink overflow holes and the change of sink strainers. Cleaning guidelines were reinforced. Since 2/2010, no ICU associated clinical isolates were found, although 20 patients became colonized, due to lapses in sink-cleaning. Transmission of EKO clones also occurred from sinks to patients in a step-down unit, and from patient to patient on a medical unit without sink contamination.

CONCLUSIONS: EKO can be a reservoir for sink-to-patient, patient-to-patient and patient-to-sink transmission of resistant *Klebsiella* spp. in hospitals. A multi-faceted approach was required to prevent the spread of hospital-acquired ESBL-producing *K. oxytoca*.

G6

A CROSS-SECTIONAL POINT PREVALENCE MULTI-HOSPITAL SURVEY OF HEALTHCARE ASSOCIATED PATHOGEN CONTAMINATION OF COMPUTER KEYBOARDS

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OBJECTIVES: Numerous studies have found that keyboards (KB) may play a role in the spread of healthcare associated pathogens (HCAP). A large cross-sectional point prevalence survey using solid agar and broth enrichment was undertaken in 4 urban hospitals to evaluate the degree of contamination of conventional keyboards.

METHODS: Using both selective agar with quantitative culture (QC) and broth enrichment (BE), 230 KBs were cultured from 2 medical, 2 surgical and 2 ICUs from 3 adult and 1 children's hospital. The same 10 keyboards were cultured 3 times using a standardized technique: 1) randomly, 2) 2 hrs post random collection and 3) post CaviWipe™ cleaning by designated personnel using a specific protocol. Differences in rates by wards were assessed by χ^2 with continuity correction.

RESULTS: On random culture, combining QC and BE, >99% of KBs were + for commensals and 58.3% (134/230) were + for at least 1 HCAP including: *S. aureus*, 16.5% (1/3 were methicillin resistant strains) and at levels of up to 10^2 cfu/ml on QC; enterococci, including vancomycin resistant strains, 29.1%; enteric gram negative bacilli (GNB), 29.6%; non-fermenting GNB, 9.1% and yeast/mould 2.2%. There was no significant difference in the relative frequency of + KB by ward. After cleaning with a CaviWipe™, 35 keyboards were HCAP +, including 3 for *C. difficile*. Using any one of the 3 cultures obtained on the same day as evidence of contamination, 67% (154/230) of KBs were HCAP +. A survey of HC workers found that there was no designated responsibility for KB cleaning or exchange.

CONCLUSIONS: This is the largest KB contamination survey conducted in Canada to date and the findings suggest that conventional KBs are highly contaminated with pathogenic organisms and may be potential vectors of transmission.

G7

COLLATERAL IMPACT OF A *C. DIFFICILLE* OUTBREAK: LESSONS FROM SURGE MANAGEMENT

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INTRODUCTION: This abstract reports the findings of a critical incident review prompted by the case of an elderly patient developing severe hypothermia following a prolonged admission to the ER as a result of the closure of 1 of 2 medical wards in the hospital due to a *C. difficille* outbreak.

METHODS: A chart review of the case was undertaken and unstructured interviews conducted with health care personnel involved in the care of the patient and the patient's family in a root causes analysis. A framework designed for managing surges was used to identify alternative crisis management strategies in future situations.

RESULTS: Process and system issues identified that contributed to the outcome included: 1. A 50% reduction in the bed capacity led to significant delays in transfers to ward beds from both the ER and ICU; 2. Techniques typically used to respond to a surge in demand were not employed; 3. Environmental exposure in the ER to cold can result from a combination of patients sleeping on litters with loose sheets instead of proper beds with linens and the cold air from; 4. It is difficult for family members to communicate with the in-patient physicians managing a patient admitted to the ER; 5. Bed-spacing internal medicine patients to off-service wards increased the demands and workload on house-staff exacerbating their decreased efficiency in work due to the outbreak conditions (all patients in isolation).

DISCUSSION: The impact of decreased system capacity can be understood by considering the equation "Surge impact = $r(d/c)^x$", where d = demand, c = capacity, 'r' = rapidity of onset of the surge event and 'x' = requirement for specialty capabilities. A proportional increase in demand or decrease in capacity will result an equivalent disruption of the 'supply & demand' balance. Most hospitals prepared to manage increases in service demands due to epidemics or disasters, despite having these plans & processes available, they often fail to recognize impact and manage decreased system capacity in the same way. The collateral impacts of this outbreak could likely have been mitigated by establishing effective command & control with an incident management system and focusing on the essential elements of 'staff', 'stuff', 'space', and communication.

G8

NOSOCOMIAL TRANSMISSION OF NEW DELHI METALLO-β-LACTAMASE-1-PRODUCING *KLEBSIELLA PNEUMONIAE* (NDM1-KP) IN A CANADIAN HOSPITAL

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INTRODUCTION: NDM-1 producing *Enterobacteriaceae* in Canadian hospitals are primarily associated with medical tourism or travel to endemic regions. We describe nosocomial transmission of NDM1-Kp in a 1,100-bed tertiary- care hospital.

METHODS: Retrospective chart review of patients colonized/infected with NDM1-Kp to describe the epidemiology and impact of infection prevention and control measures. Rectal swabs from contact patients were screened for NDM1-Kp with KPC CHROMagar. Presence of the NDM-1 enzyme was confirmed by PCR. Clonality was assessed with pulsed-field gel electrophoresis (PFGE) utilizing the restriction enzyme *Xba*1. Restriction fragment length polymorphism (RAPD) analysis of the plasmids was done with *Bgl*II.

RESULTS: Between January to October 2011, NDM1-Kp was identified in 8 patients: 2 with bacteremia, 2 with UTI, and 4 colonized. PFGE revealed 2 distinct clones, with each clone possessing a unique plasmid. The index case with clone 1 had been previously hospitalized in India. Nosocomial acquisition of clone 1 (2 wardmates and 3 roommates) and clone 2 (1 roommate) occurred. Contact precautions with cohorting were instituted. Active surveillance of contacts with rectal and urine cultures was done days 1, 7, 14 and 21 post-exposure. 3 contacts initially had negative screens but were subsequently positive weeks later. Median time to positivity among contacts was 55 days. No new cases have been identified since October.

CONCLUSION: Nosocomial transmission resulted in 6 patients acquiring NDM1-Kp from 2 patients over several months. Active surveillance extended to 3 weeks post-exposure, in addition to contact precautions and cohorting, was required to prevent transmission.

ROOM: Port Alberni
Session H

H1

CLOSTRIDIUM BOTULINUM TYPE B ASSOCIATED WITH CONSUMPTION OF WATERMELON JELLY

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OBJECTIVE: Foodborne botulism is a rare but potentially lethal paralytic illness. Here we describe the clinical and laboratory findings during the investigation of a unique case of foodborne botulism.

METHODS: A previously healthy adult female presented with symptoms consistent with botulinum intoxication (slurred speech, blurred vision). The patient required respiratory support and received botulinum antitoxin. The BC Public Health Microbiology Reference Laboratory (PHMRL), one of only two laboratories in Canada providing botulism testing, worked with the Vancouver Island Health Authority clinical and public health staff to investigate this case. Serum, fecal and food specimens were tested for presence of *C. botulinum* (culture) and its toxin (mouse neutralization assay.

RESULTS: *C. botulinum* and toxin were not detected in the patient's feces or serum, but *C. botulinum* Type B and toxin were detected in a sample of leftover watermelon jelly. Other food samples tested were negative. Watermelon jelly from the same batch showed water activity and pH values conducive to toxin production by *C. botulinum*.

CONCLUSIONS: The investigation identified an unusual food vehicle, watermelon jelly, as the cause of botulism in this outbreak and highlights collaboration between clinicians, public health, and laboratory staff during case investigations.

H2

THE EVER CHANGING ANTIMICROBIAL RESISTANCE LANDSCAPE IN CANADA: RESULTS FROM THE CANADIAN INTEGRATED PROGRAM ON ANTIMICROBIAL RESISTANCE SURVEILLANCE (CIPARS) IN CANADA

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OBJECTIVES: To describe and highlight some of the trends in antimicrobial resistance and use observed in the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) program between 2003 and 2009.

METHODS: Human *Salmonella* isolates were submitted by Provincial Public Health Laboratories since 2003. Chicken caecal or retail meat samples were collected from 2003 to 2009 across Canada. MIC values were determined by broth microdilution using the Sensititre System following the Clinical and Laboratory Standards Institute (CLSI) breakpoints. Human antimicrobial use data was obtained from IMS Brogan (2000-2009). Animal antimicrobial use is obtained from the Canadian Animal Health Institute and from sentinel farm surveillance. MDR was defined as resistance to 3 or more classes of antimicrobials.

RESULTS: Ceftiofur resistance rose in abattoir chicken *E. coli* (17% to 29%, p=0.01) and *S.* Heidelberg isolates (6% to 23%, p<0.0001) between 2003 and 2009. Ciprofloxacin resistance increase has been observed in *Campylobacter* isolated from retail chicken in BC (4% to 29%, p=0.006) and in Saskatchewan (6% to 15%, p=0.14). MDR has decreased in *Salmonella* human infections from 14.6% (2003) to 7% (2009) (p<0.001) primarily due to significant decreases in *S.* Typhimurium DT104 (p<0.0001). Analysis of antimicrobial use data has shown that 198,000kg of antimicrobials are dispensed by community pharmacies for human use, while 1.6 million kg of antimicrobials are used in the animal sector.

CONCLUSIONS: High levels of ceftiofur resistance continue to emerge in human and animal strains after the re-introduction of ceftiofur use in poultry. High variability in antimicrobial use and costs exists between provinces and between the human and animal sectors.

H3

THE NEW CARB ON THE BLOCK: THE FIRST OXA-48 CARBAPENEMASE CONTAINING ISOLATES CONFIRMED BY THE NATIONAL MICROBIOLOGY LABORATORY IN 2011

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OBJECTIVE: The OXA-48 carbapenemase is of growing concern due to its difficult detection. We describe the first isolates with OXA-48 confirmed by the National Microbiology Laboratory (NML) in 2011.

METHODS: Isolates were submitted to the NML for confirmation of a potential carbapenemase in 2011. A clinical questionnaire was distributed once an OXA-48 was confirmed. Susceptibilities were determined using Vitek2 AST-GN25. Multiplex PCR for the detecting NDM, KPC, IMP, VIM, GES, and OXA-48 carbapenemase genes was conducted. Strain typing was done using pulsed-field gel electrophoresis and multilocus sequence typing.

RESULTS: Four isolates submitted to the NML in 2011 were confirmed as harbouring OXA-48 (3 *K. pneumoniae* and 1 *E.coli*). They originated from QC (n=2), BC (n=1) and ON (n=1). Three completed clinical questionnaires indicated that one isolate caused a urinary tract infection, while two rectal swab isolates were deemed colonizers. All three patients had travelled in the previous year to India, Lebanon or Dubai, where two sought medical attention in that country. By Vitek2, only two isolates showed resistance to ertapenem, and only one isolate showed resistance to meropenem and imipenem. Sequence typing identified the 3 *K. pneumoniae* as ST395, ST147, ST831 (3 unique PFGE patterns) and *E. coli* ST38. A limitation to this report is that clinical laboratories that use automated methods to screen for carbapenem resistance may not have detected all possible OXA-48 strains.

CONCLUSIONS: We describe the first four patients in Canada with Enterobacteriaceae harbouring OXA-48. Laboratories and clinicians should be aware of the difficulties in detecting OXA-48 and its association with travel to endemic countries.

H4

THE EMERGENCE OF CIPROFLOXACIN-RESISTANT *SALMONELLA ENTERICA* SEROVAR KENTUCKY IN CANADA

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OBJECTIVE: We describe the emergence of ciprofloxacin-resistant (Cip-R) *Salmonella enterica* serovar Kentucky from human cases in Canada from 2003-2009.

METHODS: From 2003 to 2009, provincial public health laboratories submitted human clinical isolates as part of the Canadian Integrated Program on Antimicrobial Resistance Surveillance (CIPARS). MIC values were determined by broth microdilution using the Sensititre™. PFGE was performed on all Cip-R isolates and MLST was conducted on a selected number of isolates. PCR was used to determine the presence of *Salmonella* Genomic Island1 (SGI1) variants.

RESULTS: A total of 76 *S.* Kentucky isolates were identified out of 21,175 non-typhoidal *Salmonella* human isolates submitted for susceptibility testing. *S.* Kentucky comprised 66% (23/35) of all Cip-R isolates identified since 2003. Most Cip-R isolates displayed a core resistance phenotype of ampicillin, gentamicin, sulfonamide and tetracycline resistance (n=18; 78%). PFGE analysis revealed a majority of Cip-R isolates and one multi-drug resistant (MDR) ciprofloxacin susceptible (Cip-S) isolate clustered together with a percent similarity of >80% (pattern A), whereas three other MDR Cip-S resistant isolates did not belong to this cluster (patterns B and C). MLST of isolates of PFGE pattern A were ST198. PFGE pattern A were found to contain SGI1-K, SGI1-Q, and SGI1-P. Novel SGI1 variants were also identified. CIPARS animal and retail meat surveillance has not identified Cip-R *S.* Kentucky.

CONCLUSION: Cip-R ST198 MDR isolates have been described in Europe and Africa. The data strongly suggests Cip-R isolates are a result of travel to countries where resistance is endemic. Travel history should be collected in prospective studies to determine this hypothesis.

H5

SURVEILLANCE OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE AND *ACINETOBACTER BAUMANNII* IN CANADA: RESULTS OF THE CNISP 2011

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OBJECTIVE: Carbapenem-resistance complicates treatment of Gram-negative infections and is a growing concern in Canada. This report describes the results of the Canadian Nosocomial Infection Surveillance Program (CNISP) for carbapenem resistant isolates.

METHODS: From September 2010 to August 31, 2011, carbapenem-resistant Enterobacteriaceae and *A. baumannii* isolates were submitted to the National Microbiology Laboratory. A detailed patient questionnaire was administered collecting risk factors and outcome data. PCR was used to identify carbapenemase-producing isolates. Antimicrobial susceptibility was conducted using Vitek2. PFGE was conducted to determine strain relatedness.

RESULTS: A total of 141 isolates meeting the definition were identified from 11 different hospital sites. Seventy-eight cases were infections, 32 were colonizations, 19 were unknown, and the remainders were not reported. Of the infections, 35% were urinary tract, 27% were skin/soft tissue, and 10% were from blood. Forty four isolates harboured carbapenemases: *Enterobacter* spp. (4 KPC); *Klebsiella* spp. (21 KPC, 8 NDM); *E. coli* (1 GES, 1 KPC); *Serratia* spp. (6 KPC, 1 GES); *Morganella* spp. (1 NDM); *A. baumannii* (1 OXA-23). Several outbreaks were observed based on PFGE analysis including one KPC in Quebec, and two in Ontario involving NDM and KPC. Previous travel history was linked to 10 cases. Two NDM cases sought medical attention in India and one *A. baumannii* case was from a military source serving overseas.

CONCLUSION: The number of carbapenemase producing isolates identified is of concern especially in Enterobacteriaceae. Hospitals should be aware of this emerging issue and develop plans to deal with this emerging threat.

H6

CHARACTERIZATION OF VANCOMYCIN-RESISTANT ENTEROCOCCI (VRE) BACTEREMIA ISOLATES: CNISP 1999–2009

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OBJECTIVE: Vancomycin-resistant enterococci (VRE) can be associated with bloodstream infections. In this study we characterized VRE identified from blood cultures of patients over an 11-year period.

METHODS: From 1999 to 2009, enterococci were collected from across Canada in accordance with the Canadian Nosocomial Infection Surveillance Program VRE surveillance protocol. MICs were determined using broth microdilution using the automated Sensititre System. PCR was used to identify *vanA* and *vanB*. Genetic relatedness was examined using multilocus sequence typing (MLST).

RESULTS: Almost an equal number of bloodstream isolates were submitted from 2007-09 (n=42) than all previous years combined (n=43). All isolates were identified as *E. faecium*. The mean patient age was 68.3 yrs (range 1-95) and 49 isolates (60.5%) were from males. The majority of bloodstream isolates were from Western Canada (60.5%), followed by Central Canada (37.0%) and Eastern Canada (2.5%). Susceptibilities were as follows: 100% for daptomycin, linezolid, tigecycline and chloramphenicol; quinupristin-dalfopristin (96.2%); high-level gentamicin (71.6%); tetracycline (50.6%); high-level streptomycin (44.4%); rifampin (21.0%); nitrofurantoin (12.3%); clindamycin (8.6%), ampicillin (2.5%), ciprofloxacin and moxifloxacin (2.5%); and levofloxacin (1.2%). *vanA* contributed to vancomycin resistance in 90.1% of isolates and *vanB* in 9.9%. Over 11 years, 19 MLST types were observed. Beginning in 2006 there was a shift in ST types from ST16, ST154, ST17 to ST412, ST203, ST584 and other sporadic types.

CONCLUSIONS: The increase in blood infections observed since 2007 seems to coincide with the shift of MLST types. All VRE isolates remained susceptible to daptomycin, linezolid, tigecycline.

H7

ANTIMICROBIAL RESISTANCE IN PATHOGENS ISOLATED FROM CANADIAN HOSPITAL CLINICS, EMERGENCY ROOMS, MEDICAL/SURGICAL WARDS AND INTENSIVE CARE UNITS: RESULTS OF THE CANWARD 2011 STUDY

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OBJECTIVES: The CANWARD study assesses the pathogens causing infections in patients affiliated with Canadian hospitals and evaluates the prevalence of antimicrobial resistance in these isolates.

METHODS: 15 centres across Canada submitted pathogens causing infections from patients attending clinics (C), emergency rooms (ER), medical and surgical wards (W) and intensive care units (ICU) in 2011. Susceptibility testing was performed by CLSI microdilution methods.

RESULTS: 3,554 isolates were collected: 41.1%, 38.6%, 10.5%, and 9.8% from blood, respiratory, urine and wound/IV site specimens, respectively. Isolates were from patients on W 35.9%, ER 23.6%, ICU 22.6%, and C 18.0%. The most common pathogens were: *E. coli* 18.2%, *S. aureus* (MSSA) 18.2%, *P. aeruginosa* 9.3%, *K. pneumoniae* 6.4%, and *S. pneumoniae* 5.4%. The overall prevalence of MRSA, VRE, and ESBL- *E. coli* was: 19.2%, 7.8%, and 7.1%, respectively. Resistance rates (RR) for *E. coli* were: 0% meropenem (MER), 0.3% ertapenem (ERT), 0% tigecycline (TGC), 1.1% piperacillin/tazobactam (PTZ), 9.2% ceftriaxone (CTR), 11.9% gentamicin (GEN), 26.9% ciprofloxacin (CIP) and 29.3% trimethoprim / sulfamethoxazole (SXT). RR for *P. aeruginosa* were: 3.0% colistin (COL), 6.7% PTZ, 7.3% GEN, 9.7% MER, and 13.3% CIP. RR for MRSA were: 0% vancomycin (VAN), linezolid (LZD), and daptomycin (DAP), 1.9% SXT, 37.3% clindamycin, 84.7% clarithromycin, and 86.4% CIP.

CONCLUSIONS: RR for *E. coli* were lowest with MER, ERT, TGC and PTZ, while RR for *P. aeruginosa* were lowest with COL, PTZ, and MER. For MRSA, no resistance occurred with VAN, LZD, or DAP.

H8

ANTIFUNGAL SUSCEPTIBILITY OF INVASIVE *CANDIDA* ISOLATES FROM CANADIAN HOSPITALS: RESULTS OF THE CANWARD 2011 STUDY

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BACKGROUND: CANWARD is an ongoing national surveillance study that assesses pathogens causing infections in patients attending Canadian hospitals, as well as determines the prevalence of antimicrobial resistance in these isolates. Here we present the antifungal susceptibility data for candidemia isolates collected in 2011.

METHODS: *Candida* species isolated from bloodstream infections were collected from 12 participating medical centres during the 2011 study period. Antifungal susceptibility testing and interpretation was performed as per CLSI M27-S4 broth microdilution method and the recently modified breakpoints for fluconazole (FLUC), voriconazole (VORI), caspofungin (CASP), and micafungin (MICA).

RESULTS: Of the 257 *Candida* spp. collected, *C. albicans* (CA) was predominant (56.4%), followed by *C. glabrata* (CG, 15.2%) and *C. parapsilosis* (CP, 8.9%). MIC90 and susceptibility (S) values are below. Notably, CG was 56% S to CASP while MICA was 97% S, similar to the CANWARD 2010 results.

		MIC_{90} (%S)						
	No.	AMB	5FC	ITRA	FLUC	VORI	CASP	MICA
CA	145	0.5 (-)	0.5 (-)	0.06 (-)	0.25 (98)	0.03 (99)	0.06 (99)	≤0.015 (100)
CG	39	0.5 (-)	0.12 (-)	0.5 (-)	8 (100*)	0.5 (97)	0.5 (56)	≤0.015 (97)
CP	23	1 (-)	0.12 (-)	0.06 (-)	0.5 (100)	0.03 (100)	0.5 (100)	0.5 (100)

**, susceptible dose-dependent; AMB, amphotericin B; 5FC, 5-fluorocytosine; ITRA, itraconazole*

CONCLUSION: Canadian surveillance of antifungal resistance in invasive *Candida* infections will be an important long-term objective of the CANWARD program. The validation of revised, species-specific interpretive breakpoints should facilitate more accurate detection of changing resistance patterns.

ORAL PRESENTATIONS
Saturday May 5

ROOM: Junior Ballroom A
Session I

I1

CONTINUOUS MONITORING OF THE PERFORMANCE OF RUBELLA IGG AND HEPATITIS B SURFACE ANTIBODY ASSAYS USING REFERENCE CONTROLS: RESULTS OF A MULTICENTRE TRIAL

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OBJECTIVE: A multicentre trial was conducted to assess the value of using external reference controls in rubella IgG and hepatitis B surface antibody (anti-HBs) assays in determining their performance across laboratories in Canada.

METHODS: Seven laboratories using different test systems participated in the trial. Standardized reference controls for the assays were obtained from a single source and distributed for inclusion in each run. A total of 1095 rubella IgG and 1195 anti-HBs assays were performed utilizing several different lots of the respective test kits; all runs were reported valid per standard protocol. Results of the reference controls were plotted on Levey-Jennings charts and analyzed by multirule quality control (MQC) scheme as well as by a single 3 standard deviation (SD) rule.

RESULTS: With rubella IgG, all assays were 'in-control' with only one of the seven laboratories. Among the rest, the rate of 'out-of-control' assays ranged from 5.6% to 10% with an outlier at 20.3% by MQC, and 1.1% to 5.6% with an outlier at 13.4% by 3 SD rule. With anti-HBs, all assays were 'in-control' with two laboratories. Among the rest, the rate of 'out-of-control' assays ranged from 3.3% to 7.9%, with an outlier at 19.8% by MQC, and 0% to 3.3%, with an outlier at 10.5% by 3 SD rule.

CONCLUSION: Through the continuous monitoring of assay performance using standardized reference controls and quality control rules, our trial detected intra and inter-laboratory, assay system and kit lot variations in rubella IgG and anti-HBs assays. This has implications in diagnostic screening and underscores the value of using such an approach for quality control and as part of good clinical laboratory practice.

I2

HUMAN CYTOMEGALOVIRUS ANTIVIRAL DRUG RESISTANCE GENOTYPING IS A VALUABLE DIAGNOSTIC TOOL

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OBJECTIVES: Cytomegalovirus (CMV) is a herpesvirus that strikes immunocompromised patients as either a primary or recurring infection. Drug resistance to one or more of the three antivirals available to treat CMV may develop during treatment. Resistance is attributed to mutations in either the DNA polymerase gene (UL54) or protein kinase (UL97). We report the result of genotyping of 174 CMV specimens in Canada between 2004 and 2011.

METHODS: Plasma or whole blood specimens sent mostly from the provinces of AB and ON, but also from BC, SK, MB, QC, NB and NS were genotyped by 3 PCR assays, designed to amplify the entire UL54 and UL97. The sequences were analyzed using a public on-line database of all published polymorphisms and resistant mutations.

RESULTS: Approximately 40% of all samples tested carried a mutation that is predicted to cause antiviral drug resistance. Nearly 80% of the resistant mutations were found in the UL97 gene, 7% were located in the UL54 gene and the remaining 13% of samples had resistant mutations in both genes. The five most frequent UL97 mutations account for over 80% of all resistance cases whereas the mutant frequency is more evenly distributed in the UL54 gene. Polymorphisms were more common in the UL54 gene than in UL97. Several likely polymorphisms in both genes were identified that require phenotypic analysis for confirmation.

CONCLUSIONS: Mutations detected in this set of specimens are similar in distribution to what was previously reported in the literature. Genotypic testing of CMV resistance is the only practical method for routine testing of CMV resistance, but it is not suitable for the discovery of novel resistant mutations.

I3

DEVELOPMENT AND EVALUATION OF A MULTIPLEX PCR ASSAY FOR SIMULTANEOUS DETECTION OF ALL ENTEROVIRUSES AND PARECHOVIRUSES

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OBJECTIVE: Traditional methods for the diagnosis of enteroviruses (EV) and human parechoviruses (HPeV) have now largely been replaced by nucleic acid amplification tests, mainly using separate real-time RT-PCR assays. In the current study, we have developed an assay for simultaneous detection of EV and HPeV for use in diagnostic virology laboratories.

METHODS: A multiplex real-time PCR assay using MGB probes targeting the 5'NCR was designed for the detection of all EVs and HPeVs on the TaqMan platform. These assays were validated for sensitivity, specificity and accuracy. A total of 373 samples including CSF, plasma, stool, respiratory and swabs that had previously tested positive or negative for EVs from children and adults ranging in age from newborn to 97 years were retrospectively tested for EV and HPeV by the multiplex assay.

RESULTS: The sensitivity for the multiplex assay, using in-vitro transcribed RNA, ranged from 5 to 60 copies for representative enteroviruses from different species and HPeVs 1 and 3. The sensitivity was comparable between the singleplex and multiplex assays. The assays were 100% specific when tested with high copy templates of other viruses and bacteria that manifest similar symptoms, in particular rhinoviruses belonging to groups A, B and C. Using 373 samples that had been previously tested for enteroviruses, sensitivity, specificity, PPV and NPVs were 99.31%, 97.79%, 96.62% and 99.55% respectively for the multiplex assay. The Ct values for all discordant samples ranged from 35.63 to 41.15 indicating a low viral load. Human parechovirus 6 was detected in the throat swab from a one-year-old patient.

CONCLUSIONS: We have developed and validated a multiplex real-time RT-PCR assay for the detection of all EVs and HPeVs.

I4

IN-VIVO REASSORTMENT OF INFLUENZA A (H1N1)PDM09 AND SEASONAL H3N2

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OBJECTIVES: We report co-infection with influenza A (H1N1)pdm09 (pdm09) and seasonal H3N2 (sH3N2) followed by in-vivo reassortment detected in 2 nasopharyngeal swabs (NPS) from the same child.

METHODS: A 16 month-old child with influenza-like symptoms and diarrhea was admitted to hospital for rehydration on January 24, 2011. Two NPS were forwarded for influenza PCR. Total nucleic acid was extracted and tested for specific targets including matrix, sH3, sN2, pH1, pN1, sH1 and sN1 by real-time RT-PCR. Specimen was cultured in RMK cells and whole genome sequencing (WGS) conducted.

RESULTS: Both specimens were positive for sH3N2 and pdm09. Co-infection was further confirmed by partial sequencing, which detected M, NS, H1 and N1 genes of pdm09 together with sH3 and sN2 genes in both primary specimens. WGS analysis of culture supernatant suggested reassortment of H3 and N2 genes of sH3N2 with pdm09 internal genes (PB2, PB1, PA, NS, NP and M). Notably, pH1 and pN1 genes were absent in culture. Plaque assay and purification followed by WGS confirmed reassortant in primary specimen.

CONCLUSIONS: Human co-infection with pdm09 and H3N2 resulted in a reassortant virus containing sH3 and sN2 together with the internal genes of pdm09. This could result in emergence of a new subtype with high transmissibility, including pandemic potential. Laboratory-based surveillance including WGS is critical, especially when different subtypes of influenza viruses are co-circulating to allow early detection of reassortants. In-vivo experiments should be conducted to evaluate the fitness, pathogenicity and transmissibility of this reassortant.

ROOM: Junior Ballroom B
Session J

J1

CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATES FROM SUSPECTED FOOD POISONING CASES IN SASKATCHEWAN, 2001–2011

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OBJECTIVE: *Staphylococcus aureus* is rarely isolated from food-poisoning cases in Canada. The goal of this study was to characterize isolates of *S. aureus* recovered from specimens submitted to the Saskatchewan Disease Control Laboratory from suspected food-poisoning outbreaks, during the period from 2001-2011.

METHODS: *S. aureus* isolates from stools (n = 57) and food (n = 2) were typed using PFGE and *spa* typing. Toxin genes were detected by PCR and *in vitro* enterotoxin production was determined using a qualitative enzyme linked fluorescent assay.

RESULTS: Multiple isolates were recovered from 5 suspected food-poisoning outbreaks. Only one outbreak was confirmed by the isolation of *S. aureus* from stool and food samples. All 7 isolates from this outbreak shared the same *spa* type (t026) and PFGE pattern, as well as the same toxin gene profile. A second cluster was regarded as a presumptive *S. aureus* outbreak because four *S. aureus* isolates shared the same PFGE pattern and toxin gene profile, and were all non-typable using *spa*-typing, but no food

isolates were recovered. Methicillin-resistant *S. aureus* comprised 42% of non-outbreak isolates (n=39), of which 88% (21) were of the CMRSA2 cluster. All of the isolates tested possessed genes for at least one enterotoxin, although most possessed several enterotoxin genes. Eighty-three percent of isolates tested produced enterotoxin(s) *in vitro*.

CONCLUSIONS: The value of routine toxin gene profiling for food-poisoning isolates is questionable. An increasing number of isolates of MRSA were recovered from elderly patients with gastroenteritis. The significance of these isolates as a cause of enteric symptoms is questionable as CMRSA2 is commonly associated with nosocomial colonization.

J2

INVESTIGATION OF SAPOVIRUS GASTROENTERITIS OUTBREAKS IN BRITISH COLUMBIA USING REAL TIME RT-PCR, OCT 2007 TO DEC 2011

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OBJECTIVE: Sapovirus is a member of the *Caliciviridae* family and like norovirus is a gastroenteritis agent in humans. Sporadic outbreaks of sapovirus have been reported worldwide, though at a far lower rate as compared to norovirus. A study was performed by BC Public Health Microbiology and Reference Laboratory (PHMRL) for the period from Oct 2007 through Dec 2011 to determine the contribution of sapovirus to gastroenteritis outbreaks in BC.

METHODS: Viral RNA was extracted by Biomerieux EasyMag from archived stool or vomitus samples from gastroenteritis outbreaks with no previously identified etiology. Sapovirus Real-Time RT-PCR (Oka et al, 2006) was performed on extracted RNA.

RESULTS: Sapovirus was detected in 12.7% of outbreaks with no previous causative agent. Overall, sapovirus was found to be the etiological agent in 2.9% of gastroenteritis outbreaks. Annual variation in sapovirus outbreaks was observed over the study period; in 2008–2009, sapovirus accounted for 5.9% of all reported gastroenteritis outbreaks. Interestingly since July of 2009, majority of sapovirus outbreaks occurred in day care settings (87.5%).

CONCLUSIONS: In BC, sapovirus is the next leading cause of viral gastroenteritis outbreaks after norovirus. These results suggest the inclusion of sapovirus RT-PCR for investigation of outbreaks of unknown etiologies.

J3

EVALUATION AND IMPLEMENTATION OF A COMPARATIVE GENOMIC FINGERPRINTING ASSAY AS AN INVESTIGATIVE TOOL FOR OUTBREAKS OF *CAMPYLOBACTER JEJUNI* IN BRITISH COLUMBIA

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OBJECTIVE: Campylobacteriosis is the most common cause of bacterial gastroenteritis worldwide. Despite this fact, there are no rapid or cost-effective tools for investigating clusters of campylobacteriosis. To address this issue, Comparative Genomic Fingerprinting (CGF), a novel *Campylobacter* subtyping tool was recently developed and published by the Public Health Agency of Canada (PHAC). The objective of this study was to optimize and evaluate the CGF assay for use at the BC Public Health Microbiology and Reference Laboratory (BCPHMRL), and ultimately assess its use for clustering of cases during outbreak investigations of *Campylobacter jejuni* in BC.

METHODS: The CGF25 assay was optimized and evaluated for use at the BCPHMRL, with minor modifications to the published method. 86 clinical isolates from both outbreak and sporadic illnesses in BC were used for evaluation. 52 isolates were outbreak-associated, of which 27 were either lab- and/or epi-confirmed cases. An additional 34 sporadic isolates were also typed. A subset of isolates were tested in 2 different laboratories (n=27).

RESULTS: Results between labs were identical, and the assay was found to be precise, accurate, rugged, and robust. All confirmed outbreak isolates (n=27) linked via epidemiological and/or laboratory confirmation (MLST, PFGE) were also linked via CGF25. Some previously unconfirmed isolates were also grouped into clusters.

CONCLUSIONS: The CGF25 assay was determined to be a useful and cost-effective tool for clustering *C. jejuni* isolates. The assay was therefore implemented at the BCPHMRL for use during outbreak investigations of *C. jejuni*.

J4

GENOME SEQUENCING AND ANNOTATION OF PSEUDOMONAS AERUGINOSA 96: AN OUTBREAK ISOLATE THAT HARBOURS A 500-KB, SELF-TRANSMISSIBLE CARBAPENEM RESISTANCE (IMP-9) PLASMID

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OBJECTIVES: *P. aeruginosa* 96 (PA96) was isolated from Guangzhou multicentre surveillance in 2000, China. Whole genome sequencing has facilitated analysis of the outbreak strain genome and its 500 kb, IncP-2 plasmid, pOZ176, which encodes carbapenem resistance and two class 1 integrons.

METHODS: The Roche GS-FLX/454 system was used for sequencing and draft assembly of PA96 genome and plasmid DNA. A combination of bioinformatic and PCR-based approaches was used for analysis, annotation and finishing of the genome.

RESULTS: pOZ176 had a length of 500 kb and contained >600 complete coding regions with average G+C of 75% and 92% of ORFs encoding hypothetical proteins. It contains two integrons: one with *aacA4-bla*$_{IMP-9}$*-aacA4* cassettes linked to a complete Tn*402*-like *tniABQR* transposition module; and one with *aacA4-catB8a-bla*$_{OXA-10}$ cassettes and a conventional *sul1*-type 3'-conserved sequence. Large regions of the plasmid are weakly similar to pQBR103 from *P. fluorescens*. The plasmid also carries tellurite and mercury resistance operons, *pil* genes encoding a type IV secretion system, two copies of a bleomycin resistance gene, a major facilitator superfamily efflux system, a chemotaxis operon, and several insertion sequences, phage-like integrases and transposons. The core genome is highly similar to PAO1, PA14 and LESB58 with its genomic island contents are partially shared with PAO1 and PA14. PA96 is serotype O6 and has an *exoS* gene.

CONCLUSIONS: The study revealed that PA96 has gone through a series of horizontal gene transfer events, particularly in pOZ176, one of the largest IncP2 plasmids ever studied. It is first time that a *bla*$_{IMP}$ gene was found to be linked to Tn*402*-Integron.

ROOM: Port McNeil
Session K

K1

HPV FOCAL: ROUND ONE RESULTS OF A CERVICAL CANCER SCREENING TRIAL

M KRAJDEN[1,3], D VAN NIEKERK[2,3], K CEBALLOS[2,3], T EHLEN[2,3], R MARTIN[3], S PEACOCK[2,3], L SMITH[2], D COOK[1,2], G STUART[3], E FRANCO[4], A COLDMAN[2,3], G OGILVIE[1,3]

[1]BC Centre for Disease Control; [2]BC Cancer Agency; [3]University of British Columbia, Vancouver, BC; [4]McGill University, Montréal, QC

OBJECTIVES: HPV FOCAL (ISRCTN79347302) is the first North American randomized controlled trial to compare the efficacy of high-risk (hr) HPV DNA testing vs. liquid based cytology (LBC) for cervical cancer screening. Results from Round 1 of screening and 12-mo follow-up are presented.

METHODS: Women aged 25 to 65 are assigned to 1 of 2 study arms: Control: LBC screening with reflex to hrHPV for ≥ASC-US; LBC negative are re-screened at 2 and 4 yrs. Combined Safety and Intervention (HPV): hrHPV screening with reflex to LBC for hrHPV+ women; hrHPV$^-$ women are re-screened at either 2 yrs (Safety arm) or 4 yrs (Intervention arm). The outcome measure is ≥CIN3 at the exit screen in the Control and Intervention arms.

RESULTS: 15,589 of 28,000 subjects have completed Round 1 screening. Overall referral to colposcopy was 33.0/1,000 screened in the Control and 52.0/1,000 in the HPV arms. Overall, ≥CIN2 and ≥CIN3 rates were 11.0/1,000 and 4.1/1,000 in the Control vs. 14.7/1,000 and 6.7/1,000 in the HPV arms. In the Control arm, ASC-US+/HPV$^-$ women who had 12 mo follow-up LBC had no cases of ≥CIN2 detected [positive predictive value (PPV)=0%]. In contrast, among HPV$^+$/LBC$^-$ women who remained HPV$^+$ at 12-mo follow-up, the ≥CIN2 rate was 236.4/1,000 (PPV=23.6%).

CONCLUSIONS: At Round 1 of screening, overall colposcopy referral rates were higher in the HPV arms than in the Control arm. At 12-mo follow-up for Round 1 screening, the PPV in the HPV arm was substantially higher than for women in Control arm (0% vs. 23.6%), indicating the greater risk associated with persistent hrHPV infection for precancerous lesions.

K2

SELECTIVE CHAGAS BLOOD DONOR TESTING AND RESULTS OF A CHAGAS SEROPREVALENCE STUDY

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OBJECTIVE: Canadian Blood Services began selective testing for Chagas Disease in May 2010. In September 2010 a two phase Chagas Study was launched to investigate the efficacy of this testing strategy.

METHODS: Donors who answer yes to any of the Chagas risk questions on the donor questionnaire are tested for Chagas Disease using Abbot PRISM Chagas, with confirmatory testing performed by the National Reference Lab for Parasitology using immunoblot and PCR. For the Study, donors across the country answering no to all the risk questions were randomly selected for testing. Phase I included over 60,000 donors from Ontario, Manitoba, Alberta and B.C. Phase II is focused on Manitoba with a target of 30,000 donors. All repeat reactive donors are indefinitely deferred from donation and lookbacks are performed on all previous donations from all donors who confirm positive. Positive donors are interviewed to determine specific risk factors.

RESULTS:

RESULTS OF CHAGAS SELECTIVE TESTING AND CHAGAS STUDY PHASES I AND II (TO DATE)

Testing Method	# Tested	Negative	Repeat Reactive	Confirmed Negative	Confirmed Positive
Selective Testing	23,850	23,829	21	6	13
Chagas Study Phase I	62,117	62,076	41	40	1
Chagas Study Phase II	8,596	8,589	7	6	0

There were no infected recipients identified through lookback on 80 living recipients of 209 transfused products from the infected donors. Interviews revealed that the majority of donors were born in an endemic country. Those born in Canada spent time visiting relatives in rural areas, or performing missionary work in these countries.

CONCLUSIONS: The selective donor testing strategy for Chagas Disease has identified 13 infected donors while one infected donor who would not have been tested, was picked up by the Study. No cases of transfusion transmission have been confirmed to date.

K3

EPIDEMIOLOGY, CO-INFECTION AND LABORATORY TESTING OF *CHLAMYDIA TRACHOMATIS* AND *NEISSERIA GONORRHOEAE* IN SASKATCHEWAN

CE GARDHOUSE[1,2], PN LEVETT[2], GB HORSMAN[2]

[1]National Microbiology Laboratory, Winnipeg, MB; [2]Saskatchewan Disease Control Laboratory, Regina, SK

OBJECTIVES: This study compared demographics of patients who tested positive for *Chlamydia trachomatis* (CT), positive for *Neisseria gonorrhoeae* (NG), positive for both, and negative for both, and investigated the CT and NG testing patterns in the province, particularly in groups that submit multiple specimens.

METHODS: Test results and patient information from May 2009 to January 2011 were retrieved from the Laboratory Information Management System (Labware) at the Saskatchewan Disease Control Laboratory and analyzed using Microsoft Excel, SAS-EG, and GraphPad Prism software.

RESULTS: 104,624 specimens were tested for CT and NG in Saskatchewan. Positive rates for CT, NG, and CT-NG co-infection were 7.9%, 0.7%, and 0.7%, respectively. Females submitted 2.7-times as many specimens as males, however positive rates were higher in males. The average age and sex ratio of NG-positives were different than CT-positives and CT-NT co-infections. There were 19,161 patients who were tested more than once in the time period studied. Of those patients who were tested more than once, the number of tests ranged from 2 to 17. The average interval between tests was 157.6 days. Approximately 1.2% of all results occurred within 7 days of each other and 2.4% occurred within 15 days.

CONCLUSIONS: While the number of specimens tested has increased steadily for the past decade, positive rates have remained relatively constant. Repeat testing needs to be taken into consideration when interpreting laboratory-based surveillance results since they affect both positive rates and incidence rates for the province. Further study is needed to determine why patients submit multiple specimens and see multiple doctors within a short time period.

K4

DISTRIBUTION OF INVASIVE PNEUMOCOCCAL SEROTYPES IN CANADA: 2010–2011

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OBJECTIVE: With the introduction of the 13-valent pneumococcal conjugate vaccine (PCV13), changes in the distribution of invasive *Streptococcus pneumoniae* serotypes are expected. This study presents the current distribution of invasive pneumococcal serotypes identified in Canada between 2010 and 2011.

METHODS: This study includes 2335 cultures of invasive *S. pneumoniae* (IPD) submitted to the NML between April 1, 2010 and September 30, 2011. Serotyping was conducted using antisera from the Statens Serum Institute using established methods.
RESULTS: Serotypes 3, 7F, 11A, 12F, 19A and 22F represented half (n=1272) of all IPD isolates. A dramatic decrease was observed in the combined proportions of PCV13 serotypes 3, 7F and 19A among child isolates from 48% (36/75) during the 2nd quarter of 2010 to 16% (5/32) during the 3rd quarter of 2011. Increases of non-PCV13 serotypes 11A, 12F and 22F in this age group has been observed from 7% (5/75) to 25% (8/32) during this interval.
CONCLUSIONS: The current distribution of *S. pneumoniae* serotypes in Canada demonstrates that PCV13 has been effective in reducing IPD attributed to PCV13 serotypes. Continued monitoring of IPD serotypes is important to identify emerging replacement serotypes and to thoroughly assess the effectiveness of PCV13 over time.

ROOM: Port Alberni
Session L

L1
FIFTEEN YEARS OF ACUTE CHILDHOOD ENCEPHALITIS: HAVE WE MADE ANY PROGRESS?

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BACKGROUND: The identification of cause in acute childhood encephalitis (ACE) remains sub-optimal. In many series a specific cause is found in fewer than 40%. The purpose of this report is to describe 15 years of prospective encephalitis investigation (Encephalitis Registry) at SickKids, Toronto.
METHODS: All immunocompetent children ≥4 weeks of age with ACE admitted Jan 1996 to Jan 2011 underwent extensive microbiological investigation for evidence of infection with putative viral/bacterial pathogens. Encephalitis definition: encephalopathy plus ≥2 of: fever (≥38.0°C); seizure(s); focal neurologic deficits; abnormal CSF; EEG/neuroimaging compatible with ACE. Probable cause defined by: (a) detection in CSF or; (b) detection in non-sterile site or reactive serology (excluding *M. pneumoniae*) without evidence of infection with other pathogens.
RESULTS: 309 children were included. Mean age was 7.2±4.9 years; 52% were male. Evidence of infection found in 204 (66%); probable cause assigned to 138 (45%). Implicated pathogens include *M. pneumoniae* (n=22); HSV-1/2 (n=19); EBV (n=19); VZV (n=17; chickenpox = probable); HHV-7 (n=15); influenza A/B (n=14); HHV-6 (n=12); parainfluenza viruses 1-3 (n=9); enteroviruses (n=5); adenoviruses (n=3); *B. henselae* (n=3). No arboviral encephalitides occurred. There were 6 deaths; 49% of survivors had sequelae including impaired cognition (36%), focal deficits (18%) and seizures (17%).
CONCLUSIONS: Our standardized investigative approach to ACE enhanced diagnostic yield, though a probable cause was identified in only 45% of cases. This suggests that new strategies are needed to better elucidate the etiology of ACE. In assigning cause, strength of microbiologic evidence should be considered.

L2
IMPACT OF THE USE OF AN ACUTE BACTERIAL MENINGITIS ALGORITHM (ABMA) FOR EMERGENCY ROOM PHYSICIANS (ERP) AS A QUALITY IMPROVEMENT TOOL TO IMPROVE ADMINISTRATION OF DEXAMETHASONE (DM) PRIOR TO ANTIMICROBIAL THERAPY (AT)

D SABUDA[1], B DALTON[1,2], T RICH[1,2], R LALL[1,2], K ZARNKE[1,2], D GREGSON[1,2], D HOLTON[1,2], M LOUIE[1,2], M MAH[1,2], A PATTULLO[1,2], K LAUPLAND[1,2], T LOUIE[1,2], T RUMBLES[1], J CONLY[1,2]
[1]Alberta Health Services - Calgary; [2]University of Calgary, Calgary, AB

OBJECTIVES: An ABMA was introduced as a QI tool to improve administration of DM prior to AT in cases of suspected ABM. A before-after study was conducted examining the outcomes for 6 years before and 3 years after its introduction.
METHODS: An ABMA was developed for adult patients using a multidisciplinary, iterative approach and launched in 2008 across our health region. A retrospective chart based before-after study was performed using standard ICD-10 codes for ABM. Demographic data, presentation, diagnostic work-up, order of events of work-up, and timing of administration of DM and AT were entered into Excel (Microsoft Corp, 2003). The analysis focused on the outcomes of DM prior to receipt of AT and the timing of the latter using the IDSA 2004 Guideline as the gold standard. Levels of diagnostic workup were analyzed according to 3 levels of findings: clinical presentation, CSF findings, and microbiologic findings. Differences between groups were analysed using χ^2 or Fisher's exact test for categorical variables and student's *t* test for continuous variables.
RESULTS: Of a total of 88 cases, using clinical presentation alone as a criterion, 14/36 (38.8%) vs 11/52 (21.1%) received DM (p=0.07) prior to AT post algorithm, suggesting a trend towards improvement. No significant differences were seen if only microbiologically + cases were analyzed. Administration of DM occurred at a mean of 48.6 vs 49.5 min prior to SAT, , before and after the launch of the ABMA.
CONCLUSIONS: The results suggest the use of an ABMA for ERPs as a tool to improve the administration and timing of DM prior to AB for suspected ABM demonstrated a trend to improvement in the order but not the timeliness of administration. Additional efforts or alternate strategies may be required.

L3
TARGETED SCREENING OF LABORATORY SPECIMENS FOR ACUTE HIV INFECTION AMONG MEN WHO HAVE SEX WITH MEN

D COOK[1], M GILBERT[1,2], M REKART[1,2], M STEINBERG[1,3], M KWAG[1], W MEI[1], R CHOW[1], P TSANG[1], A MAK[1], M KRAJDEN[1,2]
[1]BC Centre for Disease Control; [2]University of British Columbia, Vancouver; [3]Simon Fraser University, Burnaby BC

OBJECTIVE: We assessed the performance of a diagnostic algorithm that includes pooled HIV RNA nucleic acid testing (NAT), targeted at laboratory specimens from clinic sites highly frequented by men who have sex with men (MSM), for the detection of acute HIV infection (AHI).
METHODS: Between Jan 2007 and Jun 2011, specimens submitted for HIV testing from males ≥19 yr from clinic sites with a high proportion of MSM were tested. AHI was identified using a 3rd generation HIV EIA screen test (non-reactive or reactive), together with a non-reactive or indeterminate HIV-1 Western blot, and positive HIV-1 p24 antigen (Jan 2007-Mar 2009) or HIV-1 RNA (Apr 2009-Jun 2011). From Apr 2009 to Jun 2011, 3rd generation EIA non-reactive specimens were also tested for HIV-1 RNA in pools of 24 to identify pre-seroconversion AHI.
RESULTS:

	Jan 2007-Mar 2009	Apr 2009-Jun 2011	% Change
Test Volume	14,178	16,075	+13%
All New HIV Cases	173	155	–10%
AHI Cases	15	34	+127%

Of the AHI identified after implementing pooled NAT testing, 14/34 (41%) would not have been detected by 3rd generation EIA screening. The study population accounts for ~4% of all HIV tests in the province, while yielding 65% of all AHI.
CONCLUSIONS: The use of targeted pooled NAT screening substantially increased the yield of AHI diagnoses among the study population. Targeted screening of high risk individuals with pooled NAT leads to earlier detection and significant yields of AHI, while avoiding the high cost of universal pooled NAT screening.

L4
MANAGEMENT OF *CLOSTRIDIUM DIFFICILE* INFECTION AND THE EFFECT ON MORTALITY

H HOANG, S SMITH
University of Alberta, Edmonton, AB

OBJECTIVE: *Clostridium difficile* infection (CDI) accounts for 15-25% of antibiotic-associated nosocomial diarrhea. Treatment guidelines have been developed to optimize management of this common cause of hospital morbidity. The objective of this study was to examine risk factors for CDI-related mortality, and assess the degree of adherence to treatment guidelines and their effect on mortality rates.
METHODS: Patients admitted to the University of Alberta Hospital, Edmonton, Canada who died between 2008 to 2010 with a stool sample positive for *Clostridium difficile* toxin within the preceding one month were identified. Baseline demographics and antibiotic exposure were collected to identify risk factors for CDI-acquisition. Factors including time to treatment, treating service, and adherence to the treatment guidelines were studied to determine effect on mortality.
RESULTS: 41 patients met the inclusion criteria of which 39 patients had charts available for review. 34/39 (87.2%) had documented antibiotic exposure prior to CDI-acquisition. 34/39 (87.2%) were treated for CDI. Of the 34 treated for CDI, 12 deaths were attributable to CDI. 43% of those treated >2 days after symptom-onset had a CDI-attributable death compared with only 30% of those treated ≤2 days from symptom onset (p=0.487). 20/34 (58.8%) patients were treated according to treatment guidelines with an associated trend towards decreased CDI-mortality compared to those not treated as per guidelines (25% vs. 50%) (p=0.163).
CONCLUSIONS: Treatment of CDI according to guidelines occurred in 20/34 (58.8%) patients included in this study. Those not treated according to guidelines had a trend towards higher CDI-attributable mortality. This underscores the relevance of treatment guidelines in CDI-management and highlights areas for improvement.

STUDENT POSTER PRESENTATIONS
THURSDAY MAY 3
ROOM: Grand Ballroom

SP1
CHARACTERIZING *B. CENOCEPACIA*-INDUCED NEUTROPHIL DEATH IN CHRONIC GRANULOMATOUS DISEASE

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OBJECTIVE: Patients with chronic granulomatous disease (CGD) are unable to produce phagocytic toxic reactive oxygen species (ROS) upon pathogen interaction and suffer from recurring infections. The *Burkholderia cepacia* complex (Bcc) is a group of opportunistic pathogens that cause lung infections in immuno-compromised patients. In CGD, Bcc can cause necrotizing pneumonia and sepsis and are the leading bacterial cause of death. The pathology is characterized by infiltrating neutrophils (PMN), and the species *Burkholderia cenocepacia* induces enhanced cell death in CGD PMN compared to controls *in vitro*. The objective of this study was to further characterize the mechanism leading to this PMN death.
METHODS: Primary human PMN were treated with diphenyleneiodonium, to inhibit ROS, mimicking CGD. Normal and ROS-deficient PMN were challenged with four clinical isolates of *B. cenocepacia*, and cell death was measure by flow cytometry. Intracellular bacteria were detected by lysing PMN and counting colony-forming units. Caspase activation, a hallmark of apoptosis, was examined using a FLICA detection probe to examine the rate of caspase activation. The impact of differential caspase activation was examined by pre-treating PMN with the caspase inhibitor zvad-fmk and measuring cell viability.
RESULTS: All *B. cenocepacia* isolates induced substantially more necrosis in ROS-deficient PMN compared to healthy controls. *B. cenocepacia* persisted longer in ROS-deficient PMN, in which there was a greater caspase-3 response that was not resolved as in normal PMN, but increased over time.
CONCLUSION: *B. cenocepacia* is able to survive within PMN in the absence of ROS and induce a prolonged caspase response that is associated with increased necrosis.

SP2
CHARACTERIZATION OF THE OVARIAN CANCER MICROBIOME

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BACKGROUND: Our microbiota may play a significant role in the development of chronic and neoplastic diseases. The association of ovarian cancer with pelvic inflammatory disease (PID) suggests that microorganisms, both endogenous microbiota as well as known pathogens, may lead to the development of certain subtypes of ovarian cancer.
OBJECTIVE: We will use metagenomics to compare the microbiota from three ovarian cancer subtypes: high-grade serous (HGS), endometrioid (E), and clear-cell (CC) carcinomas. Non-ovarian cancer tissue from an epithelioid sarcoma (ES) will be used as a control.
METHODS: RNA was extracted, reverse-transcribed, and sequenced on an Illumina Genome Analyzer IIx. After filtering for low quality reads and human sequences, the remaining reads were analyzed on the GenomeQuest server.
RESULTS: The microbial sequences recovered in HG, CC, E, and ES sarcoma tissue made up 0.3%, 0.05%, 1.1%, and 0.8% of the total reads, respectively. In the ovarian cancer samples, we recovered microbial sequences representing bacteria found in the vaginal microbiome and as well as those associated with PID, including lactobacilli, *Neisseria gonorrhoeae*, *Gardnerella vaginalis*, and staphylococcus, all of which were not recovered in ES tissue.
CONCLUSIONS: Many species of bacteria, phage, and viruses were recovered in ovarian cancer tissue samples but were absent in non-ovarian tissue suggesting that ovarian cancer tissue is not sterile and that these organisms may be associated with ovarian cancer. Follow-up studies using targeted PCR assays will be used to screen a larger set of samples for the presence of these microorganisms.

SP3
THE INFLUENCE OF HLA B35 AND B51 ON DISEASE PROGRESSION AMONG HIV INFECTED INDIVIDUALS IN THE PROVINCE OF MANITOBA

Y KEYNAN[1,2], Z RUEDA[3], K BRESLER[2], M BECKER[1,2], K KASPER[1,2]
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The course of HIV infection is characterized by ongoing loss of CD4 cells which determines disease progression and susceptibility to opportunistic infections. The rate of CD4 decline is determined by a combination of viral and host factors. Human leukocyte antigen genes are highly polymorphic genes responsible for expression of cellular surface molecules that present antigens to T lymphocytes. Antigen recognition and ensuing cytotoxic T cell response depend on the context of HLA genes. Several HLA-B genes have been shown to predict rate of HIV disease progression with HLA B53 and HLA B35 associated with rapid CD4 decline while B27 and B57 are associated with a slower rate. We sought to determine the HLA-B

genotypes among individuals infected with HIV in Manitoba. We present the association of HLA-B genotypes and disease progression rates among 294 individuals. Understanding the genetic background may help to understand the characteristics of the epidemic in the province.
RESULTS: 294 individuals for which HLAB typing was available along with clinical information including: demographic, co-morbidities, opportunistic infection as well as consecutive CD4 counts were included in the analysis. HLAB alleles were: 180 controls, 58 HLA B51 and 56 HLA B35. A regression model using age, HAART and HLA as independent variables. HLA B35 was associated with HR of 2.18 (CI 1.39–3.42) and HLA B51 with HR of 2.235 (CI 1.30–3.89) for progression to CD4<200.
CONCLUSIONS: HLA B35 is common in the MB patient population and similar to other cohorts its presence is associated with rapid CD4 decline. HLA B51, another common allele in the MB HIV infected population is associated with rapid disease progression. This allele has been associated with slow disease progression among patients of European descent but is shown here to correlate with fast CD4 decline.
The immune mechanisms associated with the HLA's that are predictive of rapid progression remain to be determined in the unique population of the Prairies.

SP4
EFFICACY OF BISMUTH-ETHANEDITHIOL INCORPORATED IN A LIPOSOME-LOADED TOBRAMYCIN FORMULATION AFTER INTRATRACHEAL ADMINISTRATION IN RATS WITH PULMONARY *P. AERUGINOSA* INFECTION

M ALHARIRI, A OMRI
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OBJECTIVE: To investigate the alterations in the quorum-sensing signal molecule N-acyl homoserine lactone (QS-AHL) secretion, the release of *Pseudomonas aeruginosa* (PA) virulence factors and in vivo antimicrobial activity of Bismuth-Ethanedithiol Incorporated in a Liposome-Loaded Tobramycin Formulation (LipoBiEDT-TOB) administered to rats chronically infected with PA.
METHODS: Q-AHL was monitored by a biosensor organism. PA virulence factors were assessed spectrophotometrically. Agar beads model of chronic Pseudomonas lung infection in rats were used to evaluate the efficacy of the liposomal formulation in the reduction of bacterial count. The levels of active tobramycin in lungs and kidneys were evaluated by microbiological assay.
RESULTS: LipoBiEDT-TOB was effective in disrupting the Q-AHL and significantly ($p < 0.05$) reduced lipase, chitinase and protease productions. Twenty four hours after 3 treatments, the cfu counts performed on lungs treated with LipoBiEDT-TOB were of 3 $\log_{10}$CFU/lungs comparatively to 7.4 and 4.7 $\log_{10}$CFU/lungs respectively in untreated and in lungs treated with free antibiotic. The antibiotic concentration after the last dose of LipoBiEDT-TOB was 25.1 µg/lungs while no tobramycin was detected in kidneys. However, no antibiotic was detected in lungs whereas 6.5 µg/kidneys were detected following the administration of free antibiotic.
CONCLUSIONS: LipoBiEDT-TOB reduced the production of quorum sensing and virulence factors and could highly improve the management of chronic pulmonary infection in cystic fibrosis patients.

SP5
ENHANCED ACTIVITY OF LIPOSOMAL CLARITHROMYCIN AGAINST *PSEUDOMONAS AERUGINOSA* ISOLATES FROM PATIENTS WITH CYSTIC FIBROSIS

M ALHAJLAN, A OMRI
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OBJECTIVE: The aim of this work was to investigate the efficacy and innocuity of several liposomal formulations containing Clarithromycin against clinical isolates of PA from the lung of cystic fibrosis (CF) patients.
METHODS: The liposomal clarithromycin formulations were prepared by the dehydration-rehydration method and their sizes were measured using the dynamic light scattering technique. The encapsulation efficiency of these liposomal formulations was determined by microbiological assay and their stability in biological fluid was evaluated for a period of 48hrs. The MICs and MBCs of free and liposomal formulations were determined with PA strains isolated from CF patients (CLSI). The toxicities of these liposome preparations on A549 cell lines were evaluated.
RESULTS: The average of liposomal sizes was below 222 nm in diameter with an encapsulation efficiency value ranging from 5.7±0.01% to 30.4±0.1%. The liposomes retained more than 70% of their drug content during the 48 h time-period. Interestingly, the highly resistant strains of PA became susceptible to clarithromycin when it is encapsulated in liposomal formulations. The clarithromycin-entrapped liposomes were much less cytotoxic than free drug.
CONCLUSION: The encapsulation of clarithromycin into liposomes significantly increased the *in vitro* antibacterial activity of this agent against clinical resistant strains of *P. aeruginosa*. These formulations could be used as a new strategy of drug delivery system to lower antibiotic quantity and enhance efficacy of existing antibiotics against resistant microorganisms that commonly affect individuals with cystic fibrosis.

SP6
LIPOSOMAL N-ACETYLCYSTEINE REDUCES BIOFILMS AND AFFECTS BACTERIAL GROWTH OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM CYSTIC FIBROSIS PATIENTS

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OBJECTIVE: The aim of this study was to evaluate the antibiofilm activity of liposomal N-acetylcysteine (L-NAC) and its antimicrobial effect against *Pseudomonas aeruginosa* strain from cystic fibrosis patients.
METHODS: L-NAC was prepared by the dehydration-rehydration method. The encapsulation efficiency and minimal inhibitory concentrations (MIC) of the free NAC (F-NAC) and L-NAC against clinical isolate of *P. aeruginosa* were determined by microtitre broth dilution method. In vitro time-kill studies were performed using both NAC formulations at 5, 2.5, 1.25, 0.63, 0.31, 0.16, 0.08 and 0.04 mg/ml. The effect of these formulations on biofilms production was assessed using the Calgary Biofilm Device and their cytotoxicity was evaluated on A549 cell lines.
RESULTS: The encapsulation efficiency of NAC by liposomes was measured as 37%. The MIC of L-NAC was lower (1.25 mg/ml) than that of F-NAC (5 mg/ml). L-NAC reduced the amount of biofilm by 55% at 5 mg/ml. The time-kill values of L-NAC were better than that of F-NAC. A549 cells were not affected by 24h exposure of 5 mg/ml L-NAC.
CONCLUSION: Liposomal N-acetylcysteine formulation appears to be safe and can be used as antimicrobial agent against highly resistant *P. aeruginosa* in Cystic Fibrosis patients.

SP7
SAFETY AND TOLERABILITY OF INHALED NITRIC OXIDE IN CHILDREN WITH SEVERE MALARIA

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OBJECTIVE: Based on the efficacy of inhaled nitric oxide (iNO) in experimental cerebral malaria in animal models, we are conducting a randomized clinical trial using iNO at 80ppm for adjunctive treatment of severe malaria in Ugandan children. Low-flow iNO at a concentration of 5 to 80 ppm is approved for use by the US FDA for the treatment of neonates with hypoxic respiratory failure. It is well tolerated and generally safe with

methemoglobinemia (metHg) and increased nitric dioxide (NO_2) as dose-dependent adverse reactions. We will report on the safety and tolerability in this study population.

METHODS: This prospective, randomized, blinded clinical trial compares adjunctive continuous inhaled NO (80 ppm) to placebo (room air) in Ugandan children with severe malaria. Children are excluded if their baseline metHg levels are greater than 2%. MetHg and NO_2 levels and potential drug adverse reactions are closely monitored.

RESULTS: 25 children have been enrolled to date with 10 in the iNO group. 15 out of 184 (8.2%) screened children were excluded due to elevated baseline metHg. The median peak metHg level in the iNO group was 6.6 (IQR 5.6-7.6) at 47.2 hrs (IQR 17.5-61.8) compared to 2.2 (IQR 2.1-2.6) for the placebo group. An increased rate of hyperglycemia was seen in children treated with iNO.

CONCLUSION: In our study population we found higher than expected levels of metHg at baseline, which lead to exclusion of children that would have otherwise been eligible. At iNO of 80 ppm, the median maximum metHg level is comparable to previous studies. However, the time to reach the maximum level is significantly longer than in previous reports. Further evaluation is required to determine if this difference is real and to investigate the reason for this difference.

SP8

THE EFFECTS OF UV LIGHT ON THE ANTIMICROBIAL ACTIVITIES OF CAVE ACTINOMYCETES

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OBJECTIVES: Multi-drug resistant, infectious organisms are a leading cause of death worldwide. Thus, the need for novel antimicrobial compounds is greater now than ever before. The objectives of this study were: to determine whether actinomycete strains isolated from the cave in Wells Gray Provincial Park, BC, have the potential to produce new antibiotics, and to determine whether UV light can stimulate actinomycete antimicrobial activity against several well-known, drug-resistant pathogens.

METHODS: More than 400 actinomycete strains previously isolated from Helmcken Falls cave, Wells Gray Provincial Park, BC, were subjected to preliminary screening against seven pathogens. The screening involved carrying out the "plug assay" on pathogen-seeded plates, exposing the plates to both UV and non-UV light conditions, and measuring the zones of inhibition on the plates after incubation to assess antimicrobial activity.

RESULTS: Of the more than 400 actinomycete strains tested, 99 strains showed anmicrobial activity against numerous pathogenic organisms. In particular, 26 of these strains were activated only in the presence of ultraviolet light, while the remainder was activated in non-UV, and both UV and non-UV light conditions.

CONCLUSIONS: Though the metabolites produced by these strains have yet to be determined, cave actinomycetes have the potential to yield novel antimicrobial drugs. It is possible that exposing microorganisms that traditionally live in the dark to new, lighted environments may somehow enhance their antimicrobial activity, which can then be harnessed for drug production. However, further investigation of the role of UV light in metabolite activation has yet to be made.

SP9

HUMAN HERPES VIRUS 7: TRUE CAUSE OF ENCEPHALITIS OR INNOCENT BYSTANDER?

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OBJECTIVES: Human herpesvirus 7 (HHV7) has been associated with a variety of neurological conditions in case reports and small case series. A conclusive pathogenic role has not been established. The objective of this study was to better elucidate the role of HHV7 in central nervous system (CNS) disease in children.

METHODS: Retrospective case series of children ≤18 years of age hospitalized between Jan 1996 and Dec 2011 in whom HHV7 was detected by PCR in the CSF. Most children had standardized microbiologic investigation as part of the prospective SickKids Encephalitis Registry. Cases were classified as probable HHV7 disease, or as due to alternate infectious or non-infectious causes by a multi-disciplinary team.

RESULTS: A total of 54 children had HHV7 detected in their CSF. HHV7 was the probable cause in 19 (35%), including 10 with encephalitis, 8 with meningitis and 1 who died of refractory status epilepticus. Over the 15 year period HHV7 accounted for 3% of encephalitis cases (10/330). Of 10 with encephalitis 6 had a normal outcome, 4 had persistent neurological impairments. In 35 (65%) a more plausible cause of CNS disease was identified: enterovirus (3), bacterial (2), Lyme disease (1), varicella zoster virus (VZV; 1) and tuberculous (1) meningitis; VZV (1) and EBV (1) encephalitis; demyelinating conditions (13); anti-NMDAR (1); other (11).

CONCLUSIONS: HHV7 was associated with CNS disease in children in our series. However, the observation that 65% had a clear alternate cause indicates that detection of HHV7 by PCR in CSF may be coincidental in some cases, likely due to latent infection of lymphocytes. Further study combining CSF PCR with serology may better define the role of HHV7 in CNS disease.

SP10

TICK PARALYSIS IN BRITISH COLUMBIA

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OBJECTIVE: Tick paralysis is caused by the exposure of a neurotoxin released by tick(s) during blood meals. As the disease is sporadic in the Pacific Northwest and the Rocky Mountain states of the United States, we reviewed tick submission records from the past 20 years to analyze cases of tick paralysis in British Columbia.

METHODS: Since this is a passive surveillance, we were not able to capture all the cases. Case definition is based on tick submissions along with tick paralysis data from the requisitions. Both human and animal cases during the period between 1990 and 2010 were included. Positive cases were then analyzed based on tick identification, location, host sex and age, and geo-mapped data.

RESULTS: Fifty two cases were identified, including human (21), dogs (29), llama (1), and horse (1). Four species of tick were identified: *Ixodes pacificus*, *Rhipicephalus sanguineus*, *Dermacentor albipictus*, and the dominant species, *Dermacentor andersoni* (84.6%). Case distribution of tick paralysis was found in the southern part of Vancouver Island and BC mainland, with one case from Calgary, Alberta. Most of the cases (94%) occurred during spring (March-June). For human cases, there was no sex preference but it was more common (~86%) in the younger (<10 years) and older (>50 years) age groups.

CONCLUSION: Dominant vector of tick paralysis in BC is *D. andersoni*, as found in historic data (1900-1968). Tick paralysis is seasonal and distributed across southern BC, mainly in the interior. Human data showed that tick paralysis had no sex preference but was predominant at the extreme ends of the age spectrum.

SP11

ACTINOMYCES INFECTIONS IN CHILDREN: A TEN-YEAR REVIEW

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OBJECTIVES: *Actinomyces* species are anaerobic, filamentous, gram positive bacteria that are commensals of the oropharynx, GI tract and vagina. Classically actinomycosis affects the cervicofacial, thoracic or abdominal areas, and is typically caused by *Actinomyces israelii*. The objective of this study was to review the clinical features and microbiology of pediatric *Actinomyces* infections.

METHODS: In this retrospective observational study, patients with positive cultures for *Actinomyces* species from 1999-2008 were identified and hospital charts reviewed.

RESULTS: 38 positive cultures were identified. Of these, 28 (74%) were isolated from blood and 10 (28%) from pus aspirates, biopsies or wound swabs. Of the non-blood specimens, 6 (60%) were of head and neck origin. 50% of the pus specimens were polymicrobial. Of the patients with positive blood cultures, 7 (24%) had concurrent oropharyngeal compromise, 8 (20%) GI compromise, 4 (15%) had aspiration pneumonia. Of the positive blood cultures, 1 was polymicrobial and 3 had a second organism isolated from a different site concurrently. Overall, the most common species were *A. odontolyticus* (50%), and *A. naeslundii* (28%). *A. israelii* accounted for only 5%. 90% of patients with non-bloodstream infection received appropriate systemic antibiotic treatment compared to only 55% of those with positive blood cultures.
CONCLUSION: *Actinomyces* bacteremia occurs in children, particularly in the setting of oropharyngeal, GI or respiratory mucosal compromise. The clinical significance is unclear and may at times represent transient clinically insignificant bacteremia. Furthermore *A. odontolyticus* and *A. naesludii* appear to be the predominant species isolated in this pediatric cohort as opposed to *A. israelii*.

SP12

MACROLIDE USE IN THE TREATMENT OF CRITICALLY ILL PATIENTS WITH PNEUMONIA: INCIDENCE, CORRELATES, TIMING AND OUTCOMES

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OBJECTIVES: Macrolides have been postulated to decrease mortality in community acquired pneumonia (CAP) via immunomodulatory properties in addition to their antimicrobial action. We studied the incidence, correlates, timing and mortality associated with macrolide-based treatment for critically ill patients with pneumonia – hypothesizing that macrolide use would be associated with decreased mortality.
METHODS: We prospectively enrolled a population-based cohort of critically ill adults with CAP over 2 years from 5 intensive care units (ICU) in Edmonton, Canada. Data collected included comorbidities, disease severity (APACHE II), pneumonia severity (PSI), and type and timing of antibiotics. The independent association between macrolide-based treatment at presentation and 30-day all-cause mortality was examined using multivariable Cox regression.
RESULTS: The cohort included 328 patients. 28% received macrolide-based treatments. Nursing home residence was the only significant correlate of macrolide-based treatment (15% vs. 30% for non-residents, p=0.02). 52% of patients received effective antibiotic therapy within 4 hours of presentation. Overall mortality was 16% at 30 days; 15% in those on macrolides vs. 17% for non-macrolides (aHR 0.89, 95% CI 0.48-1.65, p=0.71). Patients receiving effective antibiotic treatment within 4 hours of presentation were less likely to die than those who received delayed therapy (14% vs. 17%, aHR 0.51, 95% CI 0.27-0.96, p=0.038).
CONCLUSIONS: Macrolide-based treatment was not associated with a lower 30-day mortality among critically ill patients with pneumonia, although receipt of any effective antibiotic(s) within 4 hours was. Based on these results, choice of antibiotics is likely less important than timely treatment.

SP13

DETECTION OF OXA-48 CARBAPENEMASE: REPORT OF THE FIRST 2 CASES IN EASTERN ONTARIO

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OBJECTIVE: OXA-48 carbapenemases have rarely been reported in Canada and may be the most difficult to detect in clinical laboratories. We describe the first 2 patients with OXA-48 producing *Enterobacteriaceae* identified in Eastern Ontario.
METHODS: Meropenem and ertapenem MICs were determined by E-test or agar dilution. Isolates were investigated phenotypically with Rosco Diagnostica KPC + MBL Confirmation Kit. Multiplex PCR testing for carbapenemase genes, including bla_{OXA-48}-like genes, was performed. Isolates were compared by pulsed field gel electrophoresis (PFGE). A chart review of both patients was completed.
RESULTS: *Enterobacteriaceae* positive for bla_{OXA-48}-like genes from 2 patients were detected in the same hospital. An *E. coli* (EC) was isolated from the urine and leg wound of patient #1 who received previous medical care in Syria. This EC had an AmpC ß-lactamase phenotype and was confirmed to have the bla_{OXA-48} gene. Patient #2 recently moved from Egypt and had infection of a G-tube site with an ESBL positive EC as well as rectal colonization with both EC and *K. pneumoniae*. All 3 isolates were positive for bla_{OXA-48} gene. Ertapenem and meropenem MICs of all the above isolates ranged from 8 to >32 mg/L and ≤1 to 16 mg/L, respectively. Rosco disk testing demonstrated reduced zone diameters around all tablets (meropenem zone 12-19 mm), but no inhibition with boronic acid or dipicolinic acid. PFGE profiles of the isolates differed for both patients and there was no epidemiological link.
CONCLUSION: These are the first OXA-48 type carbapenemase identified in Eastern Ontario. Canadian laboratories should have surveillance methods in place for their detection, especially in patients from high risk countries.

SP14

FATAL PNEUMONIA DUE TO METHICILLIN SENSITIVE *STAPHLYOCOCCUS AUREUS* CONTAINING PANTON-VALENTINE LEUKOCIDIN (PVL) AND AN ELEVATED VANCOMYCIN MIC

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OBJECTIVE: When reported, higher mortality caused by MRSA *versus* MSSA may be due to resistance *per se*, virulence factors that segregate with resistance, inferior treatments for MRSA *versus* MSSA, or patient associated factors. We report a case of a previously healthy 52-year-old female who succumbed to multifocal pneumonia and sepsis due to MSSA.
METHODS: Case report and review of the literature.
RESULTS: A 52 year-old female presented to hospital with abrupt onset of malaise and rapidly progressive sepsis. She was intubated and required vasopressor support. She was empirically treated with vancomycin and ceftriaxone. Chest radiography revealed bilateral, multifocal opacities. Blood cultures grew MSSA within several hours of admission. She succumbed to her illness within 72 hours. Post-mortem examination revealed bilateral lung abscesses. There was no gross or histological evidence of infective endocarditis, other endovascular infection or extra-pulmonary infectious focus. Her *S. aureus* isolate underwent molecular characterization. The isolate belonged to *spa* type UJGFMB (Ridom t189); MLST ST188; and was positive by PCR for *pvl* and *fnbB*. PCR for *fnbA, sea, seb, sec, TSST, seg, cnaA, cnaB* were negative; vancomycin MIC as determined by E-test was 1.5 µg/ml.
CONCLUSION: Recently, decreased susceptibility to vancomycin has been observed to correlate with worse outcomes in MSSA infections. PVL has been implicated in disease severity irrespective of methicillin sensitivity. The presence of PVL and an elevated vancomycin MIC in this patient may have contributed to her severe, rapidly fatal sepsis.

SP15

EPIDEMIOLOGY OF MRSA IN PEDIATRIC AND ADULT INPATIENTS: A COMPARISON USING THE 1995-2007 CANADIAN NOSOCOMIAL INFECTION SURVEILLANCE PROGRAM (CNISP) DATA

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OBJECTIVE: The CNISP has been conducting surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) among inpatients since 1995, given its

burden and impact on the healthcare system. In the absence of data comparing the epidemiology of adult and pediatric MRSA, we aimed to compare MRSA in pediatric and adult inpatients from participating CNISP facilities.
METHODS: CNISP MRSA surveillance data from 1995 to 2007 was used. Data included: patient characteristics, clinical outcomes, and molecular laboratory testing. Descriptive statistics and non-parametric tests were used to compare pediatric and adult MRSA, where appropriate. Incidence was also calculated.
RESULTS: Overall, 1,262 pediatric and 35,907 adult MRSA cases were reviewed. Adults were more likely to be colonized than children (67.8 v. 50.9%; p<0.001) and were also more likely to harbour a healthcare vs. a community-associated MRSA (72.0 v. 42.2%; p<0.001). Skin and soft tissues was the most common site of infection for adults and children although the incidence was significantly higher in children (28.5 v. 10.8%; p<0.001). The incidence of urinary tract infections was higher in adults (3.7 v. 1.9%, p<0.001). CMRSA7 and CMRSA10 were more likely isolated from infected children (23.7 v. 2.5% and 28.5 v. 13.0%, respectively; p<0.001), whereas CMRSA2 was more likely isolated from infected adults (47.6 v. 26.4%; p<0.001). There was no difference in the proportion of patients colonized with CMRSA2 (48.5 v. 50.2%; p>0.10) in the two population subsets.
CONCLUSION: Significant differences exist in the epidemiology of adult and pediatric patients colonized and infected with MRSA. These differences may have implications for infection prevention and control strategies.

SP16

CARBAPENEMASE-PRODUCING *ENTEROBACTERIACAE* IN ONTARIO, 2008–2011

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OBJECTIVE: To describe the increased detection of carbapenemase-producing *Enterobacteriaceae* in Ontario.
METHODS: Isolates suspected to be carbapenem resistant *Enterobacteriaceae* received at Public Health Ontario Laboratories were subject to the following algorithm: isolates with confirmed MICs ≥1 µg/ml for imipenem or ertapenem, and/or ≥2 µg/ml for meropenem were tested by different phenotypic methods for carbapenemase activity detection (Modified Hodge test and, most recently, the inhibitor test*). Subsequently, molecular characterization of the carbapenemase genes was performed in the positive isolates. Molecular typing was performed by multilocus sequence typing (MLST). *Clin. Microbiol. Infect. 17:552-556, 2011
RESULTS: In the period Jan 2008-Dec 2011, the *Klebsiella pneumoniae* carbapenemase (KPC), the oxacillinase (OXA)-48, the New Delhi metallo-ß-lactamase (NDM) and the Verona integron-encoded metallo-ß-lactamase (VIM) were identified (N=73):

	Carbapenemase Type (*n*)			
Year (*n*)	KPC (*30*)	OXA-48 (*8*)	NDM (*32*)	VIM (*3*)
2008 (*4*)	4 *Kpn*	Not detected	Not detected	Not detected
2009 (*4*)	3 *Kpn*	Not detected	1 *Eco*	Not detected
2010 (*20*)	9 *Kpn*	1 *Kpn*	1 *Ecl*, 2 *Eco*, 3 *Kpn*, 1 *Mmo*, 1 *Pst*, 1 *Pre*	1 *Eco*
2011 (*45*)	1 *Cfr*, 2 *Ecl*, 11 *Kpn*	3 *Eco*, 4 *Kpn*	2 *Ecl*, 5 *Eco*, 13 *Kpn*, 2 *Mmo*	2 *Ecl*

References (n): Cfr, Citrobacter freundii (1); Ecl, Enterobacter cloacae (7); Eco, Escherichia coli (12); Kpn, K. pneumoniae (48); Mmo, Morganella morganii (3); Pst, Providencia stuartii (1); Pre, P. rettgeri (1)

Our data show that incidence of carbapenemase-producing strains has increased, particularly in the last 2 years. All the KPC-*Kpn* (n=27) belonged to ST258 or closely related. Diversity of MLST-types was observed between NDM-*Kpn* (n=16) and *Eco* (n=7) strains. *Kpn* was the prevalent species (n=48) mainly consequence of the clonal dissemination of bla_{KPC} and the polyclonal spread of bla_{NDM}.
CONCLUSIONS: Since 2008, carbapenemase-producing *Enterobacteriaceae* were increasingly observed in Ontario, particularly NDM- and KPC-producers. Their detection, associated with multidrug resistance phenotype, highlights the importance of continued surveillance to preserve the last antimicrobial options currently available.

SP17

PREVALENCE OF EXTENDED SPECTRUM ß-LACTAMASE, AMPC CEPHALOSPORINASE, AND CARBAPENEMASE PRODUCING ENTEROBACTERIACEAE IN STOOL SAMPLES SUBMITTED TO LABORATORIES IN THE OKANAGAN REGION OF BRITISH COLUMBIA – A FOLLOW-UP STUDY

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OBJECTIVES: The prevalence of resistant organisms in the community has implications for empiric therapy and screening of high-risk patients. Beta-lactamases including extended spectrum β-lactamases (ESBL), AmpC cephalosporinases (AmpC) and carbapenemases (CPM) are rapidly spreading worldwide limiting therapeutic options. This study compared the prevalence of fully expressed ESBL, AmpC and CPM in the Okanagan region of BC from 2010 to 2011.
METHODS: Routine stool samples from 4 laboratories (3 hospital/1 outpatient) were screened over the same period using cefotaxime, ceftazidime and ertapenem discs on MacConkey plates. All isolates testing intermediate or resistant (2011 CLSI guidelines) were identified and tested (MAST® discs, E Test®) to exclude ESBL, AmpC and CPM.
RESULTS: No significant difference in prevalence between 2011 and 2010 was found in recovery of ESBL (2.9% vs 2.77%) or AmpC (7.81% vs 7.25%). No CPM were recovered in either studies, although decreased ertapenem susceptibility was noted in 1 isolate of AmpC producing *E. coli*. In current study, prevalence of AmpC was higher in inpatients (IP)-10.8% than outpatients (OP)-6.39% whereas the prevalence of ESBL was lower (1.39%) in IP than OP(3.79%).
CONCLUSIONS: There has been no significant change in prevalence of ESBL, AmpC or CPM over a one year period. Continued monitoring is important given the transmissibility and therapeutic limitations of these organisms. Fully expressed AmpC are significantly more common than ESBL, may be associated with ertapenem resistance and require ongoing monitoring.

SP18

DETERMINATION OF SUSCEPTIBILITY TO FOSFOMYCIN AND TIGECYCLINE OF ENTEROBACTERIACEAE ISOLATES FROM ROYAL INLAND HOSPITAL PRODUCING EXTENDED-SPECTRUM ß-LACTAMASES

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OBJECTIVE: The recent increase in Enterobacteriaceae with extended-spectrum β-lactamases (ESBLs) has become a concern and antibiotics active against ESBLs are needed to treat infections caused by these bacteria. The objective of this study was to determine ESBL producing Enterobacteriaceae susceptible to fosfomycin and tigecycline at a regional Canadian hospital. This study is of particular importance to this region of the South Central Interior of BC, Canada as a study regarding fosfomycin and tigecycline resistance to ESBL producing Enterobacteriaceae has never been done.
METHODS: In order to determine the susceptibility of fosfomycin and tigecycline to ESBL producing Enterobacteriaceae, a fosfomycin agar disk diffusion test (Kirby Bauer susceptibility test) and a tigecycline E-test were used. The isolates were obtained from Royal Inland Hospital (RIH) in Kamloops BC, Canada. The zone of inhibition to fosfomycin and minimum inhibitory concentration (MIC) of tigecycline was determined to see whether the ESBL producing Enterobacteriaceae were sensitive to fosfomycin and tigecycline.
RESULTS: All isolates tested to date (n=49) were sensitive to tigecycline. Two isolates were resistant, and one isolate was intermediate to fosfomycin. This is an ongoing study with more isolates being collected and tested.
CONCLUSION: At the regional hospital of interest, some resistance to fosfomycin was observed among the isolates. This study demonstrates that

there are antibiotics that clinicians in the South Central Interior of BC can use as therapeutic alternatives to Carbapenem antibiotics against ESBL producing Enterobacteriaceae potentially reducing the selection of carbapenemase producing bacteria.

SP19

IN VITRO ANTIFUNGAL SUSCEPTIBILITY OF *CANDIDA ALBICANS* ISOLATES FROM ORAL CAVITIES OF PATIENTS INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS IN ETHIOPIA

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OBJECTIVE: Oral candidiasis is the most common HIV related oral lesion. Most patients are infected with a strain originally present as a commensal of the oral cavity. The chronic use of antifungal agents, in the treatment of candidiasis mainly in HIV/AIDS patients leads to the selection of strain resistant to this therapy. The objective of this study was to evaluate the *in vitro* susceptibility of *Candida albicans* to commonly used antifungal agents in Ethiopia.

METHODS: *In vitro* susceptibility tests were performed using the broth microdilution method following the National Committee for Clinical Laboratory Standards (NCCLS) M27-A guidelines. Data were then analyzed using SPSS for Windows version 16.0. Tests of proportions were done with Chi-Square, and a p value of <0.05 was considered as statistically significant.

RESULTS: A total of 42 oral *C.albicans* isolates from HIV-infected patients were included in this study. Forty-one (97.7%) isolates were determined fully susceptible to amphotericin B, 40 (95.3%) to nystatin, and 39 (92.9%) to ketoconazole and miconazole. On the other hand, the isolates showed highest rates of resistance against fluconazole (11.9%) relatively. There was little difference in the antifungal susceptibilities of *C.albicans* isolated from patients who had a history of previous antifungal therapy compared with those who had not received antifungal treatment.

CONCLUSION: The *in vitro* antifungal susceptibility testing of *C.albicans* in this study showed relatively high resistance to commonly used azoles. As with the prescribing of any antimicrobial agent, the use of a systemic antifungal drug must be justified. Efforts must be maintained to avoid inappropriate or unnecessary prescribing of these antifungal.

SP20

SPICE BACTERIA IN BLOOD CULTURES: TEN YEARS EXPERIENCE IN A LARGE TERTIARY CARE CENTRE

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OBJECTIVE: *Serratia sp.*, *Providencia sp.*, indole positive *Proteus sp.*, *Citrobacter sp.*, and *Enterobacter sp.* (SPICE) all possess inducible *amp*C beta-lactamase genes. Stable derepression and subsequent clonal spread may be promoted by overuse of extended-spectrum penicillins and cephalosporins. We reviewed ten years of blood culture results to determine whether SPICE bacteria represent a growing proportion of isolates.

METHODS: Retrospective analysis of our existing microbiology database was performed between January 2001 and December 2011. We reviewed the total number of positive blood cultures and looked into the proportion of SPICE isolated relative to members of enterobacteriaceae during the ten year period.

RESULTS: Between 2001 and 2011, we had a total of 129,029 blood cultures, 3399 (2.6%) of which were isolates of different members of the Enterobacteriaceae. Overall, the total number of SPICE bacteria isolated was 819 (0.63%). A slight increase was noted in the total number of Enterobacteriaceae isolated over the study period (263 in 2001 to 361 in 2011) but not in the percent positive. The proportion of SPICE isolated relative to enterobacteriaceae did not significantly change over the study period (21.7% in 2001 to 19.7% in 2011).

CONCLUSION: SPICE bacteria are important nosocomial pathogens responsible for various infections, including bacteremia, which can lead to significant morbidity and mortality. Although one would expect these isolates might continue to grow and spread with the extensive use of extended-spectrum penicillins and cephalosporins, our data showed that over the last decade SPICE bacteria did not cause bacteremia at increasing rates.

SP21

CLINICAL SIGNIFICANCE OF BLOOD CULTURES POSITIVE FOR *BACILLUS SPECIES*

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OBJECTIVES: Blood cultures (BCs) positive for *Bacillus species* (BSp) frequently present difficult diagnostic and treatment challenges. Our goal was to collect demographic and clinical data to characterize patients and assist in differentiating true bacteremias from contaminants. We also examined patterns of antimicrobial usage and evaluated clinical outcomes.

METHODS: We performed a retrospective analysis of patients with BCs positive for BSp at the QEII HSC in 2010. Patients were categorized as probably, possibly, or unlikely infected with BSp based on clinical and laboratory criteria. Various characteristics were compared among groups.

RESULTS: Sixty eight of 17,041 blood cultures (from 60 patients) were positive for BSp. Clinical criteria revealed 14% probable, 50% possible and 36% unlikely infected with BSp. Seventeen BCs were polymicrobial; most often with coagulase negative staphylococci. One-third (33%) of BCs positive for BSp were from the hematology service, however, less than 6% of all other positive cultures came from that service (p<0.0001). Most patients were immune-compromised (78%) and/or had an indwelling vascular catheter in place (77%). All nine with probable BSp infections were immune compromised. The most common admission diagnosis was febrile neutropenia. Over half (55%) of BSp positive BCs were drawn from an indwelling catheter, but no catheter tips were culture positive for BSp. Ninety-four per cent of BSp strains were susceptible to meropenem our empiric choice for febrile neutropenia.

CONCLUSIONS: Patients with hematological malignancy or other immune compromise are at highest risk of BSp bacteremia. It is important that BCs positive for BSp be carefully assessed especially in the immune compromised patient.

SP22

REVIEW OF A TARGETED ANTIBIOTIC-RESISTANT ORGANISM ADMISSION SCREENING PROGRAM AT A COMMUNITY HOSPITAL

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OBJECTIVE: Antibiotic-resistant organisms (AROs) lead to increased health care costs, morbidity and mortality. Targeted admission screening has been shown to be an effective and cost-efficient measure for prevention of hospital-acquired ARO colonization and infection. The objective of this study was to evaluate an ARO admission screening program for adherence and efficacy in a community hospital.

METHODS: We conducted a review of the ARO admission screening program of the Medicine, Surgery and Obstetrics inpatient care units at a community hospital. Data were collected by a combination of chart review, patient self-reported history and electronic medical records. The following characteristics were reviewed: presence, completion and accuracy of ARO screening forms.

RESULTS: Overall, screening form completion rates were high (92.8%; 77/83), but accuracy in risk assessment varied among units (range, 65-92%). Risk tended to be overestimated. Of those patients who met requirements for methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) admission screening, 64.6% (42/65) and 64.1% (41/64) of surveillance cultures, respectively, were sent within the target of 24 hours post-admission.

CONCLUSION: We observed high rates of utilization and completion of the ARO admission screening tool. However, more consistent risk assessment and interpretation, and improved timeliness of specimen collection, are required to improve the effectiveness of the hospital's ARO admission

screening program. It is recommended that Infection Control and Prevention consider unit-specific interventions to improve ARO risk assessment and timeliness of screening.

SP23

METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* DECOLONIZATION OF HOSPITALIZED PATIENTS USING TOPICAL MUPIROCIN PLUS ORAL RIFAMPIN AND DOXYCYCLINE

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OBJECTIVES: MRSA colonization of hospitalized patients is associated with risk of both MRSA infection in the affected patient and transmission to other patients. The purpose of this study was to determine the efficacy of MRSA decolonization in patients in whom it has been attempted and identify risk factors for success or failure of decolonization.

METHODS: Our institution routinely identifies patients who are colonized or infected with MRSA and, if appropriate, attempts combination oral and topical therapy for MRSA decolonization. A retrospective database analysis from 1996 to 2011 was performed and the rate of decolonization, defined as three negative MRSA swabs, was determined for each type of therapy. Follow up data was analyzed at 12 weeks, 6 months and 8 years. Patient demographics were analyzed to identify risk factors for success or failure of decolonization.

RESULTS: 1281 patients were colonized with MRSA: 25.4% were nosocomially acquired and the majority (47.5%) with CMRSA-1 type. 80.3% of patients who received a combination of topical mupirocin plus oral rifampin and doxycycline were successfully decolonized within 12 weeks of initiation of therapy, with 78.7% and 76.0% remaining negative for MRSA at 6 months and 8 years, respectively. In comparison, 12-week follow up data demonstrated decolonization in 75.0% of patients who received other oral therapy, 32.1% of patients who received topical mupirocin alone and 24.8% of patients who received no therapy. No significant patient risk factors for success or failure of decolonization were identified.

CONCLUSIONS: A MRSA decolonization regimen including topical mupirocin plus oral rifampin and doxycycline was successful in 80.3% of patients within 12 weeks of therapy and 78.7% of patients after 6 months of follow up.

SP24

CLINICAL AND MICROBIOLOGIC CHARACTERISTICS OF HOSPITAL ACQUIRED ENTEROCOCCAL BACTERAEMIA OVER A 5-YEAR PERIOD

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OBJECTIVE: Enterococcal blood stream infections (BSI) are a frequent cause of nosocomial infections. The purpose of this study was to evaluate clinical and microbiologic characteristics of enterococcal bacteremias over a five year period.

METHODS: Data regarding hospital acquired BSI is prospectively collected by the infection control department at a 750 bed tertiary care hospital. We conducted a retrospective review of medical records of 190 patients (pts) with enterococcal BSI over a five year period (January 2006 to December 2010).

RESULTS: Among the 190 isolates, 176 (92.63%) were vancomycin (VAN) susceptible and 14 (7.37%) were VAN resistant. *Enterococcus faecalis* was found in 94 (49.47%) cases and *Enterococcus faecium* in 90 (47.36%) cases. Of 50 (26.32%) primary BSI, 34 (68%) were central venous line related. One hundred forty (73.68%) secondary BSI were identified with the gastrointestinal (GI) tract as the most frequent source of infection [70 cases (50%)]. Rates of enterococcal bacteremia remained stable (1.3-1.7/10,000 pt days), but the proportion of VRE cases increased in 2010 (accounting for 19% of all cases). Common comorbidities included immunocompromised state [58 (30.53%)], diabetes mellitus [49 (25.79%) pts], recent abdominal surgery or GI endoscopy [89 (46.84%)].

CONCLUSION: Enterococcus are a common bloodstream pathogen in patients with abdominal surgery, endoscopy and those that are immunocompromised. Although VRE BSI is rare in our institution, 42% of the cases occurred in 2010 suggesting an upward trend, reinforcing the need for infection control measures and antibiotic stewardship to prevent the spread of this drug resistant bacteria.

SP25

SURVEILLANCE OF POST-PROCEDURE INFECTIONS RELATED TO TRANSRECTAL ULTRASOUND-GUIDED PROSTATE BIOPSIES AT A TERTIARY CARE HOSPITAL

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OBJECTIVE: Transrectal ultrasound (TRUS)-guided prostate biopsy is a procedure used to diagnose prostate cancer. Post-procedure infections are rare, but are considered to be a major complication. The objectives of this pilot surveillance study were (1) to establish baseline post-procedure infection rates at a single tertiary care hospital, (2) to determine the microbial etiology and (3) to establish risk factors for antimicrobial resistance.

METHODS: All TRUS-guided prostate biopsies performed at a single tertiary care hospital from July 1, 2009, to June 30, 2011, were included. Retrospective case identification occurred via medical chart review of all patients presenting to any emergency department in the region within 7 days of the procedure. Demographic, clinical, risk factor and microbiological data were collected. Combined clinical and laboratory definitions were used for urinary tract infection (UTI) and bloodstream infection (BSI). Incidence rates are reported as infections per 100 procedures.

RESULTS: The overall infection rate during the study period was 1.3% (30/2,255). The most common causative organism was *Escherichia coli* (96.7%; 29/30). Ninety-seven percent (29/30) of all the clinical isolates were ciprofloxacin-resistant. All 30 case patients were prescribed a ciprofloxacin-based prophylaxis regimen. However, 26.7% of patients (8/30) received ciprofloxacin, alone or as part of a multidrug regimen, for empiric therapy of post-procedure infection.

CONCLUSION: The observed infection rates are similar to those in the published medical literature. A change in the prophylaxis regimen is not necessary at this time, but empiric therapy prescribing practices for post-procedure infections must improve. Ongoing surveillance is required.

SP26

A STUDY OF THE MICROBIAL BURDEN OF NON-STERILE GLOVES. IS IT ADEQUATE TO CONTRIBUTE TO BLOOD CULTURE CONTAMINATION RATES?

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OBJECTIVE: Blood culture contamination can lead to unnecessary antibiotic use, prolonged inpatient stay and increased health care costs. A recent study (Kim et al. Ann Int Med. 2011;154:145-151) suggested that the use of sterile gloves can reduce blood culture contamination rates. The objective of this study was to determine the frequency of contamination of non-sterile gloves in use in our facility.

METHODS: We studied gloves (Medline Advantage, Accutouch Chemo and Sensicare Nitrile) from Medline Industries, Inc. (Mundelein, Illinois). One hundred ten gloves were sampled. After hand sanitization was performed, the top glove from previously opened packages was donned and each of the digits was applied to the surface of a trypticase soy blood agar plate. Plates were incubated for 48 hrs at 35°C in ambient air. Colony forming units (CFUs) were counted; representative colonies of different morphotypes were gram stained and identified.

RESULTS: Forty-five per cent of the gloves were culture negative. The average number of CFUs on the 60 positive gloves was 2.17 per glove. The average number of CFUs on nitrile gloves was not different from that on the polyvinyl chloride (PVC) gloves (0.95 vs 1.2). Fifty-nine of 97

morphotypes (59%) were gram-positive rods. Nine out of nine of those were identified as *Bacillus sp*.; 34 of 97 (35%) of the colonies were gram-positive cocci. Those that were further identified were coagulase negative staphylococci. Four isolates were gram-negative rods.
CONCLUSION: Although more than half of the sample gloves had bacterial growth, the average number of colonies was very low. There were no significant differences between the types of gloves sampled. The very low microbial burden on these gloves makes them an implausible source of blood culture contamination.

SP27
BUBBLE TROUBLE: INVESTIGATING THE SAFETY OF A COMMON HOSPITAL TOY FOR CHILDREN

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OBJECTIVES: Bubbles are commonly used in pediatric hospitals in therapeutic play to help relieve children's anxiety and to facilitate deep-breathing exercises. This study was undertaken to look for any contamination of the bubble solution at our facility, how it compared to other commercial soap bubble products in the market, and whether any contamination could be implicated in hospital-acquired infections.
METHODS: Seven bubble solutions were selected, including the one in use at the hospital. One hundred microlitres from each were cultured on sheep blood agar (BAP), MacConkey agar and phytone agar. Colonies from plates showing growth were sub-cultured for bacterial identification. Five new bottles of the bubble solution with positive bacterial growth were re-tested. The hospital's Infection Control database was checked for nosocomial infections due to these agents.
RESULTS: All samples taken from unopened bottles of the bubble solution in use at our facility grew *Achromobacter xylosoxidans* and *Pseudomonas pseudoalcaligenes*. No other solution showed bacterial or fungal growth. A search of the Infection Control database for the past seven years showed no evidence of ventilator-associated pneumonia, blood stream infections, surgical site infections or shunt infections with these organisms.
CONCLUSIONS: The presence of these water-borne organisms from unopened bubble solutions led us to conclude that these products were contaminated during manufacture. Based on these findings, Infection Control is recommending a change to a brand with no bacterial or fungal growth, as well as regular microbiological surveillance of these products that are not subject to microbiological standards of safety within the toy industry.

SP28
HOW MANY ASTHMATICS NEED TO BE VACCINATED TO PREVENT ONE CASE OF INVASIVE PNEUMOCOCCAL DISEASE?

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OBJECTIVE: The Advisory Committee on Immunization Practices has recommended including adult asthmatics in the high-risk category for pneumococcal (PNC) vaccination based on a 2-fold increase in risk of invasive PNC disease (IPD) in asthmatics. We aimed to determine if the number needed to vaccinate (NNV) in asthmatics warranted its addition to the high-risk category for vaccination in Canada.
METHODS: Using IPD incidence (/10,000) from published papers (4.2 in high-risk, 2.3 in low-risk asthmatics, and 1.2 in healthy people), we calculated the NNV to prevent 1 case of IPD in asthmatics aged 5-17 and 18-50 years, factoring in the proportion of PNC serotype included in vaccines (based on Quebec's data) and accounting for the possibility of waning vaccine efficacy (VE) using four scenarios.
RESULTS: Assuming a VE of 85% for PCV-13 in asthmatics, the NNV would be between 551-617 in low-risk and 302-338 in high-risk children, 227-559 in low-risk and 124-306 in high-risk adults (range depends on waning scenario). Assuming a VE of 65% for PPV-23 in asthmatics, the NNV would be 551-641 in low-risk and 302-351 in high-risk children, 226-1012 in low-risk and 124-554 in high-risk adults.
CONCLUSION: The NNV to prevent one case of IPD in low-risk asthmatics is higher than the NNV to prevent one case of IPD in high-risk conditions such as age >65 years (190-236) and HIV (106-175 for PCV-9). However, the NNVs in high-risk asthmatics may be low enough to warrant vaccination.

SP29
RISK FACTORS FOR RSV-RELATED INFECTIONS AND THEIR OUTCOMES IN ASTHMATIC CHILDREN

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BACKGROUND: Respiratory syncytial virus (RSV) is known to be associated with exacerbation of asthma in children. In this population, data on the risk factors for acquisition and outcomes of RSV infections are limited.
METHODS: We conducted a case-control study (Oct 2006 - Oct 2011) comparing RSV-positive asthmatic children (cases) to RSV-negative asthmatic controls (admitted within one month of the case), 1 case: 2 controls. Nasopharyngeal swabs were tested for RSV by direct immunofluorescence. Clinical information was obtained by chart review. Means were compared using Student's t test and medians using a non-parametric test. Proportions were compared using chi-square of Fisher's Exact test, as appropriate. Conditional multiple logistic regression was performed to calculate OR and 95% confidence intervals (CI) adjusted for the effects of other variables.
RESULTS: Fifty-three RSV-positive cases and 106 controls. Older patients (OR 0.7; 95% CI 0.5-0.9; p=0.002), and those with severe baseline asthma (based on their FEV1 values and treatment profiles) were less likely to be RSV-positive (OR 0.2; 95% CI 0.03-0.7; p=0.02). Cases were more likely to present with prolonged fever (OR 2.6; 95% CI 1.6-4.3; p=0.0002), but were less likely to be admitted to the ICU (OR 0.4; 95% CI 0.1-0.8; p=0.02).
CONCLUSIONS: In summary, the factor that was most significantly associated with RSV admissions among asthmatics was younger age. Our results indicate that RSV-associated asthma exacerbations appeared to result in a less severe clinical course of asthma compared with exacerbations due to other factors.

SP30
MCFLU2 COLD$_3$ PREVENTION: A RANDOMIZED CONTROLLED TRIAL OF VITAMIN D$_3$ AND GARGLING FOR THE PREVENTION OF UPPER RESPIRATORY TRACT INFECTIONS

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OBJECTIVE: We undertook a factorial randomized controlled trial to assess if vitamin D$_3$ supplementation and gargling could prevent upper respiratory tract infection (URTI) in McMaster University students.
METHODS: We randomized 600 students into four treatment arms: 1) vitamin D$_3$ and gargling; 2) placebo and gargling; 3) vitamin D$_3$ and no gargling; and 4) placebo and no gargling. Students completed weekly electronic surveys and submitted self-collected mid-turbinate nasal flocked swabs at study baseline, weekly, and at the onset of URTI symptoms (daily for seven days). Symptomatic students also completed an electronic symptom diary. Students were followed during the months of September and October 2010 and 2011. Logistic regression was used to conduct an intention-to-treat (ITT) analysis, adjusted for housing strata and trial year, with complete cases.
RESULTS: Of 600 participants, 471 (78.5%) completed all surveys, 85 (14.2%) opted out or stopped participating after completing at least one survey and 44 (7.3%) did not complete any surveys. A total of 149 participants reported URTI (43 events were established through adjudication). Seventy participants (23.3%) randomized to receive vitamin D3 reported URTI compared to 79 (26.3%) randomized to placebo (OR=0.73, p=0.11,

CI_{95}:0.496-1.07). Eighty-four participants (28%) randomized to gargle reported URTI compared to 65 participants (21.7%) randomized to the no gargle arm (OR=1.28, p=0.21, CI_{95}:0.87-1.90).
CONCLUSIONS: Results from our study suggested that vitamin D_3 may offer a modest protective benefit against URTI; however, this was not a statistically significant finding. Further study with a larger sample size, longer period of supplementation or a different dose may provide more insight into the role of vitamin D_3 and URTI.

SP31
SOCIAL DETERMINANTS OF ASYMPTOMATIC MALARIA ANTIGENEMIA IN TROPICAL AFRICA

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BACKGROUND: In the context of intensifying efforts toward global malaria eradication, understanding social patterns of malaria transmission will become increasingly important. Social determinants have previously been linked with child mortality but not to asymptomatic malaria carriage.
OBJECTIVE: To examine social determinants of malaria carriage in an asymptomatic pediatric population in central Africa.
METHOD: Cross sectional study of afebrile, healthy children aged two months to five years attending well-child and/or immunization visits in the North Kivu province in eastern Democratic Republic of Congo. Children were tested for malaria antigenemia by rapid diagnostic test and parents simultaneously completed survey questionnaires related to demographics, socio-economic status, maternal education, as well as bednet use and recent febrile illness.
RESULTS: Among 647 children enrolled, 19% were parasitemic (11%, 21% and 23% in Goma, Butembo and Beni, respectively; p=0.006). Increasing levels of maternal education were associated with a lower risk of malaria antigenemia in their children (p=0.001). Children from larger households, and HIV-positive children were also more likely to be parasitemic (p=0.009 and 0.046, respectively). On the other hand, socio-economic index was not statistically associated with malaria antigenemia (p=0.32). In a multivariable logistic regression model, maternal education, number of children under five in the household, and HIV seropositivity remained significant predictors of malaria antigenemia.
CONCLUSION: Children of mothers with low education level appear to be at higher risk of asymptomatic malaria carriage.

SP32
EXAMINING *C. DIFFICILE* INFECTION THROUGH A SYNDEMIC FRAMEWORK

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OBJECTIVE: It has been declared that a baseline level of *C. difficile* in any hospital is the "new normal" in Ontario. Our inability to decrease the number and severity of *C. difficile* epidemics reveals that the current approach to infection control is inadequate. A syndemic approach examines mutually enhancing epidemics sustained by disease interactions and injurious social conditions. Here, a syndemic approach is applied to the most recent *C. difficile* epidemic in the Niagara region. This analysis seeks to open up new avenues of exploration for understanding the persistence of *C. difficile* in Ontario.
METHODS: This application of syndemics theory to the Niagara case considers *C. difficile* infection's (CDI) potential interaction with Inflammatory Bowel Diseases (IBD), and the underlying social circumstances that capacitate their effects.
RESULTS: In the Niagara case, the framing of the *C. difficile* epidemic as a single disease issue led interventions to be focused only on reducing CDI, rather than interventions aimed at ameliorating the complex reality of disease interactions. Infection-control procedures were narrowly focused on eradicating the bacterium inside the hospital, rather than using a more realistic approach which views the hospital and the community as interconnected. In particular, there is the potential for inadequately cleaned ambulances to spread infection. Investigation into the socioeconomic status of victims was absent and the links between hygiene standards and hospital overcrowding and understaffing were seldom explored.
CONCLUSION: If infection control experts, as well as researchers, framed their investigations of *C. difficile* outbreaks with a syndemics framework, the spread of infection would be controlled more efficiently and unnecessary deaths prevented.

SP33
SEVERE *CLOSTRIDIUM DIFFICILE* INFECTION IN PATIENTS WITH NEGATIVE RESULTS FOR *TCDB* BY THE XPERT® *C. DIFFICILE* ASSAY

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OBJECTIVE: The real-time polymerase chain reaction (RT-PCR) amplification curve of the Xpert® *C. difficile* assay (Cepheid, CA) must have a cycle threshold (Ct) within a valid range and endpoint above a set minimum before being considered positive for tcdB. We have noted typical amplification curves that are labelled negative by the Xpert® assay with high Cts or low endpoints not meeting these thresholds. We investigated the significance of these indeterminate (IND) results.
METHODS: All non-duplicate stools with an IND Xpert® *C. difficile* assay result between 5/1/2011 and 18/5/2011 were tested by *C. difficile* toxigenic culture. Chart reviews were completed to assess if patients met the SHEA/IDSA *C. difficile* infection (CDI) case definition and symptom severity. For those who met the CDI case definition, Ct, toxigenic culture results and symptom severity were compared.
RESULTS: During the study period, 11% (387/3620) of specimens submitted for *C. difficile* testing were positive by the Xpert® assay and 1% (47/3620) were IND. Of the 47 patients with IND results, 38 (81%) met the CDI case definition, 7 of whom (18%) met criteria for severe CDI. Toxigenic stool cultures were positive for 86% (6/7) of patients with severe CDI, 23% (7/31) of patients with non-severe CDI, and 44% (4/9) of patients who did not meet CDI case definition (p=0.006). The Xpert® assay Ct threshold that identified all patients with severe CDI was ≤38.5.
CONCLUSION: The majority of patients with stools with IND Xpert® *C. difficile* assay results reported as negative by Xpert® met the SHEA/IDSA case definition for CDI. 18% met clinical criteria for severe CDI. A modified Xpert® assay Ct of ≤38.5 would permit detection of all patients with severe CDI but at the cost of a decrease in specificity. Clinical context is critical to the interpretation of this assay.

SP34
PERFORMANCE OF A COMMERCIAL URINE TRANSPORT SYSTEM FOR STABILIZING BACTERIAL GROWTH

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OBJECTIVES: Prolonged transportation of urine specimens from remote collection locations may lead to bacterial overgrowth and false culture results. Refrigeration is difficult to maintain during transportation. Boric acid preparations such as the UriSwab™ (Copan, Italy) may prevent overgrowth at room temperature. Our objective was to evaluate the effectiveness of this product.
METHODS: We compared microbial population growth in consecutive clinical urine samples received in a hospital microbiology laboratory, after storage for 24, 48 and 72 hours in UriSwab at room temperature and in sterile container at 4°C. Growth was categorically quantified and interpreted according to laboratory protocol. Percent of specimens with significant growth was compared within and between groups using logistic regression.
RESULTS: Eight hundred sixteen urine specimens were received during seven days. One hunder sixty-five specimens (20.2%) demonstrated significant growth before storage, of which 108 (65.5%) were gram-negative bacilli. There was no change in percent positives in urine stored in sterile container at 4°C for the three time points (p=0.346). OR for positivity in

UriSwab at room temperature was 1.54, 2.11 and 2.49 for the three time points, respectively (p=0.014). The percent of samples with significant growth was significantly different between sterile containers and UriSwab at all time points (p<0.001). Among 176 positive specimens developing during storage in UriSwab, 128 (72.7%) were gram-positive cocci.
CONCLUSION: There was greater percent positivity in urine stored in UriSwab at room temperature compared to urine stored in sterile containers at 4ºC. Increased inhibition of gram-positives might improve performance.

SP35
COMBING AUTOMATED URINALYSES WITH AUTOMATED MICROSCOPY TO SCREEN URINE CULTURE

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OBJECTIVE: Screening urine specimens with automated tests may prevent unnecessary culture testing.
METHODS: Consecutive urine specimens received in a university hospital lab were evaluated by automated dipstick urinalysis (Roche Urisys™) and automated flow cytometry (BioMerieux UF1000i™). Logistic regression with forward stepwise conditions was performed to compare the predictive contribution of each test and receiver operating characteristic curves to optimize cutoffs.
RESULTS: Seven hundred thirty-eight urine samples met minimum volume criteria (81.4%). Mean age was 48.3 years and 69.8% were female. 18.4% demonstrated significant growth at a 10^4 CFU/ml significance definition, and 15.2% at a 10^5 CFU/ml significance definition. With a 10^4 CFU/ml definition, bacterial counts alone explained 86.2% of the predictability in culture results, with nitrite explaining an additional 1.5%. Ideal cutoff by optimizing sensitivity and specificity equally would be 516 bacteria/µl, or 45 bacteria/µl by fixing sensitivity at 95% (specificity 40%). With a 10^5 CFU/ml definition, bacterial counts alone explained 96.1% of the predictability in culture results, with nitrite explaining an additional 1.3%. Optimal cutoff considering sensitivity and specificity equally would be 790 bacteria/µl, or 149 bacteria/µl by fixing sensitivity at 95% (specificity 60%). The model is more predictive for men than women, and more predictive for children than adults.
CONCLUSION: Automated urinalysis does not predict culture results when combined with automated bacterial count. Selection of bacterial count threshold could allow a lab to decide on workload reduction by rejecting cultures. Optimizing sensitivity over specificity would minimize false negatives.

SP36
COMPARISON OF TRADITIONAL SCREENING METHOD TO THE MIDI PARASEP® SF METHOD FOR OVA AND PARASITE EXAMINATION

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OBJECTIVES: The traditional method (TM) of screening for ova and parasites (O&P) involves two saline washes and one wash with ethyl acetate and formalin. The Midi Parasep® method (PM) requires a single saline wash, and eliminates the ethyl acetate and formalin steps. This study was designed to compare the TM with the PM in terms of detection, time for preparation (TPRE) and time for processing (TPRO).
METHODS: Forty-seven positive stool samples (SS) for a total of 66 O&P (33 – one parasite, 10 – two O&P, three – three O&P, and one – four O&P) were collected from Kelowna General Hospital and Valley Medical Laboratories (Kelowna, BC). O&P included *B. hominis*, *D. fragilis*, *G. lamblia*, *E. coli*, *E. histolytica*, *E. nana*, *Cryptosporidium* spp., *Cyclospora* spp., *S. stercoralis*, *Enterobius* spp., and *A. duodenale/N. americanus*. Seventeen negative SS were included as controls. SS were concentrated using both methods to make a permanent stained smear and wet mount and read by one technologist blinded to SS findings.
RESULTS: For SS with one O&P, TM detected 85% (28/33), versus 76% with PM (25/33). For SS with more than one O&P, TM detected 82% (27/33) versus 76% (25/33) with PM. For both methods, detection may have been compromised due to low concentration of O&P (in 8 SS) and prolonged (up to one month) time in preservative. Additionally, TM detected three O&P not initially detected in positive SS, indicating the difficulty of O&P examination when multiple parasites are present. A time analysis study demonstrated that per batch of 18 SS, TM requires 43 min more for TPRE and 67 min more for TPRO than PM.
CONCLUSIONS: Sensitivity of TM was 83%, compared to 76% for PM. There is significant reduction in TPRE and TPRO for PM, but O&P detection appears to be superior with TM.

SP37
THE UTILITY OF GASTRIC ASPIRATE IN DIAGNOSING TUBERCULOSIS IN CHILDREN

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OBJECTIVES: To determine the utility of gastric aspirates (GA) in the diagnosis of TB in pediatric patients and to identify any quality issues affecting optimal collection and diagnosis.
METHODS: A retrospective review from 1999-2011 of all TB cultures from the microbiology database. Patients' charts were reviewed for relevant clinical information and diagnosis. We reviewed number of specimens per patient, time of collection, time of receipt in lab, quality issues, positivity rate, and comparison to other specimens (eg, BALs, lung biopsy and sputum). Specimens were collected in disodium carbonate buffer (acid neutralizer).
RESULTS: 786 GAs were collected from 285 patients. GAs constituted 16.9% (786/4677) of all respiratory samples sent for TB investigation. 659/786 (83.8%) of GAs were submitted to the lab within four hours. Median TAT from collection to receipt in lab was 2.5 hr. 222/285 (77.9%) had three or more GAs. TB cultures were positive on 167 specimens from 75 patients. One hundred nineteen were respiratory specimens which included 27 (22.7%) GA. GAs were positive in nine of thirty-four (26.5%) children with culture proven pulmonary TB, five of seven (71.4%) with disseminated TB, one fo one (100%) with solitary cervical node and none of nine with suspected pulmonary TB. Forty-three patients had both sputum and GA. Six of seven sputum samples were positive and only three of seven GAs were positive. Foty-two patients had both BAL and GA. Three of four BALs were positive and only two of four GAs were positive. Six GAs specimens were rejected due to quality issues.
CONCLUSION: TAT to receipt of specimens is good and within the recommended time. There were few quality issues identified. Only 26.5% of those with culture proven pulmonary TB had a positive culture on GA. GA appeared to be less sensitive than sputum or BAL in this population.

SP38
EFFECT OF STAT GRAM STAINS ON THE ANTIMICROBIAL MANAGEMENT OF BACTERIAL MENINGITIS

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OBJECTIVE: It is generally expected that a Gram stain of cerebrospinal fluid (CSF) be treated as a stat specimen and be reported within one hour of receipt. Specimens collected overnight often require the services of an on-call technologist, which entails a significant cost to the laboratory. This may not be cost effective if the result of a Gram stain does not affect management of the patient. The objective of this study was to investigate how often a CSF Gram stain resulted in a significant change in antimicrobial management of bacterial meningitis.
METHODS: This retrospective study reviewed CSF specimens from two adult hospitals in Toronto for the years of 2000-2009. Specimens with positive results (Gram, culture or other) were selected and patient charts reviewed.
RESULTS: 10,841 CSF specimens from lumbar puncture and VP shunts were processed. Three hundred forty-nine had at least one positive test,

including 170 positive Gram stains and 175 positive cultures. Median time from specimen collection to receipt in the lab was 2:45 hours, and from receipt to Gram stain report was 1:15 hours. The majority of patients were empirically started on broad spectrum antibiotics. Antimicrobials were modified in 129 of the 349 positive cases, of which 67 changes were made were due to a culture result and seven were due to a Gram stain result. Modification of antimicrobials based on a positive Gram stain consisted only of discontinuation of some antibiotics; there were no cases where empiric therapy did not cover an organism seen on a positive Gram stain. No modifications were made due to a negative Gram stain.

CONCLUSION: There were no significant modifications of empiric antimicrobial therapy based on a Gram stain result. It may not be cost effective for a laboratory to employ an on-call technologist to process Gram stains as a stat specimen.

SP39

COMPARISON OF BACT/ALERT® FA AND FN BOTTLES TO BACT/ALERT® FA PLUS AND FN PLUS BOTTLES IN TERMS OF ORGANISM RECOVERY, TIME TO POSITIVITY AND GRAM STAIN READABILITY

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OBJECTIVES: Early detection and identification of bacteremia has significant clinical implications. This study compared recovery, time to positivity (TTP) and Gram stain (GS) readability of BacT/ALERT ® FA and FN blood culture (BC) bottles to the charcoal free BacT/ALERT ® FA Plus and FN Plus bottles.

METHODS: Forty-nine organisms; 11 Gram-positive cocci (GPC), eight Gram-positive bacilli (GPB), 12 Gram-negative bacilli (GNB), seven fastidious Gram-negative organisms (FGN), seven anaerobes (ANA) and four yeast (YST) were inoculated (500 CFU) into BC bottles. Except for aerobic Gram-negative bacilli and enteroccoci, 5 mL of human blood was also added to all bottles. GS readability was recorded on a scale of 1 to 4.

RESULTS: Three organisms failed to grow in all bottles (*A.neuii, C.jeikeium, Nocardia spp*). FA Plus recovered all other organisms while FA failed to grow *Lactobacillus spp* (clinical isolate) and *C. jejuni* (one strain-ATCC 33291). FN Plus failed to grow *F. nucleatum* (one strain-ATCC 25596) while FN failed to grow *H. parainfluenzae* (one strain-ATCC 7901). TTP was faster in FA Plus for GPC (0.7 hr) and FGN (11.42 hrs); in FN Plus for GPC (3.16 hr), GPB (4.8 hrs) and FGN (2.76 hrs): in FA for GPB (1.32 hrs), GNB (0.6 hr) and YST (0.3 hr); in FN for GNB(0.24 hr) and ANA (7.08 hrs) . Both FA Plus and FN Plus scored 3.95 for GS readability compared to 1.7 for FN and 1.8 for FA.

CONCLUSIONS: The combination of FA Plus and FN Plus BC bottles demonstrated enhanced recovery of aerobic organisms, significantly improved GS readability and TTP for GPC, GPB and FGN. TTP for GNB and YST was not significantly different between FA Plus/FN Plus and FA/FN. FN performed better in recovery and TTP of ANA. Further study is required for recovery of certain GPB in all bottles.

SP40

EVALUATION OF VITEK II AST-P612 CARD FOR DETERMINATION OF VANCOMYCIN (VAN) MINIMUM INHIBITORY CONCENTRATIONS (MIC) IN *STAPHYLOCOCCUS AUREUS* (SA) AND COAGULASE-NEGATIVE STAPHYLOCOCCI (CNS)

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OBJECTIVES: As Van is used for serious staphylococcal infections, it is important for MIC testing to be accurate. This especially applies to detection of strains with high Van MIC that are reported to be associated with therapeutic failures. This study validated the Vitek II for accuracy in Van MIC determination.

METHODS: Using 370 genetically diverse staphylococci including 270 SA [68 community-acquired (CA)-MRSA; 72 CA-MSSA; 52 hospital-acquired (HA)-MRSA; 13 HA-MSSA; 38 MR-VISA and six MS-VISA (NARSA); 20 daptomycin-non-susceptible SA courtesy, Cubist] and 100 CNS (blood isolates), AST-P612 Van MIC were compared to broth microdilution (BMD) as per 2012 CLSI where MIC 4-8 mg/L is considered intermediate (VI). Analyses were as per Cumitech 31A for essential agreement (EA), category agreement (CA), and minor errors (mE). 95% confidence intervals (95% CI) were calculated using GraphPad QuickCalcs.

RESULTS: For the 270 SA, 35 were identified as VI and 235 VS by BMD. Using P612, EA was 97% (95% CI: 94-99) and CA was 96% (95% CI: 92-97) both of which met Cumitech limits. But, 12 of the 35 VISA (34%) were called VS. This technically corresponds to a mE of 4% (95% CI: 3-8) which meets Cumitech limits, but is of such clinical and epidemiologic importance that it could be treated as a very major error rate of 34% which is outside of Cumitech limits. All 100 CNS studied were VS by BMD. Using P612, no errors were incurred (95% CI: 0-4); the EA was 99% (95% CI: <0.01-6) and the CA was 100% (95% CI: 96-100).

CONCLUSIONS: Compared to BMD, the Vitek AST-P612 technically performed within Cumitech limits. However, it missed 34% of VISA overcalling them as VS. Laboratories cannot rely solely on the Vitek AST-P612 to detect VISA.

SP41

DETERMINATION OF VANCOMYCIN (VAN) MINIMUM INHIBITORY CONCENTRATIONS (MIC) IN *STAPHYLOCOCCUS AUREUS* (SA) USING BIOMÉRIEUX ETEST (ET) AND OXOID M.I.C. EVALUATOR (MICE) GRADIENT STRIPS (GS)

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OBJECTIVES: As Van is used for serious SA infections, confirmation of Van MIC is prudent when values of 2 mg/L or more are found by automated systems such as Vitek II. This study aimed to validate ET or MICE GS for this purpose as they are easy to set-up and more readily available than reference broth microdilution (BMD)

METHODS: A comprehensive dataset of 270 SA (diverse clonal origins/resistance genotypes; CLSI Van MIC <0.5-8 mg/L) was used to test Van MICE and ET GS side-by-side on Mueller-Hinton agar (MHA-Plus, Oxoid). After 24 h at 35°C, GS were read independently by five readers, after which consensus values (where 3/5 had to agree) were compared to BMD values. Analysis was per 2012 CLSI/Cumitech 31A for essential (EA) and category (CA) agreements and minor errors (mE).

RESULTS: By BMD, 239 SA were Van-S [MIC: ≤0.5 (21), 1 (187), 2 (31)] and 31 Van-I [MIC: 4 (25), 8 (6)]. Similarly, by MICE, 201 were Van-S [MIC: 1 (1), 2 (200)] and 69 Van-I [MIC: 4 (63), 8 (6)] and by ET, 229 were Van-S [0.5 (1), 1 (85), 2 (143)] and 41 Van-I [4 (35), 8 (6)]. For MICE, the CA/EA/mE were 85.2% (95% CI: 80.4-89)/84.1% (95% CI: 79.2-88)/14.8% (95% CI: 11-19.6), while for ET, they were 94.8% (95% CI: 91.4-97)/95.9% (95% CI: 92.8-97.8)/5.1% (95% CI: 3-8.6), respectively; mE were mostly in Van-S SA for MICE (39/40) and ET (12/14).

CONCLUSIONS: In contrast to MICE which failed to meet all Cumitech limits, ET successfully met Cumitech limits for CA and EA (>90%) and its mE rate was within the combined mE/major error limit of <7%. However, significantly higher Van MIC were produced by ET (χ^2 for trend, P <0.0001) and by MICE (χ^2 for trend, P <0.0001) compared with BMD suggesting that only Van-S MIC may be used to predict Van-S in SA, and all Van-I MIC should be verified by BMD.

SP42

EVALUATION AND IMPLEMENTATION OF THE BIO-RAD VRE SELECT CHROMOGENIC AGAR AT AN INNER-CITY TERTIARY CARE MICROBIOLOGY LABORATORY

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OBJECTIVES: To evaluate the Bio-Rad VRE Select chromogenic (VRE-chrom) media compared to the currently utilized Oxoid VRE (VRE-A) media for VRE rectal screening of patients admitted to a tertiary care hospital.

METHODS: 30 isolates were utilized for verification of the VRE Select agar compared to Oxoid VRE agar, including 21 previously positive VRE rectal swabs (10 *E. faecium* and 11 *E. faecalis*), *E. faecalis* (ATCC 51299 and 29212), *E. faecium* (ATCC 51559), *E. gallinarum* (ATCC 49573) and 5 non-VRE isolates. Validation involved a parallel comparison of 447 prospectively collected rectal swabs from patients at high risk for VRE colonization. VRE-chrom were read at 24 and 48 hrs by two technologists, while the VRE-A were read at 24, 48 and 72 hrs by one technologist. Technologists were blinded and worked independently of each other. All colonies were worked up utilizing conventional microbiology techniques. VRE were confirmed by sugar utilization and vancomycin Etest.

RESULTS: All VRE isolates in the verification study grew as per the manufacturer's guidelines. Vancomycin susceptible *Enterococcus* did not grow. In the prospective validation, the performance of the VRE-chrom compared to the VRE-A was as follows: sensitivity = 95.7%, specificity = 98.6%, positive predictive value = 78.6%, negative predictive value = 99.8% and efficiency = 98.4%. Twenty-two of twenty-three VRE were alerted to infection control within 24 hrs with the VRE-chrom compared to 17/23 within 48 hrs for the VRE-A. One hundred ninety-four breakthrough colonies grew on the VRE-chrom, though 70.4% of isolates were ruled out with a rapid test.

CONCLUSIONS: VRE-chrom showed high sensitivity, specificity and negative predictive value, with quicker turn-around-time for reporting of VRE positive patients to infection control.

SP43

EVALUATION OF STREPB CARROT BROTH™ VERSUS TODD HEWITT BROTH WITH ANTIBIOTICS AND 5% SHEEP RBCS FOR DETECTION OF GROUP B *STREPTOCOCCUS* COLONIZATION IN NEAR TERM PREGNANT WOMEN

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BACKGROUND: Group B *Streptococcus* (GBS) is the most common cause of early-onset neonatal sepsis in developed countries. The CDC guidelines (2010) for prevention of perinatal Streptococcal disease stated that enrichment broth containing chromogenic substrates can facilitate the identification of GBS.

OBJECTIVE: To compare the performance specifications, cost and turn around time for GBS screening between StrepB Carrot Broth™ and Todd Hewitt (TH) broth with antibiotics.

METHODS: Four hundred eight vaginal/rectal specimens from 204 patients were collected from April 15 to June 20, 2011. Duplicate M40 swabs were collected from each patient. One swab was inoculated in (TH) broth supplemented with 5% sheep red cells with antibiotics (conventional method), and the other inoculated in StrepB Carrot Broth™ (CB). Broth tubes were incubated for 18-24 hours aerobically at 35°C then subcultured to Columbia agar supplemented with 5% sheep red cells. Change to an orange colour of the CB denoting a positive GBS was documented. From the blood agar plates, catalase negative colonies that failed to grow on bile esculin agar were tested by Streptococcal grouping latex kit to confirm GBS.

RESULTS: The sensitivity and specificity of CB compared to TH were 91.1% and 98.1% respectively. Eighty-four per cent of GBS cases showed an orange colour in the CB. The colour change was 100% specific for GBS.

CONCLUSION: StrepB Carrot Broth™ was found to be non–inferior to our current protocol as the sensitivity for GBS detection was not improved. Reporting the orange coloured broths as positive at 18-24 hours has the potential to decrease turn around time.

SP44

EVALUATION OF TWO MALDI-TOF MASS SPECTROMETRY SYSTEMS FOR THEIR ABILITY TO IDENTIFY ROUTINE BACTERIA AND NON-FERMENTATIVE GRAM-NEGATIVE BACTERIA ISOLATED FROM CYSTIC FIBROSIS PATIENTS

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OBJECTIVE: MALDI-TOF mass spectrometry (MS) uses the unique spectrum of molecular masses of each microorganism for identification. Two MALDI-TOF MS systems were evaluated for their ability to accurately identify bacteria/yeast encountered in the microbiology lab.

METHOD: Bacteria/yeast isolated and identified by standard or reference methods were tested on two MALDI-TOF MS systems: the Vitek MS (VTMS; MS-ID database) and Bruker MALDI Biotyper (BMB) systems. Routine bacteria (n=482), and collection of NF-GNB (n=159) from cystic fibrosis (CF) patients were analysed. Bacteria were sub-categorised as easy or difficult in their routine identification, and frequent or rare in their occurrence in the lab. Manufacturers' recommended procedures were used.

RESULTS: Overall, initial analysis shows there was an 91.0% and 93% agreement at the genus level and a 81.7% and 85.5% agreement at the species level with the VTMS and BMB, respectively, for routine organisms. Analysis of each sub-category was also performed. For the NF-GNB from CF patients (the majority of which were *Pseudomonas aeruginosa*, *Burkholderia spp.*, *Stenotrophomonas maltophilia*) there was a 94.3% and 96.2% agreement at the genus level and an 87.0% and 92.2% agreement at the species level for the VTMS and BMB respectively.

CONCLUSION: Both BMB and VTMS agreed with the reference identifications at the genus and species level for >80% of microorganisms analysed in this study, including the NF-GNB isolated from the sputum of CF patients. 16S rRNA PCR will be used to verify reference identifications where discrepancies were observed. MALDI-TOF MS is a potentially useful tool for the identification routine and difficult to identify bacteria in the microbiology lab.

SP45

EVALUATION OF THE DNA FRAGMENT DT-1 AND IS1311 FOR THE IDENTIFICATION OF *MYCOBACTERIUM AVIUM* COMPLEX (MAC) SPECIES IN A REAL TIME PCR ASSAY

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OBJECTIVE: The MAC Accuprobe (Gen-Probe) has been shown to be a reliable method for the identification of MAC from positive cultures. However, not all serotypes are detected and hybridization probes are not intended for direct detection. We evaluated two previously described targets, DT-1 and IS1311 (JCM. 2010. 48:4057) in a multiplex PCR assay using the Spartan DX12 for identification of MAC species.

METHODS: Primers for DT-1 (DTF- AGCACGCCATGCCAGGTC; DTR-CTAGTTGGATCGCGCCGAACAC), IS1311 (ISF- GCGTGAG R CTCTGTGGTGAA; ISR-TAAATCGTCTCGGCGGCCTG) and the probes for both targets (DT-1: 6FAM-TGCTGTCACGACACCGGGTGG; IS1311: 6FAM-CGATCAGCGGCATCGGCATTGC) were designed based on GenBank sequences. A total of 94 MAC and 40 non-MAC (five *M. gordonae*, six *M. chelonae*, nine *M. fortuitum*, four *M. kansasii*, two *M. lentiflavum* and 14 *M. tuberculosis*) previously identified clinical isolates were tested using both sets of primers and probes in a multiplex reaction. DNA was extracted by suspending the isolates from a pure culture on 7H11 agar in

sterile water, sonicated for 15 minutes and heated at 95°C for 30 minutes. The limit of detection for the multiplex PCR was determined by serial dilution of DNA extracted from strains shown to harbour DT-1 and IS1311, IS1311 only and DT-1 only.

RESULTS: Of the 94 MAC isolates, 89 (95%) were detected by PCR and five failed to amplify after repeat testing. None of the 40 (100%) non-MAC strains were detected by the multiplex PCR. The limit of detection for the DT-1/IS1311 multiplex assay was determined to be <6 fg of DNA, for the DT-1 primers in uniplex PCR 28 fg of DNA and for IS1311 primers in a uniplex PCR <18 fg of DNA.

CONCLUSION: The DT-1/IS1311 multiplex PCR assay detected the majority of MAC clinical isolates and none of the non-MAC mycobacterial species. The assay detected low levels of DNA which is desirable for detection of MAC directly from clinical samples. Despite the high specificity, 5% of the clinical isolates failed to be detected by the multiplex PCR suggesting that the DT-1/IS1311 targets may not be as universally distributed among MAC species as previously predicted. Further characterization of these strains is warranted.

SP46

CUTANEOUS CRYPTOCOCCOSIS AS A MANIFESTATION OF CRYPTOCOCCEMIA IN A SOLID ORGAN TRANSPLANT (SOT) PATIENT

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INTRODUCTION: Far from being a rare disease only a few decades ago, Cryptococcosis has grown to become a common opportunistic infection worldwide. This is attributed to the rise in immunocompromised humans including SOT recipients.

CASE DESCRIPTION: We present a case of a 63-year-old male with history of liver transplant on chronic immunosuppression, ESRD and Type 2 DM who presented to his hemodialysis session with left lower limb erythema and localised pain. Blood cultures were drawn and he was treated for probable SSTI as an outpatient with IV cefazolin. By Day 5, his blood cultures grew yeast and he was called back to the hospital. He was admitted and treated with IV cefazolin and IV fluconazole for bacterial SSTI and presumed Candida line infection. Subsequently the yeast was further characterised to be *Cryptococcus neoformans*. Given lack of improvement in the SSTI on therapy, a biopsy was taken. Tissue culture was positive for *Cryptococcus neoformans*. His CSF analysis was normal however his chest x-ray showed possible right hilar airspace disease. He was treated with a two-week course of IV amphotericin B induction therapy for cryptococcosis with pulmonary and cutaneous involvement. He was discharged home on oral fluconazole to complete rest of the course of therapy.

CASE DISCUSSION: Cryptococcosis is a serious post-transplant infectious complication affecting ~2.8% of all solid organ transplant recipients with mortality rates up to 42%. Skin is the third most common site involved in these patients, with manifestations ranging from nodular mass, abscess/ulcers, cellulitis to rarely necrotising fasciitis. Cutaneous manifestations frequently represent disseminated infection.

Thursday May 4
John Conly Innovation Academy Posters
Room: Grand Ballroom

IA1

HOSPITAL WIDE ROLL-OUT OF ANTIMICROBIAL STEWARDSHIP: A STEPPED WEDGE RANDOMIZED CONTROLLED TRIAL

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Inappropriate antimicrobial use, increasing antibiotic resistance, and lack of development of new antimicrobial agents have provided the impetus for worldwide initiatives in antimicrobial stewardship. However, there is paucity of good quality evidence to evaluate the impact of such initiatives. As a result of the positive impact our institution's Antimicrobial Stewardship Program (ASP) had in our critical care units, we rolled out our stewardship program to seven patient care services across the hospital using a stepped wedge design to provide a more rigorous evaluation of the efficacy of our interventions. As such, the objective of this study was to determine the impact of antimicrobial stewardship on antimicrobial use and *C. difficile* infection rates in seven non-intensive care medical and surgical services. A formal review of all patients on their 3rd or 10th day of broad-spectrum antibiotic therapy in seven patient care services was conducted at our institution using a stepped–wedge randomized design. The primary outcome for the preliminary 11 month analysis was a comparison of days of therapy (DOTs) of targeted antibiotics/1000 patient bed days/month during the intervention period compared to the control period. Secondary outcomes included overall and non-targeted antibiotic use and rates of nosocomial *C.difficile* infections. From November, 1, 2010 to July 31, 2011, the ASP reviewed a total of 1,042 orders with an overall suggestion and acceptance rate of 48% and 84%, respectively. Across all services, our program reduced broad-spectrum antimicrobial use by 12% (256 to 225 DOT/1000 patient days) and the number *C. difficile* infections by 59% (44 to 18 cases).Our institution's ASP hospital-wide roll-out was, therefore, successful in reducing broad-spectrum antimicrobial use and *C. difficile* infection rates.

IA2

THE MINI-MICROBIOLOGY COURSE : A SUPERIOR WAY TO TEACH INTRODUCTORY MICROBIOLOGY TO RESIDENTS

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OBJECTIVE: We radically restructured our residents' first three months of rotation in clinical microbiology to address problems and complaints. Historically, when residents first come to most microbiology laboratories they were assigned to benches and rotated throughout laboratory. There are numerous problems with this approach: The experience was not often hands-on or standardized; the work of the technologist is often impeded; some technologists are not willing or skilled teachers; individual technologists have inadequate exposure for the purposes of evaluation; faculty teaching is often out of step with bench rotations and is often inefficient when only one or two residents are in the lab at the same time; bench teaching to specific objectives is difficult and many pathogens are not encountered;

INTERVENTION: We bring residents (MM, ID and GP) to the laboratory for a 12 week mini-course. Technologist volunteers are chosen based on experience and feedback from learners. Specific goals and objectives were developed and a system-based approach is employed for bacteriology teaching. Residents perform their own work hands-on with mock samples

ensuring that all specimen types and relevant organisms are included. Afternoon small group sessions with faculty employ case-based learning relevant to the system of the week. We use a multi-headed microscope and dedicated space.

OUTCOME: The mini-course provides a cooperative learning environment where residents from different disciplines work synergistically. The focus is on hands-on learning with a dedicated highly skilled technologist ensures exposure to all relevant organisms. Faculty teaching is efficient and carefully aligned with in-lab teaching. Examination of residents to specific objectives and the evaluation of both residents and faculty is greatly facilitated. Resident, technologist and faculty feedback has been excellent. In an anonymous evaluation by residents (eight parameters, range zero to 10) there were no scores less than nine.

IA3
WITHDRAWN

IA4
MANAGEMENT OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*-POSITIVE PATIENTS IN A SPECIALIZED AMBULATORY CLINIC

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OBJECTIVE: To assess the impact of a specialized infection and prevention control (IPC) ambulatory clinic with a mandate to assist in the care of outpatients colonized with methicillin-resistant *Staphylococcus aureus* (MRSA) and provide consistent education and follow-up with the goal of improving patient outcomes and healthcare resource management.

METHODS: In April 2008, the Saint John Regional Hospital opened a clinic specifically designated for the follow-up and management of patients in the community affected by MRSA. The clinic is coordinated by a full-time registered nurse (RN) and directed by an infectious diseases physician with an operating budget of less than $150,000 per year. The clinic offers a central source of education and specialized follow-up for MRSA-positive patients, contacts and their families. MRSA status is tracked, decolonization offered as the patient's situation warrants, and screening of family members provided as indicated. The clinic also works collaboratively with local physicians and other community healthcare partners to ensure each case is managed optimally guided by individual clinical factors. The clinic is also the site of a randomized controlled trial assessing decolonization strategies.

RESULTS: The clinic has seen 395 MRSA-positive patients over a total of 1760 visits, and screened 481 MRSA inpatient contacts who had been discharged from hospital and required follow-up screening in the community. Additionally, as 11-29% of MRSA colonized patients go on to develop active infection, with the Clinic's 308 successful decolonizations, the cost savings could be as great as $500,000 – $1,200,000. Prior to its' opening, follow-up would have been required through the family physician's office at an estimated cost to the provincial Medicare of $112 per visit. Therefore, with close to 2,500 patient visits at the IPC MRSA ambulatory clinic, this translates into a further cost savings of more than $250,000 to the province over four years.

CONCLUSION: The specialized education and management provided by the clinic has served to overcome a great deal of confusion, fear and stigma that MRSA-positive patients often encounter. Improved follow-up of colonized patients and contacts, appropriate decolonization and recognized clearance alleviates pressure on inpatient resources for private rooms and transmission-based precaution-associated costs. Additional pressure on busy family physicians to follow patients specifically for their MRSA positive status has been alleviated. And finally, by the clinic's provision of timely and specialized decolonization, many MRSA infections and their associated costs have been averted.

IA5
THE INFECTIOUS DISEASE SUSCEPTIBILITY PROGRAMME: TRANSLATIONAL RESEARCH INTO HUMAN GENETIC SUSCEPTIBILITY TO INFECTIOUS DISEASES (OR "WHY IS MY PATIENT SICK?")

DC VINH
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BACKGROUND: Patients suffering from serious, unusual, recurrent or recalcitrant infectious diseases often ask, "Why is this happening to me?". Although clinicians often suspect a "problem with the immune system" in these cases, this explanation remains abstract and conceptual, rather than specific and practical. Growing evidence from work by myself and others increasingly demonstrate that these idiosyncratic cases of unusual susceptibility to infections are due to specific and demonstrable abnormalities of the immune system, mediated by defects in single genes. Further, these monogenic immunodeficiencies are increasingly recognized in adults. Identification of these genetic immunodeficiency syndromes leads to rational implementation of novel treatment approaches, including immunotherapeutics, to manage infectious diseases.

OBJECTIVE: To understand why some individuals are unusually susceptible to infectious diseases.

METHODS: The "Infectious Disease Susceptibility Programme" was established at the McGill University Health Centre to conduct translational research on human genetic susceptibility to infectious diseases. The Programme has two components:

(1) CLINIC: Patients with unusual infection diathesis (severe/refractory/recurrent) are referred for recruitment onto protocol and characterization of their clinical, microbiological and immunological phenotypes. For patients suspected of having known genetic immunodeficiency syndromes, specialized diagnostic testing in reference laboratories is pursued. For patients suspected of having novel genetic immunodeficiency syndromes, further research investigations are pursued;

(2) RESEARCH: From patients of interest and relevant family members, we have a curated tissue repository. Combining functional immunological and genetic investigations, both in my laboratory and via intra- and extramural collaborations, we strive to identify the immunogenetic basis for these novel genetic immunodeficiency syndromes.

RESULTS: To date, the Programme has identified five known genetic immunodeficiency syndromes in five unrelated subjects (Chronic Granulomatous Disease; Complement deficiency; Turner syndrome; Alpha1-anti-trypsin deficiency; Autoimmune Lymphoproliferative syndrome). Approximately 20 other subjects are robustly characterized phenotypically and undergoing in-depth investigations.

CONCLUSION: By integrating immunology and genetics to the study of infectious diseases, this Programme aims to identify and understand the critical determinants of human susceptibility to infections. Understanding these defects provides an opportunity to improve the care of these patients and to develop new treatment strategies for these infections. Ultimately, the goals of the programme are to be able to specifically answer the patient's question "Why is this happening to me?" and to then follow with "And here's what we're going to do about it..."

IA6
NASAL DECOLONIZATION OF *STAPHYLOCOCCUS AUREUS* WITH ANTIMICROBIAL PHOTODYNAMIC THERAPY

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OBJECTIVE: Traditional pre-operative strategies to decrease surgical site infections (SSIs) include nasal decolonization with mupirocin ointment and chlorhexidine washes (CHGW). In June 2011, a new program that includes preoperative CHGW and intranasal antimicrobial photodynamic therapy (aPDT) was introduced at Vancouver General Hospital. This interim-analysis describes the effectiveness of aPDT in decreasing the bioburden of intranasal *S. aureus*.

METHODS: Over a six month period, patients having cardiac-, orthopedic-, spine-, thoracic-, vascular-, breast- and neurosurgery procedures were

treated with CHGW the night prior to surgery and with nasal aPDT in the preoperative waiting area. Nasal swabs to assess colonization with *S. aureus* (including MRSA) were taken just prior to and right after aPDT; growth was graded as zero, light, moderate or heavy as per standard microbiology methods. Excluded were duplicate cases and cases within a six-week introductory period.

RESULTS: In total, 2221 patients had the selected surgeries in the interim analysis period. Three hundred thirty-nine cases within the introductory period, 17 duplicate cases and six errors were excluded, leaving 1869 cases for analysis. Patient-reported compliance with CHGW was 96.5% (1803/1869). Compliance with LPN-administered aPDT was 98.8% (1847/1869). Microbiology data was available for 1855 cases. Pre-aPDT colonization rates for MSSA and MRSA were 24.4% (453/1855) and 0.9% (16/1855), with six cases (0.6%) positive for both. Partial or complete decolonization when comparing pre and post-aPDT microbiology data was 85.0%. There was one reported adverse event.

CONCLUSION: In contrast to similar studies with CHGW and mupirocin pre-operative bundles, this program achieved very high compliance rates. aPDT is safe and microbiologically effective.

IA7

PHYSICIAN HAND HYGIENE CHAMPIONS

V LEUNG, E LLOYD-SMITH, M ROMNEY

St Paul's Hospital, Providence Health Care, Vancouver, BC

OBJECTIVE: Hand hygiene (HH) is recognized as the most effective measure for preventing healthcare-associated infections. However, despite our overall successes and implementation of a multimodal HH campaign based on the World Health Organization (WHO) toolkit, HH audits show that physicians consistently underperform when compared to other healthcare worker groups. Our objective is to significantly improve physician adherence to HH by encouraging physician developed, unit specific approaches. An improved understanding of physicians' knowledge and attitudes towards hand hygiene, and of barriers and enablers to adherence to HH will assist in developing HH programs. The establishment of Physician HH Champions in multiple medical specialties will begin a process of changing social attitudes towards hand hygiene.

METHODS: Physician HH Champions were recruited from all medical and surgical specialties at Providence Health Care. Preparation work by physicians included (i) completing WHO HH perception and knowledge surveys, (ii) watching the Hand Hygiene Video in Clinical Medicine (New England Journal of Medicine), (iii) reviewing the WHO technical reference manual for HH. Physicians were then required to attend a one hour focus group which consisted of six standardized questions and probing questions to explore physicians' barriers and enablers (structural, organizational, cognitive and social) to HH adherence. Focus group sessions were recorded and transcribed verbatim for qualitative analysis.

RESULTS: From January 1 to March 31, 2012, 103 staff physicians from medical/surgical specialties and family practice completed the current phase of the Physician HH Champions project. Survey information and themes from focus groups have been analyzed and will be presented. More importantly, many physicians have already participated in process redesign in collaboration with the Infection Prevention and Control Department to improve HH adherence.

CONCLUSION: Infection Prevention and Control teams may have the knowledge and expertise in HH, but knowledge translation and interventions are difficult to implement successfully unless there are local champions and role models. By engaging physicians to be local champions for HH, we believe that that there will be different approaches to improving HH adherence which will be sustainable and successful in all areas of the hospital. Physician HH Champions at Providence Health Care have already started affecting behavioral and cultural change among peers, medical trainees and other health care workers.

Thursday May 3
THURSDAY POSTER PRESENTATIONS
ROOM: Grand Ballroom

TP1

DISPARATE RESULTS FROM A SURVEY OF PERSONAL HYGIENIC PRACTICES (PHP) AND INFECTION CONTROL KNOWLEDGE (ICK) FROM NOVEL DESIGN (ND) VS HISTORIC DESIGN HOSPITAL WARDS (HD) HOSPITAL WARDS – EVIDENCE FOR A PURITY RISK RITUAL EFFECT

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OBJECTIVES: We explored the differences in PHP and ICK among healthcare workers (HCW) from ND (single bed rooms with enhanced hand hygiene [HH] capacity) vs HD wards (multibed rooms with poor HH capacity), which may be associated with reduced transmission of nosocomial infections.

METHODS: A two page anonymous survey of close-ended questions related to PHP/ICK was administered to 582 HCWs from seven medical units at two hospitals (one NDU; six HDU). Basic demographic data was collected on work status (full time, part time or causal), weekly work hours, patient load, age, and years of experience. Specific questions focused on when/what ICK was taught, which PHP were followed, difficulties encountered for HH, and specific scenarios related to HH, isolation precautions, personal illness, and sharps injuries. Differences between groups were analysed using χ^2 with continuity correction or Fisher's exact test.

RESULTS: A response rate of 29% was found, with the majority (67.8%) coming from nursing staff (NS). No significant demographic differences among NS and allied HCWs were found. Although 55% of NS were given core ICK in training, only 35% reported any additional training in the previous two years. NS from the ND vs HD ward encountered significantly less difficulty ($p=0.03$) performing HH but performed less well for ICK scenarios on diarrhea ($p<0.001$) and for specific PHP ($p=0.03$) that were followed >75% of the time.

CONCLUSIONS: Despite the advantages for NS on a ND vs HD ward, the performance scores for ICK and PHP were significantly less for nurses on a ND ward. With no demographic or training differences, these results suggest a purity risk ritual effect. More attention needs to be focused on the impact of purity risk ritual.

TP2

INFLUENCES ON FAMILY PHYSICIAN ANTIBIOTIC PRESCRIBING FOR UNCOMPLICATED URINARY TRACT INFECTIONS

P LAM[1], B FORD[1], C WEBSTER[2], D ZOUTMAN[1]

[1]School of Medicine, Queen's University; [2]Kingston Family Health Team, Kingston, ON

OBJECTIVE: Suboptimal antibiotic prescribing for urinary tract infections (UTIs) contributes to antimicrobial resistance, resulting in decreased antibiotic efficacy, prolonged patient morbidity and increased healthcare costs. Identifying the factors which influence inappropriate prescribing will help to develop programs to improve antibiotic use and reduce antimicrobial resistance.

METHODS: Physicians practicing family medicine in southeastern Ontario were invited to participate in an online survey which gathered demographic and practice setting information, continuing education sources, system and patient-related influences on prescribing for UTIs, and treatment of six patient-based UTI scenarios.

RESULTS: Patients having frequent UTIs and patients self-identifying symptoms as similar past UTIs were moderately or very influential in the initiation of empiric therapy in 66.7% (38 of 57) and 56.9% (33 of 58) of respondents respectively. Antibiotic allergies, urine culture and sensitivities, and patient co-morbidities were moderately or very influential in the

selection of antibiotics in 98.3% (57 of 58), 94.8% (55 of 58) and 87.7% (50 of 57) of respondents respectively. In the UTI scenarios, only 25.9% (15 of 58) of respondents appropriately treated a UTI in pregnancy with cephalexin or nitrofurantoin. 70.7% (41 of 58) of respondents appropriately managed a case of asymptomatic bacteruria and 62.1% (36 of 58) of respondents appropriately treated a TMP/SMX resistant mild pyelonephritis with a fluoroquinolone.

CONCLUSION: Inappropriate antibiotic prescribing by family physicians for community-acquired UTIs continues to be an issue in Ontario. Patient pressure and physician knowledge deficits may contribute to inappropriate prescribing. Future interventions focusing on these factors may help to decrease inappropriate antibiotic use.

TP3

STREPTOCOCCUS PNEUMONIAE COLONIZATION IN THE NASOPHARYNX OF PATIENTS INFECTED WITH THE PANDEMIC H1N1 (2009) VIRUS

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Viral and bacterial synergisms are frequently observed and discussed intensively in literature. Our study looked at the association of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Staphylococcus aureus* with pandemic H1N1 (2009) virus [pH1N1 (2009)], during the second wave, July 1-Dec 31, 2009 of the recent influenza pandemic. Nasopharyngeal swabs (NPS) submitted to the Provincial Laboratory for Public Health (Alberta) were tested for influenza virus using RT-PCR. Each pH1N1 (2009) virus positive case was matched to two pH1N1 (2009) virus negative controls based on: age (+/− 10 yrs), gender, date sample was received (+/− 2 weeks), and geographical region. Using end-point PCR assays, *LytA* and *Ply* genes were targeted to identify *S. pneumoniae*, the *BexA* gene for *H. influenzae* and the *Nuc* gene for *S. aureus*. Two Fisher's chi squared analysis were performed on 31 pH1N1 (2009) positive specimens with different sets of negative controls. First analysis had 62 pH1N1 (2009) negative controls that also had other respiratory viral pathogens. Second analysis had 42 pH1N1 (2009) negative controls that no respiratory viral pathogens. Results of first analysis showed no association between pH1N1 (2009) and *S. pneumoniae* (p=0.43). However, results of the second analysis which was based on only 42 pH1N1 (2009) negative controls showed a significant association between pH1N1 (2009) and *S. pneumoniae* (p<0.05, 95% CI). This association was not seen with *H. influenzae* or *S. aureus*. Over all, our study has shown that there is a relationship between pH1N1 (2009) virus infection and *S. pneumoniae* colonization in the NP of patients. Our study concludes that this relationship could only be identified using pH1N1 (2009) negative control specimens that contained no other respiratory viral pathogens.

TP4

PNEUMOCOCCAL CARRIAGE IN THE NURSING HOME ELDERLY IN THE NON-OUTBREAK SETTING

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McMaster University, Hamilton, ON

BACKGROUND: Though the burden of disease due to *Streptococcus pneumoniae* is high in the elderly, the incidence of *S. pneumoniae* carriage in the nursing home (NH) elderly outside of outbreak settings has not been well described.

METHODS: In December 2010, NH residents from 4 NH in Hamilton, Ontario were enrolled in a longitudinal cohort study where nasopharyngeal (NP) swabs (Copan ESwabs) were collected monthly on asymptomatic NH residents during the influenza season (January – May 2011). A validated sensitive and specific real-time PCR to detect *S. pneumoniae* was performed on the NP samples.

RESULTS: 30 NH residents were enrolled. The mean age was 87 years, 83% were female and all had at least one co-morbidity. Most (77%) were non-smokers, 23% had exposure to children and only 50% had received pneumococcal vaccination within the last five years. During the study, one patient died, none developed pneumonia and 1 had influenza. In total, 133 NP swabs were obtained on 30 residents. 20 residents had five NP swabs obtained, five had four obtained, three had three obtained and two had two obtained. Reasons for missing swabs included patient death (n=2), hospitalization (n=1) and refusal for that month (n=14). All swabs were negative for *S. pneumoniae*.

CONCLUSIONS: Despite the high incidence of infection due to *S. pneumoniae* in the NH elderly, carriage with *S. pneumoniae* outside of the outbreak setting was absent in this study. This may suggest that NH elderly are unable to control pneumococcal carriage once exposed.

TP5

SCABIES AND THE ELDERLY: WHAT COULD POSSIBLY GO WRONG?

MK CHARLES[1], D FAULDER[2], PM TOMLIN[3], K KOWALEWSKA-GROCHOWSKA[1,3]
[1]University of Alberta; [2]Alberta Health Services; [3]Provincial Laboratory for Public Health, Edmonton, AB

OBJECTIVES: Scabies occurs commonly in healthcare settings. Presentation of scabies can be atypical in elderly, demented or immunosupressed patients. Microscopical identification of mites and, less commonly, eggs, in skin samples is diagnostic. We describe an index case of scabies outbreak in a nursing home resident, diagnosed by the presence of eggs in skin scrapings.

METHODS: Skin scrapings were submitted to the laboratory in a mycology envelope and ,thus, the sample was initially processed for fungal investigations. A wet mount of the sample was later processed by the parasitology bench. Retrospective chart review of the index case and interviews with the nursing home physicians were carried out. Positive cases were defined as any nursing home resident with characteristic lesions.

RESULTS: Eggs and empty shells, but no parasites were seen in skin scrapings. The index case was a previously healthy elderly man who developed crusted scabies. His infection spread to a group of female residents with whom he shared a close contact. Outbreak duration was January-March 2011. A total of eight residents and health care workers were affected. Basic outbreak measures were taken to manage the situation. All cases responded well to 5% permethrin cream.

CONCLUSION: Our case stresses the importance of scabies as a shared pathogen in an elderly institutionalized population. The index case had crusted scabies; surprisingly, there was no underlying disease that usually accompanies this presentation. Laboratories should be sensitized to the fact that on occasions, only eggs and egg shells are present in skin scrapings. Health care workers should raise awareness of scabies as an important pathogen in view of aging of the population.

TP6

SAFETY AND EFFICACY OF POSACONAZOLE (POS) FOR THE TREATMENT OF INVASIVE FUNGAL INFECTIONS (IFI) AFTER FAILURE OF ONE PRIOR LINE OF THERAPY

S HAIDER[1], M LAVERDIÈRE[2], M POWER[3], C ROTSTEIN[4], S HUSAIN[4], J LIPTON[4], K DOUCETTE[5], P PHILLIPS[6], A GALARNEAU[7], JF POULIOT[7]
[1]Hamilton Health Sciences, Hamilton, ON; [2]Hôpital Maisonneuve-Rosemont, Montréal, QC; [3]BC Cancer Agency, Vancouver, BC; [4]University Health Network, Toronto, ON; [5]University of Alberta, Edmonton, AB; [6]University of British Columbia, Vancouver, BC, [7]Merck Canada, Montréal, QC

BACKGROUND: IFI is a common problem during AML induction or Graft Versus Host Disease (GVHD). Several options are available as first line therapies. However, second line interventions are limited by cumulative toxicities, drug interactions and potential resistance which are often encountered. POS is a triazole antifungal with broad yeast and mould coverage that possesses a favorable toxicity profile.

METHODS: A single arm phase II multicenter study of POS in patients with IFIs who failed or were intolerant to other antifungal therapy. The primary objectives of the study were to evaluate the safety (n=37) and efficacy (n=27).

RESULTS: Thirty-seven patients with proven (35%), probable (34%) and possible (31%) IFIs were included in the study. Conditions leading to

IFI were mainly hematological malignancies (36%) and stem cell transplant (27%). The study population included mostly patients refractory (70%) and/or intolerant (25%) to prior antifungal therapy. First line therapy included largely voriconazole (49%) and amphotericin B (24%). Identified pathogens were mainly *Aspergillus* (50%) and *Candida* (20%). Overall response in proven or probable IFI was 56% including 10% CR and 45% PR. More than 40% of patients entered the study with elevated liver enzymes most of whom (70%) received prior azole therapy. Of those, 75% had normalization of their liver enzymes within 12 weeks of POS initiation. Toxicities reported regardless of causality were mainly nausea and vomiting (26%) and diarrhea (26%). No grade 4 toxicities were reported.
CONCLUSION: POS was effective therapy in patients with refractory IFI previously treated with other antifungal therapy. Switching from another azole to POS was also possible even in patients with hepatic dysfunction at study start.

TP7

POSACONAZOLE UTILIZATION – AN INSTITUTIONAL REVIEW ONE YEAR AFTER APPROVAL FOR PROPHYLAXIS OF INVASIVE FUNGAL INFECTIONS IN SEVERELY NEUTROPENIC PATIENTS

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In 2010, a decision was made by the Antimicrobial Stewardship Committee in the former Capital Health (Edmonton) region to provide posaconazole prophylaxis to patients at high risk for invasive fungal infections. Criteria were developed and approved in collaboration with infectious diseases, haematology, and pharmacy divisions and included any patients with existing or anticipated ANC of 0.5 x 10^9/L or less for at least 10 days, and where posaconazole would be continued until seven days after resolution or discharge. With the patient population seen at University of Alberta Hospital, this meant we anticipated patients undergoing remission or salvage induction chemotherapy for acute myelogenous leukaemia (AML), or those with myelogenous dysplastic syndrome (MDS) would be appropriate candidates for prophylaxis. One year later, a review of where posaconazole has been used at the University of Alberta Hospital has been undertaken to assess compliance with these criteria and is presented here, looking at numbers of patients prophylaxed, costs associated with prophylaxis and rates of invasive fungal infections seen in those patients where prophylaxis was used.

TP8

INSTITUTION OF TARGETED POSACONAZOLE PROPHYLAXIS IN HIGH RISK FEBRILE NEUTROPENIA: RESULTS OF PROSPECTIVE INVASIVE FUNGAL INFECTION (IFI) SURVEILLANCE

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[1]University of Alberta; [2]Infection Prevention and Control, UAH; [3]Alberta Health Services, Pharmacy, Edmonton, AB

OBJECTIVE: To prospectively assess the burden of IFIs in a hospitalized cohort of high-risk, febrile, neutropenic patients after institution of posaconazole prophylaxis for a subset of patients. Abstract data will include Jan through Sept 2011, presentation data data will include through Dec 2011.
METHODS: Adult hematology inpatients with severe neutropenia (ANC of 0.5 x 10^9/L or less, for at least 10 days, until seven days after resolution or discharge) were followed prospectively and IFI were classified using the International Consensus Committee definitions of Definite or Probable. Posaconazole prophylaxis was instituted in Q2-4 for patients undergoing remission induction or salvage chemotherapy for AML.
RESULTS: Over Q1-Q3 of 2011 there were 83 neutropenic episodes and 1838 neutropenic days. Three IFI were observed: sinus-orbital *Neosartorya fischeri* in an aplastic anemia patient, *Candida albicans* sepsis in a CLL patient, and iliopsoas abscess with pure *Candida tropicalis* in an AML patient post colectomy for *C.difficile*. If a limited "Possible" category (neutropenic hosts, with nodules on CT, clinically treated as aspergillosis) were counted, there were six additional cases (two pre-program, three ineligible for prophylaxis, and one who received posaconazole). In Q2 and Q3 respectively, 3/7 and 10/10 eligible patients received posaconazole, with one stopping due to drug-drug interaction concerns. There were no definite or probable cases in prophylaxed patients. The 2011 IFI rate will be presented, and compared with the pre-POSA-P rate in 2010.
CONCLUSIONS: The observed IFI were outside the prophylaxis program. A "Possible" category restricted to highly suggestive CT criteria is being considered to improve concordance of infection control and clinical followup.

TP9

PITFALLS IN THE DIAGNOSIS OF INVASIVE ASPERGILLOSIS IN HEMATOLOGY-ONCOLOGY: HOW TO INCORPORATE GALACTOMANNAN AND CT RADIOLOGIC CRITERIA?

L SAXINGER, I SANDHU, G TAYLOR
University of Alberta, Edmonton, AB

OBJECTIVE: To critically review a series of six possible invasive fungal infection (IFI) in immunocompromised hosts occurring over an eight week period , with attention to the use of galactomannan (GM) testing and CT scan results.
RESULTS: Patients: four AML , one red cell aplasia , and one myelodysplasia; two (AML) presented with circulating blasts and abnormal chest imaging at four and nine days after admission, two were non-neutropenic (aplastic and myelodysplasia) and two were neutropenic for 10 and 21 days prior to IFI suspicion. No cases had positive fungal cultures. Three patients were BAL GM positive, two serum GM positive. Two patients died. Three were counted as cases by IPC surveillance.

Table 1: Description of initial radiologic findings

		EORTC CT scan criteria
1	Many small noncavitating ground glass nodules	Not met
2	Early consolidation bilaterally	Not met
3	Scattered peribronchial peripheral consolidation	Not met
4	Nodular/masslike foci LLL and RUL	Yes
5	CXR: nonspecific widespread consolidation	Not met
6	1.4 cm RUL nodule with surrounding halo	Yes

CONCLUSIONS: Both IPC criteria and clinical suspicion for IA have a low threshold, to ensure outbreak detection and appropriate patient therapy. All patients in this series received antifungal therapy for clinical suspicion, but classifying cases for surveillance and targeted prophylaxis programs remains important. CT chest findings frequently raise suspicion without meeting EORTC criteria for IFI diagnosis. BAL GM is thought to have enhanced sensitivity but higher risk of false pos results, so the finding of a sole positive BAL GM requires elucidation.

Friday May 4
FRIDAY POSTER PRESENTATIONS
ROOM: Grand Ballroom

FP1

ACTINOMYCOSIS IN A DENTIGEROUS CYST: PATHOLOGIC DIAGNOSIS OF A VERY RARE CASE IN THE MANDIBLE

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OBJECTIVE (INTRODUCTION): Many documented cases of oral actinomycosis, the infection with *Actinomyces* group of bacteria have described the classic diagnostic features which have led to an easy confirmation. The case discussed here is an extremely rare condition where presence of actinomycosis was not diagnosed clinically due to the absence of the usual features. The diagnosis was made during the histopathologic

examination of the surgically removed dentigerous cyst of the mandible. The possible mechanism of its association with the developing cyst, its diagnostic significance and changes in the conventional treatment plan are highlighted in this paper.

CASE REPORT (METHODS, RESULTS): A 24-year-old gentleman had a slow growing swelling of six months on the right lower jaw. Though it was painless initially, he had periodic episodes of severe pain later on. The pain and swelling remained even after two courses of antibiotic therapy. A full mouth radiograph showed a unilocular radiolucency in relation to the partially impacted third molar. Pathological examination of the excised specimen showed colonies of *Actinomyces* with the characteristic 'Ray fungus', surrounded by polymorphs and a fibrin wall, attached to the epithelial lining of the dentigerous cyst. The patient was put on penicillin therapy and has been asymptomatic since then.

CONCLUSION: There are only two documented cases of actinomycosis associated with oral cysts till date. In this case, the actinomycosis occurred in the lower jaw along with dentigerous cyst. Mucosal trauma from the opposing dentition at the site of the cyst possibly led to the entry of *Actinomyces* into the developing cyst. Since the cyst was associated with actinomycosis, the patient was resistant to the initial antibiotic therapy. The patient responded well to the antibiotic administration given after the pathologic diagnosis was made. The histopathologic examination holds a crucial link to the diagnosis of actinomycosis in cases such as the one presented here, as no clinical clue as to the existence of infection in relation to the cyst was available. It is therefore emphasized that oral lesions such as cysts and tumors which undergo mucosal trauma are at a potential risk of infection with *Actinomyces*, as seen in this rare intrabony presentation of actinomycosis, in a dentigerous cyst of the mandible.

FP2

THE RELATIONSHIP BETWEEN PERSISTENT AIRWAY NEUTROPHILIA AND MICROBIAL COLONIZATION/ INFECTION IN SEVERE REFRACTORY ASTHMA

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OBJECTIVES: In asthma, greater than 50% of the cost is related to the 5-10% of patients with severe asthma (SA). Neutrophil predominant asthma affects a high proportion of SA patients and is less responsive to currently clinically available therapies, which are primarily directed to eosinophil abrogation. An evaluation was made of the prevalence and types of organisms identified in SA patients with persistent neutrophilia referred to a specialist in our regional referral center.

METHODS: A retrospective chart review from a regional SA referral center in northern Alberta was performed. Data was recorded on subject demographics, asthma physiology, sputum cell counts, sputum microbiology, antibiotic treatment and acute care utilization over a 12-16 month period following an initial evaluation period.

RESULTS: A retrospective review of asthma charts between 2003 and 2009 was performed. Of 25 charts reviewed, 11 were selected based on inclusion criteria of persistent airway neutrophilia and sputum microbiology processing. Average age of this group was 48 years (SD=13.25) and 45% (5) were male. Eight (72.7%) were atopic and 10 (90.9%) patients had at least one comorbidity. There was poor correlation between airway physiology, measured by forced expiratory volume in one second and persistent neutrophilia (Pearson Correlation= −0.274, p=0.173). Infectious sputum appeared to coincide with increases in neutrophils, and antibiotic treatment coincided with reduced neutrophil levels.

CONCLUSION: Sputum microbiology should be evaluated more routinely in SA patients with persistent neutrophilia. The quantity and quality of micro-organisms isolated may be important for ongoing treatment decisions.

FP3

VARICELLA ZOSTER VIRUS CNS REACTIVATION IN THE ABSENCE OF CUTANEOUS FINDINGS: TWO CASE REPORTS WITH A REVIEW OF THE LITERATURE

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OBJECTIVES: (1) Describe two cases of Varicella Zoster Virus (VZV) CNS reactivation in the absence of cutaneous findings. (2) Review the literature describing frequency, commonalities and indications for therapy.

METHODS: A literature review was conducted to collect data on similar cases by MEDLINE search using keywords "Varicella zoster virus, meningoencephalitis, meningitis, absence of rash" and English articles were reviewed.

RESULTS: Case 1: A 67-year-old diabetic female with confusion. CSF showed WBC 381 x 10^6/L (100% monocytes) and PCR positive for VZV. VZV meningoencephalitis without rash was diagnosed. She was treated with a 14-day course of acyclovir with full recovery. Case 2: A 23-year-old healthy man with fever and meningismus. CSF showed WBC 415 x 10^6/L (88% lymphs) and PCR positive for VZV. He was diagnosed with VZV meningitis without rash and sent home without antiviral therapy and had complete resolution of symptoms. Although, VZV CNS reactivation is most typically seen in the immunocompromised host and accompanied by a rash, there have been case reports of healthy patients developing VZV CNS reactivation without rash, similar to our described cases. High quality data to guide therapy is lacking.

CONCLUSIONS: Our cases demonstrate that VZV CNS disease can occur in immunocompetent hosts with no rash and may be an underappreciated presentation that should remain on the differential for aseptic meningitis/encephalitis. The benefits of antiviral therapy are controversial; however, deaths have been reported in severe disease and because acyclovir is well tolerated, it is recommended that patients with VZV meningoencephalitis be treated. Therapy may not be necessary in uncomplicated cases of viral meningitis due to VZV as demonstrated in Case 2.

FP4

POST-OPERATIVE PELVIC ABSCESS SECONDARY TO *MYCOPLASMA HOMINIS*

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OBJECTIVE: *Mycoplasma hominis* is an infrequently encountered cause of infection in the post-operative setting. We present the case of a young woman who developed a pelvic abscess secondary to *Mycoplasma hominis* following a total abdominal hysterectomy and bilateral salpingo-oopherectomy.

METHODS: The patient was 38 years of age with a seizure disorder and history of developmental delay. She underwent surgery for an ovarian mass and elevated CA-125 level. Her surgery was uneventful and she did well until post-operative day 4 at which time she presented with fever (39.0°C), hypotension, hypoxia and a depressed level of consciousness. She required mechanical ventilation and received vancomycin and piperacillin-tazobactam empirically following collection of blood, respiratory and urine cultures. A CT scan of the pelvis on post-operative day 10 revealed an 8 × 5 cm complex fluid collection.

RESULTS: CT-guided drainage of the pelvic fluid collection was performed and the sample sent to the microbiology lab. Gram stain revealed many WBCs but no organisms. Cultures were positive for *Mycoplasma hominis*. IV azithromycin was added to her antimicrobial regimen although she continued to spike daily temperatures. Pelvic CT on post-operative day 28 revealed a persistent fluid collection. Repeat drainage of the fluid grew *Mycoplasma hominis*. Azithromycin was discontinued and levofloxacin was added, following which her fevers subsided and her infection had resolved.

CONCLUSIONS: *Mycoplasma hominis* is an under-appreciated cause of post-operative pelvic infection. This organism is typically susceptible to

fluoroquinolones and resistant to macrolides.

FP5

COMPARISION OF LOW DOSE INTRADERMAL VACCINATION AGAINST HEPATITIS B WITH ROUTINE DOSE INTERAMUSCULAR VACCINATION IN END-STAGE RENAL DISEASE PATIENTS

A TAVANAEE SANI, M NAGHIBI, H ESMAEILI, M MAHMOUDI, K MOHAMMADI
Mashhad University of Medical Sciences, Mashhad, Iran

OBJECTIVES: Haemodialysis patients have low immune response to hepatitis B (HB) vaccine**.** The route of administration plays an important role in immune response establishment. A case control study is performed to compare the efficacy of intra-dermal (ID) and intra-muscular (IM) routes for HB recombinant vaccine shots.

METHODS: This study was performed on a group of patients who were referred to the haemodialysis centres in Mashhad, Iran. After excluding patients with a history of HB vaccination, immunosuppressive therapy, positive HBS ab, HBS Ag and HCVab, 50 end-stage renal disease (ESRD) patients were recruited to the study. The patients were randomly injected by ID or IM vaccination method in each haemodialysis centre. The time of the vaccine shot was zero, one, two and six months in the two groups. In the ID group, two micrograms of Engerix B in both the right and left anterolateral forearm was applied. In the IM group, 20 micrograms of Engerix B, at two sites in deltoid muscle waw applied. HBS ab titer was measured in the third and seventh month after the HB vaccine shot.

RESULTS: After vaccination in the third month, 40% of the patients reached HBS ab levels of at least 10 mIU/ml in the ID group versus 61% in the IM group. In the seventh month after the first HB vaccine shot, 68% of the patients reached HBS ab levels of at least 10 mIU/ml in both groups, but the mean HBS ab titer in the ID group was 324 versus 277 in the IM group.

CONCLUSIONS: Although there is no significant relation between the seroconversion rate for the ID and IM method, the mean titers of HBS ab in both the third and seventh month after HB vaccination in the ID group was greater than in the IM group. Due to the low cost of HB vaccine in the low dose ID group in comparasion to the high dose IM group, it is beneficial to use low dose intradermal vaccination against hepatitis B in end-stage renal disease patients.

FP6

LESSONS LEARNED FROM THE EDMONTON STI OUTREACH LEARNING SITE: IMPLICATIONS FOR CREATING EVIDENCE-INFORMED PROGRAMS

A POETZ
National Collaborating Centre for Infectious Diseases, Winnipeg, MB

OBJECTIVE: To address the need for knowledge translation at the program level. A *Learning Site (LS)* comprising the Sexually Transmitted Infection (STI) outreach initiative was created in partnership with the National Collaborating Centre for Infectious Diseases (NCCID) and Alberta Health Services (AHS). It is intended to serve as a model for program development in other Canadian health jurisdictions.

METHODS: In-depth interviews and focus groups were conducted with 24 key stakeholders, from October 14, 2011 to February 6, 2012 in order to understand and learn from their experiences related to the LS. Inductive analysis involved open coding to derive emergent themes. Trustworthiness was confirmed by triangulating related document data from pre-2003 to 2011 and confirmation by interviewees.

RESULTS: This documentation of the experiences of the STI Outreach Team and clinic managers includes: insights that can enhance practice within Edmonton; what has worked well and what has been challenging; reflections on how gaps in programming and services have (or have not) been addressed since the initial gap identification exercise.

CONCLUSION: NCCID-supported planning tools such as the situational assessment, performance measurement framework, logic model and gap analysis have increased program quality. The Edmonton LS outreach model has been effective due to several factors: effective teamwork; a staffing model that emphasizes complementary skills; responsiveness of the team to priority issues of clients; relationships built on 3-way trust; consistency and flexibility in delivery of services; a supportive environment by host organizations for outreach activities; and a decision making model which balances autonomy and accountability.

FP7

A NATIONAL INITIATIVE ON PARTNER NOTIFICATION FOR SEXUALLY TRANSMITTED AND BLOODBORNE INFECTIONS IN CANADA

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National Collaborating Centre for Infectious Diseases, Winnipeg, MB

OBJECTIVE: Partner notification (PN) is one of the central pillars of communicable disease control in public health. Despite ongoing efforts and resources dedicated to PN for sexually transmitted and bloodborne infections (STBBIs), the incidence of STBBIs continues to rise in Canada. This calls into question the effectiveness of PN in preventing and controlling the spread of STBBIs at the population level. To address some of the existing knowledge gaps, the National Collaborating Centre for Infectious Diseases (NCCID) has embarked on a project to inform STBBI PN programs and practice in Canada.

METHODS: This project uses a mixed-methods approach to capture both "conventional" and "unconventional" evidence to address the specific knowledge needs of public health practitioners involved with STBBI PN.

RESULTS: Components of this project include 1) a review of provincial and territorial acts, regulations and protocols related to PN for STBBIs; 2) a series of Evidence Reviews on various STBBI PN topics; 3) documentation of successes and challenges of STBBI PN in local public health jurisdictions; 4) mathematical modelling for evaluating the effectiveness and cost-effectiveness of different models of PN for chlamydia; 5) a national consultation attended by public health practitioners, policy-makers and researchers; and 6) a new collaborative initiative on a national PN operational manual undertaken with relevant stakeholder organizations.

CONCLUSION: By integrating evidence from various spheres of knowledge, this project aims to provide public health practitioners (including physicians) with helpful information on promising practices for STBBI PN in the Canadian context.

FP8

EPIDEMIOLOGY OF *LYMPHOGRANULOMA VENEREUM* (LGV) IN BC, 2004-2012: INCREASED TRANSMISSION OR BETTER DETECTION OF ENDEMIC DISEASE?

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OBJECTIVE: LGV was first reported in BC in 2004 following outbreaks in Europe among men who have sex with men (MSM). Few cases were reported in 2004-2010, then 21 cases in 2011. The objective of this study is to describe the epidemiology of LGV in BC with a focus on the 2011 cases.

METHODS: Prior to May 2011, LGV confirmatory testing was performed at the National Microbiology Laboratory (NML) only by request of ordering provider; in May 2011, the Provincial Public Health Microbiology and Reference Laboratory (PPHMRL), began forwarding all positive rectal *Chlamydia* specimens to NML for LGV testing. LGV cases meeting the Public Health Agency of Canada (PHAC) case definition were identified from the provincial Sexually Transmitted Infection, PPHMRL, and PHAC databases.

RESULTS: Thirty-one cases of LGV (45% after May 2011) were reported in BC; all in MSM. In 2011 (n=21) all cases had L2b positive rectal specimens. The average age was 47 years (range 27-60) and 84% presented with proctitis and 5% with inguinal lymphadenopathy. Co-infections included HIV seropositivity (57%), gonorrhoea (24%), syphilis (10%) and hepatitis C (10%). With the exception of two epi-linked cases there was insufficient information to demonstrate other connections between 2011 cases.

CONCLUSIONS: An increase in LGV cases was detected in BC in 2011. Systematic testing of positive rectal *Chlamydia* specimens since May 2011 likely improved case finding, however other regions (Spain and UK) reported similar increases through 2010-2011 supporting increased transmission of LGV. Next steps include enhanced provincial follow-up of cases /contacts and an LGV awareness campaign for health care and laboratory professionals.

FP9

COST-EFFECTIVENESS OF 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE IN ADULTS AGED 50 YEARS AND OLDER IN CANADA

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OBJECTIVE: To assess the cost-effectiveness of vaccinating adults aged ≥50 years in Canada with 13-valent pneumococcal conjugate (PCV13) or 23-valent pneumococcal polysaccharide (PPSV23) vaccine to prevent all-cause (non-bacteremic) pneumonia (NP) or invasive pneumococcal disease (IPD) caused by *Streptococcus pneumoniae*.

METHODS: A microsimulation model was developed depicting the impact of vaccination on lifetime risk and costs of IPD and NP for an adult population aged ≥50 years in Canada (N=11,695,500). A vaccination rate of 50% was assumed for subjects entering the model. The vaccinated population was then assumed to receive either PPSV23 or PCV13; vaccine-associated risk reduction was varied by age, risk profile and vaccination history as per current NACI recommendations. PPSV23 effectiveness was based on published literature and assumed to be zero for NP; PCV13 effectiveness was based on PCV7 data in children and expert opinion. Baseline IPD and NP risks among adults were reduced to account for indirect effects from the PCV13 paediatric vaccination program.

RESULTS: Use of PCV13 in lieu of PPSV23 among Canadian adults aged ≥50 years is expected to prevent approximately 68 disease-related deaths/100,000 persons and reduce direct medical and vaccination costs by $28/patient. PCV13 was estimated to prevent lifetime total cases of bacteraemia (485 cases), meningitis (40 cases), and in-patient and out-patient NP (55,556 and 12,932 cases, respectively), yielding a per-patient gain in life-years of 10.152. These findings were largely unchanged in sensitivity analyses.

CONCLUSIONS: Under reasonable assumptions, use of PCV13 is expected to reduce the incidence of pneumococcal disease and death, and be cost-saving compared with PPSV23 in Canadian adults aged ≥50 years.

FP10

EVALUATION OF TUBERCULOSIS KNOWLEDGE GAIN IN MEDICAL RESIDENTS AFTER CORE CURRICULUM COMPETENCIES AND OBJECTIVES DEVELOPMENT – A PILOT STUDY

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OBJECTIVE: In 2003, the NIH identified a need to strengthen tuberculosis (TB) teaching for health professional students. The National Tuberculosis Curriculum Consortium (NTCC) was developed to meet this need. The University of Alberta (Edmonton, Canada) adopted the NTCC core competencies and suggested objectives to develop a curriculum for medical residents rotating through the TB service. The objective of this study was to determine whether these curriculum changes improved short-term knowledge about tuberculosis.

METHODS: Residents at the University of Alberta are offered a four week clinical elective on the tuberculosis service. A curriculum incorporating the NTCC competencies and objectives was implemented and included a resource manual, objectives, and didactic teaching sessions in addition to inpatient and outpatient clinical care. Questionnaires were designed to assess change in the level of knowledge by completing a 'before' and 'after' questionnaire.

RESULTS: Between July 2010 and June 2011, 10 residents completed before and after questionnaires. Knowledge gain was documented in all areas of core competency including TB transmission and control (42% to 56%), epidemiology (62% to 84%), diagnosis (52% to 76%), treatment (64% to 70%), issues in special populations (44% to 50%) and pulmonary and extra-pulmonary TB (44% to 64%).

CONCLUSION: This pilot study demonstrates TB knowledge gain after development of a NTCC based curriculum. Larger studies are required to confirm the observed increase in short-term knowledge gain and to investigate whether knowledge gain leads to change in clinical practice.

FP11

EVALUATION OF MLST SUBTYPING AS A SUPPLEMENTAL METHOD TO PFGE IN THE SURVEILLANCE OF *LISTERIA MONOCYTOGENES* IN CANADA

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OBJECTIVE: The detection of listeriosis outbreaks from isolated sporadic events remains an important role for Public Health Laboratories (PHLs). Currently, Canada employs serotyping and Pulsed-field Gel Electrophoresis (PFGE) to subtype *Listeria monocytogenes* and provide insight into *L. monocytogenes* distribution and epidemiology. Multi-locus Sequence Typing (MLST) additionally provides estimates of *L. monocytogenes* population structure and phylogenetic relationships while providing good discriminatory power. The objective of this study was to evaluate the use of MLST as a supplemental subtyping method for the surveillance of *L. monocytogenes*.

METHODS: MLST, as described by Ragon et al (PLOS Pathogens, 2008), was performed on historical isolates from the NML Biorepository (n=238) and prospective *L. monocytogenes* isolates received at the NML from PHLs in ON, BC, and AB during 2011 (n=74). Serotyping was performed by slide agglutination with antisera prepared at the NML. PFGE was performed and analyzed according to the PulseNet standardized protocol.

RESULTS: Phylogenetic relationships predicted by MLST correlated with the previously defined lineages and serotypes of *L. monocytogenes*. Eight isolates previously designated as untypeable by serotyping were successfully subtyped using MLST. Prospective surveillance in 2011 identified six and 10 clinical case clusters using PFGE and MLST, respectively. A total of six serotypes, 34 STs and 51 PFGE patterns were observed in 2011.

CONCLUSION: MLST correlated well with PFGE and serotyping data, while providing greater phylogenetic context. Canadian *L. monocytogenes* surveillance could benefit from the implementation of MLST as a secondary typing scheme after PFGE.

FP12

ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF *BACILLUS* SPECIES SUBMITTED TO THE NATIONAL MICROBIOLOGY LABORATORY BETWEEN THE YEARS 1992 TO 2011

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OBJECTIVE: *Bacillus* species (other than *anthracis*) are opportunistic pathogens that can cause serious infections in high-risk groups. For this reason, appropriate antimicrobial therapy is required for serious infections; however, the literature is lacking in antimicrobial susceptibility testing (AST) data for *Bacillus* species. The objective of this study was to provide information on *Bacillus* susceptibility profiles, and to examine these susceptibility profiles based upon specific species within the genus.

METHODS: *Bacillus* submitted to the National Microbiology Laboratory for bacterial ID, using standard phenotypic, chemotaxonomic and 16S rRNA gene sequencing from 1992 to 2011 were tested. AST was done using the broth microdilution method and interpretive criteria

recommended by the Clinical Laboratory Standards Institute (CLSI). Sixteen antimicrobials were tested.

RESULTS: Thirty-seven *Bacillus* species and 106 strains in total were tested; 90 clinical isolates, 15 type strains, and one reference strain. One hundred per cent of the strains were susceptible to imipenem, vancomycin, amikacin and gentamicin. The majority of strains were susceptible to tetracycline, trimethoprim-sulfamethoxazole, levofloxacin, ciprofloxacin and rifampin except for a few strains that were found to be intermediate or resistant. The greatest variability in susceptibilities was found amongst the penicillins, cephalosporins, macrolides and lincosamides, although some similarities can be found within members of the same species. No strain was completely resistant to all agents.

CONCLUSIONS: Antimicrobial susceptibility profiles vary between species and within species of *Bacillus* highlighting the importance of AST on isolates.

FP13

STUDY OF THE PREVALENCE OF SHIGA TOXIN PRODUCING *E. COLI* IN ONTARIO AND CHARACTERIZATION OF ONTARIO ISOLATES

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OBJECTIVES: Shiga toxin producing *E. coli* (STEC) can cause bloody diarrhea, haemolytic uremic syndrome and death. The prototype serotype O157 is easily detected in routine testing by its inability to ferment sorbitol. In contrast, non-O157 STEC are sorbitol fermenters and as such, difficult to differentiate from other *E. coli* that are part of normal flora. Because they are not part of routine testing, the epidemiology of serotypes other than O157 in Canada is not known. The objectives of this study were to develop a method for the detection of all STEC in human stool samples, to establish the prevalence of STEC in Ontario and to identify the virulence factors among Ontario isolates.

METHODS: Diarrheal stool samples were collected from two hospitals in Ontario. Samples were enriched for 18 hours in trypticase soy broth and screened for *stx1* and *stx2* by RT-PCR. Positive samples were plated on blood and Sorbitol-MacConkey agar to obtain isolates. Serotyping was performed at the National Microbiology Laboratory in Winnipeg. Characterization of the main virulence factors (ie, *stx* subtypes, intimin *eae* and hemolysin *hly*) was performed by conventional PCR.

RESULTS: From 1244 screened samples, 28 were positive for *stx1* and/or *2* (prevalence: 2.25%). We observed a marked difference in prevalence between urban and rural areas (1.49 % vs. 4.6%, respectively). Forty per cent of the isolates were O157 and 60% non-O157. All O157 isolates were positive for *stx2*, alone or in combination with *stx*1, while all non-O157 isolates were positive only for *stx1*. The most frequent subtypes of *stx* were *stx1a* and *2a*. 95% of the isolates were positive for the *hly* and 90% for *eae*. The most frequent serotype after O157 was O103.

CONCLUSIONS: RT-PCR for *stx1* and *2* is a reliable and sensitive method for the detection of STECs in human stools samples. We found that 60% of the STEC causing disease in Ontario are serotypes other than O157. The difference in virulence factors associated to non-O157 v. O157 STEC warrant further studies regarding severity of disease to identify risk factors associated to different strains.

Our results provide the bases for decisions on the preparedness for potential outbreaks by these emergent pathogens.

FP14

EPIDEMIOLOGY AND ANTIMICROBIAL SUSCEPTIBILITY OF UROPATHOGENS IN ONTARIO FROM 2008-2011

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OBJECTIVE: A retrospective analysis to determine the microbial etiology of urinary infections and compare them between community, hospital and nursing home populations in Ontario, and to highlight the changes in susceptibility patterns for *E.coli* from 2008-2011.

METHODS: A total of 2,801,614 urine samples were received and processed by our high volume medical laboratories in Ontario from 2008-2011.Urine cultures were processed using BBL CHROMagar Orientation (BD) starting from 2009. Prior to this, CLED media was used for urine cultures. Susceptibilities for *E.coli* were performed using Vitek 2 AST cards for gram-negative bacilli. Statistics were obtained from MEWS Epi/Stats program excluding repeats <7 days.

RESULTS: *E.coli* continued to be the most common uropathogen (51.8%) followed by *Enterococcus* spp.(21.7%) in the three populations. The third most common organism was different; Group B *Streptococcus* was the third in the community (11.9%) from 2009 -2011, and *K.pneumonia* in hospitals and nursing homes (7.4%). Nursing home uropathogens were very similar to hospitals except for *P.aeruginosa* which is more common in hospitals. *E.coli* developed increased resistance to all first line antibiotics for UTI including ampicillin, cephalothin, nitrofurantoin, trimethoprim-sulfamethoxazole and ciprofloxacin. In 2011, *E.coli* susceptibility to trimethoprim-sulfamethoxazole was 79.1%, 79.9% and 77.6% for community, hospital and nursing home population respectively, and for ciprofloxacin was 84%, 79% and 78.8%, respectively. Prevalence of ESBL *E.coli* in urines increased, with the highest rates in nursing homes.

CONCLUSION: Increase in the reporting of Group B *Streptococcus* and *Enterococcus* spp secondary to the use of chromagar. Trimethoprim-sulfamethoxazole and Ciprofloxacin are not to be used as empirical therapy for UTIs in Ontario given the increased resistance.

FP15

SUSCEPTIBILITY TO CIPROFLOXACIN, TRIMETHOPRIM/SULFAMETHOXAZOLE AND NITROFURANTOIN OF *STAPHYLOCOCCUS SAPROPHYTICUS* URINARY ISOLATES IN INTERIOR HEALTH BRITISH COLUMBIA

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OBJECTIVES: *Staphylococcus saprophyticus* is a common cause of uncomplicated urinary tract infection (UTI) in young sexually active females. Ciprofloxacin, trimethoprim/sulfamethoxazole, or nitrofurantoin are commonly prescribed for patients with UTI. This study was designed to determine whether increasing resistance is developing in *S. saprophyticus* to these antibiotics.

METHODS: *S. saprophyticus* urinary isolates were collected from six laboratories in the Okanagan region: Kelowna General Hospital (KGH); Vernon Jubilee Hospital (VJH); Penticton Regional Hospital (PRH); East Kootenay Regional Hospital (EKRH); Kootenay Boundary Regional Hospital (KBRH); and Valley Medical Laboratories (VML). Seventy-eight samples were collected over a nine month period, subcultured twice on Columbia blood agar with 5% sheep blood to ensure purity and retested with novobiocin disks (NV 5) to ensure resistance. Pure colonies were streaked onto Mueller-Hinton agar plates and tested by Kirby-Bauer method using ciprofloxacin (CIP 5), nitrofurantoin (F/M 300), and trimethoprim/sulfamethoxazole discs (SXT 25).

RESULTS: All 78 samples were found to be susceptible to all antibiotics tested.

CONCLUSIONS: Despite high antimicrobial resistance in other uropathogens, ciprofloxacin, trimethoprim/sulfamethoxazole and nitrofurantoin are still reliable agents for treating *S. saprophyticus* urinary tract infections. Routine susceptibility testing is not required for uncomplicated UTI, but should be considered for recurrences, treatment failure, and for non urinary *S. saprophyticus* isolates.

FP16

CARBAPENEM NON-SUSCEPTIBLE *ENTEROBACTERIACEAE* IN QUÉBEC: RESULTS OF A SURVEILLANCE PROGRAM

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OBJECTIVE: To assess the presence of carbapenem non-susceptible *Enterobacteriaceae* (CNSE) in Quebec and to characterize their phenotypic and genotypic resistance profiles.

METHODS: From 2010/08 to 2011/10, laboratories were asked to send all their CNSE isolates to the Laboratoire de santé publique du Québec. AmpC and metallo-beta-lactamase production were detected by Etest and carbapenemase production by the Modified Hodge test (MHT). Susceptibility profiles were determined by microbroth dilution or by E-test and resistance genes detected by PCR. The carbapenemase resistant isolates (KPC) were typed by PFGE.
RESULTS: Amongst 409 CNSE, the most frequent resistance mechanism was most likely due to AmpC overexpression associated with porin mutations (195 strains, 47.7%): 160 (82.1%) of these were *Enterobacter cloacae*. There were 87 (21.3%) carbapenemase producing isolates (CPE) from seven different species including 49 strains of *K. pneumoniae* : 83 bla_{KPC}, one bla_{NMC}, one bla_{OXA-48}, one bla_{SME} and one bla_{NDM-1}. All KPC strains were resistant to aztreonam, 55.6% to colistin and 8.6% to tigecyclin. The majority (61.4%) of KPC strains were isolated from screening specimens (rectal swabs) and 33.6% from routine clinical samples. Thirty-seven PFGE profiles were identified among the different specie, of these, two were associated with nosocomial outbreaks in Montreal. For CPE detection, sensitivity and specificity of MHT for ertapenem were 100% and 46.3% and for meropenem was 98.9% and 70.3%, respectively.
CONCLUSION: CPE are present in Québec and have been associated with nosocomial outbreaks. Their detection represents a challenge for clinical laboratories.

FP17

THE EPIDEMIOLOGY OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN A CANADIAN TERTIARY CARE FACILITY

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OBJECTIVES: To describe the epidemiologic and microbiologic characteristics of MRSA isolates at a Toronto tertiary care hospital collected over a 10-year period.
METHODS: Prospective laboratory-based surveillance of incident cases of MRSA was conducted from 2000 to 2010 at Mount Sinai Hospital. Demographic and clinical data were extracted from an infection control database and from patient charts. MRSA strains were typed by pulsed-field gel electrophoresis of SmaI-digested genomic DNA.
RESULTS: In total, 1696 incident cases of MRSA were identified. Among all MRSA cases, the majority were CMRSA-2 (48.4%), followed by CMRSA-10 (20.3%) and CMRSA-1 (14.3%). The first positive culture was from clinical specimen for 475 (28%) patients, both screening and clinical specimens for 75 (4%), and from one or more screening specimens for 1129 (67%). Of those initially positive by screening specimens, 674 (60%) had positive nasal culture, 374 (33%) had negative nasal but positive rectal culture, and 80 (7%) had MRSA from a screening wound swab only.
Compared to patients identified as colonized at admission, patients who acquired MRSA nosocomially were more likely to have a rectal swabs as their only initial positive (140/300, 47% nosocomial vs 160/610, 26% on admission), P<0.001. Age, gender, year of identification and admission from home or long-term care facility did not affect rates of nasal versus rectal positivity. Of those patients nasally colonized by MRSA, 104/674 (15.4%) subsequently had clinical MRSA isolate; of those who are only colonized rectally, 68/374 (18.2%) later had clinical isolate. Of the 414 patients who had nosocomially acquired MRSA colonization, 113 (28%) subsequently had clinical isolates.
CONCLUSION: This finding highlights the importance of rectal screening swabs in detecting MRSA colonization. Patients with nosocomially acquired MRSA were more likely to have a positive rectal swab only on initial screening. A significant fraction of patients acquiring MRSA colonization in our hospital developed an MRSA infection.

FP18

CARBAPENEM-RESISTANT ENTEROBACTERACIAE (CRE) - FINDINGS FROM 2010 TO 2011 IN A CANADIAN TERTIARY CARE FACILITY

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OBJECTIVE: To describe the epidemiologic and microbiologic characteristics of CREs over a one year period at a Canadian tertiary care centre.
METHOD: A prospective laboratory-based surveillance program of cases of CREs was started in 2010. CRE cases were initially identified by non-susceptible ertapenem MIC on Vitek 2. Upon identification of a CRE, a retrospective chart review was performed to collect clinical and demographic data for those cases occurring from January 1, 2010-December 31, 2010.
RESULTS: Twenty-six incident cases of CREs were identified in a one-year period. The patient age range was 25 to 89 years (average 57.7 years). The majority were males (17/26; 65.5%). The mean length of hospital stay was 74.7 days (range: 1-359 days). More than half of the patients were admitted to ICU (17/26, 65.3%) prior to the onset of CRE infection. Most patients had significant comorbidities, including diabetes mellitus, liver disease, renal impairment, dialysis and malignancy. The major sites of infection/colonization were urinary or respiratory tract with a small proportion involving skin and soft tissue and blood. The all-cause mortality rate in this population was high (46%). All the isolates were multidrug resistant involving 3 or more drug classes. Carbapenemase genes (two *GES* and one *KPC*) were detected in three of 26 isolates. Other beta-lactamase genes (*CMY-2, TEM, SHV, CTX-M*, and *OXA-1*) were identified in 10 isolates. The single KPC case occurred in a young, otherwise healthy traveller and was the bacteria was isolated on the day of admission.
CONCLUSIONS: In our centre, the rate of CRE infections remains very low. Patients generally had multiple comorbidities and prolonged hospital stay with significant antimicrobial exposure. Carbapenem resistance secondary to the presence of carbapenemase genes is rare.

FP19

PREVALENCE AND ANTIBIOTICS SUSCEPTIBILITY PATTERN OF EXTENDED SPECTRUM BETA LACTAMASES PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* AT A TERTIARY TEACHING HOSPITAL, RIYADH, SAUDI ARABIA

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OBJECTIVES: Prevalence and the antibiotics susceptibility of extended spectrum β-lactamases (ESBL) producing organisms widely vary in different parts of the world and among institutions. This study was performed to assess the prevalence and antibiotics susceptibility of ESBL producing *E. coli* and *K. pneumoniae*.
METHODS: Data from 1076/11241 (9.5%) confirmed ESBL producing isolates of E. coli and K. pneumoniae from various samples were collected between the years 2006 and 2010 for assessment of prevalence and antibiotics susceptibility at King Khalid University Hospital, Riyadh, Saudi Arabia. Identification and antibiotic susceptibility testing was performed by using MicroScan WalkAway 96 Plus Dried Gram-negative MIC Panel Type 39. Confirmation of ESBL was performed using CLSI method Etest® ESBL for cefotaxime/cefotaxime+clavulanic acid and ceftazidime/ceftazidime+clavulanic acid and cefepime/cefepime-clavulanate strips.
RESULTS: The majority of the isolates 680 (63.1%) were detected in urine samples followed by 184 (17.1%) in wound samples and 123 (11.43%) in sputum samples. Out of the total ESBL producing isolates there were 808 (75%) *E. coli* and 268 (25%) *K. pneumoniae*. The overall rate of prevalence of ESBLs in *E. coli* and *K.pneumonia* were 9.9% and 8.7%, respectively. *E. coli* were more resistant to ciprofloxacin (66.58%), co-trimoxazole (55.94%) and gentamicin (45.54%) and a high level of resistance for *K. pneumoniae* was observed against gentamicin (63.81%), co-trimoxazole

(61.19%) and ciprofloxacin (54.1%). Prevalence of co-trimaoxazole resistant ESBL producing *E. coli* increased from 44.62% to 63.78% and gentamicin resistant ESBL producing *K. pneumoniae* increased from 47.83% to 66.76% between 2006 and 2010. Both the organisms remained highly susceptible to imipenem.
CONCLUSION: Our prevalence of ESBL producing *E. coli* and *K. pneumonia* over years was stable; however ,it was more prevalent in *E. coli* than in *K. pneumonia*. A high resistance rate for both the organisms against commonly used antibiotics might lead to change in our antimicrobials guideline for empirical treatment and apply more strict infection control measures to minimize the spread of this resistance.

FP20

A DESCRIPTIVE AND ANALYTIC EXAMINATION OF PUBLIC HEALTH ONTARIO LABORATORY PARASITOLOGY DATA

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OBJECTIVE: The goal of the study was to describe Ontario parasite infections and identify their risk factors using routinely collected information. Additionally, the data was used to assess current protocols for parasite testing in Public Health Laboratories and to propose alternatives which optimize the allocation of laboratory resources.
METHODS: A descriptive and analytic examination of two parasitology test result data sets was undertaken. The first set, from the Public Health Ontario Laboratories–Eastern Ontario, contained 20 months of data while the second, from all Public Health Ontario Laboratories, contained eights months of data.
RESULTS: The two data sets were analyzed separately; in total 42,377 patients records were obtained. Of these, an average of 11% were multiple samples from a patient submitted on the same day with 99% having the same result, thereby suggesting that multiple specimens for a single patient may not be necessary. Across the province, 3% of parasite tests were positive; the most prevalent parasites were *Dientamoeba fragilis*, *Giardia lamblia* and *Cryptospordium* species. Age, gender and season of submission were found to be weak risk factors. For *D. fragilis*, *G. lamblia* and *Cryptosporidium* infections, a rural setting was found to have an Odds Ratio of approximately two. The strongest risk factor for parasite infection was positive response to travel, with odds ratios between five and 16 based on stratification criteria.
CONCLUSIONS: Based on the findings, six protocol alternatives founded on travel exclusion criteria and rapid high throughput screening for common parasites were considered; four of these alternatives were identified as more effective than current procedures. Future analyses on these data sets were proposed.

FP21

MICROBIAL SOURCE TRACKING OF *ESCHERICHIA COLI* WELL WATER CONTAMINATION USING BACTEROIDES FRAGILIS REAL-TIME PCR

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OBJECTIVE: In Ontario, groundwater serves as a primary source of drinking water for approximately 30% of its residents. The use of groundwater as a drinking source may pose a health risk if fecal contamination is present. The objective of this study is to reveal *Escherichia coli* (*E. coli*) contaminated areas and identify the major source(s) of fecal contamination present in well waters within multiple health unit regions in southeastern Ontario.
METHODS: Private well water samples, submitted for bacteriological analysis, that yielded a positive *E. coli* result, were further analyzed to determine the source of fecal contamination. 100 mL of each *E. coli* positive sample was subjected to membrane filtration (0.45 µm). The filter was rolled, placed in a cryovial and suspended in GITC lysis buffer for one hour at 37 °C. The DNA was extracted using an automated EasyMag DNA extractor. Real time PCR for *B. fragilis* was performed using the BacHuman, BacBovine and BacGeneral assays (1). Water data was plotted using ArcGIS v10 software.
RESULTS: Five percent of incoming water samples tested positive for *E. coli*. The predominant sources of fecal contamination are contributed by other animal species (42%). Human contamination was detected in 29% of the water samples, while bovine occurred in 5%. Both types of contamination contributed to 20% of the tested waters.
CONCLUSION: The majority of drinking water contamination for wells studied can be attributed to non-human and non-bovine sources. The results of the Bac PCR assays are spatially represented according to contamination source. Determining the microbial source may allow for targeted intervention.

1. Lee D. Quantitative identification of fecal water pollution sources by TaqMan real-time PCR assays using Bacteroidales 16S rRNA genetic markers. 2010 Applied Microbiology and Biotechnology 8:1373-1383

FP22

POOR CORRELATION BETWEEN BROTH MICRODILUTION AND E-TEST MICS FOR BETA-LACTAM NON-SUSCEPTIBLE INVASIVE *STREPTOCOCCUS PNEUMONIAE* ISOLATES

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OBJECTIVES: Invasive *S. pneumoniae* isolates with elevated beta-lactam MICs in Alberta are no longer a rare event. However, we have observed disparities between susceptibility test methods in a number of clinical isolates. The goal of this study was to compare the Sensititre and Etest methods to the reference broth microdilution (BMD) for beta-lactam non-susceptible *S. pneumoniae*.
METHODS: Twenty invasive *S. pneumoniae* isolates collected from Alberta cases (2010), characterized and serotyped at the Provincial Laboratory (Edmonton) were selected for study. These included isolates with penicillin MICs of ≤0.06 (n=10) and ≥4 (n=10) according to Sensititre. All isolates were tested for penicillin and ceftriaxone MICs using BMD and Etest. Essential agreement (EA) and category agreement (CA) were calculated using BMD as a reference.
RESULTS: The 10 strains with elevated BMD penicillin MICs were in exact (n=7) and essential (n=3; +1 dilution) agreement using the Sensititre. The Etest agreed with BMD MIC (exactly or one dilution less) for only two strains and was two dilutions lower than BMD for eight strains. Similarly for ceftriaxone MICs; Sensititre compared very well with BMD but Etest MICs were lower and not in agreement for the nine strains with elevated ceftriaxone MICs.
CONCLUSION: Despite the small size of the study, our findings suggest that Etest may "undercall" the MIC for *S. pneumoniae* isolates non-susceptible to beta-lactams. In a larger study, our results would translate to a high rate of minor errors for penicillin CLSI non-meningeal breakpoints and an unacceptably high rate of very major errors for ceftriaxone and meningeal breakpoints. As resistant serotypes become more prevalent, this test disparity will become much more significant.

FP23

COMPARISON OF GBS CHROMOGENIC MEDIUM WITH ROUTINE CULTURE METHOD FOR *STREPTOCOCCUS AGALACTIAE* (GBS) ISOLATION FROM VAGINAL/RECTAL SWABS OF PREGNANT WOMEN

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OBJECTIVES: To compare the performance of three chromogenic medium to routine culture for detecting Group B *Streptococcus* (GBS) in vaginal/rectal swabs. To evaluate the use of one of these media in our laboratory.
METHOD: Swabs from 120 pregnant patients were screened by routine culture on CNA media and chromogenic agar following incubation in LIM broth for 18-20 hours. Plates were examined after 18-24 hours of

incubation and again at 48 hours. Suspected colonies on chromogenic medium were confirmed as GBS by gram stain and pathoDx latex agglutination.

RESULTS: The performances of the three media are shown below.

bioMérieux chromID StreptoB		Dalynn Colorex StrepB		BioRad StrepBSelect	
23/23 positives detected		22/23 positives detected		23/23 positives detected	
Sensitivity	Sensitivity	Sensitivity	Sensitivity	Sensitivity	Sensitivity
24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
100%		82%	96%	100%	
Specificity	Specificity	Specificity	Specificity	Specificity	Specificity
24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
93%	89%	84%	80%	92%	90%

Twenty three of the 120 specimens tested over a six week period were GBS positive by standard culture method (19%). None of the chromogenic media detected additional isolates. The BioRad StrepBSelect media was introduced to our laboratory Based on technologist preference. In the first month of use we found 27 positive specimens (26%) in 143 vaginal/rectal swabs tested. Thirty-seven of these positives were identified at 24 hours and 1/37 at 48 hoursr. There was only one false positive requiring additional work at 48 hours. The plates are felt to be easy to read and the number of false positives, requiring additional work, remains very low.

CONCLUSION: Both the bioMérieux Media and BioRad Media performed well in this study. The BioRad Media was felt to be easiest to read by technologists in our laboratory and the change to this media has significantly improve workflow in our laboratory.

FP24

OPTIMAL USE OF MRSA*Select* FOR IMPROVED DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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OBJECTIVE: MRSA*Select*, a selective medium for methicillin-resistant *Staphylococcus aureus* (MRSA), is commonly used in MRSA screening. The objective of this study was to perform confirmatory analysis of suspected MRSA colonies on MRSA*Select* plates using both an in-house, multiplex real time PCR assay and routine microbiological methods in order to determine the positive predictive value of MRSA*Select* plates after 24 h and 48 h of incubation

METHODS: Specimens submitted for MRSA screening were routinely inoculated into MRSA*Select* pates and incubated at 35°C for 24 h or 48 h (if negative at 24 h). Suspected colonies were further assessed using microbiological methods including tube coagulase, oxacillin screening, cefotixin Kirby-Bauer (30 µg) and Phoenix PMIC/ID panel assays (BD), and by a multiplex real time PCR (mRT-PCR) using primers and probes to amplify segments of MecA, CoA and 16S rDNA genes.

RESULTS: A total of 108 MRSA*Select* positive clinical samples were identified, for which confirmatory analyses by both routine and molecular methods were performed in parallel. Suspected colonies were observed in 58 (53.7%) samples at 24 h, while the rest of the colonies appeared at 48 h of incubation. The PCR results were in 99% agreement with microbiological confirmation of MRSA (n=108). Greater than 90% of isolates positive by MRSA*Select* within 24 h were confirmed by both routine culture methods and mRT-PCR. However, only 60% of samples with suspected colonies on MRSA*Select* plates at 48 h were confirmed to be MRSA. Overall, ~77% of MRSA*Select* positive isolates were true positives. Although 40% of isolates positive by MRSA*Select* at 48 h were false positives, the longer incubation did increase the total number of true positive samples by 27%.

CONCLUSIONS: Surveillance of MRSA samples for up to 48 h using MRSA*Select* plates, followed by rapid confirmation with a molecular method can provide a high sensitivity and specificity for MRSA detection, while simultaneously reducing costs by eliminating the need for expensive molecular analysis of all samples.

FP25

MOLECULAR CHARACTERISTICS OF CONTEMPORARY *STAPHYLOCOCCUS AUREUS* BLOOD ISOLATES IN TORONTO, ONTARIO

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OBJECTIVE: The epidemiology of *S. aureus* bacteremia is changing. Risk factors associated with poor outcomes are incompletely understood. This study characterized contemporary *S. aureus* blood isolates.

METHODS: Two hundred fifty-eight consecutive, unique *S. aureus* blood isolates from three tertiary-care hospitals in Toronto collected between 1/4/2009 and 1/4/2010 were studied. Isolates were characterized by *Sma*I PFGE, *spa* typing, and virulence factor PCR (*mecA*; *nuc*; *agr*; *sea*; *seb*; *sec*; *tst*; *seg*; *fnbA*; *fnbB*; PVL; SCC*mec*). Vancomycin MICs were determined by E-test. hVISA/VISA were detected by GRD E-test.

RESULTS: Of the 258 *S. aureus*, 213 (83%) were MSSA and 45 (17%) were MRSA. The majority of the 45 MRSA isolates belonged to clonal types CMRSA2 (n=26 (58%)) or CMRSA10 (n=13 (29%)). Of the 213 MSSA isolates, 179 (84%) resembled known MRSA clones by both PFGE and *spa* typing. 6% (15/258) of all *S. aureus* (11 MRSA; four MSSA) were positive (pos) for PVL; 82% (9/11) of the PVL-pos MRSA were CMRSA10; 25% (1/4) of the PVL-pos MSSA resembled CMRSA10. Of the virulence genes tested, *sea* was more common in MSSA (n=50; 23%) than MRSA (n=2; 4%; P=0.002); no statistically significant differences were detected in other virulence factors tested. Twenty-four percent (52/258) MSSA and 29% (13/45) MRSA had vancomycin MICs ≥1.5 mg/L (P=0.51). No isolate had a vancomycin MIC >2mg/L. The GRD E-test identified two isolates as possible hVISAs.

CONCLUSIONS: MRSA represented 17% of *S. aureus* blood isolates. MSSA showed significant diversity, but many were genetically similar to known MRSA clones. MSSA and MRSA had a similar distribution of putative virulence factors and susceptibilities to vancomycin.

FP26

EVALUATION OF VITEK 2 AST-P612 CARD FOR DAPTOMYCIN (DAP) SUSCEPTIBILITY TESTING OF *STAPHYLOCOCCUS AUREUS* (SA) AND COAGULASE-NEGATIVE STAPHYLOCOCCI (CNS)

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BACKGROUND: DAP is a useful agent to treat Gram-positive infections including those due to methicillin-resistant SA (MRSA). As DAP depends on precise calcium ion content in the test medium to produce accurate MIC, testing is complex. This study evaluated Vitek 2 DAP testing of SA and CNS.

METHODS: Of 370 study isolates, 270 were genetically and geographically diverse MRSA and methicillin–susceptible SA (MSSA) and 100 were highly characterized CNS blood isolates. The MSSA/MRSA respectively, included 72/69 community-acquired and 52/13 hospital-acquired strains (consecutive blood, other clinical sites, and surveillance screen isolates), 6/38 NARSA VISA and 1/19 Cubist DAP non-susceptible (NS; >1 mg/L=NS) isolates. As per Cumitech 31A, AST-P612 card DAP MIC was compared to CLSI broth microdilution (BMD) MIC.

RESULTS: By BMD, 28 (10.4%) SA and no CNS were DAP NS. By Vitek 2, among SA, the essential agreement (EA) was 81.9% (95% CI: 76.8-86), categorical agreement (CA) was 93.7% (95% CI: 90.1-96.1), while very major (VME) and major (ME) error rates were 14.3% (95%CI: 5.1-32) and 5.4% (95% CI: 3.1-9.1), respectively. Vitek2 DAP MIC values were significantly higher than BMD in 55.6% SA by 1-4 log dilutions (P value <0.0001). For CNS, EA was 98% (95% CI: 92.6-99.9), CA was 99% (95% CI: 94-99.9), ME rate was 1% (95% CI: <0.01-6), and the VME was unable to be determined as there were no DAP NS CNS.

CONCLUSIONS: While the AST-P612 generally compared well to BMD MIC for CNS, for SA, it overestimated DAP MIC as evidenced by high ME (>3%) and low EA (<90%). As well, true DAP NS was overlooked in 3 MR-VISA leading to an unacceptable VME rate of >3%.

FP27

DETECTION OF VANCOMYCIN-RESISTANT ENTEROCOCCI (VRE) BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP): A RAPID, SIMPLE AND COST-EFFECTIVE ALTERNATIVE TO PCR

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OBJECTIVE: A number of chromogenic media have been used to improve turn-around-time to VRE isolation. However, PCR remains to be the "gold standard" for culture confirmation. The objective of this study was to develop an alternative method to PCR that is comparable in performance but faster, cheaper and can be operated in laboratories without PCR facilities.

METHOD: Seventy-five consecutive isolates presumptively identified as VRE on Dalynn Colorex VRE plates were analyzed by a conventional multiplex PCR as per standard laboratory operating procedure to detect *vanA* / *vanB* genes and by LAMP. LAMP primers for *vanA* and *vanB* genes were designed by Primer Explorer V4 software (Eiken Chemical Co.). LAMP was carried out at 62°C for 60 min followed by two min at 95°C using same DNA template used for the PCR. Isothermal amplification was done using *Bst* DNA polymerase (Lucigen) and was detected visually by the development of the turbidity and apple green color using Calcein.

RESULTS: Fifty-eight isolates were confirmed as VRE with PCR. *vanA* and *vanB* gene was present in 50 and eight isolates, respectively. Seventeen isolates were identified as non-VRE. LAMP results were 100% concordant with the PCR results.

CONCLUSION: Confirmation of VRE by the detection of *vanA*/*vanB* genes using LAMP is as accurate a molecular method as PCR. However, it is significantly faster and cheaper than the PCR. In addition, LAMP is an isothermal method and does not require an expensive thermocycler. The amplification specificity is extremely high because the LAMP requires a set of four oligonucleotide primers that recognize six distinct regions on the target DNA. Amplification of *vanA* and *vanB* genes by LAMP can be used as an alternative method for PCR for VRE confirmation.

FP28

VALIDATION OF THE CEPHEID XPERT® *VAN*A/B ASSAY FOR RAPID VANCOMYCIN-RESISTANT ENTEROCOCCI (VRE) IDENTIFICATION (ID) FROM OXOID BRILLIANCE VRE (BVRE) AND 5% SHEEP BLOOD AGARS (SBA)

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OBJECTIVES: Integral to effective infection control is rapid ID of new VRE. BVRE (Oxoid) reduced VRE ID from <7 d to <3 d, but our target is <2 d. As PCR on swabs is costly, PCR from culture using Xpert *van*A/B off-label was assessed.

METHODS: The 341 study strains included 126 retrospective isolates [retro: 45 *E. faecium* (22 *vanA*, 15 *vanB*, one *vanD*, 7 VSE); 39 *E. faecalis* (two *vanA*, 15 *vanB*, one *vanA*/*B*, 6 *vanE*, one *vanL*, 14 VS), 15 *E. gallinarum*, 10 *Leuconostoc*, seven *E. casseliflavus*, seven *Pediococcus*, three *Lactobacillus*] and 215 prospective isolates [pros: 173 *E. faecium* (110 *vanA-functional*, 15 *vanA-vanRS*-mutants, 48 VS); 32 *E. faecalis* (five *vanA* - three small colony variants, two *vanB*, two *vanE*, and one 23 VS); one *vanA E. gallinarum* and nine other]. Strains were tested off BVRE unless inhibited, while inhibited strains were tested off SBA. A dry swab was used to touch ~5 18-24 h colonies and then placed into Xpert buffer; the assay was then run as per Cepheid. Results were compared to Roche's LC VRE assay and all positives were cultured.

RESULTS: Xpert detected 155 *vanA* (24 retro, 131 pros), 32 *vanB* (30 retro, two pros), and three *vanA*/*B*; 153 were negative by both assays. Roche and culture agreed except in two of the three *vanA*/*B* VRE that were *vanA* only by Roche and on repeat Xpert. Xpert amplifications were positive in 12/33 cycles and interpretation from color-coded *vanA*/*vanB* curves was possible by 25 min (run time 47 min). Compared to Roche, sensitivities (Sn)/ specificities (Sp) of the Xpert were: *van*A 100% (95% CI 97.1-100)/100% (95% CI 97.6-100); *vanB* 100% (95% CI 87.3-100)/100% (95CI 97.5-99.98).

CONCLUSIONS: The Xpert *vanA*/*B* assay from culture was 100% Sn and Sp. It was rapid, easy to use, and required minimal hands-on and training time. ID of new VRE was reduced to <1-2 d from swab receipt, even in mixed cultures.

FP29

SUSCEPTIBILITY TO FOSFOMYCIN AND TIGECYCLINE OF ENTEROBACTERIACEAE PRODUCING EXTENDED SPECTRUM β-LACTAMASES (ESβL) AND AMPC CEPHALOSPORINASES

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OBJECTIVES: Therapeutic options are limited for infections due to extended spectrum beta-lactamase (ESBL) and AmpC cephalosporinase (AmpC) producing *Enterobacteriaceae*. This study looked at the susceptibility to fosfomycin (FOS) and tigecycline (TIG) of third generation cephalosporin resistant *Enterobacteriaceae* (3GCRE) clinical isolates from the Okanagan region of BC from June 2009 - April 2011.

METHODS: A total of 259 3GCRE (one *C.braaki*, seven *C.freundii*, nine *E.aerogenes*, one *E.asburiae*, 20 *E.cloacae*, 188 *E.coli*, six *H.alvei*, nine *K.oxytoca*, two *K.ozonae*, five *K.pneumoniae*, four *P.mirabilis*, one *P.stuartii*, one *R.aquatilis*, five *S.marcescens*) were tested. Resistance mechanisms of 3GCRE included ESBL (116), AmpC (134) and hyperproducers of K1 enzyme (9). FOS (200 µg/50 µg G6P) was tested by KB method using CLSI interpretations. TIG was tested by Etest® method using both FDA (≤2 µg/mL=S, 4 µg/mL= I, ≥8 µg/mL=R) and EUCAST (≤1 µg/mL= S, 2 µg/mL= I, >2 µg/mL=R) breakpoints.

RESULTS: Two hundred fifty-three of 259 isolates (98%) were susceptible to FOS. Resistance was noted in *E.aerogenes (1)*, *E.cloacae (2)*, *E.coli (2) and P.mirabilis (1)*. Using FDA breakpoints, 252/259 isolates (97%) were susceptible to TIG. Five isolates exhibited intermediate resistance; *C.freundii (1)*, *E.cloacae (1)*,*P.stuartii* (1) and *S.marcescens(2)* and two isolates were fully resistant (*K.pneumoniae*, *P.mirabilis*). Using EUCAST breakpoints, 243/259 isolates (94%) were susceptible to TIG. Nine isolates exhibited intermediate resistance; *E.cloacae* (2) *E.coli (1)*,*P.mirabilis (3)*, *S.marcescens (3)* and seven were fully resistant ; *C.freundii* (1), *E.cloacae (1)*, *K.pneumoniae (1)*, *P.mirabilis (1)*, *P.stuartii (1)* and *S.marcescens*(2).

CONCLUSION: The vast majority of 3GCRE remain susceptible to FOS and TIG.

FP30

VALIDATION OF ROSCO KPC+MBL DIAGNOSTIC INHIBITORS (INHIB) FOR PHENOTYPIC DISTINCTION OF CARBAPENEMASES (CARB) FROM NON-CARBAPENEMASE-MECHANISMS (NCM) IN ERTAPENEM-RESISTANT (ETP-R) *ENTEROBACTERIACEAE* (ENT)

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OBJECTIVE: Etp-R CARB+ ENT has distinct infection control implications compared to NCM-ENT. Rosco INHIB tablets were validated against CARB PCR for sensitivity (Sn) and specificity (Sp) for phenotypic distinction of CARB from NCM.

METHODS: Two hundred sixty-five ENT (Etp R=187, I=25, S=53) were

tested as per CLSI on Mueller-Hinton agar (MHA, Oxoid) using meropenem (mrp10) alone/with boronic acid (Bo), dipicolinic acid (Dp) or cloxacillin (Cl). Zones were read for ≥5 mm increases vs mrp10 to predict class A CARB (Bo^+), MBL (Dp^+), or NCM (Cl^+). Criteria to suspect CARB was modified from "mrp10 <25 mm" to "if Bo^+ or Dp^+ +/−Cl^+", and to suspect OXA48 if mrp10 <26 mm but $INHIB^-$. All had PCR (GES; KPC; NDM; VIM; IMI; OXA48) and select $PCR^-/INHIB^+$ ENT were sequenced (*ompC/F, ompK35/36*).

RESULTS: Revised criteria found: 124 $mrp10^S$ $INHIB^-/PCR^-$; 145 $mrp10^R$ [11 $INHIB^-/PCR^-$; 18 $INHIB^+/PCR^-$ (13 Bo/Cl^+; four Bo/Dp/Cl^+; one Dp^+); four $PCR^+/INHIB^-$(all OXA48); 112 $PCR^+/INHIB^+$]. Of 116 PCR^+ ENT, 23 were DP^+ only (all NDM), 88 Bo^+ only (86 KPC, 2 SME) and four KPC^+ were Bo^+/Cl^+ (one *C. freundii*, three *E. aerogenes*). For predicting CARB by Dp^+ or Bo^+ alone, % Sn (95% CI) were 100 (86-100)/98 (96-99) and % Sp (95% CI) were 96 (89-99)/100 (97-100), respectively. To detect all KPC, inclusion of any Bo^+ with Cl^+ or Dp^+ increased Sn to 100% (95-100) but reduced Sp to 91% (85-94). Use of mrp10 <26 mm for OXA48 was 100% Sn/92% Sp (95%CI wide due to study no.). Defective *ompK35/36* (three *K. pneumoniae*: two Bo/Dp/ Cl^+; one $Dp^+/GES9^+$) and *ompC/F* (seen *E. coli*: mrp10<26 mm/ $INHIB^-$) were identified.

CONCLUSIONS: Use of INHIB with revised criteria was a highly sensitive and cost effective means to detect CARB including OXA48. As $false^+$ do occur, PCR confirmation is recommended.

FP31

DISK DIFFUSION SCREENING (DDS) IN *ENTEROBACTERIACAE* (ENT) USING A MODIFIED MEROPENEM (MEM) BREAKPOINT FOR DETECTING CARBAPENEMASES-PRODUCERS (CRE)

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OBJECTIVES: Screening for CRE using ertapenem (ETP) is associated with non-specificity (Sp) due to a variety of mechanisms. This study aimed to improve Sp of CRE DDS by establishing an alternate cut-off for MEM.

METHODS: This study used 265 ENT including 149 PCR-confirmed non-CRE and 116 CRE [90 $bla_{KPC-2/3}$: 46 *K. pneumoniae* (KPN), 32 *E. cloacae* (ECL), seven *E. coli* (ECO), three *E. aerogenes*, one *C. freundii*, one *K. oxytoca*; 20 bla_{NDM-1}: 12 KPN, four *M. morganii*, two ECL, two ECO; four bla_{OXA-48}: three KPN, one ECO; two bla_{SME} *S. marcescens*]. Strains were tested with MRP10 tablet (Rosco), ETP and MEM discs (Oxoid) placed side-by-side on Mueller-Hinton agar (MHA-Plus, Oxoid). After 18hx35°C, the conservative 2011 CLSI breakpoint of <23 mm for ETP/MEM, and <25 mm for MRP10, were used to indicate screen positivity. MEM and MRP10 breakpoints were modified to maximize sensitivity (Sn) and Sp for CRE detection. Fisher's exact test compared Sp of CLSI ETP with modified MEM/MRP10 breakpoints.

RESULTS: Only ETP (range 6-22 mm) detected all CRE but with a Sp of 67%. In contrast, the % Sn/Sp for MEM and MRP10 were 97.4/89.3 and 96.6/86.6, respectively. Using breakpoint for MEM of <25 mm and MRP10 of <27 mm, CRE detection was 100% while Sp improved significantly for MEM (85%; P=0.0007) and trended towards significance for MRP10 (76%; P=0.12) vs ETP Sp of 67%.

CONCLUSIONS: Using a revised MEM breakpoint of <25 mm (closer to the EUCAST MEM breakpoint of <26 mm) or MRP10 cut-off of <27 mm, improves Sp for the detection of CRE to 85% (P=0.0007) and 76% (P=0.12), respectively, while maintaining 100% Sn. Laboratories may streamline detection of potential CRE by using these revised breakpoints.

FP32

EVALUATION OF METHODS FOR DETECTING CARBAPENEMASE-PRODUCING *ENTEROBACTERIACAE* (CRE) FROM SURVEILLANCE SPECIMENS

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OBJECTIVES: Optimal CRE detection methods are not yet established. This study compared CRE agars to ESBL agar (Oxoid) followed by CLSI Mueller-Hinton ertapenem disc diffusion (MHA, EtpDD; Oxoid) or by the Etp agar spot method.

METHODS: One hundred forty-nine non-CRE and 116 PCR-confirmed CRE [90 KPC: 46 *K. pneumoniae* (Kpn), 32 *E. cloacae* (Ecl), seven *E. coli* (Eco), three *E. aerogenes*, one *C. freundii*, one *K. oxytoca*; 20 NDM: 12 Kpn, four *M. morganii*, two Ecl, two Eco; four OXA48: three Kpn, one Eco; two SME: *S. marcescens*] were grown on ESBL agar plus Etp disc for selective pressure. From fresh 0.5 McFarland suspensions, 10 uL was: a) streaked to three lots Colorex KPC (Alere) and Brilliance CRE (Oxoid), to MacConkey with 1mg/L imipenem (McImi1), 0.5 or 1 mg/L Etp (McEtp0.5/1); b) spotted to MHA with Etp at 0.5 or 1 mg/L (EtpSpot); and c) inoculated to MHA for Etp DD. Plates were read after 18 h at 37°C (screen agars) or 18 h at 35°C (CLSI MHA-EtpDD, MHA-EtpSpot plates).

RESULTS: Only ESBL followed by MHA-EtpDD and MHA-EtpSpot with Etp0.5 or Etp1 detected 100% (95%CI: 96.2-100) CRE with 33%, 40%, and 29% growing non-CRE, respectively. Screen agar results were [% CRE detected (95% CI); No. CRE missed; % with non-CRE]: McEtp0.5 [98 (93-100); 2/20 NDM; 52]; Colorex [97 (95-99); 5/60 NDM, 3/270 KPC, 1/12 OXA48; 32]; McEtp1 [96 (90-98); 2/20 NDM, 3/90 KPC; 36]; McImi1 [96 (90-98); 1/20 NDM, 4/90 KPC; 36.2], Brilliance [91.4 (88-94]; 2/60 NDM, 21/270 KPC, 1/12 OXA48, 6/6 SME; 63].

CONCLUSIONS: Only ESBL screen agar method followed by CLSI MHA-EtpDD or the MHA-EtpSpot plate grew 100% CRE, with the MHA-EtpSpot with Etp at 1 mg/L growing the least number of non-CRE. Notably, all CRE screen agars missed some CRE while growing comparable or higher numbers of non-CRE.

FP33

ACTIVITY OF NOVEL PEPTIDES AGAINST PLANKTONIC AND BIOFILM CULTURES OF COLISTIN RESISTANT GRAM-NEGATIVE BACILLI

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OBJECTIVES: D-confirmation alpha-helical peptides have been developed that are manipulated to alter their hydrophobicity, net charge and amphipathicity properties. Charge substitutions and structure alterations on the polar and non-polar faces of these peptides produce a unique "carpeted model" mechanism of action, broad spectrum of activity, and low level of toxicity to human cells. The peptides are membrane targeted, prokaryotic specific and have prolonged half-lives. This evaluation is to investigate the activity of five peptide constructs against different gram negative bacilli with known resistance to colistin.

METHODS: Five peptides and colistin were tested; RH035, RH148, RH166, RH282 and RH 297. Colistin-resistant *E. coli*, *Serratia marcescens*, *P. aeruginosa*, and *Acinetobacter baumannii* complex were tested. MICs were determined by broth microdilution, and biofilm (MBEC).

RESULTS: Colistin MICs varied from four to greater than 500 mg/L (S. marcescens and Acinetobacter). The *S. marcescens* strain was resistant (>500 mg/L) to all peptides tested. For RH035, all other clinical strains had MICs of 16 – 32 mg/L. RH282, had similar MICS except for one *Acinteobacter* (MIC 250 mg/L). In the sessile state, all the colistin MICs

were >256 mg/L. The activity of RH 035 was reduced by one dilution against the colistin-resistant *E. coli*, but sessile MICs for the other peptides were reduced by 2-3 dilutions compared to the planktonic MICs.
CONCLUSIONS: RH035 and RH282 were the most active peptides. Planktonic MICs were similar to those for colistin. Sessile MICs for the RH035 peptide were similar to planktonic MICs for the *E. coli*. All strains had high sessile colistin MICs. It may be possible to alter the peptide structure to maintain activity in biofilms and provide alternatives for therapy.

FP34
COPAN LIQUID BASED MICROBIOLOGY AND WASP AUTOMATION ARE OPTIMIZING MICROBIOLOGY LABORATORIES WORKFLOW AND RESOURCES

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OBJECTIVE: Copan Liquid based Microbiology (LBM) and the WASP (Walk Away Specimen Processor) are optimizing workflow and resources for the microbiology laboratories. LBM devices (ESwab (ES), Fecal Swab (FS), UriSwab (US), SL-Solution (SLS), LIM, Selenite (SEL), Rappaport (RVS) and BHI broths) are allowing multiple testing from the same samples, and WASP can prepare Gram smears, plate and broths inoculation and antibiotic susceptibility testing. The objective was to demonstrate how LMB facilitate specimen's collection for multiple testing and how WASP optimises workflow and resources.
METHOD: We analyzed all testing methods performed with samples in ES for (genital, nasal, oropharyngeal, ocular, ear and wound), FS for (stool or rectal swabs), US for (urine), SLS for (sputum) and LIM, SEL, RVS and BHI enrichment or selective broths. Samples in LBM devices can be used for culture (manual or with WASP), Gram smear, detection of bacterial antigen/toxins and viral antigens and molecular assays for many pathogens and were validated for compatibility and performance with a variety of rapid bacteria antigen/toxins and viral antigens and nucleic acid, with in-house and commercial molecular assays.
RESULTS: LBM validation and utilization with the WASP and manual methods demonstrate compatibility with a variety of laboratory methods with a single sample versus test specific devices. LBM devices can be used for Gram stain, culture, antigen/toxins, molecular assays, without affecting the sensitivity of the assays, but improving workflow and resources.
CONCLUSION: The Copan LBM device and WASP are allowing microbiology laboratories to better optimize workflow, improve quality and TRT time and better utilization of staff to perform specialized molecular assays.

FP35
EVALUATION OF TWO NEW ENHANCED STREPTOCOCCAL LATEX AGGLUTINATION GROUPING KITS

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OBJECTIVES: Two newly introduced kits (MedicoDx –inter-medico and Pathodxtra-Oxoid/Remel) are now able to detect antigens A,B,C,D,F and G with a modified nitrous acid extraction method requiring no incubation. Pathodxtra offers an optional direct colony method not needing the acid extraction. This study evaluates both new kits and methods including the current PathoDx kit using 400 previously characterized *Streptococci* comprised of all Lancefield groups.
METHODS: 129 gp. A, 154 gp. B , 24 gp. C , 58 gp.D , 11 gp. F and 24 gp. G were tested using MedicoDx (MDx), PathoDxtra (PDx) and PathoDx (PD) acid extraction methods and PD and PDx direct colony methods. Time studies were performed when using all antisera/isolate and one antisera/isolate. Ease of use, cross reactions, quality of agglutination were assessed also.
RESULTS: Sensitivities were >90.5% and specificities >96% for all methods and groups tested. Slightly less specificity was achieved using PD and PDx direct methods. No cross reactions occurred using acid extraction methods. Twenty-eight cross reactions with PD occurred and 14 with PDx using the direct colony method. Direct testing was also more prone to rough and weak agglutination reactions. Time taken for acid extraction methods did not significantly vary between kits. Direct testing, compared to acid extraction, was more rapid by 60% using one antisera/isolate and 30% when using all antisera/ isolate.
CONCLUSIONS: All kits performed well including the new modification for group D. Accuracy was highest for Lancefield group A antigen detection for all kits and methods. Direct colony testing may require additional interpretive skills to be an effective method in the laboratory.

FP36
REAL-TIME EVALUATION OF URISWAB VS A DIP-SLIDE METHOD FOR TRANSPORT AND CULTURE OF URINE SAMPLES IN OUTPATIENT SETTINGS

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OBJECTIVE: Time, temperature and transport from remote outpatient facilities to testing laboratories may compromise urine culture results. This study compared urine collected by Uriswab (Copan Innovation, Brescia, Italy) with a dip-slide medium paddle method used in rural environments.
METHODS: One hundred consecutive urines submitted for culture from each of three out-patient clinics, were inoculated either to Uriswab or Dip-N Count and sent by courier to the testing lab. On receipt, the Uriswab sample was centrifuged and plated on blood and MacConkey agars. The dip-slide and plates were incubated at 35°C. Colony counts and identifications were performed. Time from collection to plating in the laboratory was noted.
RESULTS: Of the 300 urine samples collected, 111(37%) did not grow from the Uriswab sample; 72 (24%) from the dip-slide. In all, 66 pathogens were recovered from the Uriswab (35% of the 189 samples that grew). For the dip-slide, 49 pathogens were isolated (21.5% of the 228 samples that grew). There was no difference between the species isolated from each sample, except that five beta-haemolytic *streptococci* were recovered from the Uriswab that were not seen in the dip-slide cultures. Transport times from collection to processing in the testing lab varied from 15 minutes to 21 hr. There was no discernible difference in results based on delays in transport. Of the 39 (13%) samples that grew only on the dip-slide all were low colony count cultures with a single or multiple non-pathogens.
CONCLUSIONS: More pathogens were recovered from Uriswab samples than the dip slide. The Uriswab also failed to grow a large number of low colony count samples that did not contain a pathogen. The Uriswab offers a simple way of transporting urine for culture, at room temperature from remote locations.

FP37
COMPARATIVE PERFORMANCE OF TWO LIQUID AMIES TRANSPORT SYSTEMS FOR THE RECOVERY OF FASTIDIOUS BACTERIA FOLLOWING THE CLSI M40A SWAB ELUTION PROTOCOL AT ROOM TEMPERATURE

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OBJECTIVE: To compare the performance of Puritan's liquid Amies transport medium with HydraFlock® swab, non treated (P) (Puritan Medical Products Company LLC), to Copan ESwab™ transport system containing a treated flocked swab (CT) and ESwab™ transport medium with COPAN standard FLOQSwab (CS) (Copan Diagnostics Inc.).
METHOD: Viability tests were performed using Swab-Elution Method (CLSI M40-A2) on swabs held at RT for 0, 24 and 48 h. Six ATCC strains on an ongoing study were tested including two *N.gonorrhoeae* (NG), one *N. menengitidis* (NM), two *H.influenzae* (HIN), and one *S.pneumoniae* (SPN). Nine swabs of each brand were inoculated by absorbing 100 µL of an approximate 1.5×10^7 CFU/ml organism suspension. After incubation, 100 µL from each of three swab/devices from each of the three swab types was serially diluted 10-fold in 0.9 ml sterile saline. 100 µL of each dilution was pipetted onto appropriate agar plates and incubated under optimal conditions for subsequent colony counts. After 48 hrs incubation, colony counts for each swab cultured at each time point were recorded. An average CFU count was then determined from each triplicate set of swabs and each incubation

period. Performance was compared for each swab type with the 0 hr counts at the dilution that produced 30 to 300-500 colonies.
RESULTS: All three swab types produced comparable CFUs at 0 hr. Three of six strains were recovered from (P) after 48 hrs incubation; NM (1/1), HIN (1/2), and SPN (1/1) and 5/6 strains after 24 hrs. Three of six strains were recovered from (CT) after 48 hrs incubation; NM (1/1), HIN(1/2), and SPN(1/1) and 3/6 strains after 24 hrs. Two fo six strains were recovered from (CS) after 48 hrs incubation; NM(1/1) and SPN(1/1) and 2/6 strains after 24 hrs.
CONCLUSIONS: In general, based on this limited study, all three flocked swabs appear to perform comparably. Treating the flocked swabs does not appear to influence the recovery of these fastidious organisms. Further study with more isolates and different strains may be required to detect a significant difference in performance between these two systems.

FP38
UTILITY OF COPAN SL-SOLUTION FOR LIQUEFACTION OF MUCOUS IN STOOL SAMPLES USED IN MOLECULAR DETECTION OF TOXIGENIC *CLOSTRIDIUM DIFFICILE*

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OBJECTIVES: Diarrheal stool samples often contain mucous that can interfere with nucleic acid extraction. This study evaluates the ability of SL-Solution (SLS) to liquefy mucous in stools, release and preserve *Clostridium difficile* toxin genes for molecular detection.
METHODS: Adult diarrheal stool samples (n=318) negative for *C. difficile* toxin gene were re-tested using the pre-treatment of 1:10 dilutions in STAR Buffer (Roche) and the SLS. They were processed as per standard laboratory operating procedure. All specimens were extracted by EasyMAG™ (bioMerieux) and toxin genes were amplified by qualitative multiplex Real Time-PCR on the Rotorgene™ (Qiagen).
RESULTS: Of the 318 stool samples, pre-treatment with SLS resulted in detection of an additional 12 positives which were deemed negative using the STAR buffer. Twenty-one samples diluted using the STAR buffer yielded a smaller sample size of supernatant to use in the extraction process. Pre-treatment with STAR Buffer also caused plugged tips during the extraction process with EasyMAG™ resulting in 5 extraction runs being aborted and repeated.
CONCLUSION: A total of 12 new positives out of 318 samples tested were detected. That is 3.7% additional *C. difficile* positives detected using SLS and there were no runs that required repeating on the EasyMAG™ due to plugged tips.
The Copan SLS effectively liquefies mucous in stools and improves DNA extraction with EasyMAG™ with higher percentage detected. It is easy to use and eliminates the need to re-extract due to plugging of the pipettes on the EasyMAG™.

FP39
YIELD FROM MULTIPLICATE *CLOSTRIDIUM DIFFICILE* STOOL CYTOTOXICITY ASSAYS WHEN THE FIRST SPECIMEN WAS NEGATIVE

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OBJECTIVE: Although the sensitivity of *C. difficile* cytotoxicity assays is suboptimal, the value of multiplicate samples is unclear. We examined the subsequent positivity rate when the initial sample was negative and when repeat samples were submitted in the preceding week.
METHODS: The *C. difficile* cytotoxicity assay was performed using a stool filtrate on FSK or HEp-2 cells and with/without *C. difficile* antitoxin and read at four, 24 and 48 hrs. Results were extracted from the laboratory information system. A cluster was defined as two or more sample taken within seven days. Only clusters with an initial negative CDCA were included. Negative samples within 30 days after a preceding positive sample were not considered. Two random sample cluster datasets were created and analyzed by hand, and were used to validate results from an automated SAS algorithm.
RESULTS: In all, there were 131 additional positives of the 2446 duplicate (or more often) specimens received. The positivity rate for the duplicate specimens was 5.5%. This compared with an overall positivity rate for our laboratory of 11.5%. The percent of duplicates positive on the first and subsequent days in the cluster did not show an upward trend to reflect incident disease.
CONCLUSIONS: The yield from duplicate samples appears sufficiently high to justify repeated testing. At a cost of $15 per CDCA, the cost per positive when the first assay was negative was $272.73. The rate of discrepant results even on same day samples suggests technical problems with this very operator/observer-dependent assay. Sample size was too small to determine incident infection rates. More sensitive and specific *C. difficile* diagnostic tests are required.

FP40
EVALUATION OF *CLOSTRIDIUM DIFFICILE* (CD) TESTING USING MOLECULAR-BASED (MB) AND ENZYME IMMUNOASSAY (EIA) TESTS IN CLINICAL STOOL SPECIMENS FROM THE SASKATOON HEALTH REGION (SHR), SASKATOON, CANADA

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OBJECTIVE: The objective of this trial was to assess if MB tests BD GeneOhm™ (BG) and Meridian Illumigene™ (MI) were more sensitive and specific than the TechLab Tox A/B II™ (TT) EIA-based test.
METHODS: SHR stool samples submitted by a physician for CD testing between Nov '10 to Feb '11 were tested simultaneously with BG, MI and TT. Results for all samples were recorded as positive or negative for each testing platform. If all three results were concordant, then the true result was recorded as such. If any of the three results were discordant for a sample, then further testing was performed using cytotoxicity assay testing and this was used as the true result.
RESULTS: A total of 301 stool samples were tested during the study period. The prevalence of disease was 10.6%. TT displayed sensitivity (SN) of 90.6%, specificity (SP) 100%, positive predictive value (PPV) 100% and negative predictive value (NPV) 98.9%. BG and MI testing had SN 100%, SP 97.4%, PPV 82.1% and NPV 100%. The agreement of results between the two MB tests was very strong (kappa=0.97) but weaker between the MB and EIA tests (kappa=0.84). Cytotoxicity testing had higher agreement with EIA (kappa=0.95) vs MB testing (kappa=0.89).
CONCLUSIONS: Sensitivity of MB testing was higher than EIA testing, but the reverse was true of specificity. Agreement between testing methodologies and the historical gold standard for CD was higher with EIA rather than MB testing. While unexpected, these results challenge the idea of cytotoxicity testing being the gold standard, as the two MB test results had high levels of correlation. More studies are needed to assess if MB testing should become the new gold standard for CD.

FP41
EVALUATION OF THE BRUKER SEPTITYPER™ KIT AND BRUKER MALDI BIOTYPER™ FOR THE DIRECT IDENTIFICATION OF ORGANISMS FOR POSITIVE BLOOD CULTURES

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OBJECTIVES: Blood cultures are one of the most critical specimens processed by clinical microbiology laboratories. Due to the critical nature of these specimens, rapid reporting of organism identification has the potential to beneficially impact patient management. We sought to evaluate the performance and turnaround time of organism identification the Bruker SeptiTyper™ blood culture identification kit and Bruker MALDI

BioTyper for the direct identification of blood culture isolates from the BacT/Alert blood culture system.

METHODS: Blood was inoculated into bioMérieux BacT/Alert SA and/or SN bottles at the bedside and incubated on the BacT/Alert instrument. For positive cultures, a Gram stain was performed and bottles were subcultured for routine identification. An aliquot of each culture was used for direct identification using the SeptiTyper™blood culture kit and MALDI BioTyper™. Routine methods included a variety of commonly used rapid (coagulase, indole, latex agglutination) and standard biochemical tests (Vitek™ 2 and API™) depending on the organism isolated. Discordant identifications were further characterized with additional biochemical methods or 16srDNA sequencing. Turnaround time was calculated from culture positivity to reporting identification.

RESULTS: A total of 61 monomicrobial and two polymicrobial cultures were evaluated. Among monomicrobial cultures, 52 (85.2%) had a valid score (≥1.7) for identification. Of these, 100% were concordant with conventional identification. Nine (14.7%) of cultures had a low score (<1.7) or invalid test, of which all those with score ≥1.5 (4) were concordant with the final identification. Isolates with low scores and invalid tests tended to be likely contaminants or members of the *Streptococcus mitis* group. For polymicrobial cultures, the Bruker BioTyper™ correctly identified one of the isolates present with a confident score in both cultures. Turnaround time to identification was 6.5 hours from culture positivity for the Bruker BioTyper and 40.8 hours for the conventional methods ($p<0.001$).

CONCLUSIONS: MALDI-TOF MS combined with the Bruker SeptiTyper kit is a rapid and highly accurate method for direct identification of pathogens from positive blood cultures. Allowing for known limitations, the technology has the potential to dramatically reduce time from positivity to definitive identification, potentially allowing for improved patient care.

FP42

CONFIRMING *NEISSERIA GONORRHEA* ISOLATES: A NOVEL USE OF THE GENPROBE APTIMA ASSAY

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OBJECTIVE: To prospectively study the use of the Genprobe Aptima Combo 2 assay, normally used for the detection *N. gonorrhoea* RNA in genitourinary samples, as an alternative method for confirmation of *N. gonorrhea* isolates.

METHODS: After a pilot study using gonorrhea and non-gonorrhea *Neisseria* isolates was conducted to ascertain the optimal dilution method and specificity, clinical samples suspicious for *N.gonorrhea* were prospectively tested using: (1) modified rapid fermentation test (MRFT); (2) 16S PCR; and (3) Genprobe Aptima Combo 2 assay. Samples for the Aptima assay were prepared by pipetting 100ul of a 1/100 dilution of a 0.5 McFarland standard into 2.9 ml of Genprobe transport media.

RESULTS: A total of 205 suspected *N.gonorrhea* isolates were identified over six months. MRFT and 16S methods identified 201 *N.gonorrhea* isolates, with the Aptima assay also correctly identifying these isolates. Four isolates were identified as non-*N.gonorrhea* by a 16S PCR and MRFT. These four were also negative by the Aptima assay. Although prospectively, few non-*N. gonorrhea* isolates were identified, the pilot data was concordant with the prospective data that demonstrated 100% sensitivity and 100% specificity for this method as a means of confirming the identity of *N. gonorrhoea* isolates.

CONCLUSION: The Genprobe Aptima Combo 2 assay is a sensitive and specific means of confirming the identity of suspected *N. gonorrhoea* isolates.

FP43

USE OF AMPLICOR SWABS ON THE VIPER XTR FACILITATES TRANSITION BETWEEN METHODOLOGIES

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OBJECTIVES: During the transition period between old to newer methods, end users may submit previously employed specimen collection devices not intended for use with the new system. This may require concurrent use of new and old instrumentation. To facilitate the transition of chlamydia (CT) and gonorrhea (GC) testing from the Amplicor system (Roche) to the Viper XTR (BD) we validated the use of Amplicor swabs on the Viper XTR platform.

METHODS: One ml of saline diluent as added to 250 endocervical specimens collected using the Amplicor "dry" swabs. After vortexing, the swabs were discarded and the liquid specimen was tested with the Ampicor system. Residual volume was transferred to the BD Viper-XTR tubes and additional saline was added to ensure the volume was within the "fill" window. These specimens were then tested on the Viper XTR. In addition, a subset of swabs were tested using BD Viper Qx diluent vs saline and without a heating step prior to placement on the Viper-XTR.

RESULTS: Results using the Qx diluent were equivalent to those with saline. Removing the heating step prior to testing on the Viper-XTR also did not affect the results. The concordance for CT was 98.8% and 100% for GC. No residual specimen available for discordant analysis of CT results. All of the GC positive specimens had concomitant Ames swabs submitted that were culture positive.

CONCLUSION: This study validates the use of Amplicor swabs for testing CT and GC on the Viper-XTR. This will ensure that patients will not have to undergo unnecessary recollection of genital specimens during this transition phase, and could save resources that would be otherwise discarded and does not necessitate operating old and new instruments concurrently.

FP44

EVALUATION OF OUR CURRENT IN HOUSE REAL-TIME PCR CONFIRMATION SCHEME THAT SPECIFICALLY TARGETS HAEMOPHILUS INFLUENZAE OVER A 3 YEARS PERIOD (2009-2011) WHICH COVERS OVER 490 CANADIAN STRAINS

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OBJECTIVE: PCR /real-time PCR detection/confirmation methods especially for non-typeable (NT) *Haemophilus influenzae* (Hi) are very limited whether it is documentated in scientific journals or availabe from commercial sources. It is for this reason we have developed an in-house real-time PCR scheme aiming to specifically detects all Hi (typeable and NT). After three years of using this PCR detection scheme, it is time to review and evaluate it's efficiency and effectiveness.

METHODS: Our in-house real-time PCR confirmation scheme is mainly based on the use of four dual labelled (5' 6-FAM, 3'-TAMRA) TaqMan probes with degeneration designs according to our sequence survey results of the *P6* and *bexA* genes of Hi and other related *Haemophilus* species to capture all Hi (both typeable and NT) on a SmartCycler II real-time PCR platform.

For validation purposes, all confirmation results obtained by this scheme were also verified side by side with other Hi validation methods. Any result discrepancy between our scheme and validation methods would be considered as confirmation failure of the PCR scheme.

RESULTS: From 2009 to 2011, a total of 491 Canadian strains were tested by this PCR scheme and confirmation results agree completely (100%) with the results of the other validation methods for all of the strains. Of the 491 strains, 472 were identified as Hi of which 179 were typeable strains while the remaining 293 were NT strains. All 19 non *H. influenzae* strains were also successfully differentiated by this real-time PCR scheme.

CONCLUSIONS: A 100% detection and confirmation rate is highly convincing. This real-time PCR scheme is able to pick up all typeable and

NT Hi strains. It also enables us to distinguish Hi from the two important closely related *Haemophilus* species of *quentini* and *haemolyticus*. After 490+ Canadian strains were perfectly identified without a single miss, this scheme can now be more confidently served as a quick molecular detection test of Hi and is especially useful for non-culture positive Hi cases.

FP45

VALIDATION OF A REAL-TIME PCR ASSAY FOR DETECTING *BORRELIA BURGDORFERI* IN TICKS

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OBJECTIVE: The BCCDC Public Health Microbiology and Reference Laboratory has been conducting passive surveillance for *Borrelia burgdorferi* in the tick population since 1995. In order to reduce the turnaround time and increase the sensitivity of *B. burgdorferi* detection, we evaluated and validated a real-time PCR method for the detection of *Borrelia sp.* used at the National Microbiology Laboratory and incorporated an additional assay to detect PCR inhibition.

METHODS: DNA was extracted and real-time PCR was performed on the ABI Taqman 7500. The screening test, which targeted 23S rRNA of *Borrelia sp.*, was run in parallel with the custom designed inhibition control. Positive samples were then subjected to a confirmatory test which targeted the *B. burgdorferi ospA* gene. Analytical validation was based on a comparison between this method and traditional PCR sequencing of the DNA (n=50). The clinical validation was evaluated with regular and *Borrelia*-spiked ticks (n=20).

RESULTS: Analytical comparison between the real-time assay and traditional PCR sequencing showed a sensitivity of 93%, specificity of 100% and balance accuracy of 96.67%. No cross-reactivity was observed when the assay was challenged with non-*Borrelia* organisms. Eight non-Lyme *Borrelia* species were observed to be positive for the screening test but negative on the confirmatory test.

CONCLUSION: This validation study showed the Taqman assay was rapid and reproducible. The implementation of an inhibition control assay was helpful in monitoring for the presence of false negatives in complex sample matrices such as that of the tick mid-gut. This study demonstrated the assay's discriminatory power and allowed us to detect non-culturable *B. burgdorferi* in ticks.

FP46

CHALLENGES WITH LYME DISEASE DIAGNOSTICS

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OBJECTIVE: The incidence of Lyme Disease (LD) is increasing across Canada and more diagnostic testing is being performed due to increased physician and public awareness. The National Microbiology Laboratory (NML) provides LD screening and confirmatory diagnostic testing for provincial and hospital laboratories across Canada. As our scope of testing and number of submissions increases, we have encountered several challenges with LD diagnostics.

METHODS: Data from submissions to the NML from 2010 and 2011 were used to determine the percentage of positive samples based on the screening and confirmatory assays. A questionnaire was sent to all submitting labs through the Canadian Public Health Laboratory Network (CPHLN) to assess their requirements and use of NML referral services.

RESULTS: There was a 30% increase in submissions for LD diagnostics since 2009. The challenge that exists with LD diagnostics are, that often, the clinical data provided is insufficient, resulting in additional testing being performed.

LD serological tests are very difficult to evaluate and demonstrate proficiency due to the lack of well characterized serological panels.

It is difficult to determine whether the antibodies detected are the result of a past infection or a current re-infection, with the methodology presently available.

CONCLUSIONS: The demand for LD diagnostic testing at NML will continue to increase as LD becomes endemic within more areas of Canada. The creation of a LD user group would provide a forum for dialogue with our submitters, facilitate inter-laboratory sample exchanges for improved quality control and proficiency, and allow us to work towards standardization of LD diagnostic testing across Canada.

FP47

TECHLAB GIARDIA/CRYPTOSPORIDIUM CHEK EIA IS AN EFFECTIVE AND EFFICIENT METHOD FOR DETECTION OF *GIARDIA LAMBLIA* AND *CRYPTOSPORIDIUM PARVUM* IN STOOL

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OBJECTIVES: The diagnosis of *Cryptosporidium parvum* (CP) or *Giardia lamblia* (GL) infection by stool filtrate microscopy is labor intensive and not amenable to high throughput testing, which can lead to delayed turnaround times (TAT) when specimen volumes are high. In this study, the performance characteristics of stool antigen EIAs and their impact on TAT have been evaluated.

METHODS: Stool filtrates that were read by experienced technologists were tested using the TechLab *Giardia/Cryptosporidium* CHEK and Remel ProSpect T Stool EIAs. Discrepant results between EIA and microscopy were further tested by DFA (MeriFluor Crypto & Giardia). True positives were defined as positive identification by microscopy. TAT and positivity rates prior to the implementation of the EIA were compared with those after implementation.

RESULTS: The sensitivity/specificity of the TechLab and Remel EIA compared to microscopy were 94.7%/93.5% and 82.5%/93.3%, respectively. Both EIAs detected one additional GL and CS (confirmed by DFA) that were not identified during microscopy suggesting the EIA is actually a more sensitive test. Although our positivity rate before and after implementation has not changed (1.6% vs 1.3%), the average TAT has dropped substantially (85 vs. 312 hours).

CONCLUSION: The TechLab Giardia/Cryptosporidium CHEK EIA is sensitive and an efficient screening method for common stool parasites.

FP48

IMPROVED UTILIZATION OF NUGENT SCORES AFTER PHYSICIAN SPECIFIC FEEDBACK OF TESTING RESULTS WITH PEER COMPARISON

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OBJECTIVE: Many physicians view Nugent scores as part of a regular sexual health check-up; they are not. Physicians are most likely to improve utilization when they receive feedback on their testing results and when those are compared with peers.

METHODS: The intervention group included physicians who submitted ≥15 slides for Nugent scores and if their positivity rate was <33%. A letter reminded them that guidelines do not recommend testing in asymptomatic patients and informed them of their positivity rates and that of their best performing peers (in the 50% range). They were asked to review their current practice and to consider reducing the number of tests submitted. Subsequently, specimen numbers and positivity rates were compared with controls who had positivity rates in ≥33% or who sent fewer samples irrespective of their positivity rate.

RESULTS: In all, 48 letters were sent to physicians who met the selection criteria. Of those only four of 48 sent more samples in the post-intervention period. The average number of samples fell from 38 to 24 and the positivity rate increased from 18.6% to 22%. Of the 58 physicians who did not receive letters, 21 submitted more samples in the post-intervention period. The average number of specimens submitted was not statistically different (13.4 vs. 12.2%). The positivity rate in the control group fell from 30.7% to 25.4%. Using "Cliff's Delta" the effect size of the intervention was −0.48 vs a value of −0.06 in the no-letter group.

CONCLUSION: Despite selecting poor performers for the letter group, this intervention improved practices and resulted in fewer samples, more of which were positive. This constitutes a practical example of how physician feedback can be used to improve laboratory utilization.

FP49

ENTEROPATHOGENS & THEIR RESPONSE TO THERAPY IN ART NAÏVE & ART ADHERENT HIV/AIDS ADULT SUBJECTS WITH DIARRHEA

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AIMS & OBJECTIVES: To study the spectrum of enteric pathogens in ART naïve & ART adherent HIV/AIDS adult patients with diarrhea and assess the response of specific anti-diarrheal therapy on persistence/clearing of enteric pathogens identified.

MATERIALS & METHODS: Twenty-five ART naïve (group I) and 25 ART adherent (group II) HIV/AIDS adult patients with diarrhea were enrolled for intervention study. Stool samples from all subjects were examined for enteropathogens by wet mount, staining methods (gram's, modified Kinyoun's, trichrome stain), culture and ELISA (*Cryptosporidium* Ag, *Clostridium difficile* toxin, *Entamoeba histolytica* Ag). Subjects with enteropathogens were put on specific therapy as per NACO guidelines. Follow up stool samples were examined after 2-4 weeks of completion of therapy for persistence/clearing of enteropathogens. $CD4^+$ count was done for all subjects.

RESULTS: Group I had 27.8% bacterial, 55.6% parasitic and 16.7% fungal pathogens, while group II had 10.3%, 69% and 20.7% pathogens , respectively. A single pathogen was identified in 20% of subjects in group I, while it was 32% in group II. Fifty-six per cent of subjects in group I had multiple pathogens, while only 44% in group II. In group I, 47.4% of subjects had clearance of pathogens, while group II had 57.9%. Three subjects in group I succumbed during the course of study.

CONCLUSIONS: Bacterial infections were more common in the ART naive (27.8%) than the ART adherent (10.3%) group. Co-infection with multiple pathogens was more common in the ART naive (56%) than the adherent group (44%). Microbial clearance after specific therapy was less in the ART naive (47.4%) than the ART adherent group (57.9%).

FP50

DISEASE PROGRESSION AND RESPONSE TO ANTIRETROVIRAL THERAPY IN NEWLY DIAGNOSED HIV SEROPOSITIVE SUBJECTS IN A TERTIARY CARE HOSPITAL IN NORTH INDIA

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OBJECTIVE: Our study analyzes HIV disease progression and antiretroviral therapy (ART) response by measuring CD4 counts and plasma viral load (PVL) in newly diagnosed HIV seropositive subjects in a tertiary care center in New Delhi, India.

METHODS: One hundred thirty newly diagnosed HIV seropositive subjects were enrolled for the study. Subjects were categorized into three study groups; CD4 counts <200 cells/µl (group A, 43 subjects); 200-500 cells/µl (group B, 44 subjects) and >500 cells/µl (group C, 43 subjects). At recruitment, PVL estimation was done for group A subjects only. Group A cases were initiated on ART and were followed up after six months for evaluation of response to ART by measuring the CD4 counts and PVL. Group B and C cases were followed up after six months to monitor disease progression by measuring the CD4 counts only.

RESULTS: Among group A subjects, there was a statistically significant rise in the median CD4 counts after six months of ART. At baseline, PVL ranged from 2636 - >750,000 copies/ml with a median PVL at baseline of 165,000 copies/ml. At follow up, 90% of study subjects had undetectable level of viraemia (viral load of <400 copies/ml). Among group B, there was a fall in the CD4 counts at follow up as compared to baseline levels which was statistically insignificant. Among group C, a statistically significant fall in the CD4 counts was observed at follow up.

CONCLUSIONS: CD 4 count is a powerful tool to determine response to ART and also monitor disease progression in newly diagnosed HIV seropositive subjects. This study also reinforces the role of PVL estimation in judging the response to ART especially in cases of immunovirologic discordant responses. Thus PVL estimation is a vital test before switching over to second line antiretroviral therapy.

FP51

IMMUNITY TO MEASLES VIRUS INFECTION IN PRENATAL BRITISH COLUMBIANS

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OBJECTIVE: A survey for antibody to measles was conducted in BC to investigate anti-measles IgG levels by birth age in prenatal women. All age strata were assessed by Dade-Behring and reported in mIU/mL and a subset of sera were also tested by the VIDAS anti-measles IgG assay to assess the assay cutoffs of both methods.

METHODS: Routine prenatal screening sera from BC women born between 1960 and 1979 inclusive (received between January 2009 and December 2010) were retrospectively selected to assess age stratified anti-measles IgG levels. All samples were tested for anti-measles IgG by the Enzygnost Anti-Measles-virus/IgG assay (Dade-Behring) and a subset of samples was tested by the VIDAS Measles IgG Assay (Biomerieux).

RESULTS: Three hundred thirty-eight samples had antibody to measles at 337 mIU/mL or higher by both assays. A further 32 samples tested negative and 30 specimens tested equivocal for antibody to measles by both assays. Discordant results were observed for nine samples testing reactive by the Behring assay and equivocal by the VIDAS assay and 20 samples testing equivocal by the Behring assay and reactive by the VIDAS assay. Finally, of the five specimens testing non-reactive by the Behring assay, two tested reactive and three equivocal by the VIDAS assay.

CONCLUSIONS: The observations provide reassurance of the comparability of qualitative results between the Measles VIDAS and Behring Assays, and that serological immunity test conducted with the VIDAS assay are comparable to internationally accepted correlates of protection.

FP52

HCV ANTIGEN TESTING FOR THE DIAGNOSIS OF HEPATITIS C INFECTION: A COST-EFFICIENT ALGORITHM

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BACKGROUND & OBJECTIVES: Diagnosis of active Hepatitis C virus (HCV) infection is currently performed using RNA testing in North America, which is highly sensitive and specific but is associated with three major limitations: lability of RNA molecules; higher costs; and longer turn-around time as compared with commercially-available HCV core antigen testing. In the current study a new Health Canada-approved HCV core antigen assay product was evaluated for the diagnosis of HCV infection and its cost reducing potential.

METHODS: Ninety plasma specimens positive for HCV RNA along with 25 negative HCV specimens were used for HCV antigen assay. Twenty-four specimens positive for a panel of agents were used for possible cross-reactivity. Sixty-four HCV antibody-positive specimens with negative HCV RNA and indeterminate HCV immunoblot results were also employed.

RESULTS: In the first group 78/90 (86.6%) tested positive for HCV antigen with regression analysis showing no significant deviation from linearity. None of the prenatal specimens tested positive for HCV antigen. Non-specific reactions were not observed. In HCV antibody-indeterminate group, only 2/64 (3.1%) were RNA positive. In the last group, none of the HCV antibody very-low-positive specimens tested positive for HCV antigen. Both inter- and intra-run reproducibility of 100% were noted. Our cost analysis showed a minimum of 52.13% reduction in costs associated with qualitative RNA testing.

CONCLUSION: Considering the complexity of HCV infection diagnosis and a significant cost and turn-around time burden it imposes on clinical labs, HCV antigen testing seems an attractive adjunct to the current battery of laboratory diagnostics that demands more attention.

FP53

VALIDATION OF POOLED HIV POSITIVE SERA AS AN ANALYTE FOR HIV POINT-OF-CARE QUALITY ASSURANCE

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OBJECTIVE: To assess the suitability of pooled HIV positive sera for proficiency, quality control, training and competency assessment purposes.

METHODS: A pool of 42 de-identified sera that tested reactive for anti-HIV and non-reactive for HBsAg and anti-HCV on the Siemens ADVIA Centaur was prepared and serially diluted in NAT dilution matrix to 10^{-1}, 10^{-2} and 10^{-3}. The initial pool and dilutions were maintained at room temp and assessed for stability and reproducibility of test results on the Siemens Centaur and the INSTI rapid anti-HIV point-of-care test over a two week period (tested 5x/wk). Two dilutions were selected, 10^{-1} for use as a strong positive INSTI control and 10^{-2} for a weak positive control.

Results:	Homogeneity and Clarity	Centaur (Index)	INSTI HIV Test
HIV pool	Clots, turbid	>50.0	Reactive
10^{-1} dilution	Clear	>50.0	Reactive
10^{-2} dilution	Clear	>50.0	Weak reactive
10^{-3} dilution	Clear	>50.0	Non-reactive

The initial pool and the dilutions demonstrated stable sample integrity and reproducible analyser and point-of-care signals over the two week assessment period.

CONCLUSIONS: Pooled and serially diluted HIV positive sera stored at room temp for two weeks displayed stable analyser and point-of-care signals over time. Pooled sera even if diluted in NAT dilution matrix could provide a suitable challenge analyte for point-of-care quality assurance.

FP54

ROCHE COBAS® 4800 VS QIAGEN HC2® FOR HIGH-RISK HPV DNA DETECTION

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OBJECTIVE: To compare the performance of the Roche cobas® 4800 HPV Test (COBAS) and Qiagen hc2® High Risk HPV DNA Test (HC2) for detection of high-risk (hr) HPV DNA among women enrolled in the HPV FOCAL Trial (ISRCTN79347302).

METHODS: ThinPrep® cervical specimens collected at study enrollment were tested by HC2, COBAS, and the Roche Linear Array HPV Genotyping Test (LA).

RESULTS: For 6,170 specimens, agreement between HC2 and COBAS was 96.1% (κ=0.75; 95% CI 0.72-0.79). Test signals for most HC2/COBAS discordant specimens were close to the respective assay detection thresholds.

		Linear Array			hrHPV Distribution		
	N	HPV⁻ (%)	lrHPV only (%)	hrHPV* (%)	HPV 16	HPV 18	hrHPV non 16, 18
HC2⁺/ COBAS⁺	411	14 (3.4)	1 (0.2)	396 (96.4)	97	39	318
HC2⁻/ COBAS⁻	5521	4900 (88.8)	529 (9.6)	92 (1.7)	20	7	67
HC2⁺/ COBAS⁻	107	37 (34.6)	56 (52.3)	14 (13.1)	1	1	12
HC2⁻/ COBAS⁺	131	25 (19.1)	16 (12.2)	90 (68.7)	17	4	72

**hrHPV includes specimens with multiple hrHPV types*

CONCLUSIONS: Agreement between HC2 and COBAS for the detection of hrHPV was high. However, based on LA as a genotyping reference, HC2 had more false positive hrHPV detections than COBAS, and COBAS detected more hrHPV, including HPV 16 and 18, than HC2. Fifteen per cent of HPV 16 and 14% of HPV 18 infections identified by LA were not detected by either HC2 or COBAS.

FP55

INCREASED RECOVERY OF HIGH RISK HPV MRNA FROM SUREPATH LIQUID-BASED PAP SAMPLES TREATED WITH PROTEINASE K

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OBJECTIVES: SurePath™ (SP) liquid based (L-Pap) is a collection medium for cervical cytology. Storage of SP L-Pap may have a negative effect on the recovery and detection of high risk human papillomavirus (HR HPV) due to formalin-induced nucleic acid cross-linking. We sought to determine retrospectively, the effects of ProK digestion on HR HPV E6/E7 mRNA detection from SP specimens following storage at varying times and temperatures. A second prospective study group was also analyzed in two temperatures and timed scenarios.

METHODS: L-Pap samples were collected from 428 women attending a colposcopy clinic. Within 48 hours, 1mL was tested for the presence of HR HPV mRNA in an APTIMA® HPV assay (Gen-Probe). Residual samples were held at 4°C for varying time intervals (1-7 months) before being aliquoted for use in one of three ProK treatment strategies: (A) 426 samples following storage at 4°C; (B) 234 samples (132 positives and 102 negatives) 4°C storage and 10 days at RT; (C) 220 samples (126 positives and 94 negatives) 4°C storage and three days at 30°C. In the prospective study, of 170 patients, 49 positives were aliquoted in two scenarios (D) Samples held for two and five days at 30°C and (E) seven and 14 days at RT. 1 mL of sample was treated with 100 uL of ProK and a second 1 mL as an undigested control, heated for two hours at 65°C, then tested.

RESULTS: Testing of the initial SP aliquots from the 428 samples found 136 (31.8%) to be HPV positive. Following storage at 4°C (retrospective strategy A) for one to seven months, only 90 were positive. ProK increased the positives from 90 to 129 (43.3%). For strategy B, 73 to 113 (54.8%). For strategy C, 66 to 111 (68.2%). In the prospective scenarios, ProK increased the positives by 23% in (D) and 30.8% in (E).

CONCLUSIONS: The number of SP samples positive for HPV mRNA decreased during storage. Due to a substantial increase in positive samples, ProK treatment would be a useful strategy to enhance HPV results.

FP56

THE EVALUATION OF A REAL TIME RT-PCR ASSAY FOR DIAGNOSING ORTHOBUNYAVIRUS INFECTIONS IN CANADIAN PATIENTS

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OBJECTIVES: Orthobunyaviruses such as the California serogroup (CSG) viruses Jamestown Canyon virus (JCV) and snowshoe hare virus (SSHV) have been previously documented by serological procedures to cause cases of febrile and neuroinvasive disease across Canada. The objective of this study was to evaluate a real time reverse transcription (RT) – polymerase chain reaction (PCR) assay for detecting CSG virus RNA in both spiked and probable case serum and cerebrospinal fluid (CSF) specimens.

METHODS: Preparations of cultured SSHV and JCV were assessed for viable virus concentration by plaque assays to determine plaque forming units (PFU) per ml. A dilution series of viral RNA was then added to virus free plasma and CSF and a sensitivity curve generated to calculate the number of PFU detected by the RT-PCR assay being applied. Primers and probes targeting conserved regions of the S segment of SSHV and JCV viruses were utilized in a Taqman based RT-PCR run in an ABI 7900 sequence detector. Serum and CSF from 200 probable cases of SSHV and JCV infection were screened by the RT-PCR assay for viral RNA.

RESULTS: The real time Taqman assay was able to detect 0.1 PFUs in spiked samples containing SSHV and JCV RNA. The two sets of primers and probes specific for different portions of the S segment showed similar sensitivities. Screening of 200 sera and CSFs resulted in nine RT-PCR positive specimens.
CONCLUSIONS: Our results indicate that a real time RT-PCR assay has the potential to detect CSG viruses present in low concentrations in both sera and CSF. A preliminary screen of clinical samples resulted in a number of RT-PCR positives. Additional characterization of RT-PCR positives will be presented.

FP57
FLOCKED NASOPHARYNGEAL SWABS VS NASOPHARYNGEAL WASH FOR THE DIAGNOSIS OF RESPIRATORY VIRUSES IN CHILDREN

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OBJECTIVE: This study aimed to directly compare the sensitivity and tolerability of nasopharyngeal wash (NPW) and flocked nasopharyngeal swabs (FS) for respiratory virus specimen collection, in a direct prospective, cross sectional study. The ideal method of specimen collection and testing is a combination that is rapid, easy, non-distressing to the patient, inexpensive and leads to the most sensitive and specific results.
METHODS: Children, three months to16 years of age, seen in the emergency department with acute febrile respiratory symptoms were enrolled, and randomized to have either NPW or FS done first. Both tests were administered. After mucolysis, respiratory cells were washed and fixed to slides for direct immunofluorescence (DFA) for common viruses (respiratory syncytial virus (RSV), influenza A and B, parainfluenza, adenovirus and human metapneumovirus (hMPV), using commercial reagents (Chemicon and Diagnostic Hybrids).
RESULTS: Eight-nine children had both samples collected; 55 (62%) were boys. The median age was 31 months (4 months to 12 years). NPW was done first in 44 children. Viruses were detected by DFA in 68/89 (76%) of samples; 66 of the NPW samples (74%) and 68 of the FS samples (76%). RSV was detected in 35 (38%) of the samples; 34/35 were detected in both specimen types while 1 was only detected in FS. Influenza was detected in 16 specimens and parainfluenza was detected in six specimens; there was 100% concordance.
CONCLUSIONS: In these preliminary data, flocked swab performed as well as NPW; viruses were detected by immunofluorescence in 74% of NPW samples and 76% of FS samples.

FP58
EVALUATION OF RT-PCR/ESI-MS FOR THE DETECTION AND CHARACTERIZATION OF INFLUENZA VIRUSES

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OBJECTIVE: Antigenic shift among influenza dictates that surveillance is based on more than hemagglutinin typing. We evaluated base composition analyses of six influenza gene segments (PB1, PB2, PA, M, NS, NP) using multi-locus RT-PCR followed by electrospray ionization/mass spectrometry (ESI-MS).
METHODS: The Abbott PLEX-ID Flu Detection Assay uses RT-PCR/ESI-MS and a database of base compositions for the inference of influenza virus H and N subtypes. We challenged the assay with 27 isolates representing different human, mammalian and avian influenza strains consisting of seven different H and N types. Analytical sensitivity was compared to the limit of detection (LOD) of real-time RT-PCR. 'Sample' reports and 'detailed' reports were used for analyses.
RESULTS: RT-PCR/ESI-MS matched the LOD of real-time PCR but failed to detect one of the highest dilutions within a proficiency panel. Unique strains were correctly identified in 25/26 of the detected samples using the detailed report analyses. The missed identification was correctly categorized as 'other' influenza A in the sample report. However, the sample report was not helpful with 4/26 isolates indicating 'seasonal' or 'multiple matches' on swine-origin strains.
CONCLUSION: RT-PCR/ESI-MS analysis detects and characterizes most unique strains of influenza virus. The assay appears to have comparable analytical sensitivity to real-time RT-PCR. Its same-day automated approach can make novel influenza strain detection possible when DNA sequencing is otherwise impractical. RT-PCR/ESI-MS technology is suitable for influenza virus surveillance but complete analysis using the optional workstation with 'detailed' reports is required.

FP59
GENETIC ANALYSIS OF INFLUENZA A AND B VIRUSES CIRCULATING IN ALBERTA, CANADA POST 2009 PANDEMIC

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OBJECTIVE: Molecular methodologies can be used for detection, subtyping, determination of antiviral resistance and antigenic genetic drift, providing a comprehensive analysis of circulating influenza viruses (IFVA and B).
METHODS: Respiratory specimens submitted from hospitalized, community and outbreak settings between July1, 2010 and Dec 31, 2011 were included in this analysis. Detection and subtyping was performed using real-time assays. The detection of oseltamivir resistance resulting from the H275Y mutation in (H1N1) pdm09 virus was performed with a SNP RT-PCR assay. Select positive samples were sequenced for antiviral resistance in the *M2* and *NA* gene, and antigenic drift in the *HA* gene. The lineage of IFVB was determined by sequencing of the *HA* gene.
RESULTS: A total of 28,815 samples were tested and 1,181 (4.1%) samples tested positive for IFVA including 855 (72.4%) of subtype H3, 286 (24.2%) of subtype (H1N1) pdm09 and 37 (3.1%) that could not be typed because of low viral load. A total of 768 (2.7%) samples tested positive for IFVB. Positive samples representing different categories such as age groups, immune status, hospitalized or community patients, and severity of infection were characterized further. All of the (H1N1) pdm09 and H3 viruses showed the S31N amino acid change, one of the (H1N1) pdm09 sample also showed the presence of V27A; other nucleotide changes leading to oseltamivir resistance were not detected. The H3N2 viruses clustered into two distinct clades, both showed sequence drift from A/Perth/16/2009. The D222G/N polymorphisms in the HA gene of (H1N1) pdm09 was found in four samples.
CONCLUSION: Characterization of influenza viruses is important for effective treatment, infection control, and epidemiological surveillance.

FP60
CMV PCR ON THE ROCHE MAGNA PURE 96 AND LIGHTCYCLER 480

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OBJECTIVE: An internally controlled, quantitative CMV PCR was designed on the Roche Magna Pure 96 (MP96) and lightcycler 480 (LC480) instruments and compared with a previously established MPLC/lightcycler 1.5 assay for detection of CMV reactivation in transplant patients.
METHODS: Plasma samples were extracted with the MP96 Universal virus protocol with an internal control plasmid, IAC. Extracts and PCR master mix were transferred to the PCR plate by robotic liquid handling.
RESULTS: The assay was linear for viral loads above 700 copies/ml. The 95% confidence LOD was 200 cp/ml. Three hundred eighty plasma samples

previously tested on the MP-LC/LC1.5 were tested on the new system. All samples with viral loads over 100 cp/ml were positive by the new assay. Below 100 cp/ml, 18 samples were discrepant; however, these samples were below the LOD and below the threshold for clinical decision making (1000 cp/ml). All samples only positive by the new method had previously positive results within three months by the established method. A Bland Altman analysis gave a mean bias of −0.001, the XY scatter plot of the log values gave a slope of 0.9964, with a correlation coefficient of 0.963. Accordingly, the two assays gave equivalent results. No sample cross-contamination was seen when using samples with high viral loads. Reproducibility was assessed at viral loads of approximately 250 cp/ml, (CV=49%, n=25), 5000 cp/ml (CV=12.1%, n=30), and 25000 cp/ml (CV=7.8%, n=16).
CONCLUSION: The new PCR protocol using the MP96 and the lightcycler480 gave equivalent results to the established assay on the MP-LC/LC1.5.

FP61
MOLECULAR DETECTION FOR VIRUSES IMPLICATED IN BONEMARROW TRANSPLANT FAILURES

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OBJECTIVE: Human parvovirus B19 (PV-B19) has been associated with severe diseases such as hydrops fetalis in pregnant women, aplastic crisis in patients with hemoglobinopathies, and pancytopenia in immuno-deficient patients. Human herpes virus 6 (HHV6) variants A and B can cause hepatitis, pneumonitis, encephalitis in immunodeficient patients and a failure to engraft in recipients of bone marrow transplants.
METHODS: Two real-time PCR assays were designed for the detection of PV-B19, HHV6 (A and B) targeting the VP1 gene and DNA polymerase, respectively. Detection for PV was performed on the Lightcycler platform using a Taqman probe. Detection and differentiation for HHV 6A and 6B was performed on the Taqman platform using two single nucleotide polymorphism (SNP) MGB probes. These assays were validated for sensitivity, dynamic range, efficiency, specificity and reproducibility. Accuracy was determined using positive samples provided by NML.
RESULTS: Sensitivity for the detection of PV, HHV6A and 6B was five, 10 and nine copies, respectively. The assays were 100% specific when tested with high copy templates of other viruses and bacteria that manifest similar symptoms. The inter- and intra-assay variability was low and PCR efficiency was close to 100%. A panel of five PV positive samples provided by NML resulted in two discordant samples; one negative sample had a Ct of 35.83 by the in-house assay suggesting in fact a positive sample with a low viral load, and one sample with a weak band was negative by the in-house assay. Results for the detection of HHV6 were 100% accurate when a blind panel provided by NML was tested by the in-house assay.
CONCLUSIONS: We have developed and validated assays for PV and HHV6 that will be primarily used for immuno-compromised patients.

FP62
STANDARDIZATION OF THE FOLLOW-UP TESTING OF PATIENTS WITH SMEAR-POSITIVE PULMONARY TUBERCULOSIS

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OBJECTIVES: The current guidelines recommend airborne isolation of patients with pulmonary TB until they have three consecutive AFB negative respiratory specimens. This study was undertaken to a) assess the current practice followed in Manitoba with respect to submitting follow-up samples for microbiological work up and b) develop an algorithm to standardize the follow-up of AFB smear positive pulmonary TB patients in Manitoba.
METHODS: The initial diagnostic and follow-up cultures of all patients diagnosed with pulmonary tuberculosis in the year 2010 in the province of Manitoba were reviewed. The results were presented to the various stakeholders and an algorithm for follow-up testing of smear positive pulmonary TB patients was approved to be the Provincial guideline
RESULTS: There were 119 patients diagnosed with TB in 2010. Eighty-three patients had pulmonary TB. Thirty-four of the 83 had at least one initial diagnostic sample positive for AFB. Follow-up samples were submitted on 32/34 patients from day 4 to day 67. One to 24 follow up samples were submitted from these patients. Days to conversion of smear positive to negative were in the range of four to 67 days. Culture conversion to negative happened only in six (18.7%) patients and the longest time taken for the conversion was 127 days.
CONCLUSIONS: The time to conversion to smear negative depended upon the degree of positivity of the initial diagnostic sample. Culture conversion to negative was documented only in six patients. An algorithm to standardize the follow-up testing has been drawn and approved to be the Provincial guideline.

FP63
EVALUATION OF BD MGIT™ TBC IDENTIFICATION TEST FOR RAPID IDENTIFICATION OF *MYCOBACTERIUM TUBERCULOSIS* COMPLEX FROM BD MYCOBACTERIA GROWTH INDICATOR TUBE MGIT™

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OBJECTIVE: Rapid identification of *M. tuberculosis* is critical for patient care and infection prevention and control. We compared the sensitivity, specificity and ease of use of the BD MGIT™ TBc Identification Test (chromatographic immunoassay test) with Accuprobe® (GENPROBE®) *Mycobacterium tuberculosis* Complex Culture Identification Test (DNA probe test).
METHOD: Respiratory, fluid and tissue specimens were processed by NaOH-NALC decontamination and digestion, then inoculated to liquid and solid media. When the liquid medium MGIT™ was detected as positive by the BD BACTEC™ 960 Mycobacterial Detection System, the tube was removed from the instrument and an aliquot was smeared to determine growth of acid fast bacilli (AFB). AFB positive growth was then tested by TBc and Accuprobe®.
RESULTS: From September to December 2011, seven isolates tested positive with both TBc and Accuprobe® (identified by reference methods as *M. tuberculosis*). TBc was determined to be a simple procedure consisting of pipetting positive MGIT broth into a test well. Fourteen samples were negative for *M. tuberculosis* complex by both methods. These isolates (identified by reference methods) included *M. avium* complex, *M. xenopi*, *M. fortuitum* and *Tsukamurella* species. To date, all positive and negative TBc results correlated with current testing and reference methods.
CONCLUSION: Despite the small sample size of this study, our testing was 100% sensitive and specific. The BD MGIT™ TBc Identification Test can prove to be an easy, rapid method (total assay time 15 minutes) for the identification of *M. tuberculosis* complex.

FP64
SIMULTANEOUS INFECTION WITH A MULTI-DRUG RESISTANT AND PAN-SUSCEPTIBLE STRAIN OF *MYCOBACTERIUM TUBERCULOSIS* IN A MANITOBA PATIENT

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BACKGROUND: A 34-year-old HIV-positive immigrant from Africa presented with weight loss and cough. Based on CT scan findings, patient history and a 4+ AFB smear, *M. tuberculosis* (MTB) infection was suspected.
METHODS: Due to the strong suspicion of antibiotic resistant MTB infection, a sputum sample was submitted for real-time PCR detection of MTB. The DNA was also subjected to gene sequencing to detect antibiotic

resistance to isoniazid (INH) and rifampin (RIF). Upon the availability of positive culture, phenotypic antibiotic sensitivity testing was completed using the BACTEC MGIT 960 along with sequencing of the genes associated with antimicrobial resistance and MTB genotyping.

RESULTS: Real-time PCR of the sputum sample indicated the presence of MTB. PCR from the sputum sample showed no antibiotic resistance mutations. Phenotypic testing of the subsequent culture showed resistance to both INH and RIF (ie, MDR-TB strain). Gene sequencing from this culture showed a mixed result. MIRU-VNTR genotyping showed multiple alleles indicating the presence of two MTB strains in this patient. The RIF resistant strain was isolated and determined to be an MDR-TB strain. MIRU-VNTR genotyping confirmed the presence of a single strain. These laboratory results indicated that this patient was infected with both antibiotic sensitive TB and an MDR-TB. The lack of antibiotic-resistance mutations in the sputum sample likely indicates an over-representation of the sensitive strain in that sample.

CONCLUSION: This case demonstrates that the rapid identification of antibiotic resistant TB using molecular methods may be particularly challenging if the patient has simultaneous infections with drug sensitive and drug resistant strains.

FP65

MOLECULAR DETECTION OF CLARITHROMYCIN RESISTANCE IN *MYCOBACTERIUM AVIUM*

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OBJECTIVE: Clarithromycin is the first line of defence in the treatment of *Mycobacterium avium* infections. Many recent studies have suggested that point mutations at positions 2058 and 2059 of the 23rRNA gene in M. *avium* are highly predictive of clarithromycin resistance. These studies have a limited number of resistant isolates from highly clonal populations. We have evaluated sequence analysis of the V domain of the 23srRNA gene to determine whether or not it accurately identifies clarithromycin resistance in our study population.

METHODS: A blinded panel of 312 M. *avium* strains was tested for clarithromycin resistance using broth microdilution. The V region of the 23srRNA gene was amplified using PCR and the sequence analyzed to determine if mutations were present at positions 2058 and 2059.

RESULTS: Of the 312 strains tested, 51 had MIC values ≥32. All strains with MIC <32 had wild-type sequences at positions 2058 and 2059 of the 23srRNA gene. Of the 51 resistant strains tested, 12 contained mutations at position 2058, eight had mutations at position 2059. Thirty-one did not contain mutations. While the test did not identify any false positives, the sensitivity was only 39.2%.

CONCLUSIONS: For the M. *avium* isolates investigated, sequencing of the 23S rRNA gene provides poor sensitivity for identifying resistance. Further investigation is needed to determine weather or not these isolates represent mixed populations. At present phenotypic testing is still required to accurately identify clarithromycin resistance in M. *avium*.

FP66

GENOTYPING REVEALS TWO MAJOR SUBGROUPS OF *MYCOBACTERIUM XENOPI*

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OBJECTIVES: *Mycobacterium xenopi* is an opportunistic pathogen responsible for tuberculosis-like disease, and is the third most common mycobacterium isolated at the Public Health Ontario Laboratories (PHOL). Here, we describe the development of PCR- and sequencing-based methods for molecular epidemiology of M. *xenopi*.

METHODS: Identity of clinical M. *xenopi* isolates (n=55) was confirmed by rRNA sequencing. Genome sequences of one PHOL clinical isolate and the ATCC 19250^T M. *xenopi* reference strain were generated via Roche GS-FLX/454 pyrosequencing. Draft genome comparison uncovered Regions of Difference (RDs) representing sequences exclusive to individual genomes. PCR assays were developed for six RDs, including three chromosomal targets and three plasmid targets.

RESULTS: The M. *xenopi* genome contains two copies of the rRNA operon, which can be hetereogenous. Sequencing identified three ITS alleles and, in the 16S rRNA gene, two sites were polymorphic. PCR-based assays targeting the chromosomal RDs revealed two major genotypes of M. *xenopi*, with only two outlier isolates. Plasmid targets were more variable, such that 37 (66%) strains were positive for all targets, eight (14%) were negative for all targets, and the remaining 11 (20%) exhibited a mosaic distribution.

CONCLUSIONS: The presence of multiple and heterogenous copies of the rRNA operon made ITS and16S rRNA gene sequencing technically demanding and sequencing results did not correspond with RD analysis. Chromosomal RD targets divided M. *xenopi* strains into two major clusters. Distribution of plasmid RDs was more variable, but 47 (86%) isolates were positive for at least one target. The development of rapid methods for M. *xenopi* genotyping will enhance surveillance and molecular epidemiology of this emerging pathogen.

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