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C A N A D I A N J O U R N A L O F  
**INFECTIOUS DISEASES &  
MEDICAL MICROBIOLOGY**  
JOURNAL CANADIEN DES MALADIES INFECTIEUSES  
ET DE LA MICROBIOLOGIE MEDICALE

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**AMMI Canada – CACMID Annual Conference**  
April 2-5, 2014, Victoria, British Columbia

**ORAL PRESENTATIONS**

**Thursday, April 3, 2014**

<b>Session A</b>	<b>A1-A4</b>
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**Friday April 4, 2014**

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<b>Session G</b>	<b>G1-G7</b>
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<b>Session I</b>	<b>I1-I5</b>
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## ORAL PRESENTATIONS

1600-1700 Oral Presentations: Session A  
Room: Convention Centre (Esquimalt)

A01

**CLOSTRIDIUM DIFFICILE: A STUDY IN THE SEARCH FOR FEATURES ASSOCIATED WITH THE ABILITY TO CAUSE OUTBREAKS**A Wen<sup>1,2</sup>, G Broukhanski<sup>1,2</sup><sup>1</sup>Public Health Ontario Laboratories; <sup>2</sup>Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON

*Clostridium difficile* (CD) is responsible for the majority of antibiotic-associated infections acquired at hospitals. Infected patients frequently shed CD spores into the environment, making it a major transmission factor and essential to its ability to cause outbreaks. Using high-resolution typing techniques (MMLVA), we found that strains isolated during outbreaks consisted of a main strain ('outbreak', with multiple identical isolates) and several sporadic strains. The outbreak strain was commonly of NAP1 type. Previous research focused on identifying markers of a 'hypervirulent' phenotype. However, because NAP1 strains are highly variable, we sought to determine markers of an 'outbreak' strains. Outbreak and sporadic strains from eight outbreaks were cultured until sporulation had occurred. Vegetative cells morphology and sporulation rates were documented by microscopy, while germination dynamics were estimated using Terbium fluorescence assay. No statistically significant difference in morphology, sporulation and germination of outbreak and non-outbreak strains was identified. Thus, we assessed other characteristics which might promote transmissibility of CD strains, such as spore adhesion and surface features as resolved by electron microscopy. Our data indicate that CD exosporium or spore crust can be responsible for adhesion of the spore to surfaces and its persistence in a hospital environment. Further studies of strains with mutated genes responsible for these features of CD spores should identify roles of these spore's components and assist with development of diagnostic tests to differentiate strains capable causing outbreaks from sporadic strains and develop effective measures of infection control to prevent transmission of this very important hospital-associated pathogen.

A02

**MOLECULAR EPIDEMIOLOGY OF CLOSTRIDIUM DIFFICILE IN A BRITISH COLUMBIA COMMUNITY: SHIFT IN PULSED-FIELD GEL ELECTROPHORESIS PROFILES BETWEEN 2008 AND 2013**AN Jassem<sup>1</sup>, N Prystajek<sup>1</sup>, F Marra<sup>1</sup>, P Kibsey<sup>2</sup>, K Tan<sup>2</sup>, P Umlandt<sup>1</sup>, L Janz<sup>1</sup>, S Champagne<sup>3</sup>, BCAMM Members<sup>4</sup>, B Gamage<sup>5</sup>, B Henry<sup>1</sup>, L Hoang<sup>1</sup><sup>1</sup>BC Centre for Disease Control Public Health and Reference Laboratory, Vancouver; <sup>2</sup>Island Health, Victoria; <sup>3</sup>St Paul's Hospital, Vancouver; <sup>4</sup>British Columbia Association of Medical Microbiologists; <sup>5</sup>Provincial Infection Control Network of British Columbia, Vancouver, BC

**OBJECTIVES:** Toxin-producing *Clostridium difficile* causes gastrointestinal illness that can progress to sepsis and death. A 19 bp mutation in *tcdC*, the repressor of toxins A/B, is associated with hypervirulent North American pulsotype 1 (NAP1) strains that were proportionally most prevalent across British Columbia during a one-month period in 2008, at 42.4%. Here we describe the 2013 burden of health care-associated (HA) and community-associated (CA) *C. difficile* infection (CDI) in the Vancouver Island population and compare against 2008 findings from that region.

**METHODS:** *C. difficile* isolates were cultured from toxin B-positive diarrhoeal stool specimens submitted to the Royal Jubilee Hospital laboratory during a four-month period in 2013. CDI cases were reviewed for HA or

CA classification. Isolates were characterized by pulsed-field gel electrophoresis, *tcdC* genotyping and susceptibility testing.

**RESULTS:** Of the 68 CDI cases culture positive for *C. difficile*, 23 (33.8%) were CA, 45 (66.2%) were HA, and mean age was 67.8 years. On average CA cases were 9.2 years younger than HA cases, with a median difference of 21 years. Strains without a designated NAP and those of NAP other than NAP1 were most prevalent, at 44.1% each. Most (75.0%) NAP1 isolates harboured the (-18)(-1) *tcdC* mutation, while most (83.1%) non-NAP1 isolates contained wild type *tcdC*. All isolates were susceptible to metronidazole and vancomycin, although 23.5% were susceptible to clindamycin. No major strain differences between CA and HA cases were seen.

**CONCLUSIONS:** The 2013 proportion of NAP1 strains in Vancouver Island is 30.6% less than that in 2008, while *tcdC* association and susceptibility remained the same. Enhanced surveillance is necessary for identification of circulating strain types and evaluation of CA- versus HA-CDI burden.

A03

**EVALUATION OF A COLOREX C DIFFICILE FROM CHROMAGAR FOR ISOLATION OF CLOSTRIDIUM DIFFICILE FROM STOOL SPECIMENS FOR CHARACTERISATION AND STRAIN TYPING BY MOLECULAR METHODS**G Broukhanski<sup>1,2</sup>, C Lee<sup>3</sup><sup>1</sup>Public Health Ontario Laboratories; <sup>2</sup>Laboratory Medicine and Pathobiology, University of Toronto, Toronto; <sup>3</sup>St Joseph's Healthcare, Hamilton, ON

PCR is replacing EIA and culture for detection of *Clostridium difficile* (CD) toxin in clinical specimens but for typing or susceptibility testing, isolation of CD strains is essential. To evaluate performance of the Colorex *C. difficile* agar (CCD) from CHROMagar, we compared its performance to the in-house CDMN agar with regard to time to growth, ability to select for CD colonies and suitability of DNA isolated from colonies for PCR analysis as per delineated criteria. Seven stool samples, positive for toxin gene by PCR, were used for this evaluation. Specimens were directly spread using a loop on to the surface of the agar or suspensions were made in water or 50% ethanol, plated after pre-treatment and incubated anaerobically for 24 h, 48 h and for seven days. After 24 h of anaerobic growth, colonies on the CCD were clearly visible in UV light as bright spots surrounded with a fluorescent zone while UV fluorescence of CD colonies on CDMN were not readily detectable. The visible CD colonies on both types of agar had a characteristic appearance and were up to 1 mm, slightly larger on the CCD. Less CD colonies were seen on both types of plates after ethanol pre-treatment but there were significantly more of non-CD colonies on CDMN than on CCD plates when suspensions were not pre-treated. This was especially noticeable when specimens were plated directly and incubated for 48 h. Following seven days of growth, microscopy revealed lack of spores on CDMN but large number of spores on CCD, an important advantage for preservation of the isolate. There are several advantages to CCD compared to CDMN: faster isolation, ability to readily detect CD and generation of spores. Also, due to its high sensitivity (to a single cell) CCD can be used for confirmation or in place of the PCR assay.

## A04

IDENTIFICATION AND CHARACTERIZATION OF A *CLOSTRIDIUM DIFFICILE* ISOLATE WITH REDUCED SUSCEPTIBILITY TO VANCOMYCIN

A Wong<sup>1</sup>, DA Boyd<sup>2</sup>, G Golding<sup>2</sup>, M Graham<sup>2</sup>, C Frenette<sup>3</sup>, L Holmes<sup>4</sup>, K Katz<sup>5</sup>, P Kibsey<sup>6</sup>, A McGeer<sup>7</sup>, M Miller<sup>3</sup>, D Moore<sup>8</sup>, AE Simor<sup>9</sup>, K Suh<sup>10</sup>, G Taylor<sup>11</sup>, GG Zhanel<sup>12</sup>, D Gravel<sup>13</sup>, MR Mulvey<sup>2</sup>, Canadian Nosocomial Infection Surveillance Program (CNISP)

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**OBJECTIVE:** To describe an isolate of vancomycin (VAN)-resistant *Clostridium difficile*. VAN is the antimicrobial of choice for treating severe complicated *C. difficile* infection (CDI).

**METHODS:** The isolates were identified as part of the 2012 CNISP CDI surveillance. Chart reviews were conducted to determine patient characteristics. Antimicrobial susceptibility testing was conducted using Etest and agar dilution. Molecular typing was conducted using PFGE and PCR ribotyping. Whole genome sequencing (WGS) was generated using the MiSeq (Illumina).

**RESULTS:** The isolate 19B1210 displayed an MIC for VAN using Etest of 24 mg/L and 32 mg/L using agar dilution. The patient had been hospitalized for 118 days prior to the CDI and was successfully treated for the CDI with metronidazole. The isolate had a rare NAP1 PFGE type (008; PCR-ribotype 027) and two other patients with VAN-susceptible isolates were identified with the same PFGE type six weeks after the initial case in the same facility. WGS analysis of all three isolates revealed the presence of only two single nucleotide polymorphisms (SNP) in 19B1210 that were not present in the VAN susceptible isolates: 1) a G to A transition (pos 4085120) which resulted in an I301T change in a two-component sensor histidine kinase; and 2) a G to A transition (pos 4171528) which resulted in a F475S change in a putative RNA/single-stranded DNA exonuclease.

**CONCLUSION:** This is the first description of a clinical isolate with a VAN MIC of 24 mg/L to 32 mg/L. No transmission of this strain was observed. Although the clinical relevance is unknown, spread of a strain with an elevated VAN MIC leading to impaired response would be globally disastrous. Further studies are underway to examine the mechanisms explaining the elevated VAN MIC.

1600-1700 Oral Presentations: Session B  
Room: Convention Centre (Oak Bay 1)

## B01

IS THE PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA), VANCOMYCIN-RESISTANT ENTEROCOCCUS (VRE), EXTENDED-SPECTRUM  $\beta$ -LACTAMASE-PRODUCING ENTEROBACTERIACEAE (ESBL), CARBAPENEM-RESISTANT ENTEROBACTERIACEAE (CRE) AND *CLOSTRIDIUM DIFFICILE* INFECTION (CDI) CHANGING IN CANADIAN HOSPITALS? A COMPARISON OF SURVEY RESULTS IN 2010 AND 2012

AE Simor<sup>1</sup>, V Williams<sup>1</sup>, A McGeer<sup>2</sup>, G Han<sup>3</sup>, Z Hirji<sup>4</sup>, O Larios<sup>5</sup>, K Weiss<sup>6</sup>, A Kiss<sup>7</sup>

<sup>1</sup>Sunnybrook Health Sciences Centre; <sup>2</sup>Mount Sinai Hospital, Toronto, ON; <sup>3</sup>Provincial Infection Control Network of British Columbia, Vancouver, BC; <sup>4</sup>Bridgepoint Health, Toronto, ON; <sup>5</sup>University of Calgary, Calgary, AB; <sup>6</sup>Hopital Maisonneuve-Rosemont, Montréal, QC; <sup>7</sup>Institute of Clinical Evaluative Sciences, Toronto, ON

**OBJECTIVES:** A point-prevalence survey for MRSA, VRE and CDI was done in 176 Canadian hospitals in 2010. We repeated the survey in 2012 to determine if there were any changes in prevalence over time.

**METHODS:** A follow-up survey was performed in adult inpatients in Canadian hospitals with >49 beds in November 2012. Data describing the participating hospitals and patients were obtained using standard criteria and case definitions. We also obtained data for ESBLs and CREs in 2012.

**RESULTS:** One hundred forty-three (58%) eligible hospitals with 29,042 inpatients participated with representation from all 10 provinces. Seventy-nine (55%) hospitals had 50 to 200 beds, 58 (41%) had 201 to 500 beds, and six (4%) had >500 beds. The median prevalence rates of MRSA (4.3 per 100 patients in 2010; 3.9 in 2012) and CDI (0.8 and 0.9 in 2010 and 2012 respectively) did not change over time, but there was an increase in VRE prevalence (0.5 in 2010, 1.3 in 2012; P=0.04). MRSA prevalence did not vary across the country, but hospitals in eastern provinces had lower rates of VRE, whereas CDI rates were highest in Central Canada. MRSA, VRE and CDI were all predominantly health care-associated, but MRSA was more often community-associated (30%) than VRE (6%) or CDI (23%) (P<0.001). In 2012, ESBLs and CREs were reported from 71 (57%) and 10 (8%) hospitals respectively. Median ESBL prevalence was 0.7; for CRE it was 0.

**CONCLUSION:** These data provide national prevalence rates for MRSA, VRE, ESBLs, CREs, and CDI among adult inpatients in Canadian hospitals. An increase in VRE rates was observed from 2010 to 2012. CREs are currently infrequently identified in Canadian hospitals.

## B02

## CHANGING TRENDS IN VANCOMYCIN-RESISTANT ENTEROCOCCI (VRE) BACTEREMIA: THE POWER AND PITFALLS OF USING PUBLICLY REPORTED INFECTION RATES

G Garber<sup>1,2</sup>, K Adomako<sup>1</sup>, C Prematunge<sup>1</sup>, F Lam<sup>1</sup>, G Didiodato<sup>1,3</sup>, J Robertson<sup>1</sup>, C Egan<sup>1</sup>

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**OBJECTIVE:** Screening and isolation of vancomycin-resistant enterococci (VRE) colonized patients is a contested issue in health care infection prevention and control settings. In 2013, Public Health Ontario (PHO) initiated a VRE bacteremia (VRE-B) project to examine provincial trends following the cessation of VRE screening and isolation practices in some hospitals in June 2012. The project utilized publicly reported data collected by Ontario's Patient Safety Public Reporting Program (PSPR) from hospitals. The objective of this analysis was to validate the PSPR VRE data to inform our VRE project.

**METHODS:** PHO received 2009 to 2013 PSPR VRE data, which is aggregated quarterly, to identify hospitals that reported  $\geq 1$  VRE-B(s). PHO requested monthly VRE-B data via a data collection template for each respective hospital site. Hospitals validated their monthly VRE-B counts against the quarterly PSPR VRE-B count.

**RESULTS:** From 2009 to 2013, 69 of the 235 hospital sites reported  $\geq 1$  VRE-B, totalling 361 VRE-B(s). To date, 45 of the total were incorrectly reported (12%). To date, 38 hospitals (55%) with  $\geq 1$  VRE-B have participated in the data collection process and 17 (15%) of these participating sites identified incorrectly reported VRE-B within PSPR (median number of errors reported: one, range: one to 27). We are unable yet to estimate false-negative reporting and, hence, sensitivity and specificity of PSPR.

**CONCLUSION:** Because the PSPR dataset has not been previously validated, this work highlights the need for data custodians and users to develop interdependent quality control feedback processes. This is to ensure validation steps are completed for improved decision-making when relying on PSPR data.

### B03

#### A SURVEY OF INFECTION PREVENTION AND CONTROL POLICIES FOR THE MANAGEMENT OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA), VANCOMYCIN-RESISTANT *ENTEROCOCCUS* (VRE), AND *CLOSTRIDIUM DIFFICILE* INFECTION (CDI) IN CANADIAN HOSPITALS

V Williams<sup>1</sup>, AE Simor<sup>1</sup>, A McGeer<sup>2</sup>, G Han<sup>3</sup>, Z Hirji<sup>4</sup>, A Kiss<sup>5</sup>, O Larios<sup>6</sup>, K Weiss<sup>7</sup>

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**OBJECTIVE:** Infection prevention and control (IP&C) policies are designed to prevent the spread of antibiotic-resistant organisms (AROs). We describe results of a survey of IP&C policies for MRSA, VRE and CDI in Canadian hospitals.

**METHODS:** In November 2012 a point-prevalence survey of MRSA, VRE, and CDI was performed in adult inpatients at Canadian acute-care hospitals with  $\geq 50$  beds. Information was also obtained regarding institutional characteristics and IP&C policies of each participating hospital.

**RESULTS:** One hundred forty-three (58% of eligible) hospitals representing all 10 provinces participated in the survey. MRSA screening occurred at all sites; 89% of sites reported screening for VRE. Screening for VRE was less common in Western Canada (77%;  $P=0.045$ ). Targeted screening of high-risk patients on admission to hospital was most common (62% for MRSA; 70% for VRE). Universal screening for MRSA was less commonly done in western Canada (10%;  $P=0.002$ ). All hospitals used additional precautions when caring for patients with MRSA or CDI; six (4%) hospitals, all in Ontario, did not do so for patients with VRE. Few hospitals reported the routine use of antiseptic soap for patients with MRSA (28%) or VRE (17%); 14% of facilities reported routine decolonization of patients with MRSA. Enhanced cleaning of rooms and/or equipment was reported for patients with CDI (99%), VRE (82%), and MRSA (61%). Ninety-one percent of sites reported monitoring hand hygiene compliance, and 31% reported hand hygiene compliance  $>80\%$ .

**CONCLUSIONS:** Although this study was unable to determine IP&C practices, we were able to describe policies related to the control of AROs in Canadian hospitals, and demonstrated considerable variability in these policies across the country.

### B04

#### DEVELOPING A CULTURE OF EVALUATIVE RESEARCH FOR INFECTION PREVENTION AND CONTROL PRACTICE

G Garber<sup>1,2</sup>, K Adomako<sup>1</sup>, F Lam<sup>1</sup>, J Robertson<sup>1</sup>, C Prematunge<sup>1</sup>, C Egan<sup>1</sup>

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**OBJECTIVES:** Many of the recommendations and guidelines applied today in Infection Prevention and Control (IPAC) are grounded in consensus and expert opinion. This can lead to conflicting guidance and controversy due to the lack of objective scientific evidence driving the decision-making process. The goal of this presentation is to describe ways that controlled intervention clinical trials can be applied to IPAC, using vancomycin-resistant *Enterococcus* (VRE) screening and isolation as a template.

**METHODS:** Some hospitals are considering or have discontinued active screening and isolation of VRE. The rationale for change was based on the lack of clear effectiveness, substantial cost of screening procedures, and that with better infection control practices in place the impact of screening discontinuation would be minimal. Others fear that a rise in VRE exposure will lead to increasing infections and, with limited array of available treatments, will lead to increasing morbidity/ mortality. Ontario hospitals are mandated to report their VRE bacteremias. We are using the provincial hospital public reporting system to measure the impact of this practice change by comparing hospitals who maintain versus discontinue VRE screening.

**RESULTS:** We identified previous study design shortfalls including the lack of: clinical trial rigor, control groups, predetermined stopping rules and hospital IRB review. Developing a controlled trial that compares hospitals with similar demographics (eg, geography, patient mix, acuity) using a priori outcome measures can address these deficiencies.

**CONCLUSIONS:** Controlled trials in IPAC are difficult to conduct but possible. We have expertise to conduct quality IPAC clinical trials across Canada but this will require leadership of organizations such as AMMI to foster a collaborative culture of evaluative research.

## 1600-1700 Oral Presentations: Session C Room: Convention Centre (Oak Bay 2)

### C01

#### RIDA@GENE PARASITIC STOOL PANEL AND XTAG® GASTROINTESTINAL PATHOGEN PANEL FOR THE DETECTION OF COMMON INTESTINAL PARASITES

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Intestinal parasites contribute to significant morbidity and mortality in developing countries, leading to malnutrition, delayed cognitive development and death. In non-endemic countries, returning travelers, refugees and recent immigrants, and the immune-compromised can be infected with the same pathogens. We evaluated the sensitivity and specificity of two commercial multiplex molecular assays that are able to detect common stool parasites in our setting. A retrospective sample collection ( $n=101$ ) comprised of stools infected with *Cryptosporidium parvum/hominis* (CP), *Giardia lamblia/intestinalis* (GI), *Dientamoeba fragilis* (DF), and *Entamoeba histolytica/dispar* (EHD) were tested by RIDA@GENE Parasitic (RGP) Stool Panel and xTAG® Gastrointestinal Pathogen Panel (GPP). RGP includes all four targets whereas GPP lacks the DF target. Stool extraction for the RGP was performed using the Promega Maxwell® 16 platform, and for the GPP using the Abbott m2000 instrument. Testing was conducted according to manufacturer guidelines. When compared with ova and parasite (O&P) microscopy and the ProSpecT® *Giardia*/*Cryptosporidium* EIA, both RGP and GPP assays were sensitive for the detection of GL (24 of 24 [100%] for GPP; 24 of 24 [100%] for RGP) and CP (20 of 20 [100%] for RGP; 19 of 20 [95%] for GPP). Of seven specimens for EHD based on microscopy, only one (one of seven [14.2%]) was confirmed by GPP to be *E. histolytica sensu stricto*, which RGP failed to identify. The remaining six

## Abstracts

EHD were negative by GPP and RGP suggesting these are *E. dispar* observed by O&P, which do not require anti-parasitic therapy. RGP was 85.7% sensitive for the detection DF, which GPP does not identify in the panel. Both assays were 100% specific for each target. Of note, 24 specimens positive for *Blastocystis hominis* were negative in the GPP and RGP panel for any pathogen. The TAT for both GPP and RGP could be performed within 24 h with a saving in labor costs. GPP and RGP multiplex molecular assays provide a rapid, high-throughput, labor-saving alternative to traditional O&P testing and serve well as a primary screen for common intestinal parasites in the non-endemic setting.

### C02

#### THE PREVALENCE OF COMMON INTESTINAL PARASITIC PATHOGENS FROM COMMUNITY PATIENTS IN ONTARIO 2008-2012

D Lungrin, D James, D Leto, H Almohri

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**OBJECTIVE:** To determine the prevalence of intestinal parasitic pathogens (IPP) and trends over a five year period (2008 to 2012).

**METHOD:** A total of 239,475 stool samples were analysed retrospectively. Samples from Ontario residents (population 12.9 to 13.4 million) collected in SAF fixative, were submitted to the laboratory for detection of intestinal parasitic pathogens. A formalin-ethyl acetate concentration and a Kinyoun-hematoxylin stained slide were performed on all samples. An additional 8655 commercial pinworm paddle samples were received for *Enterobius vermicularis* (pinworm).

**RESULTS:** The study yielded an overall parasitic prevalence of 10.3% (n=24,611). The overall prevalence of IPP averaged 5.8% in 2008 to 4.6% in 2012 (P=0.0002). Protozoans accounted for 85.1% (n=10,239) of the IPPs while helminths were 14.9% (n=1794). Distribution of protozoans were: *Dientamoeba fragilis* 59.6%, *Giardia intestinalis* 24.7%, *Entamoeba histolytica/dispar* 11.4%, *Cryptosporidium* sp. 2.3%, *Cyclospora* sp. 2.0% and two cases of *Cystispora belli*. For helminths, pinworm represented 67.0%, *Ascaris lumbricoides* 7.4%, *Trichuris trichura* 5.6%, hookworm 4.5%, *Strongyloides stercoralis* 4.4%, *Taenia* sp. 2.3%, *Hymenolepis nana* 2.9%, *Diphyllobothrium* sp. 2.4%, *Schistosoma mansoni* 1.9%, *Clonorchis sinensis* 1.1%, *Trichostrongylus* sp. 0.5%, and *Fasciola/Fasciolopsis* 0.2%. Seasonality was observed with *Cyclospora* sp. and *Cryptosporidium* sp., peaking in mid-summer (May to July) and late summer (July to September) respectively.

**CONCLUSION:** Rates of intestinal pathogenic parasites are declining in Ontario in the past five years. *Dientamoeba fragilis* is the most common protozoan isolated in Ontario, followed by *Giardia intestinalis*. Pinworm is the most common helminth isolated.

### C03

#### ANTIFUNGAL SUSCEPTIBILITY OF INVASIVE CANDIDA ISOLATES FROM CANADIAN HOSPITALS: RESULTS OF THE CANWARD 2013 STUDY

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<sup>1</sup>Provincial Laboratory, Alberta Health Services, University of Alberta, Edmonton, AB; <sup>2</sup>Medical Microbiology and Infectious Diseases, University of Manitoba; <sup>3</sup>Diagnostic Services of Manitoba, Winnipeg, MB

**OBJECTIVE:** 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 (R) in these isolates. Here we present the antifungal susceptibility data for candidemia isolates collected in 2013.

**METHODS:** *Candida* species isolated from bloodstream infections were collected from 12 participating medical centres during the 2013 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 411 *Candida* spp. collected, *C. albicans* (CA) was predominant (49.9%), followed by *C. glabrata* (CG) (20.7%) and *C. parapsilosis*

(CP) (12.4%). Susceptibility (S) values are below. CG cases doubled from 2011 CANWARD but CA and CP remained unchanged. Azole R was detected in three CA and three CG, while echinocandin R was detected in six CG isolates from five centres.

	n	MIC <sub>90</sub> (% S)					
		AMB	ITRA	FLUC	VORI	CASP	MICA
CA	205	0.5 (-)	0.06 (98)	0.25 (100)	≤0.015 (100)	0.12 (99.5)	≤0.007 (100)
CG	85	0.5 (-)	0.25 (-)	16 (96.5*)	0.5 (-)	excluded	0.015 (93)
CP	51	1.0 (-)	0.06 (-)	1.0 (100)	≤0.015 (100)	0.5 (100)	0.5 (100)

\*Susceptible dose-dependent; - No clinical breakpoint; AMB Amphotericin B; CA *Candida albicans*; CASP Caspofungin; CG *Candida glabrata*; CP *Candida parapsilosis*; FLUC Fluconazole; ITRA Itraconazole; MIC Minimum inhibitory concentration; MICA Micafungin; VORI Voriconazole

**CONCLUSION:** The prevalence and MIC distributions of CA and CP candidemia have not significantly changed over several years of CANWARD surveillance. However, there was a significant increase in the number of CG isolates and rate of echinocandin resistance.

### C04

#### ANTIFUNGAL SUSCEPTIBILITY OF RESPIRATORY ASPERGILLUS ISOLATES FROM CANADIAN HOSPITALS: RESULTS OF THE CANWARD 2013 STUDY

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**OBJECTIVES:** 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 *Aspergillus* isolates collected in 2013.

**METHODS:** Clinical *Aspergillus* isolates recovered from respiratory specimens at 13 participating medical centres during the 2013 study period were tested against amphotericin B (AMB), itraconazole (ITRA), posaconazole (POSA), voriconazole (VORI) and caspofungin (CASP) by broth microdilution using the CLSI M38-A2 method. Growth endpoints were measured as per CLSI M38-A2 and values above the epidemiological cutoff (ECOFF) were scored as non-wildtype (non-WT).

**RESULTS:** Of the 853 *Aspergillus* isolates recovered, *A. fumigatus* represented 65% of the population; *A. flavus*, *A. niger* and *A. nidulans* comprised 8%, 10% and 2%, respectively. *A. fumigatus* isolates were recovered primarily from Clinic outpatients (66%) and Medicine inpatients (17%) from sputa (62%) and bronchoscopy (27%) specimens. Nineteen isolates (3%) of *A. fumigatus* had non-WT VORI MICs (2 mg/L to 4 mg/L), up from 0.7% in 2012. In contrast, 32 *A. flavus* (45%) isolates showed non-WT MICs to VORI but 84% tested only one dilution above the ECOFF.

**CONCLUSION:** This study marks the second consecutive year of national *Aspergillus* resistance surveillance. In the absence of clinical breakpoints, ECOFF values have been proposed to facilitate the detection of microbiological resistance. WT isolates of *A. fumigatus* were most prevalent in this study and the rate of non-WT isolates increased slightly compared to the 2012 surveillance. Overall, no significant changes were detected between the 2012 and 2013 surveillance periods.

## 1600-1700 Oral Presentations: Session D Room: Convention Centre (Saanich 1 & 2)

### D01

#### MEASLES, MUMPS AND RUBELLA VIRUS GENOTYPES IDENTIFIED IN CANADA IN 2012 AND 2013

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**OBJECTIVE:** Molecular virological surveillance of measles, mumps and rubella (MMR) aids in linking cases and outbreaks and tracking importation. Within the WHO Global Measles and Rubella Laboratory Network, high quality national laboratory surveillance plays a critical role in documenting measles and rubella elimination.

**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 identified from 85 cases: A (vaccine), B3, D4, D8 and H1. Two distinct D8 strains, one circulating in several European countries, and one linked to a measles outbreak in the Netherlands, accounted for the majority of cases. An additional D8 strain was identical to a strain circulating in Thailand. The B3 strain was identical to a strain circulating in Eastern Mediterranean countries. Two unique D4 strains were identical to strains circulating in India and Europe. The H1 strain was identical to a strain circulating in Hong Kong. **Mumps:** Nine genotypes identified from 78 cases: A (vaccine), C, D, F, G, H, I, K and N. Predominant genotype G accounted for the majority of cases. An additional G strain and all other genotypes were consistent with importation. **Rubella:** Two genotypes identified from two cases: 1A (vaccine) and 2B.

**CONCLUSION:** Mumps activity continues to provide evidence of an endemic strain, while measles and rubella genotypes reflect importation from endemic regions and verifies the elimination status in Canada. Sequencing of extended regions of the measles genome allows for further characterization of the origin of importation.

### D02

#### UPDATE ON THE MOLECULAR CHARACTERISTICS OF WEST NILE VIRUS IN BRITISH COLUMBIA

MK Lee<sup>1</sup>, S Man<sup>1</sup>, K Fernando<sup>1</sup>, T Lo<sup>1</sup>, Q Wong<sup>1</sup>, B Henry<sup>2</sup>, M Morshed<sup>1</sup>

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**OBJECTIVE:** We investigated the molecular characteristics of 2013 West Nile virus (WNV) positive strains from British Columbia (BC) and performed a historical comparison among all BC positive strains with other circulating strains in North America.

**METHODS:** All the BC WNV positive strains (10 mosquito pools from 2009, five bird isolates from 2010, one mosquito pool and one bird isolate from 2013) along with positive strains provided by the Public Health Agencies of Alberta (AB), Washington (WA) and Oregon (OR) were subjected to sequencing that targeted pre-membrane M, membrane protein M, and envelope glycoprotein E regions. Cycle sequencing was performed on the ABI Genetic Analyzer 3130xl. The sequences obtained were analyzed with Geneious software.

**RESULTS:** Phylogenetic study showed that all BC sequences, like most of the North American strains, belonged to lineage 1A. The isolates from 2009 mosquito pools and 2010 birds clustered uniquely and subgrouped together. The 2013 isolates, which were different from previous years, were closely related to the strains that circulated in WA and OR in 2009.

**CONCLUSION:** All the BC WNV detected were from the Okanagan region. However, sequencing studies indicated there were actually two

genotypic waves of WNV that circulated in this area. The unique BC strains that appeared in 2009 and 2010 did not reappear in 2013. The newly detected 2013 isolates were found to be closely related (99.8%) to the WNV isolated four years ago in neighbouring states, WA and OR. This suggests that this strain may already be established in the Pacific Northwest.

### D03

#### EVALUATION OF THE COBAS® AMPLIPREP/TAQMAN® CMV TEST FOR CMV QUANTITATIVE VIRAL LOAD TESTING

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<sup>1</sup>Providence Health Care; <sup>2</sup>University of British Columbia, Vancouver, BC

**INTRODUCTION:** Quantitative CMV viral load testing for immunocompromised patients is critical for the work-up of CMV disease. There is a lack of standardization and significant intra-laboratory variation. We assessed the performance of a commercial quantitative PCR against an in-house CMV PCR that has been validated and implemented for the past six years.

**METHODS:** Precision was analyzed with 30 replicates of a clinical sample with known copy number (8500 copies(cp)/mL, 1200 cp/mL and 140 cp/mL). Accuracy was assessed with clinical plasma in the following categories: 0 cp/mL (30 samples), <1000 cp/mL (88 samples), >1000 cp/mL (88 samples) and a WHO standard (5,000,000 IU/mL diluted 1:1000). DNA extraction was performed on the MagNA pure LC. The target for the in-house real-time PCR is the gpB gene (254 bp) and amplified on a Lightcycler. The COBAS® AmpliPrep/TaqMan® CMV Test was used as per manufacturer's instructions.

**RESULTS:** Reproducibility and accuracy was assessed against the WHO standard (5000 IU/mL), with a mean of 5393 cp/mL (coefficient of variation [CV]=23%) for the COBAS® assay and 9629 cp/mL (CV=16%) for the in-house assay. Two replicates of known copy number were repeated 30 times each on the COBAS®: 1) 8500 cp/mL – mean 4300 cp/mL (CV=22%), and 2) 1200 cp/mL – mean 729 cp/mL (CV=24%). Of the 30 replicates with 140 cp/mL by in-house PCR, 20 of 30 were called <150 cp/mL (limit of detection for the COBAS®). Accuracy of the COBAS® PCR was determined for low and high copy numbers. At copy numbers >1000 cp/mL, there was good correlation between the two assays (Spearman's coefficient of rank correlation = 0.894, P<0.0001 [95% CI 0.842 to 0.929]). At low copy numbers (<1000 cp/mL), a weaker correlation was identified (Spearman's coefficient of rank correlation = 0.679, P<0.0001 [95% CI 0.542 to 0.781]). Although copy numbers (>1000 cp/mL) trended higher for the in-house PCR, the Bland-Altman plot showed minimal spread with three outliers at viral loads >100,000 cp/mL. Samples with undetectable viral load on the in-house PCR were also negative with the COBAS® CMV test.

**CONCLUSION:** The commercial COBAS® AmpliPrep/TaqMan® CMV test was comparable to our in-house real-time PCR. Serial monitoring for individual patients' CMV viral load for should be followed using the same assay due to inherent differences between platforms and assays.

### D04

#### RAPID GENOTYPING OF HUMAN ROTAVIRUS USING A SYBR GREEN REAL-TIME RT-PCR WITH MELTING CURVE ANALYSIS

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**OBJECTIVE:** Genotyping rotavirus is critical for rotavirus vaccination program evaluations. Conventional nested PCR (cNPCR) currently used to genotype rotavirus is time-consuming and labor intensive. A simple and rapid method using SYBR Green real-time RT-PCR (SG-rtPCR) was developed and validated in this study and used to monitor circulating rotavirus in Alberta.

**METHODS:** A two-step singleplex SG-rtPCR with melting curve analysis was developed and optimized. Previously published cNPCR primers for rotavirus G (VP7) and P (VP4) typing were used in this study. Sixteen RNA samples characterized previously using cNPCR as G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] were used as positive controls. Five microlitres of diluted cDNA (1:10) synthesized by SuperScript® II

## Abstracts

reverse transcriptase was used in each reaction. Differences in melting temperatures was used to identify various genotypes and confirmed by gel electrophoresis and sequencing. The specificity of SG-rtPCR was assessed using samples positive for other gastroenteritis viruses, and the sensitivity of SG-rtPCR was compared with cNPCR. Stool samples tested positive for rotavirus using electron microscopy from 52 patients with acute gastroenteritis from January to June 2013 were genotyped using SG-rtPCR.

**RESULTS:** The newly developed SG-rtPCR assay was rotavirus-specific showing no cross-reaction with norovirus, sapovirus, adenovirus and astrovirus. The sensitivity of SG-rtPCR was comparable to cNPCR with 100% correlation of 16 samples with known genotypes. Of the 52 rotavirus positive cases, 51 (98%) were genotyped using SG-rtPCR: G1P[8] (51.9%), G3P[8] (17.3%), G9P[8] (17.3%), G12P[8] (7.7%), G2P[4] (1.9%) and an unknown GP[8] (1.9%).

**CONCLUSION:** The new SG-rtPCR assay is a fast, sensitive and specific method for rotavirus genotyping. The methodology is easy to perform and has minimal risk for PCR end-product contamination. This assay is based on singleplex PCR; a stepwise algorithm targeting the most common G and P types will reduce reagent and labour costs. The identification of an uncommon genotype, G12P[8], indicates that ongoing monitor of rotavirus genotypes is important.

### 1600-1745 Oral Presentations: Session E Room: Convention Centre (Esquimalt)

#### E01

##### EPIDEMIOLOGY OF *BORDETELLA PERTUSSIS* GENOTYPES IN ALBERTA: JANUARY 2012 – AUGUST 2012

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**OBJECTIVE:** To undertake an epidemiologic analysis of a perceived increase in *B pertussis* activity: January 1, 2012 to August 31, 2012 in Alberta, Canada.

**METHODS:** *B pertussis* testing used an IS481 real-time PCR assay. All PCR positive and indeterminate specimens were cultured and stored. Laboratory data was linked to Alberta Health Case dataset to identify case isolates. Case isolates were analyzed at National Microbiology Laboratory (NML) by Field Gel Electrophoresis (PFGE), serotyping, and Fim3 genotyping. PFGE Relatedness rules: ≤3 bands = closely related, four to six bands = possibly related, ≥7 bands = unrelated. Immunizations were extracted from Alberta's provincial immunization repository (Imm/ARI), and linked to the pertussis cases reported in the Communicable Disease Reporting System.

**RESULTS:** Following exclusions 52 case isolates gave PFGE profiles: A (n=17 [32.7%]), A1 (n=16 [30.8%]), A2 (n=1 [1.9%]), A3 (n=3 [5.8%]), A4 (n=1 [1.9%]), A5 (n=1 [1.9%]), A6 (n=1 [1.9%]), A7 (n=1 [1.9%]), B (n=1 [1.9%]), C (n=1 [1.9%]), D (n=1 [1.9%]), E (n=1 [1.9%]), F (n=4 [7.7%]), G (n=1 [1.9%]), H (n=1 [1.9%]) and I (n=1 [1.9%]). Profiles A1 to A7 were closely/possibly related subtypes of Profile A and were grouped with A as 'A' (41 of 52 [78.8%]) and were also *fim3B* genotype. All non-'A' cases were *fim3A* genotype. Cases were reported in all zones, with the South Zone reporting the highest rates. 'A' was most common in each of the zones with the distribution of the other profiles varying by zone: B, C, and D (South and Calgary), F (Central and Edmonton) and G (North). Of the 52 genotyped cases, 18 (34.6%) had a history of immunization, 28 (53.8%) were not immunized, six (11.6%) had an unknown immunization history. Twenty-five of 28 (89.2%) non-immunized cases were 'A'. Ten genotyped cases were outbreak-associated (OB), to OB A ('Profile A') and OB B (mixed).

**CONCLUSIONS:** *B pertussis* profile 'A' was most common. Of 52 cases, almost two thirds were not immunized nor had no history of immunization. OB A was due to 'A' but not all cases in OB B were closely related,

suggesting that not all cases in OB B were due to the same transmission cluster, but happened to occur within the same time period.

#### E02

##### A HIGHLY UNUSUAL LATE AUTUMN LEGIONELLA PNEUMOPHILA SEROGROUP 1 KNOXVILLE SEQUENCE TYPE 222 OUTBREAK IN AN INNER CITY NEIGHBORHOOD IN CALGARY, CANADA

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**OBJECTIVE:** To undertake an epidemiologic/laboratory analysis of an outbreak of severe *Legionella pneumophila* (LP) respiratory infection in Calgary, Alberta, November to December 2012.

**METHODS:** LP cases were defined as follows: 1) (+) urine antigen test (UAT) for LP serogroup-1 (LPS-1) with respiratory infection; 2) symptom onset on/after November 1, 2012; 3) resided/visited/worked in a 2 km radius of an inner city neighborhood ≤2 weeks prior to symptom onset. All cases were administered CDC's Legionellosis Hypothesis-Generating Questionnaire. UAT plus cultures of respiratory specimens/pleural fluid/blood and serology were collected as appropriate. LP *mip* and 16S rDNA PCR was performed on respiratory specimens from UAT (+) patients. Sequence typing (ST) was performed on LP (+) primary specimens and isolates were ST/serotyped. Water samples from potential sources were tested (American Public Health Association protocol). For case finding, all respiratory virus panel (-) lower respiratory tract patient specimens from Calgary (received 1/10/12-19/12/12) were tested by LP PCR.

**RESULTS:** Eight confirmed cases of LPS-1 were identified with symptom onset between November 20 and December 10, 2012. Five (63%) cases were female, median age 66 years (range 51 to 78 years), 50% were current smokers with underlying immune or cardiorespiratory system comorbidities. Five (63%) cases required intensive care. There was no associated mortality. No cases were travel-associated. Six UAT (+) cases were LP PCR (+) from respiratory specimens and five of eight were LP culture (+). All culture (+) isolates were LPS-1, monoclonal type Knoxville ST-222 (matching seven of seven alleles), not previously described in Alberta. One culture (-)/PCR (+) specimen matched five of seven alleles of ST-222 with PCR/sequencing failing on two of seven alleles. None of 42 water/environmental samples was LP (+). No other cases were identified from other Calgary neighborhoods. Case location geomapping identified a nine-block<sup>2</sup> area with multiple active construction projects.

**CONCLUSIONS:** This study describes an LP1 ST222 outbreak in a Calgary neighborhood temporally associated with construction. Unusual factors include prior low LP incidence, seasonality and ST.

## E03

MOLECULAR PROFILING OF HUMAN AND CATTLE SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* STRAINS IN ALBERTA, CANADA

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**OBJECTIVE(S):** Genetic determinants of virulence in Shiga toxin-producing *Escherichia coli* (STEC) and their association with diseases such as hemolytic uremic syndrome (HUS) remain largely unknown. This study establishes the virulence gene profiles for 150 STEC isolates from clinical cases and cattle in Alberta and the prevalence of these genes in strains from HUS/non-HUS cases.

**METHODS:** High-throughput microfluidic real-time PCR was used to test the presence of 44 genetic markers in clinical isolates of 91 O157 and 39 non-O157 *E. coli* as well as 20 non-O157 cattle isolates. The genetic markers include *stx*, the LEE, various O-islands and diverse type III effectors.

**RESULTS:** Each of the virulence genes tested were present in 95% to 100% of the O157 strains except *stx*<sub>1</sub>. In total, 68 (74.7%) O157 isolates were positive for both *stx*<sub>1</sub> and *stx*<sub>2</sub>, while 23 (25.3%) were positive for *stx*<sub>2</sub> only. There were 30 patients who developed HUS post-O157 infection. No significant differences in the molecular profiles of strains from HUS and non-HUS cases were observed. The mean age of HUS patients was 8.6 years, which was significantly younger than non-HUS patients (mean age 29.7 years) and of non-O157 patients (mean age 22.0 years). Many of the virulence genes were absent in non-O157 strains, particularly those from cattle isolates. Genes *eae*, *ecs1763*, *ecs1822*, *efa1*, *efa2*, *ent*, *espG*, *espJ*, *espK*, *espM1*, *espM2*, *espN*, *espV*, *espW*, *espX7*, *katP*, *nleA*, *nleB*, *nleE*, *nleF*, *nleG*, *nleH1-1*, *nleH1-2*, *terE*, *toxB* and *ureD* were significantly more prevalent in clinical than in the cattle non-O157 strains.

**CONCLUSION(S):** The virulence genes tested were present in most of the O157 strains and were less prevalent in non-O157 isolates, particularly those isolated from cattle. No significant differences were observed between the molecular profiles of the O157 strains, HUS-associated or not. However, the development of HUS seems to be dependent on age-associated factors.

## E04

MOLECULAR CHARACTERIZATION OF DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS* IN THE PROVINCE OF QUÉBEC

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**OBJECTIVES:** The increasing emergence of drug-resistant tuberculosis presents a threat to the effective control of tuberculosis (TB). The purpose of this study was to genotypically characterize resistant *M. tuberculosis* strains collected over the past 11 years in the province of Québec.

**METHODS:** A total of 225 *M. tuberculosis* strains resistant to first-line drug were isolated in Québec between 2002 and 2012. These isolates were analysed for resistance-associated mutations in regions of the *katG*, *inhA*, *rpoB*, *pncA* and *embB* genes as well *inhA* and *pncA* promoters. In addition, the first extensively drug-resistant (XDR) strain of *M. tuberculosis* isolated in 2013 from an immigrant patient was characterized by whole genome sequencing.

**RESULTS:** Of the INH-resistant isolates, 113 of 181 isolates had a mutation in *katG* (62%), 66 of 181 isolates had a mutation in the *inhA* promoter (36%), and eight of 181 isolates had a mutation in the *inhA* gene (4.4%). In combination, *katG* and *inhA* promoter accounted for 89.5% of INH-associated mutations. Twenty-three of 23 RMP-resistant isolates (100%), 45 of 48 PZA-resistant isolates (93.7%), and 14 of 15 EMB-resistant

isolates (93.3%) had mutations associated with drug-resistance in *rpoB*, *pncA* and *embB* gene respectively. For INH and PZA resistant strains, several uncommonly described mutations were found in our collection.

It is essential that molecular methods must always be compared to the gold standard phenotypic method, BACTEC MGIT 960.

**CONCLUSION:** The development of rapid molecular tests targeting these regions would drastically shorten the length of time to determine the resistance profile of most isolates from patients, and hence, allow for appropriate chemotherapy to commence weeks earlier than with phenotypic methods.

## E05

THE RE-EMERGENCE OF MULTIDRUG-RESISTANT *SALMONELLA ENTERICA* SEROTYPE NEWPORT IN CANADA: IS THERE AN ANIMAL-FOOD LINK?

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**OBJECTIVE:** This report describes multi-drug resistant (MDR)-AmpC *S. Newport* from human/food/animal sources over the past decade in Canada.

**METHODS:** Human, food and animal isolates of *S. Newport* were collected between 2003 to 2012 as part of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). Antimicrobial susceptibility was determined by broth microdilution. Multiplex PCR was used to identify extended-spectrum  $\beta$ -lactamase genes or genes associated with other resistance (*floR*, *tetA*, *sul*). Typing was conducted using pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). Plasmids harbouring CMY-2 were typed using restriction fragment length polymorphism (RFLP) and PCR-replicon typing.

**RESULTS:** A total of 1532 human isolates were collected and 89 (5.8%) were identified as MDR-AmpC. The occurrence of human MDR-AmpC varied from 9.1% (n=16 of 175) in 2003; 1.5% in 2009 (n=2 of 136); and 6.6% in 2012 (n=9 of 137). A total of 354 isolates from food/animal sources underwent susceptibility testing; 108 (30.5%) were MDR-AmpC. All human and a sub-sample of food/animal MDR-AmpC isolates (n=31) contained *bla*<sub>CMY-2</sub>. PFGE distinguished human/animal/food MDR-AmpC isolates from those with a susceptible phenotype and a sample tested by MLST were ST45. Of the 61 CMY-2 plasmids characterized, all human and the majority of animal isolates carrying CMY-2 plasmids were IncA/C, contained *floR*, *tetA*, and *sul2* and were closely related by RFLP.

**CONCLUSIONS:** Human infections caused by *S. Newport* MDR-AmpC decreased from 2003 to 2009, but have re-emerged in 2011 to 2012. Further studies are underway to identify the source of recent human MDR-AmpC cases.

## E06

EMERGENCE OF MULTIDRUG-RESISTANT *SALMONELLA* DUBLIN FROM HUMAN AND ANIMAL SOURCES IN QUÉBEC

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**OBJECTIVE:** Multidrug-resistance (MDR) in *Salmonella* is an emerging issue as it limits treatment options and can lead to transfer of the MDR



phenotype to other pathogens. This study describes the characterization of MDR *Salmonella* Dublin identified in Quebec from bovine and human sources.

**METHODS:** Bovine clinical and human isolates were collected in Quebec in 2011 to 2012. MICs were determined by the broth microdilution using the automated Sensititre system. Typing was conducted using pulsed-field gel electrophoresis (PFGE) according to PulseNet protocol. Plasmids harbouring CMY-2 were typed using RFLP and PCR-replicon typing. Whole genome sequencing (WGS) was carried out using MiSeq (Illumina) and phylogenetic analysis was conducted using bioinformatics pipelines.

**RESULTS:** Among the 26 bovine isolates, 88% (n=32) were resistant to a minimum of four antimicrobial classes. During 2011-2012, an increase in human infections with *Salmonella* Dublin was observed, with 75% (n=12) of these isolated from blood and 30% (n=5) were resistant to at least six antibiotic classes. All MDR isolates carried CMY-2. All human isolates identified prior to 2011 were susceptible. PFGE analysis of MDR and susceptible isolates from both animals and humans lacked discriminatory power. All 28 plasmids harbouring CMY-2 were closely related by RFLP, were IncA/C and encoded MDR. Phylogenetic analysis using WGS from 15 isolates differentiated MDR and susceptible isolates and MDR bovine/human isolates clustered together.

**CONCLUSIONS:** *Salmonella* Dublin is emerging as a serious threat for public health in Québec due to the predominance of the invasive form of the infection and the emergence of MDR strains. WGS analysis appears to be useful in differentiating isolates with a MDR phenotype irrespective of the source of the isolates. Further epidemiologic data is required to establish a link between MDR isolates from bovine and human sources.

## E07

### MOLECULAR SEROTYPING FOR SALMONELLA

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**BACKGROUND:** *Salmonella* is one of the most common enteric pathogens causing diarrhea. The ProvLab uses serological methods and follows the White-Kauffmann-Le Minor scheme for *Salmonella* serotyping. Serovar determination is often incomplete due to different reasons and confirmation may be required at the National Microbiology Laboratory (NML). The Check&Trace Salmonella (CTS) platform (Check-Points, The Netherlands), a commercial DNA microarray system, can fully genetically determine the serotype designation of a known *Salmonella* isolate. We evaluated the CTS platform for genetic characterization of *Salmonella* isolates in parallel to conventional serotyping.

**METHODS:** *Salmonella* subsp. *enterica* identified by conventional methods using commercial (Statens Serum Institute) or in-house antisera were tested on CTS. Isolates were assigned a 'genovar' number based on the microarray result and matched to known serovars in a database.

**RESULTS:** A total of 471 *Salmonella* subsp. *enterica* isolates were serotyped by conventional methods; 406 isolates had a complete antigenic formula and serovar determined. Of these, CTS found a matching serotype result to 349 (86.0%), discordant result to four (<1%), and a genovar only to 53 (13.1%) isolates. All but seven of the isolates in the 'genovar only' category represent serovars rarely found in the top 20 *S enterica* subsp. *enterica* in ProvLab. Of the remaining 65 of 471 isolates for which the serovar could not be determined through conventional methods (due to incomplete antigenic formula), CTS was able to determine the majority (54 of 65 [83.1%]) of serovars. These serovars were: 4,[5],12:i:- (n=29); Enteritidis (n=2); Heidelberg (n=2); Javiana (n=5); Paratyphi B var Java (n=3); Stanley (n=5); Typhimurium (n=3); and others (n=5).

**CONCLUSIONS:** CTS is an effective, rapid and easy to use platform and can generate *Salmonella* serovar results within 8 h. Using this molecular method in a diagnostic laboratory can shorten the turn-around time, especially during outbreak settings, and therefore enhance public health surveillance performed in Alberta.

## 1600-1745 Oral Presentations: Session F Room: Convention Centre (Oak Bay 1)

### F01

#### A POINT PREVALENCE STUDY TO MEASURE APPROPRIATENESS OF ANTIMICROBIAL PRESCRIBING ON GENERAL INTERNAL MEDICINE PATIENTS AS PART OF AN ANTIMICROBIAL STEWARDSHIP PROGRAM

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**OBJECTIVE(S):** To measure appropriateness of antimicrobial therapy on General Internal Medicine (GIM) patients in order to determine baseline data and areas for antimicrobial stewardship intervention.

**METHODS:** Four, single day audits were conducted by the Antimicrobial Stewardship Program (ASP) from May to December 2013 on adult patients admitted to each of three clinical teaching units at two large tertiary care university affiliated teaching hospitals. Patients had to be on at least one antimicrobial to be included in the audit. A consensus process was used to determine appropriateness of antimicrobial therapy.

**RESULTS:** A total of n=933 patients were included. On average, 36.9% of patients were on systemic antimicrobials during the audits. Of those receiving antimicrobials, 38.2% (SD ±7.1%) were judged to be suboptimal therapy. The most common reasons for suboptimal therapy were; duration too long, antimicrobial selection inconsistent with recommended empiric therapy, intravenous therapy when oral option exists, and overly broad spectrum antimicrobial therapy. The most common clinical syndrome for which antimicrobials were prescribed was pneumonia (23.4%), followed by urinary tract infection (10.8%), primary bacteremia/endocarditis (7.5%), skin and soft tissue infection (4.5%), and fungal infection (3.8%).

**CONCLUSION(S):** Over the course of four single day audits, we found a significant proportion of patients were on suboptimal antimicrobial therapy. While many ASPs focus primarily on overall antimicrobial usage, the present study provided information on the appropriateness of therapy at a patient level, a key measure, given that many ASPs aim to ensure patients receive the 'right' antimicrobial rather than simply decreasing overall usage. Information gathered from this work will be used to guide further interventions aimed to improve antimicrobial usage.

### F02

#### RISK FACTORS FOR TREATMENT OF ASYMPTOMATIC BACTERIURIA: DOES AN EDUCATIONAL INTERVENTION REDUCE UNNECESSARY TREATMENT?

**A Brooks**<sup>1,2</sup>, **N Irfan**<sup>1</sup>, **S Mithoowani**<sup>2</sup>, **D Lee**<sup>2</sup>, **A Carducci**<sup>3</sup>, **S Celetti**<sup>3</sup>, **A Cheng**<sup>3</sup>, **D Mertz**<sup>1,2</sup>

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**BACKGROUND:** Asymptomatic bacteriuria (ABU) does not require treatment in the majority of patients. We aimed to identify risk factors for inappropriate antibiotic use and to evaluate an educational intervention to reduce inappropriate use.

**METHODS:** Consecutive patients with positive urine cultures from February to April 2012 (baseline) and 2013 (intervention) were reviewed at two adult tertiary care hospitals. Nursing interviews and chart reviews were conducted to investigate new signs and symptoms of a urinary tract infection. Risk factors for inappropriate antibiotic use for ABU were identified during baseline. In January 2013, an educational intervention was provided to medical residents (monthly) in the clinical teaching unit (CTU) at one hospital, the other site's CTU served as the control.

**RESULTS:** In 2012, overall 162 of 341 (47.5%) positive urine cultures were obtained from asymptomatic patients, and 97 (59.9%) thereof were inappropriately treated with antibiotics. Risk factors identified included female sex (OR 2.0 [95% CI 1.0 to 4.0]), absence of a catheter (OR 2.4 [95% CI 1.3 to 4.5]), bacteriuria versus candiduria (OR 11.2 [95% CI 4.0 to 31.3]), pyuria (OR 2.1 [95% CI 1.1 to 4.0]), and positive nitrites

(OR 2.4 [95% CI 1.2 to 4.9]). On the intervention unit, inappropriate use of antibiotics was reduced from 10 of 21 (48%) in 2012 to two of 24 (8%; OR 0.10 [95% CI 0.02 to 0.54]). Comparison to the control CTU for the year 2013 (14 of 29, 48%) also resulted in a significant reduction in inappropriate antibiotic use (OR 0.10 [95% CI 0.02 to 0.49]). No statistical difference was found in the control site between 2012 and 2013 (OR 0.47 [95% CI 0.13 to 1.7]).

**CONCLUSIONS:** Educational sessions geared toward medical residents with a focus on previously identified factors resulting in inappropriate management of ABU were highly effective in reducing unnecessary use of antibiotics.

### F03

#### **COST OF FOSFOMYCIN COMPARES FAVOURABLY TO REIMBURSED AGENTS AS EMPIRICAL TREATMENT OF UNCOMPLICATED URINARY TRACT INFECTIONS**

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Cadillac; <sup>4</sup>Université de Montréal; <sup>5</sup>Triton Pharma Inc, Montréal,

QC

**OBJECTIVE:** Bacterial resistance to antibiotics traditionally used to treat uncomplicated urinary tract infections (uUTIs) is rising in Canada. We compared the cost per patient of including fosfomycin (an antibiotic with a low resistance profile) as an option for first-line empirical treatment of uUTI with current cost of treatment with fluoroquinolones, sulfonamides, and nitrofurantoin.

**METHODS:** A decision tree model (TreeAge Pro 2012, Williamstown, MA) was used to perform cost-consequence and sensitivity analyses to determine the financial impact of incorporating fosfomycin as a treatment for uUTI in Québec. All possible outcomes of a uUTI caused by *E coli*, *Enterococcus* species, *K pneumoniae*, *P mirabilis*, or other bacterial species (combined) treated with either fluoroquinolones, sulfonamides, nitrofurantoin, or fosfomycin were included. Weighted average cost per patient was calculated using current market share data. Deterministic one-way sensitivity analyses were conducted on variables suspected to have the most influence on expected cost per patient.

**RESULTS:** Including fosfomycin as a treatment option results in a weighted average cost per patient of \$61.38 (versus current weighted average of \$61.34). Most (96.1%) of the potential variation in cost was associated with the probability of progressing to pyelonephritis (55.8%), hospitalization for pyelonephritis (35.9%), and contracting an infection with trimethoprim-sulfamethoxazole-resistant *E coli* (4.37%). Fosfomycin was cost-minimizing versus fluoroquinolones when the probability of pyelonephritis surpassed 3.2% and probability of hospitalization due to pyelonephritis surpassed 15% (literature base case values for these variables were 4% and 20%, respectively).

**CONCLUSION:** Fosfomycin has a low resistance profile, offers a single-dose regimen, and is comparable in cost to other reimbursed antibiotics. Inclusion of fosfomycin as a reimbursed treatment option in Québec would, therefore, have a negligible impact on the provincial healthcare budget but would allow physicians to effectively treat patients with uUTIs while minimizing increasing resistance to antibiotics, such as fluoroquinolones and sulfonamides, in Québec.

### F04

#### **REVERSAL OF GONORRHEA ANTIBIOTIC RESISTANCE TRENDS IN BRITISH COLUMBIA: CAN WE SAVE THE ORAL OPTION?**

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**OBJECTIVES:** To determine antimicrobial resistance trends for cephalosporins and azithromycin before and after introducing new treatment guidelines.

**METHODS:** At the end of 2011, British Columbia changed first-line recommendations for treating uncomplicated gonorrhea from cefixime

400 mg orally for all cases, to cefixime 800 mg, and to preferring ceftriaxone 250 mg intramuscularly for men who have sex with men (MSM) or pharyngeal infections, plus azithromycin 1 g orally as co-treatment in all individuals. Data were obtained from the BC Centre for Disease Control and the Public Health Microbiology & Reference Laboratory from January 1, 2006 to September 30, 2013. Minimum inhibitory concentrations (MICs) were considered elevated if  $\geq 0.125$   $\mu\text{g/mL}$ ,  $\geq 0.64$   $\mu\text{g/mL}$  and  $\geq 1$   $\mu\text{g/mL}$  for cefixime, ceftriaxone and azithromycin, respectively ('MIC creep').

**RESULTS:** The total number of isolates evaluated was 2299. Rates of increased MICs in 2011 reached 20.2% for cefixime, 17.0% for ceftriaxone and 38.5% for azithromycin. However, rates trended back down to 2.0%, 4.7% and 13.1%, respectively, by 2013. Over this timeframe, use of the higher 800 mg dose of cefixime increased from 5% to 8% (2006 to 2011) to >50% (2012 to 2013), ceftriaxone increased from <2% to 11%, and co-treatment with azithromycin increased from 18% to 26%, to >50%. Overall, the use of 'enhanced oral therapy' including increased cefixime dosing and co-treatment with azithromycin was substantially more common in 2012 to 2013 (66%) than a switch to parenteral therapy with ceftriaxone (11%).

**CONCLUSIONS:** Increased dose cefixime plus co-treatment with azithromycin may be a sufficient strategy to overcome worrisome antimicrobial resistance trends for outpatient gonorrhea infections, preserving the oral option.

### F05

#### **SURVEILLANCE OF ANTIMICROBIAL RESISTANCE (AMR) AND ANTIMICROBIAL UTILIZATION (AU) IN CANADA: FINAL RESULTS OF A COMPREHENSIVE REVIEW AND TEN RECOMMENDATIONS TO ACHIEVE OPTIMAL CANADIAN SURVEILLANCE PROGRAMS**

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**BACKGROUND:** Surveillance of AMR and AU is needed to understand the spread and development of AMR, and for development of public health interventions.

**OBJECTIVES:** To evaluate Canadian and exemplar international surveillance programs against a rubric of surveillance system characteristics and attributes, in order to derive recommendations for optimizing Canadian surveillance programs.

**METHODS:** A systematic literature review and qualitative Canadian expert interview series identified surveillance programs and their characteristics. Key Canadian programs and exemplar international programs were selected for further evaluation. A 24-element evaluation rubric was derived from both public health literature and the expert interviews, applied by four investigators, and vetted by a multidisciplinary Steering Committee, with discordance resolved by iterative discussion.

**RESULTS:** Focusing on human surveillance, the evaluation rubric confirmed gaps in overall AMR-AU surveillance in Canada with overlapping deficiencies in key surveillance dimensions, particularly in Public Health based community AMR surveillance. Other frequent deficiencies included cumulative timeliness and availability of reports, and representativeness in both microbiologic and human populations.) The main Canadian public health programs CNISP, CIPARS and private programs CanWARD and CBSN performed well in 12 of 22, 11 of 18, 20 of 29, and seven of 21 of attributes judged to be potentially within scope, respectively.

**CONCLUSION:** Surveillance of AMR and AU in Canada lacks overall coordination and integration, with some strong elements but very important gaps. Ten recommendations for reorganization of existing resources, and gradual expansion of AMR-AU surveillance in Canadian jurisdictions will be released.

## F06

### SHIFTS IN EPIDEMIOLOGY OF BLOOD STREAM INFECTION (BSI) ON AN INPATIENT HEMATOLOGY WARD: PIPERACILLIN TAZOBACTAM RESISTANCE, AND IMPLICATIONS FOR EMPIRIC FEBRILE NEUTROPENIC TREATMENT

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**BACKGROUND:** An impression of changing epidemiology with more resistant Gram negatives prompted a review of Hematology inpatient BSI data and the adequacy of piperacillin tazobactam (P-T) as empirical febrile neutropenia (FN) Therapy.

**METHODS:** Hematology patient BSI data and antimicrobial susceptibility data acquired, from 2011 to October 2013. A 2005 to 2008 review was used as comparator data.

**RESULTS:** From January 2011 to October 2013, there were 68 bacteremias (26 in 2011, 23 in 2012 and 19 in the included portion of 2013). In 2011, 10 of 26 episodes were GNB, four were *E coli*, two P-T resistant and one ESBL. In 2012, 13 of 23 BSI were to GNB, six were *E coli*, four P-T resistant, one ESBL. In 2013, eight of 19 BSI were GNB, five *E coli*, all P-T susceptible. Other P-T resistant GNB included one each of *Klebsiella*, *Enterobacter* and *Pseudomonas* spp. Overall GNB susceptibility in this series was P-T 69%, gentamicin 93% and carbapenems 93%. Comparison with the 2005 to 2008 review (n=70) revealed notable increases in overall proportion of BSI due to GNB from 27.2% to 47.9% (76.1% increase), *E coli* from 6.1% to 22.9%. Three P-T resistant non ESBL *E coli* isolates were not clonal, by PFGE analysis. The P-T resistant *E coli* isolates occurred in four induction AML patients in 2011 to 2012, with no prior P-T exposure documented, with two sepsis related fatalities.

**CONCLUSIONS:** A significant shift to GNB was seen in Hematology inpatient BSI between 2005 to 2008 and 2011 to 2013, with increased *E coli* including an unusual P-T resistant phenotype. The FN protocol is being changed with discussion of the relative merits of aminoglycosides and carbapenems. Collaboration of IPC, Microbiology and Stewardship in elucidating local epidemiology can guide treatment protocols in epidemiologically distinct patient groups.

## F07

### BUYER BEWARE: INVESTIGATION OF AN AUTOMATED WASHER UNIT FOR CLEANING AND SANITIZING COMMODES ON AN ACUTE CARE BLOOD AND MARROW TRANSPLANT-HEMATOLOGY-ONCOLOGY WARD AND IMPLICATIONS FOR MICROBIAL TRANSMISSION

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**OBJECTIVE(S):** The efficacy of an automated washer for cleaning/sanitizing commodes on a blood and marrow transplant-hematology-oncology ward was evaluated as a potential factor in the transmission of vancomycin-resistant *Enterococcus* (VRE) and *Clostridium difficile* (CD).

**METHODS:** An automated washer was purchased in 2009 to clean/sanitize (enzymatic pre-treatment/detergent/hot water) commodes based on manufacturer data for a 4.5 log<sub>10</sub> reduction of microbial counts. Random swabs of cleaned commodes were incubated in TSB for 24 h and then plated to selective and non-selective media. The metal door was replaced with Plexiglas for observation of washing. Qualitative pre- and post-cleaning challenge experiments were conducted using Glo Germ™/ultraviolet testing. Feces seeded with CD spores (NAP-1), VRE (Harder) and *E coli* (ATCC25922) at counts of 1.2×10<sup>6</sup> CFU/mL, 1.7×10<sup>7</sup> CFU/mL and 3.5×10<sup>8</sup> CFU/mL, respectively, was applied as 7 cm<sup>2</sup> spots to textured armrests, seat topside and seat underside to determine relative load reduction pre- and post-cleaning, Semi-quantitative and broth cultures of surfaces were performed.

**RESULTS:** Initial examination of the washer revealed scaling, soiling and debris and direct observation revealed that water jets missed the seat

underside areas. Random cleaned commode surfaces were culture positive for VRE and *E fecalis*. Ultraviolet positive residue remained on all tested surfaces post-wash. Seeding experiments revealed the presence of moderate-heavy growth of VRE and *E coli* on the seat underside plus armrests and light growth of CD from all cultured sites, regardless of enzymatic pre-treatment. The textured armrests remained most heavily contaminated. Carry-over of microorganisms in the washer waterlines was also demonstrated.

**CONCLUSION(S):** The presence of pathogenic microbes on cleaned commodes, post-cleaning positive Glo Germ™ residue and post-cleaning presence of seeded VRE and CD from multiple surfaces under the conditions tested as typically observed with fecal matter contamination do not support the use of automated washers for cleaning and sanitizing commodes.

## 1600-1745 Oral Presentations: Session G Room: Convention Centre (Oak Bay 2)

## G01

### USE OF ELECTRICAL TREATMENT FOR THE RECOVERY OF MICROBIAL RIBOSOMAL RNA FOLLOWED BY RT-PCR FOR THE DETECTION AND IDENTIFICATION OF BACTERIA AND FUNGI IN BLOOD SAMPLES

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**OBJECTIVES:** Pathogen identification of sepsis by standard clinical laboratory procedure takes two to three days and requires blood culture and subculture for microbial colony formation. Although several nucleic acid amplification approaches have been attempted to identify pathogens from whole blood, time-consuming nucleic acid extraction steps are required for sample preparation. The purpose of this study was to evaluate a new platform, which by employing electrical sample treatment, prepares assay ready microbial cell lysate for reverse-transcription PCR assay to detect microbial ribosomal RNA.

**METHODS:** Human blood samples were prepared by spiking bacterial and fungal cells to whole blood collected from healthy volunteers. One millilitre of blood was transferred to a sample pre-treatment tube containing reagents for selectively lysing blood cells. Subsequently, the sample was subjected to the sample preparation process which involved electrical treatment and electrical cell lysis. The ribosomal contents of the microbial cells were thus released and used directly for detection and identification via RT-PCR. For each bacterial and fungal species, testing was repeated five times using two replicates each time. The unique feature of the method is its reliance on electrical treatment, which lasts only a fraction of a second, and enables molecular test on pathogenic microbial cells without involving nucleic acid extraction techniques.

**RESULTS:** Rapid and efficient recovery of microbial ribosomal RNA in an assay ready medium was obtained through electrical treatment, and enabled sensitive detection at a 10 CFU/mL level within a total time of about 30 min. The detection repeatability and sensitivity were demonstrated with five clinically important species of Gram-positive and Gram-negative bacteria, and fungal cells, respectively.

**CONCLUSION:** It was demonstrated that microbial cells in whole blood can be detected and identified using molecular methods without involving nucleic acid extraction and purification techniques. This is due to the unique features of the sample preparation technique which utilized electrical sample treatment and cell lysis.

## G02

## ADOPTION OF MASSIVELY PARALLEL REAL-TIME PCR ASSAYS FOR DETECTION OF VIRAL AND BACTERIAL PATHOGENS ON A NANOFLUIDIC BIOCHIP

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**OBJECTIVE:** Comprehensive molecular testing for infectious disease is a challenge given the diversity of pathogenic agents. A step-wise approach for molecular testing for multiple pathogens can impact cost, turnaround time, and potential detection of mixed infections. The objective of this work was to evaluate and adopt a massively parallel real-time PCR approach for syndrome-based detection of bacterial and viral pathogens in clinical specimens.

**METHODS:** A combination of 41 new and established real-time PCR assays specific for 36 respiratory and enteric pathogens was adapted for nanofluidic detection using the Fluidigm BioMark HD and evaluated against singleton PCR and RT-PCR assays for limits of detection and assay specificity. The nanofluidic method adaptation included a combined one-step reverse-transcription and pre-amplification process to streamline workflow. One hundred fifty-nine consecutive respiratory specimens and 341 consecutive stool specimens were tested in comparison with commercial assays.

**RESULTS:** The overall analytical limit of detection of the novel assay was comparable to singleton real-time PCR with no cross-reactions. This assay requires the same amount of reagent and target material as singleton reactions, providing a cost effective strategy for enhanced testing. For all targets, the novel approach had a combined sensitivity of 92% and specificity of 95%.

**CONCLUSIONS:** This novel approach provides fast, flexible and comprehensive syndrome-based laboratory surveillance for clinical microbiology by providing 48 or 96 discrete real-time PCR results on each of 48 or 96 specimens simultaneously without additional reagent or sample compared to traditional PCR.

## G03

## EVALUATION OF MOLECULAR ASSAYS FOR THE DETECTION OF INFLUENZA VIRUSES AND RSV IN RESPIRATORY SPECIMENS

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**INTRODUCTION:** An increasing number of molecular assays are becoming available for the rapid detection of Influenza viruses and RSV in clinical specimens. Continued evaluation of the performance of these assays is required to ensure they are performing with optimal sensitivity and specificity. The purpose of this study was to compare the RealStar Influenza S&T RT-PCR Kit 3.0 (RT-Flu) and the RealStar RSV S&T RT-PCR Kit (RT-RSV) (Altona Diagnostics, Hamburg, Germany) with the Simplexa Flu A/B & RSV Direct Kit (Focus Diagnostics, Cypress, CA, USA).

**METHODS:** Four hundred consecutive respiratory samples received in the microbiology lab were tested using both assays according to the manufacturer's instructions. Samples for the RealStar assays were extracted using the bioMérieux EasyMag extractor. For the Simplexa Direct assay, extraction, amplification and detection are performed in the self-contained Disc. For discrepant results, a fresh aliquot of the original sample was sent to Altona Diagnostics for repeat testing using the same kit version as well as a new version with a different target region. For RSV, a third independent assay was also used.

**RESULTS:** For detection of Influenza viruses, overall concordance between the two assays was 97.5% (390 of 400). Of 14 initial discordant flu samples, five were RT-Flu positive/Simplexa negative. Repeat testing confirmed the results. The other nine samples were initially Simplexa positive/RT-Flu negative. Of these, five subsequently tested positive on both versions of the RT-Flu assay, while four tested negative. For RSV, 47 (11.8%) of samples initially gave discordant results (45 RT-RSV positive/Simplexa

negative; two Simplexa positive/RT-RSV negative). Re-testing of 38 of these samples (nine had insufficient sample for re-testing) yielded the following: 10 RT-RSV positive, 27 RT-RSV negative and one RT-RSV negative/Simplexa positive. Overall concordance for RSV was 97.25%.

**CONCLUSIONS:** The performance of these assays appears good with a high degree of concordance. Discrepant analysis highlights the difficulty in trying to determine true positives and negatives despite repeat testing and the use of a third independent lab-developed assay (results not shown). The reason for the high number of discrepant RSV samples may be multifactorial and could not be easily determined.

## G04

## DETECTION OF CIRCULATING NOROVIRUS GENOTYPES: HITTING A MOVING TARGET

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**OBJECTIVE:** Similar to influenza virus, epidemic strains of norovirus emerge through antigenic drift and immune escape. Although national surveillance programs are in place to monitor norovirus epidemiology, the emergence of a new pandemic strain (GI.4.2012 Sydney) and the genetic diversity among other genotypes can be challenging for laboratories. In this study, we evaluated the analytical and clinical performance characteristics of one real-time assay and two end-point RT-PCR assays commonly used in microbiology laboratories.

**METHODS:** Lower limit of detection (LoD) was determined using 10-fold dilutions of noroviruses belonging to different genotypes. One hundred eighty-six stool specimens were tested concurrently and clinical performance of the real-time method was compared to the two conventional methods.

**RESULTS:** The real-time RT-PCR was highly sensitive and specific for the detection of all norovirus genotypes currently circulating in Canada. In contrast, the two end-point RT-PCRs displayed poor analytical sensitivity or complete failure to detect certain norovirus genotypes, which was correlated to sequence mismatches in the primer-binding sites. In an attempt to improve detection of norovirus for the end-point RT-PCRs and cover a broader range of genotypes, both end-point assays were processed concurrently and detection from either assay was considered a positive result. Concurrent testing resulted in only a modest increase in clinical sensitivity (75.0%) compared to each assay alone (62.5% and 71.9%). However, the false positivity rate increased from 1.98% and 3.36% for the end-point assays alone to 5.47% with concurrent testing.

**CONCLUSION:** Overall, this study emphasizes the benefits of a real-time method using degenerate primer and probes and provides support for routine surveillance to monitor norovirus epidemiology and ongoing proficiency testing to ensure detection of circulating norovirus genotypes.

## G05

POOLED NUCLEIC ACID TESTING INCREASES THE DIAGNOSTIC YIELD OF ACUTE HIV INFECTION COMPARED TO 3<sup>RD</sup> AND 4<sup>TH</sup> GENERATION HIV EIA

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**OBJECTIVE:** Early diagnosis of pre- and early seroconversion acute HIV infection (AHI) is important to reduce transmission during the most infectious period. We compared one 3<sup>rd</sup> generation (gen) and two 4<sup>th</sup> gen EIA assays to pooled nucleic acid testing (PNAT) for the identification of AHI.

**METHODS:** We tested 9550 specimens from males ≥18 years of age from six clinic sites attended by men who have sex with men. Specimens were tested by three HIV EIA assays: Siemens ADVIA Centaur® HIV 1/O/2 (3<sup>rd</sup> gen), Siemens ADVIA Centaur® HIV Combo (4<sup>th</sup> gen) and Abbott ARCHITECT® HIV Ag/Ab Combo (4<sup>th</sup> gen). In addition, 3<sup>rd</sup> gen

## Abstracts

negative specimens were tested by Roche COBAS® Ampliprep/COBAS® TaqMan HIV-1 Test v.2 in pools of 24 samples. Specificity of the EIAs and AHI detection sensitivity for the EIAs and PNAT were compared.

**RESULTS:** A total of 9435 (98.8%) specimens had no evidence of HIV infection, 94 (79 persons) had established HIV, nine (six persons) had pre-seroconversion AHI and 12 (nine persons) had early seroconversion AHI. Pre-seroconversion AHI cases were not detected by 3<sup>rd</sup> gen EIA, whereas two of six (33.3%) were detected by Siemens 4<sup>th</sup> gen, four of six (66.7%) by Abbott 4<sup>th</sup> gen and six of six (100%) by PNAT. All EIAs and PNAT detected all individuals with early sero-conversion AHI. EIA specificities were 99.9% (Siemens 3<sup>rd</sup> gen), 99.7% (Siemens 4<sup>th</sup> gen) and 99.2% (Abbott 4<sup>th</sup> gen).

**CONCLUSIONS:** While both 4<sup>th</sup> gen EIAs demonstrated improved sensitivity for AHI diagnosis compared to 3<sup>rd</sup> gen EIA, PNAT identified more AHI cases than either 4<sup>th</sup> gen assay. Specificities were high for all three EIAs. PNAT testing is a useful strategy to identify AHI in settings with high rates of HIV infection.

### G06

#### DUAL ENZYME IMMUNOASSAY (EIA) SCREENING FOR CONFIRMATION OF HCV INFECTION

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**OBJECTIVES:** Reflexing anti-HCV screen reactive specimens to a second manufacturer's EIA has been shown to improve anti-HCV screening specificity. We evaluated the performance of a dual EIA algorithm to verify screening EIA reactivity for the diagnosis of HCV infection.

**METHODS:** Specimens from September 2012 to April 2013 were screened with the Siemens ADVIA® Centaur™ HCV Assay (Centaur). Reactivity was categorized by signal/cutoff (s/c) ratios: non-reactive (NR) (s/c <0.80); equivocal (EQ) (s/c 0.80 to 0.99); weakly reactive (WR) (s/c 1.00 to 10.99); and strongly reactive (R) (s/c ≥11.00). Specimens with Centaur s/c ratios ≥0.80 were also tested by Abbott ARCHITECT® Anti-HCV Assay (Architect), except when the individual had documentation of a previous dual EIA reactive result.

**RESULTS:** A total of 105,026 specimens were tested.

Centaur	n	Architect supplemental				% positive
		NR	EQ	R	Prior reactive	
NR	99,204					
EQ	114	80	4	24	6	26.3
WR	798	220	11	318	249	71.1
R	4910	5	0	2202	2703	99.9

NR Nonreactive; EQ Equivocal; WR Weakly reactive; R Strongly reactive

**CONCLUSIONS:** Supplemental testing helps rule out false reactivity in specimens with equivocal or weakly reactive primary screen results, but adds little value for specimens with high screening s/c ratios. HCV RNA testing is still necessary to confirm or rule out active HCV infection among patients who are anti-HCV screen seroreactive, and this analysis is in progress.

### G07

#### SEROPROTECTION AGAINST EMERGING AND RE-EMERGING INFLUENZA VIRUSES: RESULTS OF AN AGE-BASED SEROSURVEY, BRITISH COLUMBIA, 2013

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**NZ Janjua<sup>1</sup>, G De Serres<sup>4</sup>, M Petric<sup>2</sup>, M Krajden<sup>2</sup>, D Kobasa<sup>5</sup>, Y Li<sup>5</sup>**

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**OBJECTIVE:** To assess seroprotection against emerging and re-emerging influenza viruses using an age-based serosurvey in British Columbia, Canada, May 2013.

**METHODS:** A community-based sample of approximately 500 anonymized residual sera (approximately 50 per 10-year age band) was assessed by hemagglutination inhibition assay for antibody to pandemic H1N1 (A/California/7/2009), recent human seasonal H3N2 (A/Victoria/361/2011), emerging swine-origin variant H3N2v (A/Indiana/10/2011) and novel avian-origin H7N9 influenza viruses. Seroprotection was defined as antibody titre ≥40.

**RESULTS:** Overall age-standardized seroprotection was 46% (95% CI 42% to 51%) for H1N1, 29% (95% CI 24% to 33%) for H3N2, 37% (95% CI 32% to 41%) for H3N2v and none for H7N9. Seroprotection to H1N1 was lowest (<20%) in young children <5 years of age and highest (≥60%) in school-aged children and elderly adults ≥70 years of age, but lower in adults 20 to 39 years of age (45% to 50%) and 40 to 69 years of age (35% to 40%). Seroprotection to H3N2 showed a similar peak in school-aged children but was comparably lower (<30%) in adults and pre-school children. Elderly adults also lacked the substantial seroprotection to H3N2 (<40%) observed for H1N1. Conversely, seroprotection to H3N2v peaked (≥60%) in adults 20 to 39 years of age but was substantially lower in children <10 years of age (<10%) and 10 to 19 years of age (<30%), and older adults (<40%). Although none of the tested sera showed seroprotection to H7N9, 10 had detectable titres ≥10, of whom three had titres ≥20, and all were ≥60 years of age.

**CONCLUSIONS:** Pre-school children and young and middle-aged adults remain most susceptible to pandemic H1N1. While partial population immunity may lessen the pandemic threat of emerging H3N2v, broad susceptibility to H7N9 suggests this novel virus would pose a threat if adapted for efficient human-to-human spread.

## 1600-1745 Oral Presentations: Session H Room: Convention Centre (Saanich 1 & 2)

### H01

#### URINE COLLECTION SYSTEMS THAT ARE DESIGNED TO PRESERVE URINARY PATHOGENS DURING TRANSPORT: EFFECT OF SYSTEM AND TRANSPORT TIME ON BACTERIAL SURVIVAL

**K Gauchier-Pitts<sup>1</sup>, R Rennie<sup>1</sup>, L-A Turnbull<sup>1</sup>, C Oosterhoff<sup>2</sup>**

<sup>1</sup>University of Alberta Hospital, Edmonton; <sup>2</sup>Red Deer Regional Hospital, Red Deer, AB

**OBJECTIVES:** We compared two transport devices, Uriswab (Copan Italia) with BD Urine Collection tubes (Becton Dickinson) for urine transport for culture. Both transport tubes contain preservative – boric acid and sodium formate.

**METHODS:** A total of 103 urines were collected for routine microbiological culture either close to the testing laboratory or at a remote site (additional urines are being collected to improve sample size). Urine was transferred to the BD Vacutainer tube (4 mL) and also dipped with the Uriswab (approximately 1.5 mL). The two preservative tubes were transported (1 h to 48 h) at ambient temperature for comparative culture. Urine from the two samples was plated with a 1 µL loop onto blood agar and chromogenic agar and incubated overnight at 35°C. Colony counts were performed and identification of species was made from each sample. Results were compared to original culture results.

**RESULTS:** Transport time did not influence recovery of bacteria from Uriswab or BD Vacutainer. Sixty samples did not grow from Uriswab; 56 did not grow from BD. Twenty-five Uriswab samples and 31 BD samples had mixed growth of non-uropathogens at 1 to 10×10<sup>6</sup> cfu/L. Nineteen uropathogens were isolated from Uriswab and 16 from BD. For Uriswab there were 10 *E coli*, one *Klebsiella sp.*, one *Proteus sp.*, one *S agalactiae*, one *S aureus* and five enterococci. For BD there were nine *E coli*, one *Proteus sp.*, one *S agalactiae*, one *S aureus* and four enterococci. The enterococci were only isolated at 1×10<sup>6</sup> cfu/L from both systems.

**CONCLUSIONS:** Uriswab and BD vacutainer urine transport devices maintain equivalent viability of urinary pathogens over prolonged transport to testing laboratories. Uriswab had fewer low colony count mixed cultures.

**H02****COMPARISON OF BD MAX MRSA ASSAY USING A COMBINED NASAL/PERIANAL ESwab TO NASAL AND PERIANAL SWABS CULTURED ON CHROM AGAR AND BROTH ENRICHMENT MEDIA****C Ellis, W Ciccotelli****Grand River Hospital, Kitchener, ON****OBJECTIVE:** To assess the diagnostic capabilities of the BD MAX nasal MRSA assay when using a combined nasal/perianal collection on an Eswab.**METHODS:** Patients classified as MRSA negative/positive by Infection Control had separate nasal and perianal rayon swabs collected followed by a combined nasal/perianal Eswab (Copan). The Eswab was tested on the BD MAX using the MRSA nasal assay. The Eswab and rayon swabs were streaked on MRSA Select Chrom agar (BIO-RAD) and then inoculated into 6.5% NaCl broth enrichment, incubated for 24 h then subcultured to MRSA Select Chrom agar. MRSA chrom agar was incubated for 24 h and examined for typical pink colonies.**RESULTS:** A total of 169 patients were tested. The pooled broth enrichment results (gold standard) were compared to the results from the direct PCR assay (Eswab) and Chrom agar (Eswab and rayon swab). Sensitivity, specificity, PPV and NPV are as follows: BD MAX PCR assay 80.6%, 88.8%, 80.6% and 88.8%; Eswab culture 82.3%, 100%, 100% and 90.7%; and the rayon swabs were 69.4%, 100%, 100% and 84.9%, respectively. The lower specificity with direct PCR detection was attributed to detection of a non-viable organism, lower detection threshold or *Staphylococcus aureus* possessing a dropped SCCmec cassette.**CONCLUSION:** The BD MAX PCR nasal MRSA assay when pooled as a nasal/perianal collection was comparable to direct culture using the Eswab. Both outperformed standard rayon swabs in terms of sensitivity. Although PCR detection had more false positives, its rapid turn around time allowed for earlier determination of potential MRSA colonization.**H03****DETECTING MYCOBACTERIUM AVIUM COMPLEX IN RESPIRATORY SPECIMENS BY A DUPLEX QPCR USING THE ABI 7900 FAST REAL-TIME PCR SYSTEM****N Lowther<sup>1</sup>, D Eisler<sup>1</sup>, A McNabb<sup>1</sup>, P Tang<sup>1,2</sup>****<sup>1</sup>BC Centre for Disease Control, PHSA; <sup>2</sup>University of British Columbia, Vancouver, BC****OBJECTIVES:** To adapt the conventional PCR assay of Park et al targeting the 16-23S rRNA internal transcribed spacer region (ITS) for the identification of mycobacterial species to a duplexed real-time PCR assay. This adaptation will allow the duplexed assay to detect both the MPT64 gene found in *Mycobacterium tuberculosis sensu stricto* and a region of the ITS gene unique for the *Mycobacterium avium* complex (MAC) directly from clinical specimens or from cultured isolates. The goal is to use this qPCR duplex assay as a replacement for the commercial GenProbe Accuprobe TM hybridization assay currently in use in the Mycobacteriology Laboratory for the identification of MAC isolates.**METHODS:** The sensitivity and specificity of the primers were assessed by conventional PCR against a panel of 206 known mycobacterial and non-mycobacterial isolates. The PCR results were compared with the identification based on the in-house *hsp65* sequencing results. 98% of the ITS amplicons were successfully sequenced in-house using the primers of Park et al with identification matching that of *hsp65*. A MAC qPCR Singleplex was then evaluated on an ABI 7900 Real-time Fast PCR System. All of the known MACs were detected, but there were seven false-positive non-MACs. Using this data we modified the forward primer of Park et al, developed two reverse primers MAR and MIR to detect *M avium* and *M intracellulare*, respectively, and developed a probe to detect the ITS target of MAC. The new set of primers and probes greatly improved the specificity of the assay. The performance of the qPCR duplex was assessed using DNA lysates from a total of 283 respiratory specimens; including a double-blind, parallel study of 102 clinical samples. All known MACs were detected and there was only one false-positive non-MAC. Compatibility of the MAC ITS primer/probe set

with the MTBC MPT64 primer/probe set originally established was determined for the duplex, with no loss of sensitivity and no cross-reactions between the two targets.

**CONCLUSIONS:** This novel qPCR duplex designed in-house to detect both MTBC and MAC in one test will facilitate an accurate and early diagnosis of mycobacteriosis by eliminating the need for the culture dependent commercial Accuprobe method to detect MAC infection.**H04****DEVELOPMENT OF A REAL-TIME PCR ASSAY FOR THE DIFFERENTIATION OF *STREPTOCOCCUS PNEUMONIAE* AND *STREPTOCOCCUS PSEUDOPNEUMONIAE*****T Lee<sup>1</sup>, K Adie<sup>1</sup>, A McNabb<sup>1</sup>, L Hoang<sup>1,2</sup>, P Tang<sup>1,2</sup>****<sup>1</sup>BC Centre for Disease Control; <sup>2</sup>University of British Columbia, Vancouver, BC****OBJECTIVES:** The identification of clinically relevant *Streptococcus* species, particularly *Streptococcus pneumoniae*, *S pseudopneumoniae*, and *S mitis*, can be difficult when using biochemical methods and 16S rDNA sequencing. In order to find a quicker method to discriminate these three species we designed a real-time PCR assay to identify these species.**METHODS:** Using the type strains of *S pneumoniae*, *S pseudopneumoniae*, and *S mitis*, we designed primers and probes to a variable region of the autolysin A (*lytA*) gene for use in a real-time PCR assay. The assay consisted of a single set of primers that amplified a 178bp fragment of *lytA* specific to *S pneumoniae* and *S pseudopneumoniae* combined with probes specific to each of these two species. *S mitis* group isolates referred to the BC Public Health Microbiology Reference Laboratory (PHMRL) between the period 2011 to 2013 for identification or serotyping were tested using this assay.**RESULTS:** A total of 83 *S mitis* group isolates, including 14 *S pneumoniae* isolates referred to the reference lab for serotyping, were tested by our real-time PCR assay. Of the 83 *S mitis* group isolates submitted, 68 were unable to be identified at the species level by biochemical and 16S rDNA testing. When tested using our real-time PCR assay, three isolates were identified as *S pneumoniae* and three isolates were identified as *S pseudopneumoniae*. All referred *S pneumoniae* isolates were confirmed as *S pneumoniae*. No cross reactivity or non-specific amplification occurred with other *S mitis* group species.**CONCLUSIONS:** *S pseudopneumoniae* is present in the population serviced by the BC PHMRL. Using a duplex real-time PCR assay for *S pseudopneumoniae* and *S pneumoniae*, these otherwise difficult to differentiate species can now be identified. With the ability to identify *S pseudopneumoniae*, its prevalence and clinical relevance in the PHMRL client population can now be examined.**H05****COMPARISON OF VIRAL TRANSPORT MEDIUM AND AMIES TRANSPORT MEDIUM WITH CHARCOAL FOR THE DETECTION OF *BORDETELLA PERTUSSIS* BY PCR****L Zhou, L Hoang, M Petric****BC Centre for Disease Control, Vancouver, BC****OBJECTIVE:** To compare the effects of using Viral Transport Medium (VTM) against Amies Charcoal Transport Medium (ACTM) for the detection of *Bordetella pertussis* by polymerase chain reaction (PCR).**METHOD:** A suspension of *B pertussis* at 0.5 McFarland (1.5×10<sup>8</sup> CFU/mL) standard was subjected to a serial 10-fold dilution series from 1.5×10<sup>8</sup> CFU/mL to 1.5×10<sup>-2</sup> CFU/mL, of which 200 µL was inoculated onto each of three swabs, one ACTM and two VTM collection vials, and incubated at 4°C for 24 h. The ACTM swab and one of the VTM swabs were extracted using the InstaGene bacterial DNA extraction protocol, while the second VTM swab was extracted using the MagMAX viral RNA extraction protocol. All three extracts for each dilution were tested by PCR and the Ct values were compared. We also inoculated 200 µL of each dilution onto Oxoid Bordetella Selective Medium and incubated these at 35°C for 72 h for colony count.**RESULT:** After 24 h incubation, *B pertussis* was detected from VTM at 1:10<sup>6</sup> dilution (Ct of 36.03) and from ACTM at 1:10<sup>5</sup> dilution (Ct of 34.74) using the InstaGene extraction protocol. Detection of *B pertussis* in

## Abstracts

VTM using the MagMAX extraction method is at 1:10<sup>6</sup> dilution (Ct of 38.84). After 96 h incubation, *B pertussis* was detected from VTM at 1:10<sup>5</sup> dilution (Ct of 32.79) and from ACTM at 1:10<sup>7</sup> dilution (Ct of 36.55) using the IntaGene extraction protocol and at 1:10<sup>5</sup> dilution (Ct of 36.47) using the MagMAX extraction protocol.

**CONCLUSION:** The use of a single VTM for detection of both viruses and *B pertussis* by nucleic acid amplification would not only be more economical in supplies and work flow, but also less traumatic for the patients. Preliminary results show a potential for VTM after 24 h incubation. Further clinical evaluation is required.

### H06

#### EVALUATION OF THE TBcID ASSAY FOR THE DETECTION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX ORGANISMS DIRECTLY FROM LIQUID CULTURE

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**OBJECTIVE:** The rapid and accurate differentiation of *Mycobacterium tuberculosis* complex (MTBC) organisms and non-tuberculosis mycobacteria (NTM) from AFB-smear positive cultures is essential for the appropriate management of both MTBC and NTM disease. A rapid, immunochromatographic assay for the identification of MTBC directly from liquid culture, the TBcID assay, has been introduced by BD Canada. We have evaluated this assay to determine if it is a viable option for routine detection of MTBC organisms from liquid culture.

**METHODS:** Eighty-eight MTBC and 28 NTM cultures (representing 11 species) were tested using the Genprobe Accuprobe assay, real-time PCR and the TBcID kit (BD). Judicial IDs were determined using gyrB/16s rRNA gene sequencing. Results were collated and compared for all methods tested.

**RESULTS:** Of the 88 MTBC MGIT cultures tested, 86 of 88 tested positive for the presence of MTBC using the TBcID assay. Negative testes were confirmed by repeat testing. All of the NTM cultures tested negative for the presence of MTBC. Real-time PCR provided accurate results for 116 of 116 cultures. Accuprobe results were available for 88 of 116 cultures, with sensitivity and specificity of 100%.

**CONCLUSION:** The TBcID assay was able to provide 97.7% sensitivity and 100% specificity for the AFB positive cultures tested. Further investigation into the reason the two MTBC positive cultures were not identified is required. Overall, the TBcID assay is rapid and easy to use. While it identifies MTBC directly from liquid culture, there is no specific identification of NTM that might be present in the culture. There is a relatively low false-negative rate, more data needs to be collected to determine the cause and implications of these results.

### H07

#### ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST) BY BROTH MICRODILUTION (BMD) USING CLSI GUIDELINES FOR CORYNEBACTERIUM ISOLATES REFERRED TO THE NATIONAL MICROBIOLOGY LABORATORY

**K Bernard**<sup>1,2</sup>, **Al Pacheco**<sup>1</sup>, **T Burdz**<sup>1</sup>, **D Wiebe**<sup>1</sup>

<sup>1</sup>National Microbiology Laboratory; <sup>2</sup> Department of Medical Microbiology, University of Manitoba, Winnipeg MB

**INTRODUCTION:** CLSI guidelines for AST of *Corynebacterium* and allied genera (M45-2A, Table 5) have existed since 2006 based on the BMD method. AST data for such taxa have only been infrequently published, usually describing use of methods other than BMD and, for older publications, describing AST data derived from strains identified by phenotypic not genotypic methods. Here, 466 *Corynebacterium* strains from 2006 to 2013 underwent AST after identification using genetic and other standard methods.

**METHODS:** Identification was done using 16SrRNA and *rpoB* gene sequencing, except for *C diphtheriae* received for toxin production assay testing. Older isolates were also tested biochemically, with some by

MALDI-TOF. AST was performed as described by CLSI guidelines using Trek Sensititre panels GPN3F and STP6F. Multidrug resistance (MDR) was defined as being resistant to >3 drug classes.

**RESULTS AND DISCUSSION:** A total of 466 strains, 430 assignable to 37 valid species, as well as 36 *Corynebacterium* possibly representing 17 new species, were studied. All were susceptible to vancomycin and linezolid. Nearly all were susceptible to daptomycin (99.6%) and quinupristin-dalfopristin (95.3%), with >85% of isolates being susceptible to rifampin, tetracycline, gentamicin and meropenem. MDR was observed for some or most *C afermentans*, *C amycolatum*, *C aurimucosum*, *C auris*, *C coyleae*, *C frenesi*, *C glucuronolyticum*, *C jeikeium*, *C pilbarensis*, *C propinquum*, *C pyruniciproducens*, *C resistens*, *C riegelii*, *C striatum*, *C tuberculo-stearicum*, *C urealyticum*, *C ureicelerivorans* and two unassigned taxon groups, with resistance to >5 drug classes being observed for unique strains of bolded species. A small percentage of 144 *C diphtheriae* studied were resistant to several drug classes, with most being non-susceptible to cephalosporins. In summary, commonly-recovered *Corynebacterium* species in Canada can be or usually are, MDR. AST should be performed for all clinically relevant isolates in order to best evaluate for precise treatment options for patients.

## 1110-1225 Oral Presentations: Session I Room: Convention Centre (Esquimalt)

### I01

#### APPLICATION OF MULTIPLE MOLECULAR METHODS FOR SUBTYPING CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* AND *ENTEROBACTER CLOACAE* ISOLATES FROM MULTIPLE HOSPITALS

**Y Chang**<sup>1</sup>, **C Ng**<sup>1</sup>, **E Brodtkin**<sup>2</sup>, **B Mac**<sup>2,3</sup>, **D Purych**<sup>2,4</sup>, **Da Boyd**<sup>5</sup>, **K Fakharuddin**<sup>5</sup>, **MR Mulvey**<sup>5</sup>, **L Hoang**<sup>1</sup>

<sup>1</sup>BC Public Health Microbiology & Reference Laboratory, Vancouver; <sup>2</sup>Fraser Health Authority; <sup>3</sup>Surrey Memorial Hospital, Surrey; <sup>4</sup>Royal Columbian Hospital, New Westminster, BC; <sup>5</sup>National Microbiology Laboratory, Winnipeg, MB

**OBJECTIVE(S):** Since 2010, BCPHMRL has been performing genotyping analysis of carbapenem-resistant and intermediate isolates forwarded from BC microbiology laboratories. A recent cluster of *Klebsiella pneumoniae* and *Enterobacter cloacae* New-Delhi metallo-beta-lactamase-1 (NDM-1) isolates in a BC region was identified. We here evaluate the different strain subtyping methods utilized to best suggest strain relatedness.

**METHODS:** Resistance characterization using a multiplex PCR assay targeting carbapenem-resistant Enterobacteriaceae (CRE) genes, extended-spectrum beta-lactamases (ESBLs), and AmpC beta-lactamases was completed on NDM-1 *K pneumoniae* and *E cloacae* isolates from 2008 to 2013 from four hospitals. Pulsed-field gel electrophoresis (PFGE) and NDM-1 plasmid fingerprinting using *Bgl*II was also carried out on a subset of isolates. These data were reviewed against case history and infection control information.

**RESULTS:** Of the 59 cases in this cluster, *K pneumoniae* (n=34 [58%]) has been the predominant species isolated followed by *E cloacae* (n=15 [25%]). Thirteen cases had travel history to CRE-endemic countries. Results of molecular analyses on 35 of these cases revealed that isolates of *E cloacae* had a high genetic similarity by PFGE (>90%) and NDM-1 plasmid analysis (≥80%) as well as beta-lactamase gene profiles with the exception of one *E cloacae* that had a unique pattern and positive travel history. In contrast, the *K pneumoniae* contained multiple beta-lactamase gene profiles and the PFGE patterns were diverse.

**CONCLUSION:** Genotypic profiling by beta-lactamase gene, PFGE, and NDM-1 plasmid analysis were consistent for *E cloacae* compared to *K pneumoniae* isolates in this study. The high mobility of NDM-1 plasmids may complicate the molecular epidemiologic analysis of isolates during CRE clusters.

## I02

## EVALUATION OF THE SS-LACTA™ TEST FOR THE RAPID DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES IN ENTEROBACTERIACEAE FROM PERIANAL SWABS

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## I03

## EVALUATION OF THREE CHROMOGENIC MEDIA FOR THE DETECTION OF VANCOMYCIN RESISTANT ENTEROCOCCI (VRE)

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**OBJECTIVES:** To evaluate the performance of Oxoid Brilliance VRE agar and Bio-Rad VRESelect chromogenic agars at 24 h, 36 h and 48 h and to compare them to the currently used Colorex chromogenic media.**METHODS:** A total of 500 rectal swabs for VRE surveillance were inoculated in parallel to Oxoid Brilliance VRE, Bio-Rad VRESelect and Colorex media. The Oxoid Brilliance VRE and Bio-Rad VRESelect were read at 24 h, 36 h and 48 h and the Colorex was read at 36 h. Reading of the plates was blinded. All colonies regardless of pigment were investigated. VRE were confirmed by Gram stain, catalase, PYR, Vitek 2 GP ID, BHIV6 and vancomycin E-test.**RESULTS:** There were a total of 50 VRE isolates detected. Colorex, Oxoid Brilliance VRE and Bio-Rad VRESelect detected 22 (44%), 38 (76%) and 34 (68%) VRE respectively. At 24 h, 34 of 38 isolates were detected on Oxoid Brilliance versus 20 of 34 isolates on Bio Rad VRESelect.

		24 h	36 h	48 h
Oxoid Brilliance	Sen/Sp	68%/93.1%	74%/87.6%	76%/85.6%
VRE Select	Sen/Sp	40%/95.1%	56%/94.2%	68%/92.9%
Colorex VRE	Sen/Sp	–	44%/95.6%	–

Sen Sensitivity; Sp Specificity

Colour was not reliable on either media to speciate *E faecalis* and *E faecium*.**CONCLUSIONS:** Both Oxoid Brilliance and Bio-Rad VRESelect performed better at 36 h than 24 h incubation. The Oxoid Brilliance VRE detected more VRE at 36 h than Bio-Rad VRE select. However it had more breakthrough growth of suspicious colonies that proved not to be VRE at 24 h, 36 h and 48 h incubation.

## I04

## USE OF WELLCOLEX COLOUR SHIGELLA (WCS) LATEX AGGLUTINATION TO DETECT SHIGELLA AMONG NON-LACTOSE FERMENTORS (NLF) IDENTIFIED (ID) BY MALDI-TOF AS ESCHERICHIA COLI

BM Willey<sup>1,2</sup>, C Vermeiren<sup>3</sup>, G Ricci<sup>4</sup>, C Watt<sup>5</sup>, P Lo<sup>1,2</sup>, AE Simor<sup>5,6</sup>, SM Poutanen<sup>1,2,6</sup><sup>1</sup>Mount Sinai Hospital; <sup>2</sup>University Health Network; <sup>3</sup>Shared Hospital Laboratory, Toronto; <sup>4</sup>William Osler Health Sciences Centre, Markham; <sup>5</sup>Sunnybrook Health Sciences Centre; <sup>6</sup>University of Toronto, Toronto, ON**OBJECTIVES:** MALDI-TOF is unable to distinguish *Shigella* from *E coli*. This study evaluated the ability of the WCS, a simple 2 min latex agglutination test, to identify *Shigella* to species level among NLF identified by VITEK MS PLUS (VMS; bioMérieux) as *E coli*. The WCS contains two latex pools with mixed red and blue particles each sensitized to one of four *Shigella* species but not to *E coli*.**METHODS:** A total of 238 study isolates derived from four Toronto Area laboratories were tested by the WCS following kit instructions. These included 114 retrospective isolates with conventional ID (n=87 *Shigella*: *S flexneri* [n=50], *S sonnei* [n=29], *S boydii* [n=7], *S dysenteriae* [n=1] and 27 NLF *E coli*) and 124 prospective NLF *E coli* from primary MacConkey agar cultures identified by VMS ID. IDs were blinded to prevent bias.**RESULTS:** Correctly, 50 of 50 *S flexneri* reacted blue and 29 of 29 *S sonnei* reacted red in Reagent-1, while seven of seven *S boydii* reacted blue and one of one *S dysenteriae* reacted red in Reagent-2, for an overall sensitivity for *Shigella* detection of 100% (95% CI 94.9% to 100%). Conversely, zero of 151 NLF *E coli* reacted with Reagent-1 red or Reagent-2 latexes, but two reacted reproducibly with Reagent-1 blue latex (suggesting *S flexneri*). Both were *E coli* by VITEK 2 GNI, API 20E, and were non-reactive with Remel *Shigella* anti-sera, and showed auto-agglutination after 48 h. Specificities (95% CI) for *S flexneri*, *S sonnei*, *S boydii* and *S dysenteriae* latexes were 98.9% (95% CI 96% to 99.96%), 100% (95% CI 97.8% to 100%), 100% (95% CI 98% to 100%), and 100% (95% CI 98.1% to 100%), respectively. **CONCLUSIONS:** The low-complexity WCS proved to be a highly accurate, rapid and cost-effective way to resolve the inability of MALDI-TOF to distinguish *Shigella* from *E coli*. A negative result rules out *Shigella* without the need for additional testing. Positive results should be confirmed by conventional methods.

## I05

## EVALUATION OF A NOVEL ENTEROCOCCOSEL ENRICHMENT BROTH IN COMBINATION WITH A CHROMOGENIC VRE PLATE WITHIN A REGION OF HIGH VRE PREVALENCE

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Gamma-Dynacare Medical Laboratories, Ottawa, ON**OBJECTIVES:** VRE screening has become a controversial topic of discussion amongst health care workers. Our laboratory has been challenged with screening for VRE in a high prevalence region with a positivity rate of 21% in 2013. The streamlining challenges were diagnosing these high numbers of positive VRE within a reasonable time frame and having a method able to reduce non-VRE *E faecalis* breakthrough growths. To enhance, we co-developed a broth able to grow VRE at a high yield and reduce suspect breakthrough growth.**METHODS:** A total of 858 VRE screens were inoculated onto Oxoid Brilliance chromogenic VRE directly (-B) followed by immersion into enrichment broth for further overnight incubation. The incubated broths were also subcultured onto Brilliance VRE plates (+B). -B plates were



## Abstracts

incubated for 26 h to 32 h and held refrigerated until +B could be read simultaneously at a similar plate incubation time. Both sets of plates were examined for presence and amount of VRE growth, colour, subculture requirement, and breakthrough growth. A positive was deemed such by one or both methods.

**RESULTS:** One hundred forty-five of 858 swabs were VRE positive with 100% sensitivity using +B. One hundred thirty-eight (95% sensitivity) positives were recovered using -B. Specificity was 87% -B versus 93% +B. All +B yielded 3+ growths while -B was uniformly graded from +/- to 3+.

**CONCLUSIONS:** In our situation, added overnight incubation of broths did not affect TAT, in fact had better TAT due to a reduction in subcultures; easier workup with better yields of growth with +B; higher sensitivity and specificity with +B; 17% less use of media and reagents with +B, while not needing extra staff due to a recent increase of VRE.

## 1110-1225 Oral Presentations: Session J Room: Convention Centre (Oak Bay 1)

### J01

#### COMPARISON OF CONTINUOUS RANDOM ACCESS TESTING FOR *C TRACHOMATIS* ON A PANTHER® INSTRUMENT TO BATCH TESTING ON A COBAS® 4800 SYSTEM

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**OBJECTIVE:** Comparison of Aptima® Combo 2 on Panther and cobas CT/NG on cobas 4800 for *C trachomatis* (CT) diagnosis.

**METHODS:** Self-collected vaginal swabs (SCVS) and first-void urine (FVU) were tested spiked and unspiked with CT by AC2 on a Panther (Hologic | GenProbe) and cobas CT/NG on a cobas 4800 (Roche). Discordants were tested by a third test. Pre-analytical interactions, reagent and sample preparation and loading, in-process, post-analytical and maintenance hands-on times were measured. Time to results, automation times, hands-on time per sample and mandatory return visits were calculated for 96 and 192 tests.

**RESULTS:** Probit calculations of SCVS and FVU levels of detection (LOD<sub>50</sub>) were -8.1 and -7.9 for AC2 respectively, compared to -5.9 and -7.1 for cobas CT/NG. There was no amplification inhibition. Prevalence was 9% (53 of 575) using a patient infected status of positivity in both assays or in a single assay confirmed by another. Sensitivities of AC2 and cobas 4800 on SCVS were 96.2% and 84.6% (P=0.016); 88.0% and 81.1% with FVU. Specificities were 98.4%-100%. Total hands-on time for 96 and 192 tests were 21 min and 33 min on Panther, and 40 min and 98 min on cobas 4800; hands-on times per sample were 13 s and 10 s on Panther compared to 26 s and 31 s on cobas 4800. Results were reported continuously for 96 and 192 tests at 3 h 51 min on Panther compared to two batched reports on the cobas 4800 at 4 h 23 min and 6 h 8 min. Overall maintenance was similar.

**CONCLUSIONS:** AC2 demonstrated higher analytical and clinical sensitivities than cobas CT/NG. Panther demonstrated half the amount of total hands-on time than cobas 4800, with greater efficiency per patient specimen.

### J02

#### GENOTYPING OF *LYMPHOGRANULOMA VENEREUM* CHLAMYDIA SEROVARS AT THE NATIONAL MICROBIOLOGY LABORATORY

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**OBJECTIVE:** *Lymphogranuloma venereum* (LGV) is a genital infection caused by the particularly aggressive *Chlamydia trachomatis* serovars L1, L2

and L3. LGV used to be endemic only in tropical countries and cases in Canada were due to importation. However, in 2003, an outbreak with the novel variant L2b started in men-who-have sex-with-men (MSM) in the Netherlands and spread quickly through Europe, Australia and North America. Chlamydia L2b is now endemic in MSM in Canada and diagnosis of LGV requires genotyping of all suspected chlamydia-positive cases. Exact diagnosis of LGV is crucial since prolonged antibiotic therapy is required to cure the infection and prevent sequelae.

**METHODS:** The National Microbiology Laboratory (NML) has been receiving chlamydia-positive specimens from Quebec, Ontario and BC from suspected LGV cases. Specimens are rapidly screened by real-time PCR and those that are positive for LGV are genotyped at the *ompA* gene to determine the exact serovar.

**RESULTS:** The NML received 299, 433, 790 and 782 specimens for LGV typing and, of those, 18, 34, 38 and 59 were positive in the years 2010, 2011, 2012 and 2013, respectively. Of the 149 LGV specimens, 137 were of the endemic variant L2b, while 11 were of serovar L2. There is a clear rising trend in the number of cases which may be due to both spreading of the diseases and improved detection.

**CONCLUSION:** LGV is now endemic in MSM in Canada and its incidence is on the rise and, perhaps, underestimated. Prompt diagnosis and therapy can reduce morbidity and spreading of LGV and therefore aggressive screening and surveillance in all Canadian provinces is advisable.

### J03

#### A PRELIMINARY MOLECULAR-BASED ASSAY FOR THE DETECTION OF ANTIMICROBIAL RESISTANT *NEISSERIA GONORRHOEA*

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**OBJECTIVE:** Reported cases of gonorrhoea have increased over the last decade in Canada but cultures available for antimicrobial susceptibility testing have decreased due to the rise of Nucleic Acid Amplification Testing (NAAT) for diagnosis. Approximately 70% of gonorrhoea cases are diagnosed by NAAT in Canada yearly and for those cases, there is no antimicrobial resistance data available. Novel methods to monitor antimicrobial resistance in *Neisseria gonorrhoeae* directly from NAAT samples would aid in surveillance and treatment recommendations.

**METHOD:** Real-time (RT-) PCR assays were developed to detect genes/single-nucleotide polymorphisms (SNPs) associated with antimicrobial resistance. Seven targets were selected including six associated with cephalosporin, azithromycin and ciprofloxacin resistance (*ponA*, *mtrR*, *penA*, *gyrA*, *parC* and *porB*) and one *N gonorrhoeae* specific gene (*porA*) as positive control. The 83 *N gonorrhoeae* and 12 non-gonococcal species tested (in duplicate) included a range of MICs for the drugs of interest. DNA extracted from 10 NAAT samples were also tested.

**RESULTS:** Using DNA sequence as the Gold standard for each loci, RT-PCR assays detected SNPs associated with antimicrobial resistance in the 83 *N gonorrhoeae* as follows: *ponA* and *parC*, 100% agreement; *gyrA*, 98.7%; *mtrR* and *penA*, 98.7%; *porB*, 97.6%. SNP detection was successful and *N gonorrhoeae* multi antigen sequence types (NG-MAST) were determined for eight of the 10 NAAT specimens tested. Assays were 100% reproducible when tested in duplicate and the gonococcal specific target *porA* achieved 100% specificity.

**CONCLUSIONS:** The increased use of NAAT for the detection of *N gonorrhoeae* decreases the ability to understand antimicrobial resistance

trends. In this study we demonstrated a RT-PCR assay that has high sensitivity and specificity to known antimicrobial resistance markers. Preliminary work using NAAT specimens is encouraging. It is hoped we will be able to adopt this assay from NAAT specimens in our national surveillance program and to optimize gonorrhea treatment.

## J04

### A NEW RISE IN AN OLD DISEASE: LGV IN BRITISH COLUMBIA 2004-2013

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**BACKGROUND:** Lymphogranuloma venereum (LGV) is a sexually transmitted disease caused by the L1, L2, L2a, L2b or L3 subtypes of *C trachomatis* (CT). Historically, it was an old-world disease presenting primarily with inguinal lymphadenopathy, but it may also present as proctitis if rectally acquired. We here review the provincial surveillance data in BC between December 2004 and 2013, as well as the spectrum of clinical presentations.

**METHODS:** Patients were included if they had probable or confirmed LGV infection. Since 2010, all rectal specimens that had a positive CT nucleic acid amplification test (NAAT) result were forwarded to a reference laboratory for LGV genotyping. Patients diagnosed with LGV were treated and active contact tracing was conducted through public health.

**RESULTS:** Between 2004 and 2013, there were 71 cases of LGV, genotype L2b, reported in BC. Except for two, all were male (five bisexual, 63 MSM and one unknown). The average age was 43.6 years. The majority (52 of 71) were self-described as Caucasian. Forty-four were co-infected with HIV and one was co-diagnosed with syphilis. Most cases presented with a non-specific proctitis, three were diagnosed on rectal biopsy after referral to gastroenterologists for rectal symptoms, with one being mistreated for Crohn's disease. Presentation involving inguinal lymphadenopathy was rare.

**DISCUSSION:** LGV now occurs primarily as proctitis among MSM in BC. Rectal CT NAAT testing is crucial in diagnosing rectal LGV, especially among MSM. LGV should also be suspected in rectal inflammatory lesions in MSM where diagnosis may be delayed by misdiagnosis as inflammatory bowel disease.

## J05

### WHOLE-GENOME SEQUENCING OF *NEISSERIA GONORRHOEAE* ISOLATES WITH REDUCED CEPHALOSPORIN SUSCEPTIBILITIES COLLECTED IN CANADA BETWEEN 2001 AND 2012

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**OBJECTIVE:** A large-scale, whole-genome comparison of Canadian *N gonorrhoeae* isolates with high-level cephalosporin MICs to demonstrate a novel genomic epidemiology approach to investigate strain relatedness and dynamics.

**METHODS:** *N gonorrhoeae* isolates include 23 susceptible isolates; 95 isolates with cefixime and/or ceftriaxone MICs ranging from 0.063 µg/mL to

0.5 µg/mL; and one European reference strain with high-level cefixime (≥4 µg/mL) and ceftriaxone (2 µg/mL) resistance. Whole-genome sequencing (WGS) was performed using Illumina MiSeq. FastQC was used to analyze sequence quality, SNPs were determined against reference genome NCCP11945 using SMALT, de novo assembly was performed using SPAdes and assembled contigs were annotated with Prokka.

**RESULTS:** The 119 strains consisted of 39 *N gonorrhoeae* multi-antigen sequence types (NG-MAST, ST) types that grouped into nine phylogenomic clades labelled A-I. Specific ST types were strongly associated with individual phylogenomic clades. Elevated ceftriaxone MICs (≥0.032 µg/mL) were observed in 86 of 91 (94.5%) of Clade A, C, D, and F isolates, whereas all 13 Clade B isolates had low MICs (≤0.008 µg/mL). Isolates in Clades G, H and I (n=8) had moderate ceftriaxone MICs 0.008 µg/mL to 0.032 µg/mL. The *penA* mosaic genotype represented 51% (n=65) and *penA* type XXXIV was most predominant (n=60). Other mutations associated with elevated cephalosporin MICs or resistance to fluoroquinolones were identified: *mtrR* A deletion, (n=95); *ponA* L421P, (n=98); *porB* mutations, (n=95); *gyrA* or *parC*, (n=99). Accessory genomic content represented approx. 6.5% of the *N gonorrhoeae* genomes, often associated with the extrachromosomal pNGTCD08107/pEP5289 plasmid.

**CONCLUSIONS:** The clades generated from WGS analysis correlated strongly with NG-MAST. WGS data offers further differentiation and enhanced discriminatory power in the analysis of the very clonal *N gonorrhoeae*. As the costs associated with WGS decline and analysis tools are streamlined, WGS will provide more thorough understanding of isolates and contribute to epidemiological studies, improved surveillance and optimized treatment.

## 1110-1225 Oral Presentations: Session K Room: Convention Centre (Oak Bay 2)

### K01

#### THE NATIONAL NOTIFIABLE DISEASES DATABASE (NDDDB): A NEW RESOURCE FOR PUBLIC HEALTH

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**OBJECTIVES:** Effective notifiable disease (ND) management requires the active participation and cooperation of all health care professionals. As part of a critical examination of Canada's Federal, Provincial and Territorial (FPT) ND reporting legislation, we learned: 1) jurisdiction-specific information concerning ND reporting requirements and case definitions are not easily accessible or comparable; 2) there is significant variability between jurisdictions for ND legislation and regulation. In response, we created an easy-to-use database of ND lists and reporting requirements for all 14 Canadian FPT jurisdictions. The NDDDB has two primary objectives: 1) to provide a central location where FPT ND lists and legislation can be easily retrieved and compared; 2) to stimulate discussion about ND case definitions and reporting criteria across Canada.

**METHODS:** Through an ongoing process of policy review and consultation, we determined: a) appropriate content for the online database; b) content organization; and c) a user-friendly interface. In addition, our ongoing knowledge dissemination and translation activities will develop awareness and encourage use of the database by public health policy makers and practitioners. User feedback and web-based measurement tools have been incorporated to evaluate database utility and effectiveness.

**RESULTS:** Preliminary feedback from public health officials and other stakeholders regarding usability and prospective utility has been very positive. Ongoing evaluation and feedback from users will inform future updates and upgrades to the database.

**CONCLUSION:** ND policy and practice varies considerably between jurisdictions in Canada. Public health policy makers and practitioners may benefit from having easy access to ND information that is jurisdiction specific and comparable.

## K02

**IMPACT OF CONJUGANT PNEUMOCOCCAL VACCINE PROGRAMS ON RATES OF INVASIVE PNEUMOCOCCAL DISEASE (IPD), 2001–2013**

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**OBJECTIVES:** PCV7 vaccination resulted in declines in IPD due to PCV7 serotypes (STs), but increases in IPD due to non-conjugate vaccine STs. Our objective was to monitor the effectiveness of routine infant PCV programs against IPD in southern Ontario.

**METHODS:** PCV7 was authorized in 2001; in Ontario, publicly funded PCV7 was introduced in 2005, PCV10 in 2009 and PCV13 in 2010. PCV13 introduction included a catch-up dose for children 12 to 36 months of age. Population-based surveillance for IPD was performed; all isolates were serotyped at a central lab. Clinical/vaccine data were collected by chart review and patient/MD interview.

**RESULTS:** From 2001 to 2013, 960 pediatric (<15 years of age) IPD cases were identified. 149 (15%) had chronic illness predisposing to IPD. A total of 386 (41%) had pneumonia, 327 (35%) bacteremia without focus and 91 (9%) meningitis. Case fatality rate was 2% (21 deaths). In 2013, 19A (four of 28, 14%) and 22F (four of 28, 14%) were the most common STs. From 2009 to 2013, among vaccine-eligible children (VEC), incidence of PCV13/not7 ST IPD decreased from 14.8 per 100,000 per year to 1.6 per 100,000 per year (IPD due to all STs decreased from 27.0 per 100,000 per year to 6.7 per 100,000 per year). In 2012/2013, 56% (19 of 34) of PCV13 ST cases occurred in VEC: five were unvaccinated (four ST19A, one ST3); seven (five 19A, two 7F) had received a PCV7 series, but missed PCV13 catch-up; three had incomplete PCV13 vaccination (one received PCV13 at two and four months of age and developed IPD (ST19A) at 11 months of age; two (ST19A, ST3) received only one dose of PCV13 after three doses of PCV7/10). A five-year-old child developed empyema (ST5) after two PCV10 and two PCV13 doses. A two-year-old child developed bacteremia without focus (ST19A) after four PCV13 doses.

**CONCLUSIONS:** Implementation of the PCV13 program was associated with prompt reductions in the rate of PCV13/not7 disease in vaccine eligible children.

## K03

**BABESIA AND HEPATITIS E SEROPREVALENCE IN CANADIAN BLOOD DONORS**

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**OBJECTIVES:** Blood operators maintain vigilance for emerging infectious disease risks to the blood supply. In recent years, the protozoan parasite, *Babesia microti* has been recognised as a significant cause of transfusion transmitted disease in the northeastern United States. Hepatitis E (HEV), commonly seen in the developing world, is now known to occur endemically in North America, and has caused more than 10 cases of post-transfusion hepatitis worldwide.

**METHODS:** The target was to recruit a total of 14,000 consenting blood donors (10,000 from Canadian Blood Services (CBS) from Manitoba, Ontario, and Atlantic Canada and 4000 from Héma Québec (HQ). Babesia antibody testing was performed by Indirect Fluorescence Antibody (IFA) assay at Imugen Laboratories, Norwood, MA, USA. The National Microbiology Laboratory (NML) performed hepatitis E polymerase chain reaction (PCR) in pools of 50 on all donors. Hepatitis E antibody testing (IgG) was done on a subset of 4000 donors using the Wantai HEV IgG ELISA. All donors filled out a questionnaire on possible risk factors.

**RESULTS:** CBS: All 10,062 CBS donors have tested Babesia antibody negative. Out of 3071 donors tested for HEV and anti-HEV, there are zero HEV PCR positives, and 95 of 1773 donors tested HEV IgG antibody positive (5.36% seroprevalence). HQ: To date, all 3581 HQ donors tested for

*Babesia* antibody, have been negative and 52 of 750 tested for anti-HEV have been positive (6.1%).

**CONCLUSIONS:** Based on these results, exposure to *Babesia* appears to be rare in Canada, at least in blood donors. Hepatitis E antibody prevalence is consistent with that seen in other limited serosurveys in North America. There is no evidence that these donors carry transmissible virus.

## K04

**A PROSPECTIVE COMPARISON OF ROTAVIRUS AND NOROVIRUS SEVERE GASTROENTERITIS FOLLOWING THE IMPLEMENTATION OF A PUBLICLY FUNDED ROTAVIRUS VACCINE PROGRAM IN QUÉBEC**

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**OBJECTIVE:** Rotavirus (RV) and norovirus (NV) are leading causes of severe pediatric gastroenteritis (GE). We compared severity of RV, NV and GE of unknown etiology (GEUE), following the implementation of a RV vaccination program.

**METHODS:** Prospective, active surveillance for acute GE among children eight weeks to three years of age was initiated at two Quebec emergency departments. Participant demographics, clinical features, and stool specimens were collected; stools were tested for RV (ELISA), NV (PCR), and sapovirus (SV, PCR). Risk and absolute differences (95% CIs) comparing RV and GEUE with NV cases were calculated for several disease severity outcomes using generalized linear regression, with adjustment for age centered at 16 months.

**RESULTS:** From February 2012 to September 2013, 521 patients were successfully recruited, and complete information was available for 408 participants. Of these, 133, 81 and 12 tested positive for RV, NV, and SV, respectively; 182 cases were GEUE. RV activity peaked in May (2012) and April (2013), versus February (2012/2013) for NV. Mean age was 22 months (RV) and 16 months (NV/GEUE). Among NV cases, baseline risk of fever was 44.4% (95% CI 33.6% to 55.2%), 4.7% (95% CI 0.2% to 9.3%) for dehydration and 9.2% (95% CI 2.4% to 16.0%) for hospitalization. Compared with NV cases, RV cases had a risk difference of 35.4% (95% CI 22.4% to 48.4%) for fever, 18.4% (95% CI 9.3% to 27.4%) for dehydration, and 12.5% (95% CI 2.6% to 22.4%) for hospitalization. In a 24 h period, RV patients reported 3.1 (95% CI 1.5 to 4.7) greater diarrhea episodes compared to NV cases (baseline: 5.2 [95% CI 4.0 to 6.4] episodes), but no difference in vomiting. GEUE had 2.1 (95% CI 0.1 to 4.0) less vomiting episodes than NV cases (baseline: 6.8 [95% CI 5.2 to 8.5] episodes), but a 27.9% (95% CI 15.3% to 40.5%) higher risk of fever. **CONCLUSIONS:** RV cases were clinically more severe than NV cases; severity of NV and GEUE cases were similar.

## K05

**HOSPITALIZATION DUE TO RESPIRATORY SYNCYTIAL VIRUS (RSV) INFECTION IN IMMUNOSUPPRESSED ADULTS: A RETROSPECTIVE COHORT STUDY**

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**OBJECTIVES:** To evaluate characteristics and outcomes in immunosuppressed patients (ISP) hospitalized due to RSV infection.

**METHODS:** We conducted a retrospective cohort study of adults admitted to four hospitals between September 2012 and June 2013 with RSV infection diagnosed by polymerase chain reaction. Ninety-five patients were identified; nine were excluded: six with nosocomial infection and three whose infection was not the cause of their admission. Main outcomes were length of stay (LOS), need for intensive care (ICU) or mechanical ventilation (MV), and all-cause mortality. Chi-square and Fisher's exact test were used for analysis.

**RESULTS:** Twenty-seven of 86 (31%) hospitalized patients were ISP; 15% due to disease alone, 59% due to therapy and 26% due to both. Median age was 62 years among ISP versus 81 years among immunocompetent patients

(ICP). There was no difference in the rate of chronic lung disease or smoking, but ISP were less likely to have cardiac disease (33% versus 61%;  $P=0.02$ ). There was no difference in the rates of most common symptoms/signs (cough, shortness of breath, sputum production, fever, weakness, wheezing). Among ISP, mean LOS was 10.2 days, 11% required ICU care and 7% MV; 4% died. Cardiovascular complications occurred in one ISP, versus 20% ICP. Bacterial co- or super-infections were present in 26% of ISP versus 10% of ICP ( $P=0.06$ ). Ninety-six percent of ISP versus 70% of ICP received antibiotics ( $P=0.01$ ); 30% of ISP received ribavirin.

**CONCLUSIONS:** RSV infection is associated with extended hospital stay, ICU care and mortality in ISP. Presenting signs and symptoms are nonspecific. ISP hospitalized for RSV tended to be younger with less cardiac comorbidity, may be more likely to have bacterial co- or super-infection, and were more likely to receive antibiotics than ICP.

## 1110-1225 Oral Presentations: Session L Room: Convention Centre (Saanich 1 & 2)

### L01

#### DETECTION OF NON-O157 *ESCHERICHIA COLI* IN SASKATCHEWAN USING CHROMOGENIC CULTURE MEDIUM AND PCR SCREENING

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**OBJECTIVE:** Non-O157 *Escherichia coli* infections are difficult to identify using current culture approaches. In this study, a chromogenic culture medium for non-O157 *E coli* was evaluated.

**METHODS:** All stools received for bacterial culture at the Saskatchewan Disease Control Laboratory during a six month period (June 1 to November 30, 2013) were cultured on a chromogenic selective medium (STEC agar, Alere Canada) in addition to the normal range of culture media. Pure cultures of all suspected STEC colonies were identified using standard methods and characterized by PCR (for *stx1*, *stx2* and *eae*) and serotyping.

**RESULTS:** A total of 1151 stool specimens were cultured. *E coli* O157 was recovered from two specimens. Two hundred suspect STEC colonies were picked, of which eight colonies (from seven patients) were *stx* and *eae* PCR positive. The serotypes isolated were O103:H25, O26:H11 (3), O111:NM, O145:NM, O69:H11. One of the *stx*-positive isolates (O69:H11) was not agglutinated by a polyclonal 'big six' antiserum. All isolates were *stx1* positive; one isolate (O111:NM) was also positive for *stx2*. All isolates produced unique PFGE profiles. The available epidemiological data supported the conclusion that these were sporadic cases. Of the colonies agglutinated by the polyclonal antiserum, 22 were *stx*-negative. These isolates belonged to 18 different serotypes that were not included in the polyclonal antiserum.

**CONCLUSIONS:** Non-O157 STEC were more commonly isolated than were O157 strains. Stool culture using STEC medium led to the isolation and screening of many suspect colonies, which generated significant extra workload. Use of a polyclonal 'big six' antiserum generated false-positive agglutinations and did not detect all STEC. Screening of suspect colonies by *stx* PCR identified non-O157 STEC infections in seven patients.

### L02

#### POOLED NUCLEIC ACID AMPLIFICATION TEST FOR SCREENING OF STOOL SPECIMENS FOR SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI*

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**OBJECTIVES:** Shiga toxin-producing *Escherichia coli* (STEC) is a significant cause of enteric illness worldwide. Reported cases of STEC likely

underestimate the true burden owing to diagnostic methods that under-detect non-O157 STEC. The current CDC recommendation for STEC identification is to screen all stools submitted for enteric pathogen detection for Shiga toxins. We evaluated the feasibility of a pooled nucleic acid amplification test (NAAT) as an approach for large-scale STEC screening of stool specimens.

**METHODS:** Following specimen enrichment in BHI broth and nucleic acid extraction, a duplex real-time PCR was used to detect *stx1* and *stx2* from randomly chosen, previously resolved stool specimens. Pooling was performed before or after specimen enrichment with a single positive and nine negative stools ( $n=30$ ) and evaluated for sensitivity and accuracy, the latter by change in cycle threshold ( $\Delta Ct$ ).

**RESULTS:** The sensitivity of stool specimen pooled NAAT was 80%, with  $\Delta Ct$  ( $\pm SE$ ) of  $7.04 \pm 1.01$  and  $5.95 \pm 1.00$  for *stx1* and *stx2*, respectively. Inhibition was not correlated with original positive specimen Ct. The sensitivity of enriched specimen pooled NAAT was 100%, with  $\Delta Ct$  of  $2.93 \pm 0.51$  and  $3.19 \pm 0.32$  for *stx1* and *stx2*, respectively. Ct ranges of positives used for stool and enriched sample pools were 14.88 to 38.58 and 12.83 to 34.92.

**CONCLUSIONS:** Two pooled NAAT approaches were evaluated for use in large-scale STEC screening of stool samples. While pooling of pre-enriched specimens resulted in a loss of test sensitivity and large Ct shifts, all positive samples were detected with minimal Ct changes when enriched samples were pooled. NAAT pooling of enriched specimens is proposed as a solution for STEC screening as it simplifies pool resolution, workflow, and is cost-efficient.

### L03

#### HEAT-RESISTANT SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC) – A NEW EMERGING PATHOGEN?

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**BACKGROUND:** In 2012, a STEC O157 outbreak occurred in Alberta and contaminated, undercooked, tenderized steak was suspected as the source. The objectives of this study were to screen for the presence of heat-resistant STEC and study the effect of NaCl on STEC.

**METHODS:** A total of 178 STEC O157 (human) and non-O157 (human and cattle) isolates were screened for heat resistance. Cultures were grown in Luria Broth (LB) in the presence of 1%, 2% and 4% NaCl; heat-treated at 60°C for 15 min and 30 min in a thermocycler/water bath and subsequently plated onto LB/sheep blood agar plates to determine the cell viability. Survival curves of selected heat-resistant strains were determined.

**RESULTS:** None of clinical isolates survived the heat treatment when grown in 1% NaCl LB as compared with 21% of the cattle isolates. After 15 min in 2% NaCl LB, the survival rate was at 51% for the O157 human isolates and 33% and 37% for the non-O157 STEC isolates from human and cattle. Following 30 min of heat treatment, the survival decreased to 14%, 16% and 11% respectively. When 4% NaCl LB was used, the clinical O157 and non-O157 STEC have a survival rate of 25% after 15 min but the data remained unchanged for the cattle isolates (37%). After 30 min of exposure to heat, the survival rate of O157, clinical and cattle non-O157 STEC dropped to 18%, 4% and 5% respectively. The survival curves further support this observation. Reproducibility results were obtained when experiments were conducted using the water bath.

**CONCLUSION:** Heat-resistant STEC were found to survive up to 30 min at 60°C, more so at 2% NaCl LB than 1% and 4%. Circulating water baths provide even heating and larger volume of cell suspension for heat-resistant testing and thus result in more consistent data.

## L04

**TWO-STEP IDENTIFICATION OF SHIGA-TOXIGENIC *ESCHERICHIA COLI* (STEC) WITH CHROMOGENIC MEDIA (CGM) AND ENZYME IMMUNOASSAY (EIA) FROM HUMAN STOOL SPECIMENS****K Tan<sup>1</sup>, B Walker<sup>1</sup>, L Hoang<sup>2</sup>, P Kibsey<sup>1</sup>**<sup>1</sup>Island Health, Victoria; <sup>2</sup>BC Centre for Disease Control Public Health and Reference Laboratory, Vancouver, BC

**INTRODUCTION:** More efficient and effective methods to detect STEC infections are needed. PCR-based methods are sensitive and specific. However, such assays are variably performed due to technical or cost limitations. CGM and EIA for STEC have recently been introduced, and each has its limitations. We evaluated a two-step approach using CGM followed by confirmatory EIA.

**METHODS:** Stool specimens were inoculated on Colorex STEC CGM (CHROMagar, Paris, France) as part of the routine workup for enteric pathogens. PCR for Shiga-toxin genes (*stx1* and *stx2*) were performed on the same specimens if: CGM was positive; the specimen was suggestive of bloody diarrhea; or on physician request. STEC isolates were recovered and serotyped, and subsequently, Shiga Toxin Quik Chek EIA (TechLab, Blacksburg, VA, USA) was performed. CGM and EIA results were compared against PCR and serotype results.

**RESULTS:** Over a 16-month period, 9635 specimens were processed by CGM. PCR was positive for 51 individual patient samples, of which 69% were non-O157 serotypes. CGM was evaluated on the first 3349 specimens, and found to have a sensitivity of 94.1%, specificity of 97.6% and positive-predictive value (PPV) of 20.5%. EIA was performed on 175 CGM-positive isolates, of which 44 were PCR-positive. On these, EIA sensitivity was 95.5%, and PPV was 100%. Together, the CGM-EIA sensitivity and specificity were 89.8% and 100% respectively.

**CONCLUSION:** While the implementation of CGM improved our detection of non-O157 STEC, it increased the number of STEC PCR performed by three- to fourfold due to the high false-positivity rate of CGM. Adding STEC EIA as a confirmatory step overcomes this limitation. This two-step method is a promising approach.

## L05

**SHIGA-TOXIN PRODUCING *ESCHERICHIA COLI* (STEC) INFECTIONS IN THE PROVINCE OF QUÉBEC: RESULTS OF 13 YEARS OF SURVEILLANCE PROGRAM****S Bekal<sup>1</sup>, F Doualla-Bell<sup>1</sup>, C Gaulin<sup>2</sup>, G Gravel<sup>3</sup>, C Tremblay<sup>1</sup>**<sup>1</sup>Laboratoire de santé publique du Québec, Sainte-Anne-De-Bellevue; <sup>2</sup>Ministère de la santé et des services sociaux, Québec;<sup>3</sup>Ministère de la santé et des services sociaux, Montréal, QC

**OBJECTIVES:** Shiga toxin producing *Escherichia coli* (STEC) is an important cause of serious human gastrointestinal disease which may lead to complications such as hemolytic-uremic syndrome (HUS). We describe the characteristics and trends of STEC infections in Québec from 2000 to 2013.

**METHODS:** Data on serotypes, PFGE and clusters obtained from the provincial surveillance programme as well as epidemiological data were examined. Thrombotic thrombocytopenic purpura (TTP) and HUS data were obtained from the Quebec reportable system.

**RESULTS:** Over the past 13 years, 96% of the 2145 O157 STEC infections notified to the public health notification system of Quebec were confirmed at the Quebec public health reference laboratory. O157 STEC infections decreased dramatically over time, from 422 reported cases in 2000 to 61 cases in 2013. Forty-one percent of all STEC infections of the province affected the population aged between 18 to 64 years old. Children  $\leq 5$  years of age and elderly population ( $\geq 65$  years of age) presented with the lowest incidence rate. Numerous clusters were observed during the period between 2000 and 2004. While we observed a decrease in their duration and size since 2007, we also assisted to a net decrease in sporadic cases' incidence from 0.91 cases per 100,000 per year in 2007 to 0.48 cases per 100,000 per year in 2013. Intriguingly, the rate of HUS and TTP reported is rising since 2009.

**CONCLUSIONS:** The integration efforts of (i) active surveillance and cluster investigations developed over the years in Quebec and Canada (ii) improvement of food control by industry (iii) food safety education for

consumers may have contribute in the overall O157 STEC infection incidence decrease since 2000. However, other non-O157 STEC serotypes may be responsible for the HUS and TTP increases and should be diagnosed to get a complete portrait of STEC infections in Québec.

## POSTER PRESENTATIONS

Poster Presentations:  
Thursday April 3 and Friday April 4  
Room: Convention Centre (Carson Hall)

Poster Viewing:  
Thursday April 3, at 1100-1300  
Friday April 4, at 1100-1300

Presenters at Posters:  
Thursday April 3, at 1100-1230  
Friday April 4, at 1100-1230

## Student Poster Presentations (SP01-SP45)

## Poster Presentations (P01-P59)

## P01

**COMPARISON BETWEEN INTRATHECAL ANTIBODY PRODUCTION AGAINST NEUROTROPIC VIRUSES IN PEDIATRIC AND ADULT ONSET MULTIPLE SCLEROSIS****K Haratian, P Fallah**

Faculty of Medicine, Alborz University of Medical Sciences, Karaj, Alborz, Iran

**INTRODUCTION:** Multiple sclerosis (MS) is an inflammatory demyelinating disease of central nervous system (CNS) that is thought to be caused by a combination of genetic and environmental factors. Now, considerable evidence has associated Epstein-Barr virus (EBV) infection with disease development. The objective of the study was to assess the frequency and intensity of intrathecal antibody production against EBV as compared to other neurotropic viruses in pediatric and adult onset MS.

**METHOD:** In a cohort of 25 childhoods, 35 adult onset MS patient, 15 children and 12 adult with other CNS disorders, paired CSF and serum samples were studied. Frequency and intensity of intrathecal antibody production against EBV as compared to measles, rubella, varicella zoster (VZV) and herpes simplex virus (HSV) were analyzed by determination of virus-specific CSF-to-serum antibody indices (AI).

**RESULTS:** Intrathecally synthesized EBV antibodies were detectable in 26% pediatric and 10% adult onset MS patients, compared to frequencies ranging in both groups from 10 to 53 %.median AIs for EBV were lower than those for all other viruses, with more than twofold higher median AI for measles, rubella and VZV .

**CONCLUSION:** Our results do not rule out the possibility that EBV is involved in the pathogenesis of MS by triggering diverse cellular immune mechanisms, but they argue against a direct pathogenic role of EBV-targeted humoral immune response within the CNS.

## P02

**COMBINATION USE OF ANTIVIRAL AND ANTI-INFLAMMATORY AGENTS AGAINST LETHAL INFECTION OF AVIAN INFLUENZA VIRUS H5N1 IN ANIMAL MODEL**

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**OBJECTIVE:** To evaluate whether co-administrations of antiviral and anti-inflammatory agents may improve survivals of influenza virus H5N1 infected animals.

**METHODS:** We challenged BALB/c mice with 1000 LD<sub>50</sub> of influenza A/Vietnam/1194/04. Survival, body weight, histopathology, inflammatory markers, viral loads, T lymphocyte counts and responses to neutralizing antibody were documented in infected mice treated individually or in combinations with zanamvir, celecoxib, gemfibrozil, and mesalazine. In order to imitate the real life scenario, treatment was initiated at 48 h following viral challenge.

**CONCLUSION:** There were significant improvements in survival rate ( $P=0.02$ ), survival time ( $P<0.02$ ) and inflammatory markers ( $P<0.01$ ) in the group treated with a triple combination of zanamvir, celecoxib and mesalazine when compared to zanamvir alone. Zanamvir with or without immunomodulators reduced viral load to a similar extent. Insignificant prolongation of survival was observed when individual agents were used alone. Significantly higher levels of CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes as well as less pulmonary inflammation were also found in the group receiving triple therapy. Zanamvir alone reduced viral load but not inflammation and mortality. The survival benefits of adding celecoxib and mesalazine to zanamvir could be due to their synergistic effects in reducing cytokine dysfunction and preventing apoptosis. Combinations of a neuraminidase inhibitor with these immunomodulators should be considered in randomized controlled treatment trials of patients suffering from H5N1 infection.

## P03

**GENETIC DIVERSITY OF EPSTEIN-BARR VIRUS IN THE SETTING OF TRANSPLANT RELATIVE TO NON-TRANSPLANT SETTINGS: PROOF-OF-PRINCIPLE**

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**INTRODUCTION:** There is growing interest in the role of Epstein-Barr virus (EBV) genetic diversity and clinical outcomes, including susceptibility to post-transplant lymphoproliferative disorder (PTLD).

**OBJECTIVES:** 1) To compare EBV strains obtained from transplant patients versus patients with infectious mononucleosis by sequencing the EBNA1 and EBNA2 genes; 2) To evaluate next generation sequencing (NGS) as a tool to detect EBV DNA within a genomic DNA sample, and evaluate its genetic variation.

**STUDY DESIGN:** Sanger sequencing of EBNA1 and EBNA2 was used to compare single nucleotide variations (SNVs) from samples taken from transplant patients versus those with infectious mononucleosis. For NGS, we sequenced EBV DNA from a healthy EBV-seropositive individual on a HiSeq 2000 instrument. Data were then mapped to the EBV reference genomes (AG876 and B95-8).

**RESULTS:** The number of SNVs for EBNA2 was significantly higher than for EBNA1 within comparable coordinates. For EBNA2 there was median of 8.5 SNV among four transplant samples compared with 4.5 among the mononucleosis samples ( $P=0.02$ ). In addition, for EBNA2 there was a non-statistically significant trend for the grouping of samples for Thymine versus Cytosine alignments, affecting three of four transplant samples versus one of six infectious mononucleosis samples ( $P=0.19$ ). EBNA1 showed little variation between samples. For NGS, we identified 640 and 892 variants at a genome-wide unadjusted  $P$  value of  $5 \times 10^{-8}$  for AG876 and B95-8 genomes, respectively.

**CONCLUSIONS/SUMMARY:** First, we identified differences in SNVs among transplant patients compared with infectious mononucleosis patients. Second, using NGS, one can identify EBV DNA in total genomic DNA from a blood sample and evaluate its genetic variation. These results provide the framework for further characterization of EBV strains and related outcomes in settings such as organ transplantation.

## P04

**DETERMINATION OF THE BIOLOGICAL FORM OF HUMAN CYTOMEGALOVIRUS DNA IN THE PLASMA OF SOLID ORGAN TRANSPLANT RECIPIENTS**

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**OBJECTIVE:** Despite using a new international reference standard for the calibration of CMV DNA measurement, inter-assay result harmonization remains elusive. The biological form of CMV DNA in plasma (virions versus free DNA and fragmented versus unfragmented DNA) is uncertain. We studied CMV DNA biological forms in solid organ transplant recipient (SOTR) plasma and determined the impact of CMV DNA fragmentation on quantitative real-time PCR (CMV-QPCR) results.

**METHODS:** An assay combining DNase I digestion and CMV-QPCR was developed to differentiate CMV free DNA from encapsidated virions in plasma. CMV free DNA was quantified by subtracting virion DNA (after digestion) from total DNA (without digestion). A total of 104 serial CMV-positive plasma samples collected from 20 SOTR were tested. Four SYBR and two Taqman QPCR assays targeting different CMV genes with amplicons of variable length were used to quantify and assess CMV DNA fragmentation in 14 SOTR plasma samples. Assays were validated to ensure similar sensitivity.

**RESULTS:** The optimized DNase I assay degraded  $\geq 99.9\%$  of the CMV DNA in 1:4 diluted plasma. 98.8% to 100% of the CMV DNA was free DNA in all plasma samples regardless of the CMV donor/recipient serostatus. CMV virions were not detected in 88 samples and found in extremely low levels ( $\leq 1.2\%$  virions) in 16 samples. Assays using smaller targets always quantified CMV DNA higher than assays with larger targets, indicating the CMV DNA was fragmented. The log<sub>10</sub> (copies/mL) result difference was 0.52 and 0.57 between two Taqman and two SYBR assays (using 81 bp and 138 bp MIE gene targets) respectively and 1.04 between two SYBR assays (using 70 bp and 585 bp gB gene targets).

**CONCLUSION:** CMV DNA in SOTR plasma is almost exclusively free DNA, not infectious virions, and fragmented. CMV DNA in plasma is likely not infectious; the size of the amplicon may result in quantitative bias and variability in CMV DNA QNAT assay results.

## P05

**HCV CLEARANCE IS ASSOCIATED WITH IMPROVED IMMUNE PHENOTYPE**

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**BACKGROUND:** The immune mechanisms underlying cure of chronic viral infections are incompletely understood. In the case of chronic HCV infection, small studies suggest a role for adaptive immunity in effective viral clearance, however they are confounded by exogenous interferon administration. The objective of this study was to assess the immune response during clearance of a chronic human viral infection during and after all oral antiviral HCV therapy.

**METHODS:** Sixty HCV GT-1 subjects were treated with sofosbuvir and ribavirin for 24 weeks. Comprehensive peripheral blood T cell, B cell, and NK cell immunophenotyping was performed on 34 patients (22 SVR and 12 relapsers) at baseline, day 10, end of treatment (EOT), and week 48 (24 weeks post therapy). HCV-specific immune responses were quantified by IFN- $\gamma$  ELISpot and multiparameter intracellular flow cytometry after stimulation with comprehensive overlapping HCV peptides.

**RESULTS:** All individuals in this substudy achieved viral suppression at the end of therapy. Markers of T cell exhaustion decreased at

## Abstracts

EOT (17.2%±2.4% versus 14.1%±1.65%, P=0.01; 3.22%±0.8% versus 2.1%±0.33%, P=0.008), and were significantly lower than EOT 24 weeks after therapy (8.4%±2.11%, P=0.005; 0.7%±0.3%; P=0.01). NK cells expressing KIR2DL2 or KIR3DL1 were lower at EOT (10.2%±3% versus 3%±3.5%, P=0.01; 3.1%±0.4% versus 2.2%±0.1%; P=0.009, respectively). Exhausted B cell subsets were significantly lower at EOT (13.4%±1.4% versus 7.2%±2%; P=0.009), however, increased after viral recurrence in relapsers (week 48 relapsers 15.9%±3% versus SVR 7.8%±0.8%; P=0.001). At EOT, polyfunctional HCV responsive T cells were more frequent than at baseline (15.1%±4.6% versus 2.1%±2%; P=0.01). 17 of 22 (77%) cured patients had increased HCV specific responses at end of treatment and 24 weeks post treatment, while only two of 12 relapsers had augmented HCV specific responses ( $\chi^2=11.6$ ; P=0.0006).

**CONCLUSIONS:** Inhibition of HCV replication by a direct-acting antiviral regimen is associated with increased HCV specific immunity and improved immunophenotype beyond duration of therapy. Lack of HCV specific response is associated with poor treatment outcome, suggesting that immune responses may play a role in viral clearance.

## P06

### PERFORMANCE OF TWO AVIDITY ASSAYS, BIO-RAD AVIDITY AND SEDIA LAG AVIDITY, TO ESTIMATE RECENT HIV INFECTION IN QUÉBEC

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**OBJECTIVE:** The present study aims to evaluate and calibrate two IgG avidity assays to measure recent HIV infection: Sedia HIV-1 LAg-Avidity EIA (Sedia Biosciences, Portland) and Genetic Systems Bio-Rad avidity assay (Hercules, CA).

**METHODS:** A total of 992 specimens from patients with documented HIV infection were tested. Longitudinal specimens (n=582) obtained from 150 seroconverted patients enrolled in the FRQ-S primo infection cohort were used to determine the recency period (Kaplan-Meier method). A total 420 specimens from individuals with established HIV infection were tested to investigate false recency rate (FRR).

**RESULTS:** Although Biorad exhibits a higher rate of recent infections within four months when compared to Sedia, this rate did not appreciably decline during the first year:

Days post infection	Sedia (cutoff 1.50)		Biorad (cutoff 30%)	
	Recent	Established	Recent	Established
40-124	69 (58%)	49 (42%)	109 (93%)	8 (7%)
40-185	121 (40%)	183 (60%)	260 (86%)	43 (14%)
40-246	141 (32%)	295 (68%)	356 (82%)	76 (18%)
40-856	156 (27%)	426 (73%)	434 (75%)	141 (25%)

When taking into account longitudinal specimens, Sedia average recency period was 168 days (95% CI, cutoff: 1.50) and BioRad, 291 days (95% CI, cutoff: 30%). Using established HIV infection specimens, FRR for Sedia and Bio-Rad was estimated at 6% (26 of 420) and 10% (43 of 420), respectively.

**CONCLUSIONS:** Our data suggest Sedia to be more suitable than Biorad in identifying recently-infected individuals. A testing Algorithm that sequentially uses Bio-Rad followed by Sedia avidity tests will be evaluated to determine the feasibility of its implementation in the Quebec surveillance program.

## P07

### LYME DISEASE IN NOVA SCOTIA: HOW MANY HUMANS HAVE BEEN INFECTED?

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**BACKGROUND:** Over the past 20 years, *Ixodes scapularis* ticks have been identified with increasing frequency in Nova Scotia (NS) and are now identified as endemic in at least six regions. As a result, the number of human cases of Lyme disease (LD) in NS has increased, with most cases occurring in localities where *I scapularis* populations are endemic.

**OBJECTIVE:** To understand the risk of LD for people living throughout NS.

**METHODS:** A seroprevalence survey for antibodies to *Borrelia burgdorferi* was conducted from June to August 2012. To provide a representative provincial estimate, a convenience sample of residual sera submitted for diagnostic testing was used. Serum samples were selected from all regional hospitals in each health district proportional to the population age and sex distribution. Sera were screened using a commercially available EIA (*B burgdorferi* ELISA II, Wampole Laboratories, Princeton, NJ) and positive and equivocal results were sent to the NML for testing using C6 ELISA and Western blot testing. A positive IgG Western blot was considered conclusive evidence of previous infection.

**RESULTS:** A total of 1855 sera were submitted and screened. Of these, 215 (11.6%) were sent to the NML for further testing. Only 17 of these were positive or equivocal on the C6 EIA and only two had indeterminate IgG Western Blots, resulting in a population seroprevalence of 0.11% (95% CI 0.01 to 0.43%). One of the indeterminate results came from an area not known to be endemic for Lyme disease.

**CONCLUSION:** While LD is an emerging infection in NS, the estimated number of Nova Scotians who have evidence of infection is low.

## P08

### EVALUATION OF PAB LYME EIA FOR THE DIAGNOSIS OF BORRELIA BURGDORFERI INFECTION

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**OBJECTIVE:** Lyme disease is a tick-borne infectious disease, which is caused by *Borrelia burgdorferi* and is found in several provinces of Canada. Diagnosis of Lyme disease is based on multiple factors and criteria with serology being the most important and convenient at the present time which is based on a two-tiered method: an EIA followed by an Immunoblot. In this study a newly Health Canada-approved product for Lyme disease screening was evaluated.

**METHODS:** A total of 161 serum specimens were tested using PAB Lyme EIA (Mikrogen) that uses three recombinant proteins, according to manufacturer's instructions on residual sera collected and sent to CPL for purposes of routine diagnosis. Results were compared with our current C6 peptide ELISA kit (Immunitics) as well as those performed by immunoblot (IB) at NML. Panels included: Lyme IgG positive (n=45), Lyme IgG negative/IgM positive (n=40), C6 positive/IgM and IgG negative (n=20), C6 negative (n=40), cross-reaction panel (n=16), precision panel (three sera in triplicate on two separate runs).

**RESULTS:** The major and minor categorical agreements for the first four panels were: 91% and 95%, 100% and 95%, 100% and 95%, 97% and 100%. Only one minor cross-reaction observed with CMV-IgM positive serum on cross-reaction panel. Intra- and inter-run CV% were 7.64% and 2.92%, respectively.

**CONCLUSION:** Overall, this kit has acceptable agreement with C6 peptide ELISA although further detailed investigations into the patients'

history showed that PAB Lyme EIA though being highly specific but suffers from slightly suboptimal sensitivity making this product a less desirable choice for screening purposes.

## P09

### A NOVEL REAL-TIME PCR ASSAY PANEL FOR THE DETECTION OF COMMON RESPIRATORY PATHOGENS IN A CONVENIENT, STRIP-TUBE ARRAY FORMAT

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**OBJECTIVE:** Commercial multiplex assays, built on different chemistries and platforms are widely available for simultaneous detection of multiple pathogens that cause respiratory infections. However, these assays are expensive and often lack sensitivity compared to real time PCR testing. In this study, we compared the performance characteristics of an in-house real time PCR assay panel with a common commercial (Qiagen Resplex) assay.

**METHODS:** Primers and probes for several respiratory pathogens were either selected from the literature or newly designed. Following optimization of individual PCR assays, primer and probe (PP) mixes were prepared in large volume and dispensed into two sets of 8-strip tubes using an automated liquid handling system (Perkin Elmer), and stored at  $-80^{\circ}\text{C}$ . For specimen analysis, PP mixes were thawed; sample extracts were mixed with QuantiTect RT mastermix (Qiagen), and dispensed column-wise into 16 wells of a 96-well plate. PP mixes were then added to the wells using a multichannel pipette, and PCR reactions were performed simultaneously in an ABI7500 instrument.

**RESULTS:** PCR assays included simplex assays for *Streptococcus pneumoniae*, *Bordetella pertussis*, adenovirus, human metapneumovirus, influenza A, parainfluenza 3, respiratory syncytial virus, enterovirus, rhinovirus and bocavirus. Duplex assays included *Mycoplasma pneumoniae/Chlamydia pneumoniae*, Influenza B/Internal control, Parainfluenza 1/2, Coronavirus 229E/OC4, and Coronavirus NL63/HKU1. A total of 583 samples were used for individual validation of all of these assays and most assays had >90% agreement with the reference methods (viral culture, DFA or PCR). For clinical validation, 34 nasopharyngeal wash (NPW) specimens, which were previously tested by Resplex were re-assessed by the newly developed real-time PCR assay panel. As parts of the panel, a total of 612 PCR assays were performed for 18 PCR targets that matches with the Resplex assay. The overall, observed agreement with Resplex assay was ~95% ( $\kappa = 0.713$  [95% CI 0.618 to 0.808]). In total, 73 pathogens were identified in these samples by our in-house PCR panel, which was 55.3% higher than that of Resplex assays. Interestingly, we noted that >90% of pathogens that were undetectable by the Resplex assays had higher  $C_T$  values (>30) by real-time PCR. The cost of the in-house assay is approximately 75% lower than the commercial assay and required less than 10 min of hands-on time per sample, apart from the initial effort required for producing PCR-ready aliquots of PP mixes in PCR strip-tubes.

**CONCLUSION:** Our novel respiratory PCR panel is a convenient, rapid and inexpensive assay that is more sensitive than the Resplex assay for the detection of common respiratory pathogens.

## P10

### VALIDATION OF A PLAQUE REDUCTION NEUTRALIZATION TEST FOR RUBELLA: PERFORMANCE COMPARISON WITH VIRUS-SPECIFIC IGG EIAs

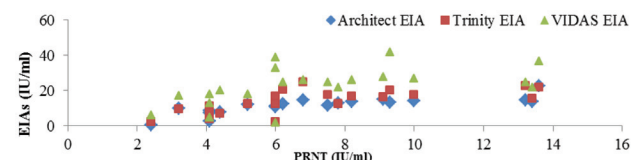
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**OBJECTIVE:** The main objective was to develop and validate a plaque reduction neutralization test (PRNT) for the quantification of rubella antibodies.

**METHODS:** A PRNT was developed by combining two CDC previously described methods. Vero cells and rubella HPV-77 virus were used. An immunoenzymatic staining was performed using two monoclonal antibodies (to capsid and E1 proteins). PRNT results were converted into IU/mL using the Kärber formula. A panel of three controls and 19 specimens was tested with PRNT and three different EIAs.

**RESULTS:** The validated PRNT confirmed the result of the WHO international standard (80 IU/mL). Among 17 PRNT negative specimens, 13 were equivocal and four negative with Architect; 13 were low positive, two equivocal and two negative with Trinity; and 14 were low positive, one equivocal, and two negative with VIDAS. Architect EIA showed the best correlation with PRNT ( $R^2=0.80$ ) followed by Trinity EIA ( $R^2=0.68$ ), then by VIDAS EIA ( $R^2=0.54$ ).



**CONCLUSION(S):** We successfully developed and validated a PRNT for the quantification of rubella antibodies. Contrary to this gold standard method, titres observed using EIAs may have led to misclassification of non-immune individuals.

## P11

### IMMUNE BIOMARKERS PREDICTIVE OF RESPIRATORY VIRAL INFECTION IN ELDERLY NURSING HOME RESIDENTS

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**OBJECTIVE:** To determine if immune phenotypes associated with immunosenescence predicted risk of respiratory viral infection in elderly nursing home residents.

**METHODS:** Elderly residents  $\geq 65$  years from 37 nursing homes in four Canadian cities were enrolled in September 2009, 2010 and 2011, and followed for one influenza season. Immunocompromised residents and those not vaccinated against influenza were excluded. Peripheral blood mononuclear cells were obtained and analysed by flow cytometry for T-regs and CD8 T-cell subsets: naive (CCR7+CD45RA+), terminally differentiated (TD) (CCR7-CD45RA+) and senescent (CD28-CD57+). Nasopharyngeal (NP) swabs were obtained and tested for respiratory viruses in symptomatic residents. Using a Cox proportional hazards model adjusted for age, sex and frailty, we determined the relationship between each immune phenotype and time to viral infection.

**RESULTS:** A total of 1072 residents were enrolled. Median age was 86 years and 72% were female. 87 NP swabs were positive for viruses: influenza (24%), RSV (14%), coronavirus (32%), rhinovirus (17%), human metapneumovirus (9%) and parainfluenza (5%). High TD CD8 T-cells were associated with increased risk of infection (HR 1.59 [95% CI 1.02 to 1.59]); the results with high senescent CD8 T-cells were similar (HR 1.62 [95% CI 1.03 to 2.54]). Low naive CD8 T-cells were not associated with increased risk of infection (HR 0.71 [95% CI 0.42 to 1.22]). High T-regs were associated with reduced risk of infection (HR 0.49 [95% CI 0.27 to 0.90]).

**CONCLUSIONS:** In elderly nursing home residents, high CD8 TD and senescent T-cells were predictive of increased risk of respiratory viral infection. High T-regs were predictive of reduced risk during the ensuing influenza viral season.



P12

WITHDRAWN

P13

### VALIDATION OF HOLOGIC GEN-PROBE APTIMA COMBO 2 ASSAY FOR *CHLAMYDIA TRACHOMATIS* IN EYE SPECIMENS

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**OBJECTIVE:** Nucleic Acid Amplification Test (NAAT) for *Chlamydia trachomatis* (CT) has been shown in many clinical studies to have higher sensitivity and specificity than non-amplification methods, but is not Health Canada-approved for non-genital specimens. In this study, we compare the APTIMA NAAT assay for CT in eye specimens against our current method, direct fluorescent antibody (DFA) staining.

**METHODS:** Eye specimens submitted to BCCDC Bacteriology and Mycology laboratory between June 2008 and December 2013 were tested by both NAAT and DFA methods. Gen-Probe APTIMA Combo 2 Assay was used as the NAAT assay and MicroTrak CT Direct Specimen Test from Trinity Biotech was used for DFA examination. A positive NAAT result was confirmed using the APTIMA CT Assay. Confirmed positive results were followed up for clinical correlation.

**RESULTS:** A total of 283 eye specimens were received and analyzed. Eighty-six percent (244 of 283) were CT negative and 6% (17 of 283) were CT positive by DFA. Two percent (five of 283) were reported as inconclusive and 6% (17 of 283) had insufficient material on the DFA slides. For NAAT, ninety-three percent (263 of 283) were CT negative and 7% (20 of 283) were CT positive. Sensitivity of CT NAAT is 100% (17 of 17) with reference to DFA method. Three samples were found to be CT positive by NAAT with confirmation and CT negative by DFA. Follow-up of these patients confirmed consistent clinical findings.

**CONCLUSION:** Fifteen percent (three of 20) of the CT positives were missed using the DFA detection technique. APTIMA Combo 2 Assay with confirmation demonstrated a more sensitive and specific performance than the DFA protocol for eye specimens.

P14

### DETECTION OF CIRCULATING RESPIRATORY VIRUSES IN RIYADH USING A MULTIPLEX REAL-TIME PCR

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Respiratory viruses are very common in causing severe respiratory illnesses with a significant mortality and morbidity annually. Detection of these viruses is very critical for patient management and infection control. To date, many viral infections are unreported or misinterpreted, and thus very little information is available about single and multiple respiratory viral infections in Riyadh. Here we investigated the most circulating respiratory viruses which cause severe illnesses and admissions in a tertiary hospital during the winter season of 2013-2014 by a multiplex molecular method. Nasopharyngeal aspirate or Nasal swab samples from patients admitted to the hospital with severe respiratory illnesses were screened for viral infections using the anplex™ II RV16 Detection kit (Seegene) and real-time PCR instrument. A total of 73 samples were tested for respiratory viral infections, and we found that 59 (80.8%) samples were positive and 14 (19.2%) samples were negative. Among the positives 42 (71.2%) cases were admitted due to a single viral infection, whereas 17 (28.8%) cases were hospitalized due to multiple viral infections. Interestingly, infection with rhinovirus was the highest infection with 34 (57.6%) samples positive followed by influenza A, respiratory syncytial virus B, and enterovirus infections with 10 (16.9%) samples positive, 10 (16.9%) samples positive, and nine (15.3%) samples positive, respectively. Further, rhinovirus was the most predominant virus detected as single infection or multiple infections. In conclusion, most of the admitted cases were due to a single respiratory viral infection, and the rhinovirus was the most frequent virus causing severe illness during the study period.

P15

### MOLECULAR DETECTION OF *PNEUMOCYSTIS JIROVECI* BY REAL-TIME PCR

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**BACKGROUND:** *Pneumocystis jirovecii* (PJ) is an opportunistic pathogen causing pneumonia with a significant morbidity and mortality among immunocompromised patients. Real-Time PCR is known to be more sensitive than DFA and two main gene targets have been used for PCR detection. However, the increased sensitivity of PCR can detect colonization in patients that do not have clinical disease. The present study examined sensitivity and limit of detection (LOD) for mitochondrial large subunit (mtLSU) rDNA and Cyclin-dependant Kinase (CDC-2) gene targets using real-time PCR and compared to patient risk factors and clinical presentation.

**METHODS:** One-hundred and forty three consecutive bronchoalveolar lavage (BAL) specimens sent for routine testing for PJ by DFA were used for PCR. DNA from 1 mL of BAL was extracted and purified using EasyMag (BioMereux) after pretreatment with lyticase. Five microliter of eluate was used as the PCR template. The PCR targets were the mitochondrial large subunit (mtLSU) rDNA and Cyclin-Dependant Kinase (CDC-2). The lambda DNA was used as an internal control (IC). Primers and TaqMan probes were synthesized by BioSearch Technologies. Genes were amplified and detected using the Qiagen QuantiTect Multiplex PCR kit containing respective primers and TaqMan probes in a Rotorgene 6500 (Qiagen). Sensitivity of PCR and lower limit of detection (LOD) was determined by using a serial dilution of known concentration of target template and by calculating the copy number. A retrospective chart review was performed for patients with discordant results between the two PCR targets and/or DFA.

**RESULTS:** Of 143 specimens tested, 125 were negative for PJ by PCR. Fourteen specimens were positive by both PCR methods. Four specimens were negative by CDC-2 target were positive by mtLSU rDNA and had Ct values >36. Only four specimens were DFA positive. Four DFA positive specimens had mtLSU rDNA Ct values between 12 and 27. The CDC-2 method detected 100 copies of the gene whereas mtLSU rDNA detected 10 copies. The nine patients that were positive by mtLSU and CDC-2 but negative by DFA had the following characteristics: 78% had multiple risk factors for PCP, 67% had bilateral reticular disease and 34% were started empirically on PCP treatment. In contrast, the four patients that were positive by mtLSU only, only 25% had risk factors for PCP and 50% did not have respiratory symptoms

**CONCLUSION:** Sensitivity of detection of PJ by real-time PCR depends on the selection of the amplification target. The detection by mtLSU rDNA target is ten times more sensitive than the CDC-2 target; however, appears to be less specific. Patients negative by DFA but positive by CDC-2, may reflect disease or colonization in high risk patients. Further elucidation to differentiate colonization and disease is required.

P16

### MULTIPLEX REAL-TIME PCR DETECTION OF HERPES SIMPLEX VIRUSES 1 AND 2, VARICELLA ZOSTER VIRUS AND HUMAN BETA-GLOBIN

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**OBJECTIVE:** In 2009, the BC Public Health Microbiology & Reference Laboratory (PHMRL) switched from cell culture to nucleic acid extraction and detection of herpes simplex viruses (HSV) 1 and 2 and varicella zoster virus (VZV) in a two-tube duplex qPCR. This resulted in decreased turnaround time (TAT) and labour and consumables costs and increased HSV-1, HSV-2 and VZV detection sensitivities by 11%, 9% and 7.5%, respectively. In 2012, to further improve cost and workflow efficiencies, all targets were multiplexed into a 4-plex qPCR reaction for simultaneous

detection of HSV-1, HSV-2, VZV and  $\beta$ -globin (a sample quality marker and endogenous internal positive control for PCR inhibition).

**METHODS:** After optimizing the 4-plex qPCR assay and confirming that constituent reactions were as efficient as those in duplex, 422 clinical samples were blindly tested in parallel by duplex and 4-plex qPCR.

**RESULTS:** The 4-plex qPCR maintained the duplex qPCR's one-day TAT and specificity (with equal or slightly increased sensitivity) and further saved time, labour, consumables and reagents. Although DNA extraction costs were identical for both assays, the estimated per-sample PCR cost (based on current reagent and consumables pricing) decreased from \$1.63 to \$0.90. Compared to the duplex assay, 4-plex qPCR decreased repeat testing of viral target-negative samples by more sensitively detecting  $\beta$ -globin.

**CONCLUSIONS:** The 4-plex qPCR assay is at least as sensitive as the two-tube duplex and, at estimated test volumes of 30,000 samples per year, would decrease PHSA's herpes and VZV testing costs by at least \$22,000 annually.

### P17 DOES THE BD VIPER HSV-QX ASSAY REQUIRE A GREY ZONE?

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**OBJECTIVE:** The Viper HSV-Qx assay was recently approved for the detection and differentiation of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2); however, approval was limited to swabs from anogenital sites. Using swabs from various anatomical sites, this study compared the performance of HSV-Qx to a commercial real-time PCR on the LightCycler instrument (HSV-LC).

**METHODS:** 276 swabs (115 anogenital, 91 oral and 70 miscellaneous) were evaluated by HSV-Qx and HSV-LC, and discordant results were resolved using a third commercial molecular method. For HSV-Qx, the maximum relative fluorescence units (MaxRFU) distribution for each target was evaluated to ensure the recommended cutoff for positivity ( $\geq 125$ ) was appropriate.

**RESULTS:** HSV-Qx demonstrated a slight reduction in specificity for HSV-1 at 98.6% compared to 100% for HSV-2 or both targets with HSV-LC. For HSV-1 and HSV-2, the sensitivity of HSV-Qx (98.6% and 100%) was greater than HSV-LC (94.6% and 97.1%). When MaxRFU values were analyzed for HSV-Qx, 99.3% of the HSV-2 results could be classified as either negative with values between 0 and 49 or as positive with values  $>800$ . For HSV-1, only 93.1% of results fell within these two categories, and a larger number of results ( $n=19$ ) fell between 50 and  $<800$ . Of note, MaxRFU did not correlate with viral loads and signal reproducibility was poor (inter-experimental % CVs ranging from 25% to 173% for HSV-1 and seven to 117 for HSV-2).

**CONCLUSIONS:** This study demonstrated that the Viper HSV-Qx assay is fairly accurate for detection and differentiation of HSV from a variety of swabs; however, a 'grey zone' may be required for results falling between 125 and 800 MaxRFU.

### P18 RAPID DETECTION OF ROTAVIRUS IN FECAL SPECIMENS BY PCR DURING ACUTE GASTROENTERITIS AMONG CHILDREN YOUNGER THAN SIX YEARS OF AGE

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**INTRODUCTION AND OBJECTIVES:** Human rotavirus infections are considered as main cause of acute viral gastroenteritis, occurring primarily among children  $<2$  years of age. This study illustrates the development of real-time PCR assays for the detection of rotavirus A, and rotavirus C from stool specimens.

**METHODS:** Two hundred stool samples from pediatric patients exhibiting symptoms of diarrhea and/or vomiting were examined. PCR results were compared with those of virus detection by electron microscopy and latex agglutination antigen detection. The incorporation of an internal-control RNA that was spiked into individual stool extracts functioned as an internal validation for the reporting of PCR-negative results.

**RESULTS:** Rotavirus C was not detected by real-time PCR in the patient stool samples examined. Real-time reverse transcription-PCR resulted in 111% increases in the rates of detection of rotavirus A, compared with latex agglutination testing. Sequencing of a proportion of the rotavirus strains identified only genotype G1 rotavirus in circulation within the patient cohort examined.

**CONCLUSION:** The results highlight the significance of rapid molecular methods for the routine screening of stool samples in hospital laboratories to provide rapid definitive diagnoses.

### P19 TYPE DISTRIBUTION OF HUMAN PAPILLOMAVIRUSES IN NON-FAMILIAL BREAST CANCER PATIENTS IN IRAN

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**BACKGROUND:** Our ex-clinical findings have verified that human papillomavirus infection may have an association with non-familial breast cancer development. This study directed to find a better understanding of human papillomavirus (HPV) type distribution in some urban areas of Iran.

**METHODS:** Archival Mammary paraffin-embedded tissue specimens and axillary lymph nodes of 68 confirmed breast cancer patients were utilized in this retrospective study. This project was based on the results of high sensitive Multiplex polymerase chain reaction (Multiplex-PCR) using the specific primers covering different low, intermediate and high-risk types and southern hybridization for the detection of HPV DNA in non-familial breast cancer patients.

**RESULTS:** Twenty-eight of 68 samples tested positive for HPV DNA (41.2%). Among these 28 samples, 18, eight and just two were infected by high-, potentially high- and low-risk HPV respectively. The most coverable HPV genotypes in this round were 11, 16, 18, 31, 33, 35, 45, 51, 52, 58 and 70. Occurrence of single and multiple infections was about 26 (93%) and two (7%) respectively. The patients who were infected with high-risk HPV were younger than the rest and their histopathological results showed more proliferative as observed by the specific Ki-67 staining and S-phase/proliferative fractions. Genotypes 16 and 18 were the most HPV types (23 of 28) recognized in cases with single infection (82%).

**CONCLUSION:** According to the results, HPV's specially types 16 and 18, make an acceptable role in malignancy development such as breast cancer. These data suggest that using HPV vaccine prepared against mentioned types of virus may accelerate the prevention or decrease of breast cancer rate in Iran.

### P20 COMPARISON OF NUCLEIC ACID EXTRACTION FROM STOOL SAMPLES AMONG FOUR AUTOEXTRACTORS AND THEIR PERFORMANCE FOR DETECTION OF GASTROENTERITIS VIRUSES USING REAL-TIME RT-PCR

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**OBJECTIVES:** The major challenge for detection of viral nucleic acid (NA) in stool is the presence of inhibitory substances, which could suppress NA detection and cause false negative results. We compared four commercial NA autoextractors and reagents, and investigated their performance using a real time RT-PCR gastroenteritis virus panel (PCR-GIVp). **METHODS:** Archived clinical stool samples ( $n=200$ ) were used for NA extraction with EasyMag/NucliSens, KingFisher mL/MagaZorb, Arrow/Stool DNA, and KingFisher Flex/ExtraStar. Five enteric viruses including

## Abstracts

norovirus (NoV), rotavirus (RV), adenovirus (AdV), sapovirus (SaV) and astrovirus (AsV) were assayed using PCR-GIVp. Sensitivity and precision were assessed with positive samples. Salmon DNA was used as an internal control to measure inhibition.

**RESULTS:** Salmon DNA was detected in all negative samples, indicating no inhibition occurred. Precision and sensitivity were comparable in all four methods. Of the 200 specimens, 90% were concordant including NoV (n=32), RV (n=26), AdV (n=31), AsV (n=18), SaV (n=10), mixed viruses (n=4) and negative (n=60). Total discordant results were detected in 10% (19 of 200) of the samples, and approximate half had a high ct (>35, low viral load). The EasyMag and KingFisher Flex systems resulted in SaV being detected in 50% fewer samples compared to the KingFisher mL and Arrow, accounting for half of the discordant results.

**CONCLUSIONS:** The PCR-GIVp results showed no obvious difference among the four methods, except with SaV. As the instruments have different capacities, processing times, and reagent costs, instrument selection depends on the individual laboratory's testing volume, workflow and budget.

### P21

#### COMPARISON OF TWO MOLECULAR TYPING METHODS FOR *STREPTOCOCCUS PNEUMONIAE*

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**OBJECTIVE:** Two molecular methods for the typing of *S pneumoniae* (Spn) have been developed by the Centers for Disease Control and Prevention (CDC) based on multiplexed conventional PCRs (MP-PCR) or triplex real-time PCRs (TP-PCR). To date, no studies have compared these two molecular typing methods.

**METHODS:** The performance of MP-PCR and TP-PCR were verified using previously characterized Spn isolates (n=45) and a variety of other organisms. The limit of detection (LoD) for each assay was determined using dilutions of quantified Spn. For clinical evaluation, 1770 nasopharyngeal (NP) swabs were obtained from patients with or without invasive pneumococcal disease (IPD) that were screened for Spn using two real-time PCRs targeting *lytA* and *cpsA*. Typing was performed on specimens that were *lytA*- and *cpsA*-positive (considered Spn-positive).

**RESULTS:** MP-PCR and TP-PCR were highly specific, but their LoDs (~6700 copies/mL and ~4800 copies/mL, respectively) were less sensitive than the screening PCR assays (*lytA* ~950 copies/mL; *cpsA* ~550 copies/mL). Of the 132 NP swabs identified as Spn-positive, 15 were assigned a type by MP-PCR alone since it has the ability to identify a larger number of different Spn types compared to TP-PCR. However, TP-PCR identified an additional 19 types in specimens that had low bacterial loads due to its increased sensitivity. Using an algorithm-based approach with both typing methods, a type could be assigned for 69 (52.3%) Spn-positive specimens. A large proportion of Spn-positive specimens remained untypeable: 33 had DNA concentrations that fell below the LoD for both assays and 30 displayed strong screening results suggesting types that were not included in either typing method.

**CONCLUSIONS:** While the molecular methods for typing of Spn are valuable tools for epidemiologic studies, further optimization is required to achieve sensitivities equivalent to molecular detection methods and typing methods should be expanded to identify and discriminate between all Spn types.

### P22

#### ANTIMICROBIAL SUSCEPTIBILITIES AND MULTI-ANTIGEN SEQUENCE TYPES (NG-MAST) OF *NEISSERIA GONORRHOEAE* ISOLATED IN CANADA, 2008-2012

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**OBJECTIVE:** *Neisseria gonorrhoeae* have acquired resistance to many antimicrobials and developed decreased susceptibility to third generation cephalosporins limiting treatment options for physicians. In this report, we describe the antimicrobial resistance profiles and molecular types circulating in Canada over the previous five years.

**METHODS:** *N gonorrhoeae* that were resistant to at least one antimicrobial were submitted to the NML from the provincial public health laboratories between 2008 and 2012. Denominator data for calculation of percent resistance was collected from the provinces. Antimicrobial susceptibility was performed to eight antimicrobials using agar dilution. NG-MAST sequence typing was performed on all isolates from 2010-2012.

**RESULTS:** A total of 5282 *N gonorrhoeae* were submitted to the NML between 2008 and 2012. Decreased susceptibility to cefixime (minimum inhibitory concentrations, MIC  $\geq 0.25$  mg/L) was identified in 0.5% (18 of 3907) of isolates in 2008, increasing to 4.2% (140 of 3360) in 2011; and then decreasing to 2.2% (68 of 3036) in 2012. Decreased susceptibility to ceftriaxone (MIC  $\geq 0.125$  mg/L) was identified in 0.6% (24 of 3907) of isolates in 2008, increasing to 7.3% (218 of 2970) in 2010; and then decreasing to 5.5% (168 of 3036) in 2012. Penicillin resistance during 2012 was detected in 20.3% (615 of 3036) of the isolates; tetracycline in 30.3% (920 of 3036); erythromycin in 23.1% (702 of 3036); ciprofloxacin in 28.5% (866 of 3036) and azithromycin in 0.9% (26 of 3036). A total of 249 sequence types (STs) were identified in 2010 with ST1407, ST3150 and ST3158 being most common, representing 13.3%, 11.3% and 9.0% respectively. In 2011, 238 STs were identified with ST1407, ST3307 and ST3550 being most common at 15.3%, 9.3% and 5.9% respectively. In 2012, 258 STs were identified with ST1407, ST2400 and ST3150 representing 11.1%, 7.3% and 6.6% of the strains, respectively.

**CONCLUSIONS:** Continued surveillance of antimicrobial susceptibilities and sequence types of *N gonorrhoeae* is necessary to inform treatment guidelines and mitigate the impact of antimicrobial resistance in gonorrhoea.

### P23

#### CARBAPENEMASE DETECTION IN CLINICALLY RELEVANT *ENTEROBACTERIACEAE* AND *PSEUDOMONAS AERUGINOSA* BY MALDI-TOF MS

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**OBJECTIVE:** Carbapenemases are an increasingly common mode of resistance for carbapenem-resistant Enterobacteriaceae. The aim of this study was to evaluate the accuracy of matrix assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS) to determine the presence of carbapenemase.

**METHODS:** A blind study, consisting of a diverse mix of clinical isolates of Enterobacteriaceae and *Pseudomonas aeruginosa* that express one of eight different carbapenemases (n=204), and non-carbapenemase-producing isolates that are mostly non-susceptible to carbapenems (n=45), was performed via an assay containing the bacterium and imipenem. The degradation of imipenem was monitored using the Autoflex III MALDI-TOF MS, at 300 m/z, over a four-hour incubation period. The Carba-NP assay, with imipenem, was performed on a subset (n=64) of the isolates, representing all eight types of carbapenemases.

**RESULTS:** MALDI-TOF MS detected imipenem hydrolysis from 99% of the strains exhibiting carbapenemase activity, from all types of carbapenemase (202 of 204). In contrast, Carba-NP assays identified activity from 85% of the carbapenemase-positive isolates (45 of 53), and could not consistently identify activity from OXA-48 or GES-type carbapenemase-producing strains. We detected carbapenemase activity in one *Klebsiella pneumoniae* isolate identified as carbapenemase-negative by PCR, and also negative in the Carba-NP assay.

**CONCLUSIONS:** MALDI-TOF MS can detect carbapenemase activity with high specificity/sensitivity from bacteria harbouring diverse carbapenemases. It has greater sensitivity than the Carba-NP assay, as MALDI-TOF MS can detect the presence of carbapenemases with low hydrolyzing activity (eg, OXA-48, GES-5).

## P24

### USE OF CHROMOGEN SPECIFICITY IN URINE AGARS TO REDUCE MALDI-TOF WORKLOAD ASSOCIATED WITH IDENTIFICATION (ID) OF *ESCHERICHIA COLI*

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**OBJECTIVES:** The development of 'burgundy-pink' (BP) colonies on urine chromogenic agars is claimed by manufacturers to be highly-specific for *E. coli*. To reduce the number of urine isolates requiring MALDI-TOF, this prospective study determined whether observation of BP colonies was sufficient for *E. coli* ID.

**METHODS:** 1 uL from 2500 consecutive urines was inoculated by Copan's WASP to 5% Sheep Blood (BA), MacConkey w/o CV (MAC), Brilliance UTI Clarity (UTI; Oxoid), CPS4 chromID (CPS; bioMérieux) and Colorex Orientation (COR; Alere). Definitions of significant growth (SG), non-SG (NSG) or mixed (MG) cultures were as per clinical laboratory BA/MAC algorithm. Quantities, colours and sizes of all colony types were noted at 16 h to 20 h by persons blinded to one another's results, and ID was by VITEK MS PLUS (VMS, bioMérieux). Analyses included: 1) all BP isolates, and 2) all *E. coli* regardless of colour from SG, NSG and MG cultures.

**RESULTS:** ID was done on 2584 isolates overall. Of these, 814 (31.5%) were BP-coloured (CPS 282, UTI 270, and COR 262); VMS found all 814 BP-coloured isolates to be *E. coli* resulting in a BP-specificity of 100% (95% CI 99.7 to 100) for all chromogenic agars combined. All 282 CPS *E. coli* were BP (CPS BP-sensitivity [Sn] 100% [95% CI 98.4 to 100]), while 10 of 280 (3.6%) UTI *E. coli* were 'cream-translucent' (CR) (UTI BP-Sn: 96.4% [95% CI 93.5% to 98.1%]), and four of 286 (1.4%) COR *E. coli* were CR and 20 (7%) were light-BP (COR BP-Sn 91.6% [95% CI 87.8% to 94.3%]). The proportion of *E. coli* detected as BP on CPS (100%) was significantly higher than on UTI (96.4%; P=0.0009) or COR (91.6%; P<0.0001).

**CONCLUSIONS:** This study demonstrated that observation of BP colour on these urine chromogenic agars was 100% species-specific for *E. coli* and sufficient for its ID.

## P25

### VITEK MS PLUS (VMS) IDENTIFICATION (ID) PERFORMANCE WHEN CHALLENGED WITH MIXED BACTERIA (MB) OR MIXED YEAST (MY) CULTURES

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**OBJECTIVES:** VMS from short incubation subcultures (3 h to 6 h incubation) of positive blood cultures (BC) provides fast ID of BC isolates. As BC may be polymicrobial, VMS accuracy from mixed cultures (MC) was determined.

**METHODS:** A total of 376 MB and 56 MY tests were performed on VMS. 243 MB included a mixture of two isolates and 133 included a mixture of three isolates of different combinations of 24 ATCC strains of commonly isolated species. The 56 MY included mixtures of two isolates using seven ATCC strains of *Candida* spp. (*albicans*, *glabrata*, *krusei*, *guilliermondii*, *lusitaniae*, *parapsilosis*, and *tropicalis*).

**RESULTS:** Thirty-one of 376 MB produced "Bad spectrum during analyses" (BSDA) or No ID results. Of 345 MB with ID, 340 (98.6%; [95% CI 96.6% to 99.5%]) had high-confidence ID, 293 with one ID correct (186 from two-strain MB, 107 from three-strain MB) and 47 with two ID correct (38 from two-strain MB, nine from three-strain MB), while five (1.5% [95% CI 0.5% to 3.5%]) were low-confidence ID that would be rejected. No misidentifications occurred with high confidence. Six of 56 MY produced BSDA or No ID, and of 50 MY with ID, 49 (98% [95% CI 88.5% to 99.9%]) had high-confidence correct ID (39 with one correct ID, 10 with two correct ID), while one (2% [95% CI <0.01% to 11.5%]) MY resulted in an incorrect ID of *Debaryomyces polymorphus* with 98% confidence, a species not typically isolated from humans that would prompt confirmatory testing in most laboratories prior to reporting.

**CONCLUSIONS:** VMS produced reassuring results from MC. 98.6% of MB and 98% of MY with an associated ID from VMS produced correct ID with high confidence. Only 0.2% of all ID were incorrect with high confidence. This suggests that VMS ID from 3 h to 6 h subcultures of positive BC will be highly accurate even with MC.

## P26

### COLORIMETRIC DETECTION OF 3RD GENERATION CEPHALOSPORIN RESISTANCE (3GCR) IN ENTEROBACTERIACEAE (ENT): A RETROSPECTIVE EVALUATION OF THE RAPID BIO-RAD BLACTA TEST (BLT)

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**OBJECTIVES:** This study determined whether faster susceptibility reporting could be achieved using the BLT to detect 3GCR clinical isolates by means of detecting a colour change reflecting hydrolysis of a chromogenic cephalosporin.

**METHODS:** A total of 305 ENT including 35 (11.5%) 3GC-susceptible (S) and 270 (88.5%) 3GCR with diverse chromosomal (chr) and plasmidic (pl) beta-lactamases (BL) [class A extended spectrum (A-ESBL), class C (C-BL), class D (D-ESBL), carbapenemases (CP)] were sub-cultured onto 5% sheep blood agar (Oxoid) and tested by BLT in a blinded fashion. ENT included two *C. freundii*, eight *E. aerogenes*, 44 *E. cloacae*, 89 *E. coli*, 12 *K. oxytoca*, 129 *K. pneumoniae*, six *M. morgani*, five *P. mirabilis*, one *P. stuartii*, four *R. ornithinolytica*, five *S. marcescens*. A yellow to red colour change noted in 15min was considered BLT+. Colour was noted again at 30 min.

**RESULTS:** Of 305 ENT, 225 (80.7%) were BLT+ (red<15 min), 12 (3.9%) were delayed+ (red >15/<30 min), nine (3%) were indeterminate (IND) (orange <15/<30 min), and 59 (19.3%) were negative (neg) (yellow >15/>30 min). IND results were considered neg. All BLT+ and delayed+ ENT were 3GCR due to A-ESBL, C-BL and/or CP, some plasmid-mediated alone or others with hyper-produced chr A- or derepressed chr C-BL. There were no false +. False-neg included 13 ENT with derepressed

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chr C-BL +/- porin changes (nine were IND and four were BLT-neg) and 20 ENT that carried pla-mediated C-BL (11 CMY2, seven DHA1) or D-ESBL (2 OXA1) (all BLT-neg). BLT sensitivity and specificity for detection of 3GCR using a 30 min cut-off was 87.8% (95% CI 83.3% to 91.2%) and 100% (95% CI 88.2% to 100%), respectively.

**CONCLUSIONS:** BLT accurately detects most 3GCR by 15 min, and is further improved by extending the final read-time to 30 min with no associated false positives.

### P27

#### PILOT PROSPECTIVE EVALUATION OF THE BLACTA TEST (BLT) FOR PREDICTING 3<sup>RD</sup> GENERATION CEPHALOSPORIN RESISTANCE (3GCR) IN SHORT-INCUBATION BLOOD CULTURE ISOLATES (SIBCI) OF *ESCHERICHIA COLI* (EC), *KLEBSIELLA PNEUMONIAE* (KP), *KLEBSIELLA OXYTOCA* (KO) AND *PROTEUS MIRABILIS* (PM)

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**OBJECTIVES:** This study evaluated detection of 3GCR in SIBCI incubated for approximately 3 h to 6 h using the BLT where hydrolysis of a chromogenic cephalosporin by 3GCR  $\beta$ -lactamases (BL) produces a yellow-red colour change (BLT+) within 15 min.

**METHODS:** A total of 153 SIBCI were tested by BLT immediately following VMS ID as EC, KP, KO or PM and results were compared to VITEK 2 N213 AST and/or Double Disk Test results. BLT-sensitivities (Sn) and specificities (Sp) for detecting class A extended spectrum BL (A-ESBL) and 3GCR overall were calculated; the impact on 3GCR reporting-time was compared to routine susceptibility testing.

**RESULTS:** A total of 153 BLT were performed on 103 (66.9%) EC, 37 (24%) KP, five (3.3%) KO, eight (5.2%) PR. Twenty-two (14.3%) were BLT+ (red), 131 (85.1%) BLT-negative (yellow), and one (0.7%) BLT-indeterminate (IND) (orange). One hundred percent (95% CI 84.2% to 100%) of the BLT+ were 3GCR [11 A-ESBL EC (seven CTX-M, four TEM/SHV), seven A-ESBL KP (four CTX-M, three TEM/SHV), two A-ESBL KO (two CTX-M), two KO K1/OXY]. The single BLT-IND and four of the 131 (3.1%) BLT-negative isolates were 3GCR due to Class C  $\beta$ -lactamases. Sn and Sp for A-ESBL and 3GCR overall were 100% (95% CI 82.5% to 100%)/98.5% (95% CI 94.4% to 99.9%) and 81.5% (95% CI 62.8% to 92.3%)/100% (95% CI 96.4% to 100%), respectively. Compared with routine testing, 3GCR was reported based on BLT+ results 24 h earlier in 83.3% of cases (P<0.0001). BLT also identified an A-ESBL (TEM/SHV) KP that was otherwise missed due to overgrowth of a 3CG susceptible KP.

**CONCLUSIONS:** This pilot found the BLT to be a rapid and low-complexity method that is highly accurate in its prediction of A-ESBL and 3GCR from SIBCI. Through interim reporting of BLT+ results, patients would have benefited from appropriate therapy for 3GCR 24 h earlier than with routine testing.

### P28

#### EVALUATION OF THE VALUE OF TESTING CIPROFLOXACIN SUSCEPTIBILITY IN *SALMONELLA* SP BY CONCURRENT NALIDIXIC ACID DISK DIFFUSION AND CIPROFLOXACIN DISK DIFFUSION USING THE NEW REVISED CLSI DISK DIFFUSION BREAKPOINTS

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**OBJECTIVE:** To determine if there is any value added to the use of nalidixic acid disk diffusion (DD) together with ciprofloxacin disk diffusion (DD) using the revised CLSI breakpoints for *Salmonella*.

**METHOD:** DD was performed on Mueller Hinton agar with ciprofloxacin and nalidixic acid disks on 101 *Salmonella* isolates representing 31 species. Interpretation was based on revised CLSI breakpoints. Ciprofloxacin Etest was performed on 20 isolates that tested I or R to nalidixic acid and/or ciprofloxacin.

## RESULTS:

# Isolates	CIP DD	NAL DD	CIP Etest
81	S	S	-
2	S	I	S
11	I	R	I

CIP Ciprofloxacin; NAL Nalidixic acid; DD Disk diffusion

**CONCLUSION:** Ciprofloxacin disk diffusion using revised CLSI breakpoints detected 18 *Salmonella* species with reduced susceptibility. The addition of nalidixic acid disk diffusion did not detect any additional isolates. Ciprofloxacin disk diffusion may overcall reduced susceptibility as six isolates testing ciprofloxacin intermediate were ciprofloxacin Etest susceptible. Ciprofloxacin disk diffusion results alone using the new CLSI breakpoints are more in agreement to ciprofloxacin Etest interpretations (MiE 6) than nalidixic acid KB disk diffusion used alone (MiE 15) or in combination with ciprofloxacin (MiE 17).

### P29

#### VALIDATION OF BD BACTEC™ PLUS PLASTIC AEROBIC/F RESIN MEDIUM USING RECOMMENDED REFERENCE ATCC STRAINS SEEDED BLOOD CULTURE AND BD BACTEC™ FX, 9240 AND 9120 INSTRUMENTS

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**OBJECTIVE:** To evaluate the performance of the new BD BACTEC™ PLUS plastic aerobic/F resin medium using the current BD BACTEC™ standard glass aerobic/F resin medium seeded with recommended reference ATCC strains and BD BACTEC™ FX and 9240/9120 instruments.

**METHODS:** The study set up seeded cultures with ten ATCC strains in both plastic and glass BD resin bottles that mimic actual clinical specimens. The test bottles (plastic) were in parallel with Control bottles (glass) to perform the evaluation in BACTEC™ FX as well as between new BACTEC™ FX and old 9240/9120 instruments. For each ATCC strain, a 0.5 McFarland ( $1.5 \times 10^8$ ) bacterial suspension was made and diluted serially to  $\sim 10^2$ ; 0.1 mL of  $10^2$  suspensions was inoculated to a blood agar plate for colony count and a pair of glass and plastic media bottles stimulated with human blood before loading into BACTEC instruments. Time for detection (TTD in h: min) and morphology of growth of each organism were also monitored.

**RESULTS:** Among twenty pairs of aerobic glass bottles versus plastic bottles, 13 glass bottles have slight quicker TTD than those of plastic bottles, with differences from 0:08 to 1 h. Four pairs showed the same TTD while three pairs showed that glass bottles had longer TTD from 0:08 to 0:32. BD FX showed comparable TTD to BD9120 and BD9240 for each ATCC strain seeded blood culture; any differences were marginal except for the fungus *C tropicalis* – ca 24 h earlier TTD was found in glass bottle culture. No differences of morphology of growth for each organism in three models were seen.

**CONCLUSION:** Both plastic and glass BACTEC™ PLUS Aerobic/F media performed similar TTD, growth and morphology in detecting positive blood cultures seeded with ten ATCC organisms in three models of BD instruments. Further study for fungal blood culture in BD systems may be warranted.

### P30

#### IN VITRO BETA-LACTAM SUSCEPTIBILITY TESTING OF *STREPTOCOCCUS PNEUMONIAE* USING LIOFILCHEM GRADIENT DIFFUSION COMPARED TO CLSI BROTH MICRODILUTION

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**OBJECTIVES:** We compared a novel gradient diffusion susceptibility test method to broth microdilution (BMD) for determining beta-lactam minimum inhibitory concentrations (MICs) against clinical isolates of *Streptococcus pneumoniae*.

**METHODS:** *S pneumoniae* isolates collected between 2011 and 2013 were grouped based on penicillin MICs of  $\leq 0.12$  mg/L to 0.25 mg/L, 0.5 mg/L to

1.0 mg/L and  $\geq 2.0$  mg/L. A standard inoculum was used for each strain to test amoxicillin (AMX), ceftriaxone (CRO), meropenem (MER) and penicillin (PEN) MICs by BMD and Liocheffim® (Alere) gradient diffusion test strips. Essential agreement (EA) and categorical agreement (CA) were compared to BMD using CLSI M100-S23 breakpoints.

**RESULTS:** In total, 91 clinical isolates were tested. The EA values for AMX, CRO, MER, and PEN were 87%, 71%, 96% and 86%. The majority of MICs in EA were either in exact agreement or one dilution lower than BMD for AMX, MER, and PEN. In contrast, 26% of isolates tested two dilutions lower than BMD for CRO, which contributed to a CA of 64% and 81% and a very major error (VME) rate of 13.1% and 0% using meningeal and non-meningeal breakpoints, respectively. CA values for AMX, MER, and PEN were 90%, 68%, and 91%/81% (meningeal/non-meningeal breakpoints), respectively. VME rates for AMX, MER, and PEN were 0%, 1.1%, and 4.4%/0% (meningeal/non-meningeal breakpoints).

**CONCLUSION:** This is one of the first studies to demonstrate the performance of the Liofilchem test for beta-lactams and *S pneumoniae* isolates. The experience with the CRO strip and the potential for reporting resistant isolates as susceptible in clinical cases of meningitis is concerning and warrants further investigation.

### P31

#### EVALUATION OF SELECTIVE MEDIA FOR THE DETECTION OF CIPROFLOXACIN-RESISTANT *ENTEROBACTERIACEAE* (CR-E) FROM RECTAL SPECIMENS

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**OBJECTIVES:** Urosepsis caused by CR-E is an important complication of transrectal ultrasound-guided prostate biopsy (TRUSPB). Identifying Identifying colonization with CR-E prior to the procedure in order to modify antimicrobial prophylaxis may decrease subsequent infectious complications. This study was performed to evaluate the ability of two ciprofloxacin-containing media to detect CR-E from rectal swabs.

**METHODS:** Fifty rectal swabs obtained for ESBL screening, were plated onto MacConkey agar with 1 µg/mL of ciprofloxacin from two suppliers (Oxoid, Nepean, ON; Alere, Santa Maria, CA), and on MacConkey agar not containing antibiotic. After incubation in ambient air at 37°C for 18 h to 24 h, lactose fermenting colonies and oxidase negative non-lactose fermenting colonies were identified using the Vitek 2 GN ID card (bioMérieux); antimicrobial susceptibilities were determined with the Vitek 2 AST-N208 card.

**RESULTS:** Of the 50 specimens tested, nine (18%) of the swabs grew CR-E (all *E coli* with ciprofloxacin MIC  $\geq 4$  µg/mL). All CR-E grew on the Oxoid media; eight grew on the Alere media. Alere media failed to identify a resistant organism in which only one colony grew on the corresponding Oxoid plate. Both media were highly selective; only a single non-CR-E isolate, a yeast, grew on an Alere plate. One isolate of an ESBL-producing CR-E (*E coli*) was recovered on both media.

**CONCLUSIONS:** Direct planting of rectal specimens on media containing 1 µg/mL ciprofloxacin was able to identify CR-E in 18% of specimens. These media may be helpful to screen patients for CR-E prior to TRUSPB. Additional studies, including a larger number of specimens, would be helpful in determining the utility of these selective media for detection of CR-E.

### P32

#### STUDY OF BIOMIC V3 AUTOMATED VERSUS MANUAL READING OF MICROSCAN ESBL PLUS PANEL RESULTS

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**OBJECTIVE:** To compare the use of the BIOMIC V3 Microbiology System (Giles Scientific Inc, Santa Barbara, CA, USA) automated well reader to manual reading of MicroScan ESBL Plus panels (Siemens Canada, Mississauga, ON).

**METHODS:** Ninety-three sequential unselected clinical isolates of *Enterobacteriaceae* were screened for potential ESBL production using ESBL Plus panels, between November 2010 and October 2012. Quality control testing was performed using the ATCC strains recommended by the manufacturer. After incubation, plates were read manually, then read in a BIOMIC V3 Microbiology System automated well reader. BIOMIC automated turbidity test results and interpretations were compared to manual results. Data were verified using ANSI/ASQC Z1.4 sampling plans.

**RESULTS:** 1209 drug-organism combinations (DOC) were read. A total of 1177 (97.4%) were within  $\pm 1$  dilution and 1101 (91.1%) were an exact MIC match. 325 QC DOC were read, of which 96.9% were within  $\pm 1$  dilution and 93.2% were an exact MIC match.

**CONCLUSIONS:** The BIOMIC V3 provided accurate turbidity readings and interpretations of MicroScan ESBL Plus panel results. All panel images were saved routinely and were available for review if necessary, for example to compare sequential isolates from the same patient. As an alternative to manual reading, the BIOMIC V3 provides more standardized turbidity reading and eliminates potential variation between different technologists.

### P33

#### COMPARATIVE PERFORMANCE OF ESWAB AND M40 IN EXTREME TRANSPORT CONDITIONS

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**OBJECTIVES:** From remote collection sites, swabs may be subjected to low temperature conditions and prolonged transportation. We compared Copan ESwab to Copan M40 swab performance in stabilizing bacterial populations.

**METHODS:** Swab elution method (CLSI M40-A, 2003). Acceptable performance was less than 3 log decline or 1 log overgrowth (eg, 10<sup>6</sup> CFU to 10<sup>3</sup> or 10<sup>7</sup> CFU).

**RESULTS:**

	M40 hours of storage with acceptable growth			ESwab hours of storage with acceptable growth		
	-30°C	4°C	22°C	-30°C	4°C	22°C
<i>S pneumoniae</i>	72	72	72	72	72	72
<i>S pyogenes</i>	<24	<24	<24	72	72	48
<i>P aeruginosa</i>	48	72	<24	<24	<24	72
<i>H influenzae</i>	<24	24	72	72	72	72
<i>B fragilis</i>	72	72	72	24	72	72
<i>N gonorrhoea</i>	72	48	72	<24	<24	48
<i>P anaerobius</i>	72	72	72	72	72	72
<i>P acnes</i>	<24	72	48	72	72	72
<i>P melaninogenica</i>	72	72	72	72	72	48

**CONCLUSIONS:** Most swab failure is due to overgrowth. Swabs transported at temperatures below 4°C should be rejected. Swabs transported at 4°C may lose fastidious organisms by 24 h. ESwab preserves better than M40 at 22°C, and may be accepted after up to 48 h transportation.

### P34

#### A SIMPLE SPECTROPHOTOMETRIC METHOD TO MEASURE FLOCKED SWAB ABSORPTION AS AN ALTERNATIVE TO COMPLEX RESEARCH METHODS

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Previous studies have focused on research methods such as scanning electron microscopy (SEM), radioactive tracer (RT), and zeta potential (ZP) measurements as well as water and protein absorbance of whole flocked swabs to determine their absorption characteristics. Based on the CLSI M40A document Roll Plate method, experiments were conducted to

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validate the performance of spectrophotometric (OD) measurements after removal of HydraFlock swabs Puritan Medical Products and nylon flocked swabs Copan Diagnostics Inc, immersed in 100 µL of standardised bacterial inocula in a microtitre plate for 30 s. Twelve flocked swabs of each type were assessed using three serially diluted suspensions. Subsequently, 25 µL of a red dye was added to each well to visually compare the differences in volumes. Finally, the absorption of 100 µL of viscous lactophenol cotton blue dye by both flocked swab types was compared visually.

**RESULTS:** *Optical Density measurements*

Concentration	Avg OD	Range	Concentration	Avg OD	Range
Pur 105	-0.0039	-0.001 to -0.005	Cop105	+0.026	+0.012 to +0.045
104	-0.0019	-0.000 to -0.005	104	+0.025	+0.014 to +0.036
103	-0.0036	-0.000 to -0.007	103	+0.024	+0.009 to +0.032

*Blank average optical density (avg OD) - 0.0006*

*Red dye assessment:* Copan swabs appeared to have more leftover inoculum. *LPCB dye absorbance test:* The Puritan swab buds were approximately 90% blue while Copan's swabs were approximately 60% blue.

**CONCLUSIONS:** The OD measurements for HydraFlock and Copan flocked swabs relative to the blank suggests there is left over inoculum after absorption with the Copan flocked swab but not the Puritan swab. The OD method is in line with the 100µL volume the CLSI M40A document recommends, while the other absorption method saturates the entire swab with 1.0 mL of water or serum and requires sophisticated instruments and training to perform as does SEM, RT and ZP methods. The OD method is rapid, less tedious and requires less training and expensive equipment than the SEM, ZP or the RT methods. However, results of the OD method are in agreement with those of SEM and ZP methods. The OD method, the red dye visual assessment and the LPCB dye uptake experiments show that the HydraFlock has a greater propensity to absorb fluids than Copan's nylon flocked swab.

### P35

#### CLINICAL UTILITY OF REFLEXIVE THROAT CULTURE ON NEGATIVE RAPID ANTIGEN TESTS FOR THE DIAGNOSIS OF GROUP A STREPTOCOCCAL PHARYNGITIS IN ADULTS

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**OBJECTIVES:** Appropriate diagnosis and treatment of Group A streptococcal (GAS) pharyngitis aids in ameliorating symptoms, reducing transmission and preventing post-streptococcal complications. Recent guidelines for the diagnosis of GAS pharyngitis in adults are conflicting; laboratory guidelines recommend that negative rapid antigen detection test (RADT) results be confirmed by throat culture while some clinical guidelines suggest that negative RADTs do not require confirmation. The latter approach is based on the lower prevalence of GAS pharyngitis in adults and the rarity of rheumatic fever in industrialized countries. However, the lower sensitivity of RADT in comparison to culture or molecular assays may reduce the detection of clinically significant infections.

**METHODS:** To assess the utility of back-up cultures for confirmation of negative RADT results, we retrospectively analyzed the charts of 750 patients, aged 13 or older, with a negative RADT and positive GAS throat culture between January 1, 2000 and December 31, 2011.

**RESULTS:** Modified Centor scores  $\geq 2$  were observed in 56% of patients with negative RADT and positive GAS culture. Furthermore, 76% of these patients had a moderate or heavy bacterial burden ( $\geq 2+$ ). RADTs failed to detect some patients with serious complications of GAS pharyngitis: 29 (3.9%) had peritonsillar abscesses and two (0.27%) were diagnosed with acute rheumatic fever. Providers found culture results useful, either for initiating antibiotic therapy or confirming a clinical diagnosis. Antibiotic treatment was administered to 69.7% of the patients, and in 45.1% of cases was initiated only after the culture results were reported.

**CONCLUSIONS:** Our observations demonstrate that back-up cultures can be clinically useful when RADT results are negative. RADTs fail to

detect a substantial number of adult patients with significant GAS pharyngitis that can benefit from treatment.

*Interim data from this abstract was previously presented at the 113<sup>th</sup> American Society for Microbiology General Meeting, May 18 to 21, 2013, in Denver, Colorado, USA.*

### P36

#### EFFECT OF MINIMIZING SALIVARY CONTAMINATION BY SELECTIVE PROCESSING OF SPUTUM PLUGS ON SPUTUM MICROBIOLOGY

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**INTRODUCTION:** Bacterial and fungal culture yields from unselected whole expectorate of sputum in most laboratories vary from 10% to 15%. We examined 1) whether minimization of salivary contamination will improve the culture yield and 2) the correlation between the intensity of airway neutrophilia and the airway pathogens.

**METHODS:** Fifty consecutive outpatient sputum specimens from patients with infective exacerbation of asthma, COPD, bronchiectasis or chronic cough were split and processed by two methods. One portion was examined under inverted microscopy to select sputum plugs (SP), quantitative cell count was performed and the plugs sent for bacterial and fungal culture. The other sample was sent for routine culture without selective processing. Specimens were inoculated to standard media for bacterial and fungal culture. Identification was performed using standard biochemical reactions and the Vitek2™ (BioMerieux) system.

**RESULTS:** Forty specimens were available for analysis. For routine culture, 12 of 40 (30%) were rejected based on presence of  $>10$  epithelial cells/lpf compared to 0% for SP and two of the rejected specimens grew significant pathogens from SP. Non-pathogenic bacteria were isolated in 16 of 27 versus 12 of 27 and significant pathogens in 12 of 27 versus 18 of 27 specimens for routine culture versus SP respectively. Significant pathogens detected only by culture of SP included: *P aeruginosa* (n=2), *M catarrhalis* (n=1), *H influenza* (n=1), *S maltophilia* (n=1), *Acinetobacter* (n=1), *Achromobacter* (n=1), and *S aureus* (n=1) versus routine culture which detected an additional *Haemophilus* (n=1) and *S pneumonia* (n=1). Fungal pathogens were isolated in 18 of 50 of SP. Of the 34 SP that grew a known bacterial pathogen or fungus, 28 were associated with a neutrophilic bronchitis (total cell count  $>10$  million cells/g and neutrophils  $>65\%$ ). The mean total cell count was higher in bacterial versus fungal bronchitis:  $32.1 \times 10^6/g$ , SD 33.3 versus  $28 \times 10^6/g$  and the neutrophil differential mean was 88% versus 79%. Samples with 'non-pathogenic bacteria' also had intense neutrophilic bronchitis (mean total cell count  $21.6 \times 10^6/g$ ).

**CONCLUSION:** Selection of sputum samples by inverted microscopy to minimize salivary contamination decreases the rejection of specimens and increases the identification of significant pathogens in patient in routine clinical practice. Quantitative neutrophil counts may help clarify the pathological relevance of bacteria reported as non-pathogenic.

### P37

#### WITHDRAWN

### P38

#### CANWARD 2013: ANTIMICROBIAL RESISTANCE IN PATHOGENS ISOLATED FROM CANADIAN HOSPITAL CLINICS, EMERGENCY ROOMS, MEDICAL/SURGICAL WARDS AND INTENSIVE CARE UNITS

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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:** Fifteen tertiary-care 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 2013. Susceptibility testing was performed by CLSI microdilution methods.

**RESULTS:** A total of 3516 isolates were collected: 41.3%, 38.8%, 10.5% and 9.4% from blood, respiratory, urine and wound/IV site specimens, respectively. Isolates were from patients on W 35.8%, ER 23.9%, ICU 21.6% and C 18.7%. The most common pathogens were: *E coli* 18.6%, *S aureus* (MSSA) 18.0%, *P aeruginosa* 11.0%, *K pneumoniae* 6.5%, *S pneumoniae* 5.3% and *H influenzae* 5.1%. Resistance rates (RR) for *E coli* were: 0% for meropenem (MER), ertapenem (ERT) and tigecycline (TGC), 0.6% piperacillin/tazobactam (PTZ), 7.8% gentamicin (GEN), 10.9% ceftriaxone (CTR), 21.8% ciprofloxacin (CIP) and 25.8% trimethoprim/sulfamethoxazole (SXT). For *P aeruginosa*, RR were 1.3% colistin (COL), 7.3% PTZ, 7.3% GEN, 13.6% CIP and 14.1% MER. RR for MRSA were: 0% vancomycin (VAN), linezolid (LZD), tigecycline, and daptomycin (DAP), 1.8% SXT, 29.4% clindamycin, 72.5% CIP, and 74.3% clarithromycin. Overall, the prevalence of MRSA was 20.2%.

**CONCLUSIONS:** In Canada, resistance rates for *E coli* remain lowest for MER, ERT, TGC and PTZ, while for *P aeruginosa*, rates are lowest with COL, PTZ and GEN. No resistance was observed in MRSA with VAN, LZD, TGC or DAP.

### P39

#### REDUCTION OF CLOSTRIDIUM DIFFICILE INFECTION (CDI) RATES THROUGH STEWARDSHIP: DIFFICULTY ENGAGING PRESCRIBERS, WITH IMPLICATIONS FOR STEWARDSHIP PROJECT DESIGN

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**BACKGROUND:** The Mazankowski Alberta Heart Institute (MAZ) experienced elevated CDI rates in spite of IPC interventions. Antibiotic (Abx) exposure and decreased stomach acidity are well recognized risks for CDI. In the absence of a formal stewardship program, an intervention in cardiovascular surgery (CVS) based on prescriber recorded indication for prescribing was implemented.

**OBJECTIVES:** 1) To assess whether recording the indication for use would improve utilization patterns due to increased awareness of indications, reflection process, and implied scrutiny; and 2) to collect intervention outcome data including form completion rate, antimicrobial and PPI utilization, clinical data, and CDI rates.

**METHODS:** Forms were placed on charts of patients admitted to cardiovascular surgery (ICU and ward) from May 21, 2013 to July 15, 2013, and collected, with chart review, at discharge. An education and awareness campaign targeted ward staff, physicians, and surgeons. A panel of blinded investigators assessed antibiotic guideline concordance with a local/national tool, Bugs and Drug 2013.

**RESULTS:** Abx: 18 forms (9.8%) of 192 were completed, for 185 patients. There were 144 Abx orders, 60% concordant with guidelines. The most common Abx were ciprofloxacin, vancomycin and metronidazole, for SSTIs (30.2%), suspected urinary tract infections (11.9%) and suspected *C difficile* infections (10.3%). A reduction in utilization conferring an estimated cost savings of \$8,500.

**CONCLUSION:** In this CVS setting, prescriber engagement proved impossible and a more directive stewardship project design is required. Although prescriber form completion was <10%, a modest reduction utilization was observed, hypothesized to be due to enhanced awareness and implied scrutiny.

### P40

#### PREVALENCE OF EXTENDED-SPECTRUM BETA-LACTAMASE, AMPC CEPHALOSPORINASE, AND CARBAPENEMASE PRODUCING ENTEROBACTERIACEAE OVER A FOUR-YEAR PERIOD IN STOOL SAMPLES SUBMITTED TO LABORATORIES IN THE OKANAGAN REGION OF BRITISH COLUMBIA, CANADA

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**OBJECTIVES:** Beta-lactamases producing Enterobacteriaceae including extended-spectrum-beta-lactamases (ESBL), AmpC cephalosporinases (AmpC) and carbapenemases (CPM) confer resistance to a wide variety of Beta-Lactam antibiotics, resulting in limited therapeutic options. This study compared the prevalence of fully expressed ESBL, AmpC and CPM in the Okanagan region of British Columbia from 2010 to 2013.

**METHODS:** 800 routine clinical stool samples (submitted for bacterial culture or *Clostridium difficile* assay) from three hospital laboratories were screened over four consecutive years using cefotaxime (30 µg), ceftazidime (30 µg) and ertapenem (10 µg) discs. All isolates testing intermediate or resistant (CLSI M100-S23) were identified (biochemical tests and automated panels) and tested for ESBL, AmpC and CPM mechanisms of resistance (MAST® discs, E Test®).

**RESULTS:** The prevalence of fully expressed AmpC remained consistent throughout the study period and was the predominant mechanism of resistance in both inpatient (IP) and outpatient (OP) populations (2010, 7.25%; 2011, 7.81%; 2012, 7.25%; 2013, 8.13%). No significant change was seen between 2010 and 2011 for ESBL (2.77% to 2.9%; P=0.88) however the prevalence of ESBL producing organisms increased in 2012 (4.25%; P=0.109) and 2013 (4.125%; P=0.173), notably due to an increase in OP ESBL prevalence (from 3.8% [2011] to 5.5% [2013]; P=0.09).

**CONCLUSIONS:** ESBL increase over the past two years is consistent with the increased prevalence of ESBL worldwide. Fully expressed AmpC is twice as common as ESBL in the Okanagan region. Continued surveillance for CPM is indicated given its propensity for transmission and limited therapeutic options.

### P41

#### INCREASE IN PREVALENCE OF EXTENDED-SPECTRUM β-LACTAMASE (ESBL) PRODUCING GRAM-NEGATIVE BACILLI (GNB) ISOLATED FROM PATIENTS WITH INTRA-ABDOMINAL INFECTIONS IN CANADA FROM 2008–2012

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**OBJECTIVES:** Antimicrobial-resistant GNB are increasing worldwide, largely due to ESBLs. The Study for Monitoring Antimicrobial Resistance Trends (SMART) is a global surveillance study monitoring the susceptibility of GNB isolated from intra-abdominal infections (IAI). This study reviewed antibiotic susceptibilities of IAI GNB from participating Canadian centres from 2008-2012.

**METHODS:** GNB from IAI were collected annually from each centre following the SMART protocol. Clinical Laboratory Standards Institute standards were used to determine broth microdilution MICs for amikacin (AK), cefoxitin (CFX), cefotaxime (CFT), ceftriaxone (CAX), ceftazidime (CAZ), cefepime, ampicillin-sulbactam (A/S), piperacillin-tazobactam, ertapenem (ETP), imipenem (IMP), ciprofloxacin (CP) &



## Abstracts

levofloxacin (LVX), and phenotypic ESBL production for *Escherichia coli*, *Klebsiella pneumoniae*, *K oxytoca*, and *Proteus mirabilis*.

**RESULTS:** A total of 2,224 GNB were collected including *E coli* (46%), *K pneumoniae* (16%), *Pseudomonas aeruginosa* (10%), *Enterobacter cloacae* (8%) *K oxytoca* (4%), and others (16%). From 2008 to 2012, the proportion of ESBL-producing (ESBL+) GNB rose significantly from 3.8% to 11.3% ( $P=0.003$ ,  $\chi^2$  test for trend), driven largely by community-associated ESBL+ *E coli*, which rose from 0% to 16.8% ( $P=0.0007$ ,  $\chi^2$  test for trend). ESBL+ *E coli* had reduced susceptibility to A/S, CFT, CAX, CAZ, CPE, CP and LVX compared with ESBL-negative isolates, but remained susceptible to AK, ETP and IMP.

**CONCLUSIONS:** From 2008 to 2012, there was a significant increase in ESBL+ GNB from IAI in Canada, with an associated reduction in treatment options, that was primarily driven by an increase in community-associated ESBL+ *E coli*.

### P42

#### USE OF SPOT AUDITS TO MEASURE APPROPRIATENESS OF ANTIMICROBIAL THERAPY ON GENERAL INTERNAL MEDICINE AS PART OF AN ANTIMICROBIAL STEWARDSHIP PROGRAM

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**OBJECTIVE:** To measure appropriateness of antimicrobial prescribing on General Internal Medicine (GIM) patients longitudinally.

**METHODS:** Four, single day audits were conducted approximately two months apart on all adult patients admitted to three GIM inpatient wards at two tertiary care academic hospitals. Patients were included if they were receiving antimicrobials on the day of the audit. The audit was conducted by an Infectious Diseases (ID) trained physician or pharmacist using a standardized data collection form for data entry. Each auditor reviewed microbiology, laboratory investigations, clinical documentation, and pertinent diagnostics available on the electronic patient record. Suboptimal antimicrobial use was assigned using pre-defined categories based on adherence to local best practices documents, published national guidelines, as well as clinical and laboratory information for the patient. A consensus panel (consisting of an ID physician, two ID pharmacists, a GIM physician, and a GIM pharmacist) later reviewed the data to ensure internal reliability.

**RESULTS:** An average of 221 patients were reviewed for each of the four audits, which occurred between May and December 2013. Approximately one third of these patients were receiving antimicrobial therapy. Total time invested for each audit was approximately 15 h, in addition to the 2 h consensus meeting.

**CONCLUSION(S):** Serial spot audits are a novel approach to measure the appropriateness of antimicrobial therapy. Despite a significant cost in terms of time invested, this approach allows antimicrobial stewardship programs an ability to determine patterns of antimicrobial use and misuse with minimal electronic infrastructure. This information can be used to guide future educational interventions, provide feedback to clinicians, highlight areas for improvement, and monitor outcomes from antimicrobial stewardship interventions.

### P43

#### CARRIAGE OF CIPROFLOXACIN-RESISTANT ENTERIC ORGANISMS IN PATIENTS UNDERGOING TRANSRECTAL ULTRASOUND-GUIDED BIOPSIES OF THE PROSTATE (TRUSBP): WHAT WE FOUND AND HOW WE FOUND IT

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**OBJECTIVES:** The study was performed to 1) evaluate the performance of the 'Colorex Orientation' with 2 ug/mL from Alere<sup>R</sup> Canada for identification of ciprofloxacin-resistant (cip-R) organisms, 2) determine the prevalence of rectal carriage of cipR organisms prior to undergoing TRUSBP and 3) determine if patient-collected swabs would yield results comparable to nurse-collected swabs.

**METHODS:** The chromogenic plates were inoculated with: 28 cip-R resistant and 28 cip-S organisms, 20 stool specimens seeded with cip-R organisms, 20 random stool specimens and 10 random rectal VRE swabs. Over 21 months, rectal swabs were collected from patients scheduled to undergo TRUSBP. A smaller group of patients participated in a self versus nurse-collected swab collection study.

**RESULTS:** Of the 28 cip-R isolates, two isolates (*S aureus* and *E faecalis*) failed to grow. Of the 20 cip-R isolates that were used to seed stools, one isolate (*E faecalis*) did not grow. The TAT of the test was 24 h and the hands-on-time was 10 min. Carriage of cip-R coliforms was identified in 60 of 293 (20.5%) of patients. All cip-R isolates were found to be fully susceptible to amikacin and imipenem. Of 56 patients, cip-R organisms were isolated from 11 of the self-collected versus 13 of the nurse-collected swabs. Identification and susceptibilities of isolates were confirmed by microbroth dilution using the BD Phoenix system.

**CONCLUSIONS:** The medium accurately identifies the presence of cip-R organisms from rectal swabs. Greater recruitment is required to determine if self-collection of swabs is as accurate as nurse-collection of swabs. The prevalence of carriage of cip-R enteric organisms in a local population of males undergoing TRUSBP of the prostate is 20.5%.

### P44

#### WITHDRAWN

### P45

#### IMPACT OF A BORDERLINE ZONE ON THE CONCORDANCE OF TUBERCULIN SKIN TEST AND INTERFERON GAMMA RELEASE ASSAY RESULTS AMONG A LOW-RISK COHORT IN MARITIME CANADA

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**OBJECTIVES:** With regards to the interpretation of interferon gamma release assay (IGRA) results, the 2013 Canadian Tuberculosis Standards acknowledge that a borderline zone value should be interpreted cautiously. In this study, the impact of the borderline zone on clinical interpretation of IGRA results is examined among a cohort at low-risk for tuberculosis (TB) infection and exposure. Among this cohort a high proportion of negative confirmatory IGRA results is anticipated and hence a high discordance between positive tuberculin skin test (TST) and the IGRA result.

**METHODS:** Among a cohort of individuals who were deemed low-risk for TB infection, the IGRA was performed following a positive TST. Data was obtained from a database of all IGRA testing performed with the QuantiFERON-TB Gold In-Tube in the maritime provinces of Canada from August 2009 to February 2012. Proportions of TST and IGRA agreement were analysed for those in/outside the borderline zone (TbAg-Nil 0.20 IU/mL to 1.00 IU/mL). A two-sample test of proportion was used to analyze the difference in concordance between groups.

**RESULTS:** Of the 551 patients contained in the database, 170 (30.9%) TST-positive cases were classified as low-risk and had both IGRA and TST induration values for analysis. Concordant results were noted in 38.9% ( $n=44$  of 157 [95% CI 31.2% to 46.5%]) of cases with IGRA values outside the borderline zone versus 50.0% ( $n=13$  of 26 [95% CI, 30.8% to 69.2%]) within the borderline zone ( $P=0.29$ ).

**CONCLUSION:** The difference in concordance between those in and outside the borderline zone was not statistically significant though concordance was higher in the borderline zone than would be expected clinically. The lack of significance may be due to the small sample size, particularly among those in the borderline zone. The results of this study support the need for cautious interpretation of low-risk individuals with positive TST and borderline IGRA values.

## P46

### COMPARISON OF CHICAGO SKY BLUE (CB) AND CALCOFLUOR WHITE (CW) STAINING METHODS FOR THE DIAGNOSIS OF DERMATOMYCOSIS (DE) AND ONYCHOMYCOSIS (ON)

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**OBJECTIVES:** There are very limited studies published on the comparative performance characteristics of the CB and CW staining procedures for the detection of fungal elements in skin and nails specimens. This study evaluated the sensitivity and specificity of the CB and CW staining methods for detecting fungal elements in skin and nail specimens from patients with a clinical diagnosis of DE and/or ON from across Saskatchewan, Canada.

**METHODS:** Sixty-two skin and 135 nail samples of suspected DE and/or ON were prospectively studied from February to July 2012. Each sample was divided equally for each of the following assays: CB and CW smears and cultures. CB and CW smears were examined under light and fluorescent microscope, respectively, for the presence fungal elements including hyphae/arthroconidia, yeast cells/pseudohyphae. Fungi were identified by conventional laboratory procedures. Culture, performed using standard mycological media, was used as a gold standard for determining the sensitivity and specificity of each staining method.

**RESULTS:** For skin specimens, the sensitivities and specificities of CB and CW stains were 88.9% and 96.2%, and 88.9% and 96.2%, respectively. For nail samples, the sensitivities and specificities for CB and CW stains were 92.7% and 76.6%, and 95.2% and 68.8%, respectively. Dermatophytes were the most common causative agents (70%), followed by non-dermatophytes (24%) and yeasts (6%) in this study. *Trichophyton rubrum* was the predominant species (80%) isolated.

**CONCLUSION:** The CB preparation can provide a rapid and accurate method for diagnosing DE and ON in this study population, with the use of a light microscope.

## P47

### COMPARISON OF SABOURAUD DEXTROSE AGAR WITH CYCLOHEXAMIDE, CHLORAMPHENICOL AND GENTAMICIN MEDIA PREPARED BY THREE DIFFERENT SUPPLIERS

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**OBJECTIVE:** Sabouraud dextrose agar with cycloheximide, chloramphenicol, and gentamicin (Sab+CCG) medium is used in the mycology laboratory for the selective isolation of pathogenic fungi (eg, Dermatophytes) from clinical superficial specimens, which normally may harbour bacteria and saprophytic fungi. The objective was to compare the performance of this media from different suppliers; Bio-Media, Oxoid and Dalynn.

**METHODS:** 97 clinical specimens including skin scraping, hair and nail were evaluated. The three media were inoculated in parallel. QC was performed with each lot as per the usual procedure. Fungal growth was divided into Dermatophytes, opportunistic and contaminant fungi. Overgrowth of bacteria was evaluated.

**RESULTS:** The rate of growth was similar for the three media. Positivity rate for dermatophytes was 24.7%, 19.6% and 16.5% on Bio-Media, Dalynn and Oxoid respectively. Growth of bacteria was 4.2%, 9.3% and 7.2% respectively. Colonies on Bio-Media and Oxoid had more pigmentation compared to Dalynn.

Recovery rate	Bio-Media (%)	Dalynn (%)	Oxoid (%)
<i>Trichophyton rubrum</i>	17.5	13.4	10.3
<i>T mentagrophytes</i> complex	4.1	3.1	4.1
<i>T tonsurans</i>	2.1	2.1	1.0
<i>E floccosum</i>	1	1	1
Opportunistic fungi	7.2	5.2	6.2
Contaminant fungi	1	3.1	1

**CONCLUSION:** Sab+CCG from Bio-Media showed better isolation and growth of dermatophytes with lesser bacterial contamination.

## P48

### EMMONSIOSIS IN SOUTH AFRICA – CLINICAL AND LABORATORY FEATURES OF 27 CASES

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**OBJECTIVE:** The first 13 cases of human infection with a novel *Emmonsia* spp. in HIV-infected patients were recently described in South Africa. This report elucidates further clinical and laboratory characteristics of 27 additional cases.

**METHODS:** Multicentre, retrospective chart review of cases identified by a national surveillance system of dimorphic fungi.

**RESULTS:** The median age of patients was 37 years and fourteen (55%) were male. Cases came from Western Cape (n=15), Eastern Cape (n=5), Free State (n=2), Gauteng (n=4), and Mpumalanga (n=1) provinces. Twenty-five (93%) were HIV-positive, (median CD4 = 17 cells/mm<sup>3</sup>). At presentation, 14 of 22 (62%) were on antiretrovirals. One HIV-negative patient had a renal transplant; the other, a 52-year old male with an emmonsia brain abscess, had no prior medical history. Five patients (19%) had a history of proven TB (range six to 21 months prior to presentation). All patients had disseminated disease at presentation. Fever was documented in 88% (16 of 18), cough in 75% (15 of 20), and skin involvement in 92% (22 of 24). Chest radiographs were abnormal in 71% (15 of 21). Common laboratory abnormalities included anemia (Hb<120 g/L; 86% [19 of 22]); and elevated cholestatic liver enzymes (89% [16 of 18]). Ultrasound demonstrated hepatomegaly in four patients, splenomegaly in one, and hypochoic splenic lesions in another. Empiric TB therapy was started in 76% (19 of 25) but in none of them was TB confirmed. In 12 cases, yeasts were visualized on histopathology: nine skin biopsies, and one each from liver, lung, and brain. *Emmonsia* spp. was cultured from blood (n=9), bone marrow (n=9), and skin biopsy (n=7), and one each from lung, brain, and lymph node; speciation was confirmed by PCR. Seventeen (63%) patients died.

**CONCLUSIONS:** This case series extends our knowledge regarding a newly recognized human *Emmonsia* species causing disseminated disease in predominately, but not exclusively, immunocompromised patients in South Africa.

## P49

### EVALUATION OF YEAST MIC STRIPS (LIOFILCHEM, ITALY) FOR ANTIFUNGAL SUSCEPTIBILITY TESTING

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**OBJECTIVES:** Antifungal susceptibility testing is frequently required to optimize antifungal treatment for invasive fungal infections. The purpose of this study was to evaluate the MIC strips (MICS) for yeast susceptibility testing comparing to YeastOne® Sensititre Panels (SENS; Thermo Scientific) and Vitek 2® (bioMérieux) YS01 Susceptibility cards (VTK1). **METHODS:** Forty-six *Candida* spp. were tested for susceptibility testing against six antifungal agents [Amphotericin B (AB), Anidulafungin (AND), Fluconazole (FLU), Micafungin (MF), Posaconazole (POS), and Voriconazole (VOR)] by MICS and SENS. These isolates were also tested for AB, FLU, and VOR by VTK1. The results were interpreted using CLSI M27-S4 guidelines for AND, FLU, MF and VOR, and using EUCAST 2013 for AB and POS. The susceptibility results of MICS were compared with the results obtained by SENS and VTK1 as category agreement (CA), minor discrepancy (mD), and major discrepancy (MD)

## Abstracts

**RESULTS:** AB – MICS showed 19 MD compared with both VTK and SENS for a CA of 59%. FLU – MICS showed 2 mD with both SENS and VTK1 for a CA of 95.3%. POS – MICS showed three MD compared with SENS for a CA of 93.5%. VOR – MICS showed CA of 100% compared with both VTK1 and SENS. AND – MICS showed 2 mD and 1 MD for a CA of 93% compared with SENS. MF – MICS showed three MD (all *C glabrata*) and 2 mD compared with SENS for a CA of 88.3%.

**CONCLUSIONS:** MICS can be used reliably for FLU, VOR, and AND susceptibility results. Resistant MF results should be confirmed with alternative method.

MICS improved workflow compared to SENS and can be used as an alternative method for yeast susceptibility test for AND, FLU, MF and VOR.

### P50

#### INTER-LABORATORY COMPARABILITY STUDY OF VORICONAZOLE THERAPEUTIC DRUG MONITORING

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**OBJECTIVES:** The clinical utility of voriconazole (VORI) therapeutic drug monitoring (TDM) for managing invasive fungal infections (IFIs) is improving and there is an interest for a Canadian TDM proficiency program for clinical laboratories. We conducted an inter-laboratory comparability study of VORI TDM performed by Canadian laboratories using a shared series of VORI concentrations.

**METHODS:** The central test site reconstituted VORI powder in methanol (10 mg/L stock) and prepared the VORI samples in purchased human plasma or serum matrix. Expected concentrations of 0.3 mg/L, 0.6 mg/L, 1.25 mg/L, 2.5 mg/L and 5.0 mg/L were shipped on wet ice to participating sites, which were blinded to the concentrations. Sites performed TDM in duplicate on three separate runs on all samples according to their laboratory procedures. VORI concentrations were verified externally by a reference laboratory. Data analysis included standard error of the mean (SEM) and percent coefficient of variation (CV).

**RESULTS:** A reference centre assayed the samples as 0.24 mg/L, 0.41 mg/L, 0.98 mg/L, 2.0 mg/L and 4.02 mg/L. Six of seven sites used liquid chromatography-based methods and one used a bioassay. SEM values for all standards ranged from 0.01 mg/L to 0.18 mg/L. All sites achieved %CV values <15%, with the exception the bioassay site and the three lowest concentrations (SEM  $\leq$ 0.07 mg/L). A scatter plot of all VORI standard test values versus reference values showed a very high correlation ( $R^2=0.9993$ ).

**CONCLUSION:** Established procedures for VORI TDM in seven Canadian laboratories were highly concordant across the therapeutic range. These assays are sufficiently accurate and precise to support clinical practice and guideline development for managing IFIs.

### P51

#### AN UNUSUAL CASE OF GRAM-NEGATIVE MENINGITIS

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A 58-year-old woman with a history of alcohol abuse was brought to the emergency department (ED) after being found unresponsive at home. She had been unwell for one day with fever, headache, vomiting and non-bloody diarrhea. She had no sick contacts and no history of travel. She had a pet dog but no other animal exposures. She had no previous admissions to hospital or regular medications.

In the ED she had a temperature of 39.8°C, heart rate of 110 beats/min and a Glasgow Coma Scale of 11. Physical examination revealed nuchal

rigidity and meningismus with no focal neurologic deficits. The rest of the exam was normal.

Piperacillin-tazobactam and ciprofloxacin were given after initial labs and blood cultures were drawn. Complete blood count showed a white blood cell (WBC) count of  $16.5 \times 10^9/L$ . A rapid HIV test was negative. Urinalysis was normal. A non-contrast computed tomography (CT) head was normal. Lumbar puncture showed WBC count of  $2108 \times 10^6$  cells/L, red blood cell count of  $114 \times 10^6$  cells/L, protein 2.34 g/L and glucose 1.1 mmol/L. CSF Gram stain showed pleomorphic Gram-negative coccobacilli.

Intravenous ceftriaxone, ampicillin and vancomycin were started. Blood cultures were negative. The organism seen on CSF Gram stain failed to grow after aerobic/anaerobic incubation. She completed 21 days of ceftriaxone uneventfully. CSF was sent for 16S rRNA PCR and returned with an unexpected result.

### P52

#### SEEING RED: A CURIOUS SKIN MANIFESTATION

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A 44-year-old female day 6 post-allogeneic matched unrelated stem cell transplant for relapsed AML presented with Erythroderma of both hands and feet in the context of febrile neutropenia. She had undergone induction chemotherapy with danorubicin and cytarabine with three rounds of consolidation chemotherapy (with high dose cytarabine), with evidence of relapse. This was followed by re-induction chemotherapy with NOVE-HiDAC and subsequent Stem Cell Transplant (SCT) related conditioning with Cyclophosphamide and Total Body Irradiation. In the context of her febrile neutropenia she had a catheter associated *Serratia marcescens* bacteremia and Typhilitis involving the distal ileum and right colon.

She had marked redness over the right dorsal aspect of her hand that was warm to the touch but non-tender, and two flaccid vesicles on the left hand that had been present since day 2 after her SCT. Over the next two weeks, progressive, non-tender, weeping erythroderma of both hands bilaterally to the mid-forearms and the toes of both feet was noted despite broad spectrum antibiotics (Figure 1, 2).

Topical 1% hydrocortisone cream was applied bid to her hands and feet. A week later, the crusting and desquamation had resolved and the erythema had almost completely disappeared (Figure 3).

This case illustrates the importance of associating the temporal timing of skin rashes in the context of an immunocompromised host considering both infectious and non-infectious etiologies.

### P53

#### A CASE OF *LECLERCIA ADECARBOXYLATA* BACTEREMIA IN A NEUTROPENIC PATIENT

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**OBJECTIVES:** To report a case of *Leclercia adecarboxylata* bacteremia in an immunocompromised patient with serous ovarian cancer.

**METHODS:** A review of medical records.

**RESULTS:** A 71-year-old female had a past medical history of hypertension, coronary artery disease and stage III serous ovarian cancer, currently day 7 post-chemotherapy. She was allergic to penicillin, streptomycin, sulfa and flagyl. Patient presented with a two-day history of malaise, chills, nausea, vomiting. She was otherwise afebrile and denied any symptoms of cough, shortness of breath, chest pain, abdominal pain, dysuria or diarrhea. On admission, she was afebrile with stable vital signs except heart rate of 122 beats/min. Her absolute neutrophil count was  $0.3 \times 10^9$  cells/L. Lactate was 4.8 mmol/L. Creatinine and liver enzymes were normal. She had normal urinalysis and chest x-ray, and perianal abscess was ruled out by ultrasound. Physical examination was normal except a very tender perianal fissure. Her PICC line was removed, the PICC line site showed no obvious signs of infection. Blood cultures obtained from PICC line and the peripheral vein were both positive for *Leclercia adecarboxylata* which was susceptible to  $\beta$ -lactams, quinolones, trimethoprim/sulfamethoxazole and

aminoglycosides. Patient was on intravenous tazocin and ciprofloxacin for the first four days, and ciprofloxacin (750 mg PO q12h) was chosen to complete the total treatment of 14 days based on the susceptibility profile of MIC=0.2. Filgrastim 300 µg was given daily for three days subcutaneously. Her repeated blood cultures were negative 48 h after the start of antibiotics and her neutropenia resolved on the fifth day of admission. Patient was discharged home after seven days of hospital stay.

**CONCLUSION:** *Leclercia adecarboxylata* is a rare pathogen to cause bacteremia mostly in immunocompromised patients, with only 16 cases reported in English so far. Here we reported a case of bacteremia caused by *Leclercia adecarboxylata* in a neutropenic patient that was successfully treated with catheter removal and 14 days of antibiotics.

#### P54

##### FOODBORNE BOTULISM: URGENT COLLABORATION AND CONSULTATION REQUIRED

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**OBJECTIVE:** Foodborne botulism is a severe neuroparalytic disorder resulting from ingestion of preformed neurotoxin produced during the growth of *Clostridium botulinum* in contaminated food. Incubation is short, usually 12 h to 48 h. The intoxication presents with gastrointestinal symptoms, blurred or double vision, dry mouth, dysphagia and descending, symmetrical flaccid paralysis. Death may occur due to respiratory failure. Here we describe the clinical and laboratory findings during the investigation of a case of foodborne botulism suspected to be linked to consumption of home-canned green beans.

**METHODS:** A previously healthy, afebrile adult female presented with symptoms consistent with botulinum intoxication. The night prior to illness, she had consumed home-canned green beans. Serum, fecal and food specimens were tested for *C botulinum* and its toxin by culture and bioassay.

**RESULTS:** *C botulinum* Type A was detected in the patient's feces following heat treatment and culture but *C botulinum* toxin was not detected in the patient's serum. No toxin was detected in the food samples tested. pH values of several jars of green beans were conducive to toxin production. The patient required respiratory support and was given botulinum antitoxin prior to laboratory confirmation.

**CONCLUSIONS:** Botulism is a Reportable Communicable Disease and requires urgent consultation with public health. While laboratory confirmation is lengthy and not necessary for patient management, it provides a basis for public health interventions. This case highlights the required collaboration between clinicians, laboratories and public health agencies.

#### P55

##### A FIVE-YEAR ASSESSMENT OF PRIVATE WELL OWNER SUBMISSION BEHAVIOURS IN SOUTHEASTERN ONTARIO

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**OBJECTIVE:** Well owners are responsible for the bacteriological potability of their drinking water and current literature is lacking in private well water submission information. The objective of this study was to investigate well owner submission behaviours over a five-year period, in the context of provincial guidelines and factors promoting or deterring submission.

**METHODS:** Using five years of data (2008 to 2012) from a public laboratory, private well water submissions from unique properties were descriptively and statistically analyzed utilizing ANOVA and odds ratio tests.

**RESULTS:** Well owner behaviour was affected by adverse water results, with the majority of well owners re-testing their well after a contamination event. Furthermore, well owners with a previous contaminated water sample had different future submission behaviours (in the same and future

years) than owners without an adverse test result. The distance to the nearest water sample drop-off location was associated with the number of times well owners submitted well water samples for testing, with more frequent submissions from wells located closer to a drop-off site. Overall, approximately 80% of well owners did not meet current Ontario guidelines in any given year and only 1% met the guidelines in all five years of the study.

**CONCLUSIONS:** While well owners exhibited an appropriate reaction to receiving notice of contaminated drinking water samples, private well water submission guidelines are alarmingly disregarded in Ontario. Accessibility to testing may be a major factor contributing to non-compliance, and thus, additional drop-off sites should be considered.

#### P56

##### WITHDRAWN

#### P57

##### THE BRAINSPAN LEARNING GAME: WHAT'S IN IT FOR INSTRUCTORS?

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**BACKGROUND:** A multiplayer learning game was developed for use in the preclinical courses at our medical school. The resource was developed from two perspectives: student and instructor. Students use the game to review course concepts and to test themselves for exam preparation. Instructors have different needs and this poster describes how the game system can be used to improve teaching practices.

**METHODOLOGY:** Instructors can make games to review lectures and by asking students to complete the game of the day, get good feedback about potential problems and levels of understanding. Instructors can generate reports of several types that help steer the teaching process; individual student performance on game questions is one parameter useful for identification of students who need more attention. Instructors can also generate reports on the subcategories of the major subject and see how students are doing in different areas of the course, which can lead to a change in teaching practices if deficiencies are found. A major useful function of the game system is the ability to make a quiz game and receive an item analysis on individual multiple choice questions, helping standardize the quality of the course examinations. This game system was used as a tool for an assignment: students were required to submit multiple choice questions, all students answered the questions and the file was sent for item analysis. In this manner, standardized criteria were applied for assessment of assignment quality.

**CONCLUSIONS:** Use of game systems for feedback and assessment is an easy way for instructors to interact with students outside of class. Added advantages include the ability to assess student performance and areas of difficulty.

#### P58

##### A CLINICAL OVERVIEW OF TIGECYCLINE USE AT A TERTIARY CARE TEACHING ORGANIZATION OVER 5 YEARS

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**OBJECTIVES:** The appropriateness of tigecycline utilization in Canada is unclear, given evidence showing increased mortality in pneumonia (HAP/VAP). The purpose of this review is to determine the appropriate utilization of tigecycline.

**METHODS:** Retrospective review of patients receiving tigecycline for more than 1 day was completed from May 2009 – December 2013 at Hamilton Health Sciences. Mortality was defined as death during therapy or within 30 days of discontinuation.

**RESULTS:** A total of 53 patients were included in the study; four patients were excluded due to insufficient data. The mean length of tigecycline treatment and patient age was 15.1 days (range one to 180 days) and

61.1 years respectively. The most common indications included complicated intra-abdominal infections (cIAI) (n=14 [26%]), HAP/VAP (n=11 [21%]), endovascular infection (n=7 [13%]) and complicated skin and soft-tissue infection (n=4 [7.5%]). In total, 14 (26.4%) patients treated with tigecycline expired: four of 11 (36%) of patients with HAP/VAP, two of three (66%) patients with *C.difficile* infection, three of seven (43%) with endovascular infection, three of 14 (21%) with cIAI, one of one (100%) with an infected prosthetic joint and one of three (33%) with sepsis. VRE was the most common pathogen treated (n=12 [23%]). The majority of patients (91%) received an antibiotic prior to tigecycline (mean duration 15 days). Allergy to antibiotics was documented for 27 (51%) patients with 10 (19%) being allergic to multiple classes.

**CONCLUSIONS:** Tigecycline is used for a variety of indications, typically after previous extensive antibiotic therapy, and to treat VRE. From this data the mortality rate was found to be 26.4%. Whether this is due to treatment failure of tigecycline or the complex nature of these infections remains unclear.

## P59

### MICA\*008 ALLELE: A GENETIC PREDISPOSING FACTOR FOR RESISTANCE AGAINST CHLAMYDIA TRACHOMATIS INFECTION

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**OBJECTIVES:** Most patients infected by *Chlamydia trachomatis* (CT) are asymptomatic and untreated. This infection may affect male fertility by damaging the sperm. Sperm parameters and proportion of DNA fragmentation can be impaired. After infection with CT, NK activation signals begin through interactions of its receptors with molecules like MHC class I (MICA/B). Accordingly, relation of MICA gene polymorphism and infertility in CT infected men was investigated.

**METHODS:** Eight hundred patients with poor sperm parameters were selected for *Chlamydia*. Molecular detection following ELISA test were performed for *Chlamydia* detection. PCR-SSP method by seven primers were used to detect MICA\*008 alleles. Moreover, DNA Fragmentation Index (DFI) was evaluated by SCSA. Control group was 30 normal males.

**RESULTS:** Among 800 patients, 62 (8%) were CT positive, 32 (52%) symptomatic and 30 (48%) asymptomatic. Frequency of MICA\*008 allele was higher in control group than patients infected with CT (P<0.05). There was no significant difference between the allele frequency of control and symptomatic patients (P=0.193), but it was higher in control group than in asymptomatic patients (P<0.05). The frequency of MICA\*008 was higher in asymptomatic than in symptomatic patients (P=0.010). DFI in CT infected group is statistically higher than that of in control group (P=0.013).

**CONCLUSION:** Given the prevalence of infection and high frequency of asymptomatic patients among patients with poor sperm parameters, screening for infection is essential in order to avoid the adverse effects. The presence of MICA\*008 allele product may reduce the susceptibility of the host to be infected with CT and those having this protein are more resistant to *Chlamydia* infection. MICA gene is a good candidate gene to determine the host potential genetic predisposition toward resistance against pathogens. The increase of DNA fragmentation in patients with CT is greater than the influence on classical semen parameters and result in a decreased fertility capability.

## Student Poster Presentations

### SP01

#### STRAIGHT FROM THE COW'S MOUTH (CLINICAL VIGNETTE POSTER)

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Vesicular skin lesions following animal contact can represent a classic, but uncommon zoonotic infection.

A previously healthy 21-year-old female agriculture student presented to the emergency department with a two week history of two discreet vesicular lesions to the second and third digits on the dorsum of her left hand. One week prior to the onset of the lesions she had been at a farm where she had contact with goats and also had her ungloved hand inside the mouth of a baby calf during a feeding. No animals had any lesions nor were unwell. The lesions started one week after this exposure. She was systemically well with no history of fever. She also complained of left wrist tenderness as well as left elbow tenderness. She has never had a symptomatic herpetic infection in the past.

On examination the patient appeared well. She was afebrile with normal vital. Please refer to Figure 1 (picture not attached) for the picture of the lesions found on the patient's left hand. In addition, she had mild swelling of the left wrist but no erythema or limitation of movement. She also had some small palpable epitrochlear lymph nodes on the left arm.

Previous HSV1/2 PCR swabs of the lesions were negative. A further diagnostic test was performed and the diagnosis of her vesicular lesions was made.

### SP02

#### PREDICTORS OF ASYMPTOMATIC CLOSTRIDIUM DIFFICILE COLONIZATION ON HOSPITAL ADMISSION

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**BACKGROUND:** *Clostridium difficile* (CD) is the leading cause of health care-associated diarrhea and can result in asymptomatic carriage. Rates of asymptomatic CD colonization on admission range from 1.4% to 13.3%. Factors previously associated with colonization on admission include prior hospitalization, use of antibiotics, corticosteroids, chronic dialysis, and prior CD infection (CDI). The objective of this study is to evaluate host and bacterial factors associated with colonization on admission.

**METHODS:** Data from the FRSQ consortium study was used for analysis. The prospective study provided data for 5236 patients from six hospitals in Quebec and Ontario studied over 15 months from 2006 to 2007. For all patients, demographic information, known risk factors and potential confounding factors were collected. Stool or rectal swabs were obtained for culture on admission, then weekly until discharge. Pulsed field gel electrophoresis was performed on the isolates. The presence of serum antibody against CD toxins A and B was measured.

**RESULTS:** A total of 212 (4.05%) patients were colonized with CD on admission, and 5024 patients were not. Multivariate logistic regression analysis adjusting for hospital site showed that hospitalization within last 12 months, use of corticosteroids, hemodialysis, prior CDI, and presence of antibody against toxin B were associated with CD colonization on admission. Among patients with CD colonization on admission, 84.4% had non-NAP1, non-NAP2 strains.

**CONCLUSIONS:** Our study showed that recent hospitalization, use of corticosteroids, hemodialysis, prior CDI, and presence of antibody against

toxin B were risk factors associated with CD colonization on admission. The majority of patients with colonization on admission carried non-NAP1 and non-NAP2 strains.

### SP03

#### WITHDRAWN

### SP04

#### CHARACTERIZATION OF PENICILLIN-RESISTANT (PRSP) AND MULTI-DRUG RESISTANT (MDR) *STREPTOCOCCUS PNEUMONIAE* (SPN) IN CANADIAN HOSPITALS, 2007–2012

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**OBJECTIVES:** The goal of this study was to characterize PRSP collected from blood and respiratory infections across Canada in 2007 to 2012.

**METHODS:** SPN strains were obtained from Canadian hospitals as part of the CANWARD study. Antimicrobial susceptibility testing using CLSI methods identified PRSP strains (MIC  $\geq 2$   $\mu\text{g/mL}$ ) for further study. PRSP were serotyped by the Quellung method, tested for genetic relatedness by PFGE, virulence due to the presence of pili (PI-1 and PI-2), macrolide resistance mechanisms *mef*(E) and *erm*(B) and mutations in penicillin-binding proteins (PBP) 1A, 2B and 2X.

**RESULTS:** A total of 68 (3.5%; n=1941) PRSP were identified in 2007 to 2012. Isolates demonstrated concomitant resistance to several antimicrobials, including cefuroxime (97.1%), trimethoprim-sulfamethoxazole (91.2%) and clarithromycin (75.0%). 73.5% of isolates demonstrated a MDR phenotype. Serotypes included 19A (n=30; 44.1%), 19F (n=11; 16.2%), 9V (n=11; 16.2%), 23F (n=6; 8.8%), 14 (n=4; 5.9%), 35B (n=3; 4.4%), 22F (n=1; 1.5%) and non-typeable (n=2; 2.9%). PFGE revealed six clusters of isolates. Four were associated with PMEN clones Taiwan<sup>19F-24</sup>, Spain<sup>23F-1</sup>, Spain<sup>9V-3</sup> and England<sup>14-9</sup>. Serotypes 19A, 19F, 9V, 14, 35B and 22F were associated with PI-1, while only 19A and 19F were associated with PI-2. Macrolide resistance was associated with the presence of *mef*(E) (16.2%), *erm*(B) (8.8%) or both *mef*(E) and *erm*(B) (55.9%). Sequencing of PBP genes 1A, 2B and 2X revealed amino acid substitutions in key motifs.

**CONCLUSIONS:** PRSP isolates in Canada demonstrate MDR, piliation and possess dual *mef*(E)/*erm*(B) genes. These organisms, containing mutated PBPs, are associated with the spread of well-described international clones across Canada. Continual surveillance is necessary to assess the evolution of PRSP in Canada.

### SP05

#### WITHDRAWN

### SP06

#### PRACTICES SURROUNDING OUTPATIENT ANTIBIOTIC THERAPY FOR INTRAVENOUS DRUG USERS: A SURVEY

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**OBJECTIVES:** Although outpatient parental antimicrobial therapy (OPAT) has become a common practice, there is no clear Canadian standard of care with regard to discharging intravenous drug users (IVDUs) from hospital with intravenous (IV) access and little is known about current physician practices.

**METHODS:** An electronic survey was distributed to members of the Association of Medical Microbiology and Infectious Disease (AMMI) Canada. Members were eligible if they had treated IVDUs with four or more weeks of IV antibiotic therapy.

**RESULTS:** Seventy of 583 members (12%) physicians responded to the survey. Seventy-four percent indicated that they had discharged one or more IVDUs from hospital for outpatient IV therapy. A greater proportion of physicians in Western provinces (British Columbia, Alberta, and Manitoba) than the Atlantic provinces and Quebec reported they

frequently or sometimes discharged patients with IV access (65.2% versus 16.6%); the trend was reversed in respondents who reported seldom or never discharging them (26.1% versus 83.3%). Only 11.6% of those surveyed reported access to an outpatient program or clinic designed for IVDUs. The greatest reported barriers to discharge were unstable housing, active IV drug use, and insufficient patient supervision. 31.8% of respondents felt that inpatient IV antibiotic treatment was best for IVDUs, 56% felt that in and outpatient treatment were both reasonable options, and 12.1% felt that outpatient treatment was the best option.

**CONCLUSIONS:** Despite the lack of guidelines, most physicians surveyed have discharged IVDUs from hospital with IV access for antibiotic administration. Further research on outcomes of OPAT in IVDUs is warranted to determine the safety of this practice and to address the infrastructure needed for such programs.

### SP07

#### MOLECULAR EPIDEMIOLOGY AND VIRULENCE CHARACTERISTICS OF EXTENDED-SPECTRUM $\beta$ -LACTAMASE-PRODUCING *ESCHERICHIA COLI* (EC) ISOLATED IN CANADIAN HOSPITALS FROM 2007 TO 2012

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**OBJECTIVE:** To assess the prevalence, patterns of antibiotic resistance, and molecular characteristics of ESBL-producing EC isolated from Canadian hospitals.

**METHODS:** 5951 EC were collected from January 2007 to December 2012 as part of the ongoing CANWARD national surveillance study. Antimicrobial susceptibility testing was performed according to CLSI guidelines and putative ESBL-producers were identified. All putative isolates were characterized by PCR and sequencing to detect resistance genes and the key virulence markers associated with extraintestinal pathogenic EC (ExPEC). PFGE and phylogenetic grouping were carried out to assess clonal spread. The EC ST131 clone was identified by an allele-specific PCR for the *pubB* gene.

**RESULTS:** The prevalence of ESBL-EC [2007: 3.4%, 2012: 7.6%] increased significantly between 2007 and 2012. Antimicrobials demonstrating the greatest activity against ESBL-EC in this study were colistin, amikacin, ertapenem, and meropenem, while 78.8% of ESBL-EC were multidrug resistant. Isolates were generally unrelated by PFGE (<80% similarity); however, ST131 was identified among 56.9% of ESBL-EC. ESBL-EC belonged to phylogenetic groups B2 (62.8%) > D (22.3%) > A (14.9%). In total, ST131 comprised 89.9% of all phylogenetic group B2 isolates identified. ExPEC was identified among 94.8% of ST131 isolates, in comparison to only 50.6% of non-ST131 isolates ( $P < 0.001$ ). CTX-M-15 was the dominant genotype in ESBL-EC (66.5%).

**CONCLUSIONS:** The prevalence of ESBL-EC increased significantly from 2007 to 2012 in Canada. This trend was primarily driven by an increase in the proportion of ST131 isolates among ESBL-EC. The ST131 clone is associated with CTX-M-15, phylogenetic group B2, and ExPEC.

### SP08

#### PREVALENCE AND CHARACTERISTICS OF *ESCHERICHIA COLI* AND *SALMONELLA* SPP. IN THE FECES OF WILD URBAN NORWAY AND BLACK RATS (*RATTUS NORVEGICUS* AND *RATTUS RATTUS*): IMPLICATIONS FOR PUBLIC HEALTH

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**OBJECTIVES:** There is widespread concern that contamination of the environment and foodstuffs with rat feces can cause foodborne illness in people; however, few studies have identified fecal pathogens in rats. Our objective was to determine the prevalence and characteristics of *Escherichia coli* and *Salmonella* spp. in a population of urban rats.

**METHODS:** Colonic contents from trapped rats underwent culture for *E coli* and *Salmonella* spp. Isolates were tested for antimicrobial resistance as per the protocols of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). *Salmonella* isolates were serotyped and colonic contents underwent PCR-based screening for *E coli* intestinal pathogenicity genes with serotype identification for positive samples.

**RESULTS:** *E coli* and *Salmonella* spp. were detected in 397 (62.7%) and three (0.5%) of 633 rats, respectively. Of the 397 *E coli*-positive rats, 15 (3.8%) were positive for *Stx 1*, *Stx 2*, and *eae*. *E coli* serotypes identified included O145, O103, O26, and O45. Forty-seven (11.8%) and 27 (6.8%) of 397 *E coli* isolates showed reduced susceptibility to  $\geq 1$  and  $\geq 3$  antimicrobials, respectively, and two isolates were CTX-M extended spectrum  $\beta$ -lactamase resistant. Twenty-three different *E coli* resistance patterns were identified. *E coli* serotypes and resistance patterns demonstrated significant geographic clustering. The *Salmonella* serotypes identified were Derby, Indiana and Enteritidis. The S Enteritidis isolate was susceptible to all antimicrobials tested whereas the other two isolates showed reduced susceptibility to  $\geq 3$  antimicrobials.

**CONCLUSION(S):** *Salmonella* was rarely isolated from wild rats. *E coli* isolation was more common and included multidrug resistant and pathogenic strains. Geographic clustering of resistance patterns and serotypes may indicate common-source exposure or propagation of strains among rats. Comparison of these results to similar studies in urban and agricultural settings indicates that rat fecal microbiota is strongly tied to the environment in which they reside.

#### SP09

### CHRONIC OTITIS MEDIA DUE TO NONTUBERCULOUS MYCOBACTERIA: A CASE OF SUCCESSFUL TREATMENT WITH TOPICAL BORIC ACID (CLINICAL VIGNETTE POSTER)

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**OBJECTIVE:** Nontuberculous mycobacteria (NTM) are an increasingly recognized cause of chronic otomastoiditis in children with tympanostomy tubes. Treatment of this infection is challenging, usually requiring prolonged systemic antibiotic therapy combined with surgical debridement. There are no previous reports of topical boric acid as a therapeutic option for this infection.

**METHODS:** We present the case of a two-year-old male with NTM otomastoiditis who was successfully managed with topical boric acid. A literature review on the treatment of NTM otomastoiditis was performed via PubMed using the search terms: "nontuberculous mycobacteria", "otitis", "otitis media", "mastoiditis", "otomastoiditis", "treatment", "boric acid".

**RESULTS:** A two-year-old boy had tympanostomy tubes placed for recurrent otitis media. He subsequently developed chronic otorrhea which was refractory to ciprofloxacin drops. Middle-ear cultures grew *Mycobacterium abscessus/bolletii/massiliense* complex. Further questioning revealed that he had been swimming in the family's spa. After results of antibiotic susceptibility testing, he was begun on clarithromycin and linezolid. Imaging revealed non-coalescing bilateral mastoiditis. After seven months of systemic antibiotics, he had only mild improvement in symptoms and his cultures remained positive. Antibiotics were stopped and weekly topical boric acid was initiated. After one month of therapy, he showed significant decrease in otorrhea. Treatment of NTM otomastoiditis is not well-defined. Canadian TB Standards 7<sup>th</sup> edition recommend four to six months of therapy with two active agents combined with surgical debridement for skin and soft-tissue as well as bone and joint infections due to NTM. Topical boric acid powder has been shown to be effective in chronic suppurative otitis media caused by typical bacterial pathogens, but no data exists for nontuberculous mycobacteria.

**CONCLUSION:** Chronic otitis due to NTM is difficult to treat with combination systemic antibiotic therapy. Topical boric acid should be considered as an adjunct to treatment as soon as the diagnosis is established.

#### SP10

### THE CASE OF THE NON-RESPONDING OSTEOMYELITIS (CLINICAL VIGNETTE POSTER)

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A healthy, immunized two-year-old Indian-born male, in Canada since infancy, presented with a two-week history of right foot swelling and limp. Xrays showed cortical destruction of the calcaneus. MRI showed flexor myositis, bone marrow edema in the talus, and calcaneus, with calcification and T2 hyperintensity. He was treated for osteomyelitis with IV Cefazolin. Repeat MRI showed worsening necrosis, calcaneus sinus tract formation and subcutaneous abscess. After eight weeks, surgical debridement showed copious amounts of gelatinous tissue in the calcaneus, concerning for caseous granulomatous tissue rather than pyogenic osteomyelitis. Histology showed AFB negative granulomatous material, TB PCR positive, and mycobacterial culture positive.

The patient and his parents' chest radiographs were normal, and all were TB skin test negative. He received the BCG vaccine at three days of life. Empiric treatment with isoniazid, rifampin, ethambutol, and pyrazinamide was commenced, until the isolate returned pyrazinamide resistant. Speciation revealed *Mycobacterium bovis* BCG. Pyrazinamide was discontinued and levofloxacin initiated.

He was continued on the rifampin, and isoniazid for a year. Ethambutol was discontinued after three months and levofloxacin after seven months. In follow-up at 12 months, he has achieved radiographic resolution and remains well.

**DISCUSSION:** BCG complications include local and disseminated abscesses, lymphadenitis, and osteomyelitis. The incidence of osteomyelitis is 0.39/million vaccinated newborns. Onset of the osteomyelitis is approximately one year after vaccination (range three to 26 months).

The presentation of BCG osteomyelitis is often non-specific, and diagnosis is delayed after failure of routine antibiotics for osteomyelitis. Often microbiologic results are required, and a pyrazinamide resistant pattern can be an early clue to the identification of BCG *M bovis* prior to final culture results. The optimal treatment for BCG osteomyelitis isn't established; generally triple-regimen therapy is used.

**CONCLUSION:** Clinicians should consider BCG osteomyelitis in the BCG vaccinated child with failing to respond osteomyelitis. The sensitivity pattern indicating pyrazinamide resistance can indicate BCG *M bovis*. Oral antituberculosis chemotherapy, in addition to surgical debridement shows a favourable prognosis.

#### SP11

### CHANGING TRENDS IN $\beta$ -HEMOLYTIC STREPTOCOCCAL BACTEREMIA IN MANITOBA, CANADA: 2007-2012

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**OBJECTIVE:** European surveillance studies have reported increasing incidence of bacteremia due to  $\beta$ -hemolytic group G streptococci (GGS), but no studies have evaluated trends in  $\beta$ -hemolytic streptococcal bacteremia in North America.

**METHODS:** Data was compiled from the microbiology laboratories of Health Sciences Centre and St. Boniface General Hospital in Winnipeg from January 2007 to December 2012. We tallied all positive blood cultures and calculated the proportion attributable to  $\beta$ -hemolytic streptococci ( $\beta$ HS) by Lancefield grouping. *S pneumoniae* isolations from blood culture and the results of clinically submitted throat swabs that isolated  $\beta$ HS over the same period were reviewed as comparators.

**RESULTS:** During the study period, there were a total of 19,864 positive blood cultures. Of these, a total of 1025 (5.16%) episodes of bacteremia attributable to  $\beta$ HS were identified, which included 425 GAS isolations (2.03%), 339 GBS (1.71%), 62 GCS (0.31%), and 199 GGS (0.95%) (Table 1). A significant increase in the proportion of bacteremia attributable to  $\beta$ HS in general was observed over the study period (Figure 1; 6.32% in 2012 versus 4.02% in 2007 [P<0.0001]; with a significant linear trend test [LTT], [P<0.0001] suggesting that these results were representative of

the trend study period); and GGS (1.49% versus 0.43% [ $P < 0.0001$ ; LTT,  $P < 0.0001$ ]) and GCS (0.58% versus 0.13% [ $P = 0.0068$ ; LTT,  $P = 0.0105$ ])  $\beta$ HS in particular. Bacteremia attributable to GAS, GBS and *S pneumoniae* were unchanged. There were no changes in the distribution of  $\beta$ HS groups in throat swabs.

**CONCLUSIONS:** Bacteremia attributable to GGS and GCS increased in Manitoba over the study period. No analogous change occurred in the  $\beta$ HS causing oro-pharyngeal infection. Further study of the factors underlying these changes is required.

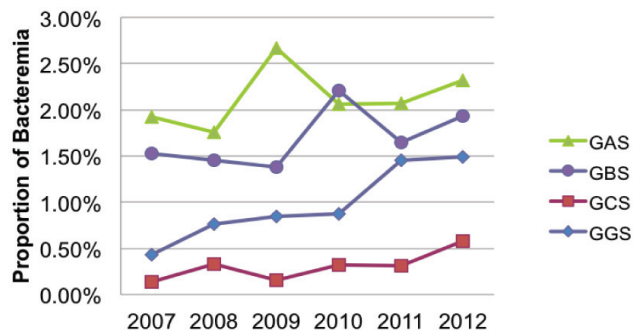


Figure 1) Proportion of bacteremia due to  $\beta$ HS groups, from 2007 to 2012.

### SP12 BLINDED COMPARATIVE PERFORMANCE AND EFFICIENCY OF URINE CHROMOGENIC AGARS

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**OBJECTIVES:** Chromogenic agars may reduce workload and cost. No study has compared efficiency between products. The role of concurrent non-selective media is unclear.

**METHODS:** A total of 572 consecutive urine specimens received over four days at a tertiary care laboratory were applied simultaneously to blood and MacKonkey agars (BM, Oxoid, reference method), BBL CHROMagar® with TSA biplate (BD, Becton Dickinson) and Colorex Orientation® (Alere) with TSA (Oxoid), and incubated for 18 h at 35°C. Identification was performed according to laboratory protocol, by blinded technologists without prior chromogenic experience.

**RESULTS:** Of 572 specimens, 10 were rejected for incomplete data. One hundred twenty-one of 562 (21.5%) were reported as significant growth by the reference method. Categorical agreement for Alere was 82.0% (95% CI 78.6% to 85.0%) with major disagreement rate 3.4% (1.8% recommending overtreatment, 1.6% recommending undertreatment). Categorical agreement for BD was 77.2% (95% CI 73.6% to 80.5%) with major disagreement rate 4.8% (3.0% recommending overtreatment, 1.8% recommending undertreatment). Mean workload per specimen and per significant positive was 3.03 (95% CI 2.77 to 3.28) units and 8.07 (95% CI 7.49 to 8.65) units for BM, 2.25 (95% CI 2.08 to 2.41) units and 4.54 (95% CI 3.91 to 5.17) units for Alere and 1.91 (95% CI 1.72 to 2.12) units and 4.64 (95% CI 3.96 to 5.32) units for BD. Mean total cost per specimen for identification only was \$3.97 (95% CI 3.51 to 4.42) for BM, \$2.94 (95% CI 2.67 to 3.21) for Alere, and \$2.53 (95% CI 2.23 to 2.82) for BD. The concurrent nonselective plate was beneficial for identification.

**CONCLUSIONS:** Alere trended to greater accuracy. Both were significantly less work and cost than reference method.

### SP13 STAPHYLOCOCCUS AUREUS AND STREPTOCOCCUS PNEUMONIAE NASAL COLONIZATION RATES AMONG PATIENTS WITH LABORATORY CONFIRMED INFLUENZA VIRUS INFECTION

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**OBJECTIVES:** Influenza complicated by bacterial pneumonia, most often due to *S pneumoniae* (SP) or *S aureus* (SA), is associated with considerable morbidity and mortality. In this study, we determined the rates of nasal colonization with SA and SP in patients with laboratory confirmed influenza.

**METHODS:** Frozen influenza positive nasopharyngeal (NP) or mid-turbinate (MT) swabs were obtained from the Toronto Invasive Bacterial Diseases Network (TIBDN) during the 2012-2013 influenza season. Genetic material was extracted and compared to frozen genetic material from respiratory virus negative MT swabs obtained at Sunnybrook Health Sciences Centre. Specimens were analyzed by real time-PCR for SA or SP by *nuc* or *ply* amplification respectively; *nuc*-positive specimens were also analyzed for *mecA* and Panton-Valentine leukocidin (PVL).

**RESULTS:** Of 330 NP/MT specimens, SA was detected in 18 of 74 (24%) of ICU admissions, 17 of 80 (21%) of ward admissions, 13 of 86 (15%) of outpatients and 14 of 90 (15.6%) of patients with no respiratory virus detected ( $P = 0.36$ ). SP was detected in 17 of 74 (23%) of ICU admissions, six of 80 (7.5%) of ward admissions, eight of 86 (9.3%) of outpatients and five of 90 (5.5%) of patients with no respiratory virus detected ( $P = 0.002$ ). The *mecA* gene was detected most often in the ICU group in nine of 80 (12%), while it was detected eight of 80 (10%) of ward patients, four of 86 (4.7%) outpatients and six of 90 (6.7%) patients in the respiratory virus negative group. PVL was detected in only two of 80 (2.5%) of specimens, all from ward patients.

**CONCLUSIONS:** Rates of SA and *mecA* were slightly higher among hospitalized patients with laboratory confirmed influenza. The rate of SP among ICU-admitted patients was higher than that detected in other groups.

### SP14 C THE DIFFERENCE: STRATEGIES FOR IMPROVING EARLY APPROPRIATE ISOLATION IN SUSPECTED *C DIFFICILE* CASES

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**OBJECTIVE:** *C difficile* shedding is most prominent during diarrhea. Patients suspected of infection should be properly isolated prior to confirmation. Our objective was to obtain a clinically significant increase in proper isolation in medicine patients who have the *C difficile* toxin assay ordered.

**METHODS:** Intervention 1 was posters that educated the difference between the isolation signs. Intervention 2 was a change in the on-line order entry system to generate a reminder to flag the patient in the system. Intervention 3 was an automatic printout of a gastrointestinal (GI) precaution poster when *C difficile* test labels are generated with online ordering. Intervention 4 was an automatically printed set of isolation instructions for unit clerks and nurses generated with online ordering.

**RESULTS:** In baseline 1, those appropriately isolated were 15 of 61 (25%). By intervention 3, this improved to 20 of 48 (42%),  $P = 0.0912$ . After an approximate six month period with no new interventions, baseline 2 showed a durable response. Intervention 4 resulted in increase from baseline 2 of 43 of 77 (56%) to 41 of 54, (76%);  $P = 0.0297$ . Overall, compared to baseline 1, the effects of all the interventions revealed an increase in correct isolation from 25% to 76%,  $P < 0.0001$ .



## Abstracts

**CONCLUSIONS:** Our simple, system-wide interventions resulted in a significant and durable improvement in GI isolation among patients suspected of *C. difficile* infection. Proper isolation is a multistep process involving multidisciplinary groups.

### SP15

#### TO SCREEN OR NOT TO SCREEN FOR CARBAPENEM-RESISTANT ENTEROBACTERIACEAE IN RECIPIENTS OF OUT-OF-COUNTRY MEDICAL CARE: A COST ANALYSIS

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**OBJECTIVE:** A key risk factor for carbapenem-resistant Enterobacteriaceae (CRE) acquisition is the receipt of out-of-country medical care (OCMC). We sought to determine the proportion of admitted patients who received OCMC in the previous 12 months, assess their CRE colonization status, and estimate the direct hospital and laboratory costs associated with a screening and pre-emptive isolation (PreIso) program.

**METHODS:** A screening OCMC questionnaire was administered at all hospitals in a large health region in July/August 2012 and March/April 2013. Screening for CRE colonization was done by rectal swab or stool sample using CHROMagar™ KPC media. Direct laboratory costs included the costs of media, PCR confirmation of positive results (estimated 0.5% prevalence) and technologist time. Direct costs for PreIso including housekeeping, nursing, and isolation supplies, were extrapolated from published Canadian data adjusted by a Bank of Canada correction factor and applied for an expected three days of isolation. Costs for loss of private room revenue and infection control practitioner time were excluded.

**RESULTS:** Of 29,020 admissions screening questionnaires were administered to 15,029 patients (52%) of which 505 (3.4%) were OCMC recipients. None of 317 (63%) patients screened were CRE colonized. Extrapolation to a full year yielded 2960 OCMC recipients requiring PreIso at a cost of \$642,320/year (\$217/patient) and laboratory costs of \$27,232/year (\$9.20/patient screened). Total direct costs associated with screening and PreIso for CRE would be \$669,552/year or \$45,240/patient identified based on an estimated 0.5% prevalence in our setting.

**CONCLUSION:** Our point-prevalence study did not identify any CRE colonized patients. Using conservative direct costs only, excluding private room revenue loss, a screening and PreIso strategy has significant resource implications, with an estimated cost of over \$45,000/patient identified, and raises serious questions about this strategy in a fiscally challenged healthcare environment.

### SP16

#### MANAGEMENT AND OUTCOMES IN PATIENTS WITH STAPHYLOCOCCUS AUREUS BACTEREMIA AFTER IMPLEMENTATION OF AN AUTOMATED INFECTIOUS DISEASES CONSULT: A BEFORE/AFTER STUDY

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**OBJECTIVES:** It has been previously shown that infectious disease (ID) consultation increases adherence to guidelines and decreases mortality for patients with *Staphylococcus aureus* bacteremia (SAB). This retrospective study assessed the impact of mandatory ID consultation for SAB in a Canadian setting.

**METHODS:** We retrospectively reviewed all consecutive adult patients at two tertiary care teaching hospitals in Hamilton, ON, who presented with a first episode of SAB. Mandatory ID consults for SAB were implemented on January 1, 2012. We compared SAB cases in 2011 (control group) with those in 2012 (intervention group). Outcomes included adherence to the Infectious Diseases Society of America (IDSA) guidelines and components hereof, as well as patient outcomes.

**RESULTS:** There were 128 SAB cases in 2011 and 124 in 2012. The majority were methicillin-susceptible (97 of 128 [75.8%] and 100 of 124 [80.6%], respectively). When palliative cases were excluded, ID involvement increased significantly from 91 of 123 (74.0%) in 2011 to 100 of 116 (86.2%) in 2012 (OR 2.2 [95% CI 1.1 to 4.3]; P=0.02), with a significant decrease in the median time to ID involvement from two to one day (P=0.001). Amongst patients surviving a minimum treatment course (greater than 13 days), there was a significant improvement in adherence to IDSA guidelines in 2012 (65 of 102, 63.7% versus 77 of 96, 80.2%; OR 2.3 [95% CI 1.2 to 4.4]; P=0.01). Mortality and SAB relapse rates were similar.

**CONCLUSION:** Creating an automated ID consultation for SAB led to an increase in involvement of ID, a significant decrease in time to ID involvement, and better adherence to IDSA guidelines. The study was not sufficiently powered to detect significant changes in mortality and SAB relapse rates.

### SP17

#### NOSOCOMIAL GRAM-NEGATIVE BACTEREMIA IN A GENERAL SYSTEMS INTENSIVE CARE UNIT: EPIDEMIOLOGY, ANTIMICROBIAL SUSCEPTIBILITY PATTERNS, TREATMENT AND OUTCOMES

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**OBJECTIVES:** Nosocomial gram negative bacteremia in critically ill patients is associated with significant morbidity and mortality. In this study we describe the microbiologic and epidemiologic characteristics of nosocomial gram-negative bacteremia in a large tertiary care general systems intensive care unit from 2004 to 2012.

**METHODS:** All patients admitted to the University of Alberta Intensive Care Unit who had a hospital-acquired (according to CDC/NHSN definitions) gram-negative bacteremia from January 1, 2004 to December 31, 2012 were reviewed for microbiological etiology, antimicrobial susceptibility and treatment. Patient charts were reviewed to determine the source of infection, treatment adequacy and outcome.

**RESULTS:** Eighty-five nosocomial gram-negative bacteremias occurred in 80 patients. Infection rate was 1.4/1000 patient days. Admitting diagnoses included respiratory failure, organ transplant, post-surgical, burns and multi-trauma. Thirty-day mortality was 50%.

The most common source of bacteremia was pneumonia. Eight percent of bacteremias were central line associated bloodstream infections. *Escherichia coli* followed by *Pseudomonas aeruginosa* were the most common pathogens isolated. Only 50% of *E. coli* isolates and 63% of *P. aeruginosa* isolates were susceptible to ciprofloxacin.

The most commonly prescribed antibiotics were piperacillin-tazobactam (40%) and anti-pseudomonal carbapenems (35%). Only 6% of patients received inadequate empiric treatment. All cases of inadequate empiric treatment were associated with multidrug resistant microorganisms such as *Stenotrophomonas maltophilia* and *P. aeruginosa*.

**CONCLUSIONS:** Nosocomial Gram-negative bacteremia is associated with high mortality in critically ill patients. Resistance to ciprofloxacin was common. Despite this most patients received adequate empiric antimicrobial therapy. Empiric treatment regimens should be based on unit-specific data.

### SP18

#### VALIDATION OF SUSCEPTIBILITY TESTING RESULTS FROM ORGANISMS ISOLATED DIRECTLY FROM COLOREX ORIENTATION® AGAR

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**OBJECTIVES:** Chromogenic media offer the potential for more rapid identification of urinary pathogens while improving workflow by rapid elimination of contaminated culture. In 2012, an evaluation of multiple chromagars determined that Colorex Orientation Agar (CLX: Alere) provided the most accurate and reliable results when compared to standard

urine culture (SUC). In order to incorporate the CLX into the lab workflow, this study was performed to validate the accuracy of susceptibility results of colonies taken directly from CLX.

**METHODS:** A total of 119 organisms (67 Enterobacteriaceae [ENT], 45 Gram-positive cocci [GPC] and seven *Pseudomonas aeruginosa* [PA]) were sub-cultured onto CLX and set up directly to Vitek 2® (bioMérieux) automated susceptibility testing (AST) panels and recorded as susceptible (S), intermediate (I) or resistant (R). Each AST result was compared to the SUC AST result and categorized as total agreement (TA), minor disagreement – mD (no change in report), major disagreement – MD (change in report, not clinically significant), and very major disagreement – VMD (clinically significant change in report).

**RESULTS:** Of 1266 different antibiotic (AB) combinations tested for ENT, 12 mD and 3 MD were detected for a TA of 98.8%. For GPC, there were 825 AB combinations tested, and 1 VmD, 1 MD and 1 VMD were seen giving a TA of 99.6%. For PA there were 125 AB combinations with 100% TA. Overall TA for all AB/organism combinations in this study was 99.2%.

**CONCLUSIONS:** Based on these results, susceptibility testing can be performed accurately and reliably directly from colonies grown on CLX. Incorporation of CLX for work-up of urinary tract pathogens is anticipated to improve turn-around time to reporting of both identification and susceptibility results.

### SP19

#### EFFECTIVENESS OF ROTAVIRUS VACCINATION AGAINST SEVERE ROTAVIRUS INFECTION AMONG CHILDREN AGED <3 YEARS IN QUÉBEC

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**OBJECTIVE:** The Province of Quebec implemented a publicly funded, routine, childhood rotavirus (RV) vaccination program using Rotarix® vaccine in November 2011. We estimated vaccine effectiveness (VE) against RV gastroenteritis (GE) severe enough to present to an emergency department (ED).

**METHODS:** Prospective, active surveillance for acute GE among children aged eight weeks to three years was initiated at two Quebec EDs. Participant demographics, clinical features, vaccination status, and stool specimens were collected and tested by ELISA. Patient age was calculated as of November 1, 2011, to determine age at program implementation; children aged ≥15 weeks were considered ineligible for RV vaccination, and excluded. We used a modified case-control “test-negative” design, where cases were RV-positive and controls, RV-negative, among persons with severe GE. Cases were matched to up to five controls on symptom onset date (±30 days). Conditional logistic regression was used to calculate odds ratios (OR) and 95% CIs for the effect of vaccination on risk of severe RV GE, after adjustment for age, ED location, and hospitalization as a marker of clinical severity. VE estimates were calculated as (1-OR)\*100.

**RESULTS:** From February 2012 to September 2013, 521 patients were successfully recruited, and complete information was available for 408 participants. Of these, 201 were eligible for RV vaccination and did not receive RotaTeq® vaccine; 26 (12.9%) were RV positive. Mean age was 12.7 (95% CI 10.5 to 15.0) versus 9.8 months (95% CI 8.8 to 10.7) among cases and controls, respectively. Overall, 16.0% of cases had received ≥1 vaccine dose compared to 75.7% of controls; 4.7% of patients with ≥1 dose were RV positive. VE of RV vaccine was 75.0% (95% CI 60.8 to 85.7%) for a one-dose, 91.7% (95% CI 81.5 to 97.0%) for a ≥1-dose, and 92.3% (95% CI 81.7 to 97.4%) for a two-dose regimen.

**CONCLUSIONS:** Rotarix® vaccination is highly effective in the prevention of severe RV GE disease.

### SP20

#### POST-SPLENECTOMY IMMUNIZATION AT TERTIARY CARE FACILITIES IN HAMILTON, ONTARIO

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**BACKGROUND:** Despite national recommendations for post-splenectomy patients to get vaccinated for Pneumococcus, *Hemophilus influenzae* B (HiB) and Meningococcus, adherence to these recommendations in a Canadian setting remains unknown.

**METHODS:** We reviewed all asplenic patients admitted to Hamilton Health Sciences, Hamilton, ON, between March 2009 to November 2012.

**RESULTS:** Of the 125 patients that could have been vaccinated, vaccination rate in hospital post-splenectomy was 55% for Pneumococcus, 59% for HiB, and 58% for Meningococcus. When these vaccinations were stratified by indication for splenectomy, we found that traumatic splenectomy had the highest vaccination rates overall (30%), typically ordered by general surgery. Of the 69 patients who received pneumococcus vaccination, only two patients received Pnevnar 1<sup>st</sup>, 24 did not have the type of vaccine specified and the rest received Pneumovax.

**CONCLUSIONS:** Our institution did not consistently follow national recommendations for vaccination for asplenic patients. Vaccination is conducted in <60% of patients, and Pnevnar is rarely used despite the recommendation to prefer it over Pneumovax. Our next step is to implement a vaccination order set for those post-splenectomy to improve compliance. Also, a post-discharge information sheet will be given to these patients with instructions for their family physician, advising what vaccines were given and the schedule for subsequent vaccinations.

### SP21

#### VALUE OF AN ACTIVE SURVEILLANCE POLICY TO DOCUMENT CLEARANCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS AND VANCOMYCIN-RESISTANT ENTEROCOCCUS AMONGST IN-PATIENTS WITH PROLONGED ADMISSIONS

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**OBJECTIVES:** In-patients known to carry methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) are typically kept in contact precautions (CP) as part of infection prevention and control measures. Discontinuing CP once patients have cleared MRSA or VRE carriage can improve patient care and reduce costs, but there is little data to guide optimal means of screening for clearance. We evaluated the impact of an active surveillance policy to identify clearance of MRSA or VRE in known carriers with prolonged admissions.

**METHODS:** We reviewed all consecutive in-patients with a length of stay of 30 or more days and who were known, or newly detected, carriers of MRSA or VRE at three acute care hospitals over a one year period. Until clearance (defined as three negative consecutive swabs at least one week apart) or discharge, our policy at these hospitals requests that carriers are weekly screened for clearance for the first two months, then monthly for three months and thereafter every six months.

**RESULTS:** A total of 365 admissions were included, 132 (36.2%) patients carrying MRSA, 196 (53.7%) carrying VRE and 37 (10.1%) carrying both. We found 11.2% of MRSA carriers cleared during their hospital stay after a median of 23 days (IQR 14 to 39 days), and 18.0% of VRE carriers cleared after a median of 26.5 days (IQR 13 to 45.5 days). We estimated 2151 patient-days in CP saved compared to the alternative of continuing CP indefinitely for carriers. Cost calculations estimated overall savings of \$289,112.00 over the study period of one year.

**CONCLUSIONS:** Active surveillance of known MRSA and VRE carriers to detect clearance during prolonged hospital stays reduced isolation days and costs.

## SP22

## EMERGENCY DEPARTMENT AND INPATIENT MANAGEMENT OF FEBRILE INFANTS UNDER SIX WEEKS OF AGE: PERCEIVED UTILITY OF RESPIRATORY VIRUS TESTING

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**BACKGROUND:** Well appearing febrile infants with viral illnesses cannot be easily distinguished from those with occult life-threatening infections. This study sought to assess current approaches to febrile infants <6 weeks of age, and determine how respiratory virus testing influences management.

**METHODS:** A scenario-based survey describing two hypothetical cases of febrile infants without an infectious focus three and five weeks of age was sent to tertiary care pediatric ED and hospitalist physicians at a single center. Participants were asked multiple-choice questions on management decisions with and without results of respiratory virus testing. Chi-square testing was used to compare proportions.

**RESULTS:** Response rate was 93% (n=32 ED, 22 hospitalists). Investigations most commonly performed for both three- and five-week-old infants were urine culture, urine analysis, CBC and blood culture. Lumbar puncture was performed less frequently in five-week old infants (69% versus 98%, P<0.001), and by hospitalists compared to ED physicians (50% versus 81%, P<0.01). Respiratory virus testing by nasopharyngeal swab (NPA) did not differ between age groups (67% in both), however hospitalists performed NPA testing more frequently than ED physicians (82% versus 56%, P<0.01). Infant age impacted admission decisions (three versus five weeks, 100% versus 72%; P<0.001), but positive NPA did not. Overall, positive NPA decreased empirical antibiotic treatment (P<0.01). Among hospitalists, 82% would discharge five-week-old infants in ≤24 h with a positive NPA and otherwise negative workup; admission duration among three-week infants was highly variable.

**CONCLUSIONS:** Management of febrile infants <6 weeks of age differs between ED and hospitalist physicians and by infant age. Respiratory virus testing could potentially reduce empirical antimicrobial treatment and permit earlier discharge. Establishment of guidelines for this patient population is warranted.

## SP23

## DESCRIPTIVE ANALYSIS OF HBV AND HCV POSITIVE PATIENTS REFERRED FROM A REFUGEE CLINIC

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**OBJECTIVES:** To: 1) describe the characteristics of refugees referred to our hepatitis clinic; and 2) compare characteristics of refugees with HBV to a control HBV population.

**METHODS:** A retrospective chart review of New Canadians' Health Clinic (NCHC) referrals to the Hepatitis Support Program (HSP) from April 2007 to January 2013 was performed. A 1:1 case-control analysis was performed comparing refugees with HBV to randomly selected controls from a prior study looking at HBV genotype epidemiology.

**RESULTS:** NCHC referred 68 cases; 57 HBV, 10 HCV and one HBV-HCV. Median age was 29.5 (IQR 24.5 to 38.0); 64.7% were male. Most were born in Asia (45.6%) or Africa (47.1%). Few had identifiable risk factors, beyond birth in an endemic country. In total, 12 (17.6%) were treated; two received tenofovir and four entecavir for HBV, while six with HCV received peginterferon and ribavirin. Of the cases with HBV (58), 16.1% were HBeAg positive and median HBV DNA was 1590 (IQR 52 to 18,800). Most (79%) were inactive carriers, with 1.8% immunotolerant, 15.8% immune-active and 3.4% HBeAg negative disease. Dominant HBV genotypes were A (22.4%) and C (24.1%). Compared to controls, refugees were younger (median 27.5 versus 34 years; P=0.0016), more likely to have been born in Africa (57.7 versus 27.6%; P=0.005) and less likely to have genotype B (8.1% versus 19%; P=0.05). The clinical phase of infection, AST, ALT, bilirubin, INR, HBV DNA and proportion treated were not significantly different between cases and controls.

**CONCLUSIONS:** Refugees from NCHC represent about 3% of hepatitis B referrals, but about 10% of our HBV referrals over this timeframe. Refugees with HBV differed from controls in birth region and genotype which may impact natural history and treatment outcomes. Further studies are needed to document the epidemiology of HBV and HCV in refugees to Canada and engage these patients in care.

## SP24

## REPLACING THE STAPHYLOCOCCAL PROTEIN A (SPA) GENE ALTERS ITS BIOLOGICAL CHARACTERISTICS, BEHAVIOR AND VIRULENCE IN A HIGH-VIRULENCE COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (CA-MRSA) STRAIN USA300

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**OBJECTIVES:** USA300 is a predominant CA-MRSA strain which has caused significant morbidity and mortality, and has disseminated widely worldwide. Currently, the information about the virulence, the transmissibility and the origin of this pathogen is limited. We therefore sought to evaluate the role of the *spa* gene in the virulence of CA-MRSA strain USA300, and constructed a chimera clone by replacing the USA300 *spa* with the *spa* from a low-virulence *S aureus* strain.

**METHODS:** A high-virulence CA-MRSA strain USA300 (*spa* t008) and a typically low-virulence hospital-associated MRSA strain CMRSA6 (*spa* t037) with different genetic backgrounds (ST8 and ST239, respectively) were selected. A chimera clone was constructed by replacing the USA300 *spa* with the CMRSA6 *spa* using homologue recombination. Expression of the *spa* gene was confirmed by western blot. The chimera clone was evaluated by its growth curve, biofilm formation, and virulence using the *Caenorhabditis elegans* nematode infection model, and compared with the wild type (WT) donor strains.

**RESULTS:** The growth rate of the chimera clone was significantly lower than WT-USA300 strain but higher than the WT-CMRSA6 strain. The chimera clone had a 3.4-fold increase in biofilm formation compared to the WT-USA300 strain, bringing it to the same level as the WT-CMRSA6 strain biofilm production. The chimera clone had reduced nematocidal activity at 20% compared to 82% for the WT-USA300 strain (P<0.001) and 5% for the WT-CMRSA6 strain (P<0.001) at day 5, respectively, in the *C elegans* nematode model.

**CONCLUSION:** Replacing the *spa* gene of a highly virulent *S aureus* strain altered its biological characteristics, behavior and virulence. Our results further corroborate our hypothesis that *spa* plays an important key role in the pathogenicity of the CA-MRSA strain USA300.

## SP25

## WITHDRAWN

## SP26

## INFECTIONS CAUSED BY ACTINOMYCES NEUII: A CASE SERIES AND LITERATURE REVIEW OF AN UNUSUAL BACTERIUM

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**OBJECTIVES:** *Actinomyces neuii* is a Gram-positive bacillus rarely implicated in human infections. This study analysed recent *A neuii* infections in Alberta, Canada, and reviewed the literature regarding this unusual pathogen.

**METHODS:** Five cases of *A neuii* infection were identified in 2013 in Alberta, Canada. Samples were cultured aerobically and anaerobically. A predominant Gram-positive coryneform bacillus with no branching was isolated in each case. Testing was initially done with API-Coryne

(bioMérieux) and isolates were sent to the Provincial Laboratory for further testing. MALDI-TOF (bioMérieux) and/or DNA sequencing were used to identify isolates.

**RESULTS:** Isolates were catalase positive Gram-positive bacilli which grew equally aerobically and anaerobically. Identities were confirmed by MALDI-TOF (five of five) and DNA sequencing (four of five). All patients (46 to 85 years of age) had soft tissue infections; when possible, incision and drainage was done followed by a course of antibiotics. Agents used included cephalexin, ertapenem, ciprofloxacin and clindamycin. All had agreeable outcomes. Of the 100 cases in the literature of *A. neuui* infection (zero to 94 years of age, mean age 49 years), most involved soft tissue or atheromas. However, the organism was found in a range of infections, including genitourinary, eye, and prosthetic infections. Isolates were susceptible to beta-lactams, vancomycin, clindamycin and tetracycline.

**CONCLUSIONS:** While *A. neuui* is infrequently recognized, it has been implicated in a diverse array of infections. Increased use of MALDI-TOF will lead to higher rates of identification; thus, understanding the pathogenic potential of this coryneform bacterium and its typical susceptibility profile will aid clinical decision-making.

### SP27

#### URINARY TRACT INFECTION CAUSED BY AN UNUSUAL PATHOGEN IDENTIFIED USING CYSTINE-LACTOSE-ELECTROLYTE DEFICIENT MEDIA AND MATRIX-ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY (CLINICAL VIGNETTE POSTER)

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**OBJECTIVES:** A 47-year-old female presented to her general practitioner with a history of increasing confusion. We report an interesting and unusual case of urinary tract infection, highlighting important clinical and microbiological considerations.

**METHODS:** Urine was collected for urinalysis and cultures the day of presentation and two and five days prior. Urine was cultured using the Uricult® Trio (Orion Diagnostica, Oy, Finland) dip slide method with Cystine-Lactose-Electrolyte Deficient (CLED), MacConkey and β-glucuronidase-producing *E. coli* detection medium. Besides routine culture setup, isolates were cultured in O<sub>2</sub> and CO<sub>2</sub> on chocolate agar, a Martin Lewis agar, a thioglycollate broth and a fastidious organism broth. Isolates were also cultured anaerobically in fastidious anaerobic agar and thioglycollate broth. Identification was done using Vitek® MS matrix-assisted laser desorption ionization-time of flight mass spectrometry and 16s rRNA sequencing. Antibiotic susceptibility testing was done based on CLSI 2013 guidelines.

**RESULTS:** Urinalysis on the day of presentation and two days prior, demonstrated white cell clumps and bacteria. Three consecutive urine cultures grew 10<sup>8</sup> cfu/mL of a Gram-negative cocci on CLED media. The isolate did not grow on MacConkey, blood, chocolate or Martin-Lewis agar, but grew anaerobically. Appropriate antibiotic treatment was given and the patient's confusion resolved.

**CONCLUSION:** This case demonstrates the role of an unusual organism in urinary tract infection, along with its presentation and pathogenesis. Specialists in microbiology and infectious diseases must be aware of the ability and limitations of their local laboratory's urine culture method to detect these pathogens.

### SP28

#### CARBAPENEMASE PRODUCING ORGANISMS IN BC, 2008 –2013

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**BACKGROUND AND OBJECTIVES:** Carbapenemase-producing organisms (CPOs) pose an increasingly serious therapeutic and infection control problem worldwide, including in Canada. Since 2010, a province-wide phenotypic screening and PCR-based testing for CPOs were implemented in our province. A review of these organisms, from 2008 to May 2013, was performed with the objective to characterize associated risk factors, resistance mechanisms, and possible person-to-person transmission and institutional spread.

**METHODS:** Data on pertinent patient demographics and resistance mechanisms genetically encoded by the isolates, as well as antibiogram profiles were reviewed and descriptive analysis of their numbers and resistance mechanisms was carried out. Pulsed-field gel electrophoresis (PFGE) profiles for available *E. coli* and *K. pneumoniae* isolates were obtained and analysed using the BioNumerics software.

**RESULTS:** We found that CPOs have been increasing in numbers over time, from one isolate from one patient in 2008 (retrospectively detected) to 84 isolates from 63 patients by May of 2013. *K. pneumoniae* was the most commonly isolated species, with NDM being the most commonly found resistance mechanism. Although the isolates overall lacked clonality, apparently related clusters of organisms were seen.

**CONCLUSIONS:** Rising numbers of CPOs demonstrate the need for tight infection control practices. Presence of apparently related clusters of organisms argues towards evidence of inter-patient organism transmission both within and across institutions. Additionally, there is a possibility of transmission of mobile elements between organisms, which needs to be further addressed.

### SP29

#### CHRONIC NECROTIZING ASPERGILLOSIS IN A DIABETIC PATIENT USING MARIJUANA

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**OBJECTIVES:** 1) To describe a case of chronic necrotizing pulmonary aspergillosis (CNPA) in a type 1 diabetic patient; 2) to determine potential sources of the patient's infection by culturing his marijuana and nebulizer; and 3) to review the literature for cases of invasive aspergillosis in patients with diabetes and marijuana use.

**METHODS:** A patient history, physical exam and chart review were done as well as a Medline search for cases of invasive aspergillosis associated with diabetes and marijuana use.

**RESULTS:** A 29-year-old diabetic male who uses vaporized marijuana presented with shortness of breath and pleuritic chest pain. CT chest showed a left pneumothorax, and left lower lobe (LLL) consolidation with cavitation, likely communicating with the pleura. His pneumothorax persisted despite a chest tube requiring surgery. LLL wedge resection cultures grew *A. rugulosa* confirmed by ITS DNA sequencing, a *Penicillium* species and a non-sporulating fungus. Pleural fluid grew *A. fumigatus*. Pathology showed hyphae invading into the parenchyma and pleura. Immunologic workup was negative. His infection was likely acquired through marijuana use as his marijuana cultures grew various fungi including a *Penicillium* species, *A. versicolor*, and *A. ochraceus* while vaporizer cultures were negative. He was started on voriconazole with subsequent weight gain and cessation of night sweats. Literature review confirmed diabetes as a risk factor for invasive aspergillosis. There are several reports of patients with invasive aspergillosis and marijuana use.

**CONCLUSIONS:** Our patient had LLL CNPA and a fungal empyema. Fungal infections should be considered in the differential for pulmonary disease in diabetic patients, particularly those who use marijuana.

### SP30 WITHDRAWN

### SP31 WITHDRAWN

### SP32 IN VITRO BETA-LACTAM SUSCEPTIBILITY TESTING OF STREPTOCOCCUS PNEUMONIAE BY COMMERCIAL METHODS COMPARED TO CLSI BROTH MICRODILUTION

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**OBJECTIVES:** Elevated beta-lactam MICs in *S pneumoniae* are becoming more frequent and we have observed discordance between beta-lactam susceptibility test methods in these isolates with higher MICs. We evaluated four commercial susceptibility test methods compared to broth microdilution (BMD).

**METHODS:** *S pneumoniae* isolates collected between 2011 and 2013 were grouped based on penicillin MICs of  $\leq 0.12$  mg/L to 0.25 mg/L, 0.5 mg/L to 1.0 mg/L and  $\geq 2.0$  mg/L. The same inoculum of each strain was used to test penicillin, amoxicillin, ceftriaxone and meropenem MICs by BMD, Etest®, Vitek® 2 AST-ST01 (bioMérieux), M.I.C.Evaluator™ (M.I.C.E.) and Sensititre™ (Thermo Fisher). Essential agreement (EA) and categorical agreement (CA) were compared to BMD using CLSI M100-S23 breakpoints.

**RESULTS:** From 91 clinical isolates tested, the EA of all test methods was  $\geq 90\%$  for penicillin and ceftriaxone. CA for Sensititre™ compared to BMD for all antibiotics was  $>90\%$  except for penicillin (oral breakpoint, CA=89%). CA for ceftriaxone (meningitis breakpoint) and Vitek® 2, Etest® and M.I.C.E.™ was 76%, 77% and 68%, respectively, with 2.2%, 1.1% and 1.1% of the 91 isolates tested by these methods were considered very major errors. CA for amoxicillin and meropenem with Etest® and M.I.C.E.™ were 77% and 85% respectively.

**CONCLUSION:** EA for all susceptibility test methods was high compared to BMD, while CA was unacceptably low. Most concerning is the potential for reporting ceftriaxone resistant isolates as susceptible in clinical cases of meningitis using gradient diffusion methods. The clinical significance of these observations requires further investigation.

### SP33 PSEUDO-OUTBREAK OF MYCOBACTERIUM FORTUITUM DUE TO CONTAMINATED ICE MACHINES

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**OBJECTIVES:** We observed a 10-fold increase in isolation rates of *M fortuitum* from respiratory specimens over a three-month period. The majority of these isolates were from patients in contact with one of three wards at a tertiary care hospital. An epidemiologic investigation was undertaken to identify the source.

**METHODS:** Environmental samples were collected from water/ice machines and tap water on affected and uninvolved wards as well as the Microbiology laboratory. All specimens were cultured with the use of a continuously monitored broth system for mycobacteria isolation. Positive samples for mycobacteria were sent to the regional Public Health Laboratory for identification. Case and control as well as environmental isolates were compared by genotyping using ERIC-PCR. A retrospective chart review was conducted to evaluate clinical impact.

**RESULTS:** *M fortuitum* was isolated from 20 respiratory samples from 17 patients. Surveillance cultures obtained from uninvolved wards were negative for mycobacteria. *M fortuitum* was isolated from three ice machines on affected wards but not from tap water or water in the Microbiology laboratory. ERIC-PCR based typing revealed that patient isolates were identical or closely related, with the exception of one patient previously colonized. Isolates from environmental cultures were identical or closely related to

patient isolates. All but one case was thought to represent transient colonization. One patient was changed from an anti-tuberculosis treatment regimen to a regimen for *M fortuitum*; this resulted in clinical progression of tuberculosis that was later microbiologically confirmed.

**CONCLUSION:** The *M fortuitum* pseudo-outbreak was due to contaminated ice machines. A negative clinical impact was observed in one patient.

### SP34 CLINICAL SIGNIFICANCE AND CORRELATION BETWEEN EBV QUALITATIVE PCR ON PLASMA AND WHOLE BLOOD EBV VIRAL LOAD IN HAEMATOPOIETIC STEM CELL TRANSPLANT (HSCT) PEDIATRIC PATIENTS

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**OBJECTIVES:** To evaluate the clinical significance and correlation between EBV qualitative PCR positivity in plasma and EBV viral load in whole blood in HSCT patients.

**METHODS:** Retrospective chart review of patients who underwent HSCT at the Hospital for Sick Children from January 2010 to December 2013.

**RESULTS:** One hundred and ten patients (68.2% male) with HSCT, age ranging from one month to 17.33 years (median age 8.5 years) were analyzed. Acute myeloid leukemia and acute lymphoblastic leukemia accounted for 39% and 20.1% respectively. Recipient pre-transplant EBV seropositivity was 90%. Donor serostatus was not done. None of the patients had a whole blood EBV viral load above a detection limit of  $\geq 1000$  copies/mL pre-transplant. Antiviral prophylaxis was given in 77.3%, primarily for CMV and HSV seropositivity. Six of 110 (5.5%) were positive for EBV in plasma. Of these, two had whole blood viral loads of  $\geq 100,000$  copies/mL and four had  $< 100,000$  copies/mL; remaining patients were negative for EBV in plasma. The association between plasma positivity and an EBV viral load of  $\geq 100,000$  copies/mL in whole blood was statistically significant at  $P=0.0073$ . Two patients developed EBV disease, both with elevated transaminases and with viral load values  $\geq 100,000$  copies/mL plus plasma positivity. Patients were followed-up for one to 36 months (median 7.5 months).

**CONCLUSION:** Plasma PCR positivity was an uncommon event ( $< 6\%$  of subjects). Quantitative EBV viral PCR level in whole blood of  $\geq 100,000$  copies/mL correlated with EBV PCR positivity in plasma. Further studies will be conducted to evaluate whether this correlation is applicable to other patient populations.

### SP35 ANTIBACTERIAL ACTIVITY OF STEARYLAMINE- CONTAINING LIPOSOMES AGAINST *HELICOBACTER PYLORI*

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**OBJECTIVE:** *Helicobacter pylori* infects half of the world population causing gastritis, peptic ulcer or gastric cancer. Standard therapies often fail in populations where prevalence of *H pylori* infection is high. Cationic liposomes are reported to have antimicrobial activity to some bacteria and protozoans at concentrations that are nontoxic to mammalian cells. This study investigates the antimicrobial activity of different concentrations of stearylamine-containing liposomes against *H pylori*.

**METHODS:** Cationic liposomes containing egg phosphatidylcholine, cholesterol, stearylamine (molar ratio 7:3:1) were prepared by membrane extrusion. Liposome sizes were assessed using the Malvern Zetasizer Nano S. Recovered phospholipid content was assayed using the Stewart assay. *H pylori* A64, a clinical isolate, was cultured in 3.7% brain heart infusion (BHI)/0.5% yeast extract (YE)/5% horse serum (HS) agar plate for 24 h at 37°C under microaerobic conditions. Growth was suspended in BHI/YE/HS broth ( $OD_{600}=0.1$ ) and incubated with shaking at 120 rpm for 24 h in the absence and presence of increasing concentrations of cationic liposomes (0  $\mu\text{g/mL}$ , 25  $\mu\text{g/mL}$ , 50  $\mu\text{g/mL}$ , 75  $\mu\text{g/mL}$ , 100  $\mu\text{g/mL}$  and

125 µg/mL). Aliquots were taken at 0.5 h, 3 h, 6 h and 24 h, then serially diluted and plated for cfu/mL determination.

**RESULTS:** Liposome sizes were uniformly distributed (mean = 130 µm) and contained 88.6% of total phospholipid from the original preparation. *H pylori* growth was inhibited by 86%, 90% and 97% after 0.5 h, 3 h and 6 h, respectively. Decreased inhibition (77%) was observed after 24 h (stationary phase).

**CONCLUSION:** Stearylamine-containing cationic liposomes have antibacterial activity against *H pylori*, and offer potential for a new *H pylori* therapy, although selectivity must be addressed to minimize alteration of the gut microbiome.

### SP36

#### WITHDRAWN

### SP37

#### PHYLOGENETIC ANALYSIS OF DISTINCT HEPATITIS C VIRUS GENOMIC REGIONS FOR IDENTIFICATION OF TRANSMISSION CLUSTERS IN BRITISH COLUMBIA

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**BACKGROUND:** Hepatitis C virus (HCV) exhibits high intrahost and global genetic diversity, the degree of which varies across the viral genome. Sequence analysis of highly conserved regions including the HCV Core and NS5b polymerase, is used to monitor HCV evolution and spread while sequencing more variable regions such as the HCV Envelope genes and hypervariable region (HVR)-1 has been used to identify recent transmission events.

**OBJECTIVE:** To identify potential HCV transmission clusters in BC by sequencing various regions of the HCV genome in patients determined to be recently infected.

**METHODS:** HCV RNA was extracted from 240 HCV positive sera and the region extending from HCV Core to HVR1 was PCR-amplified. The amplicons were sequenced, sequences were aligned using MUSCLE and a maximum-likelihood tree was constructed. A patristic distance cutoff of 0.03 was used to extract phylogenetic clusters. A subset of 95 partial NS5b sequences was used for comparison.

**RESULTS:** A total of 149 Core-HVR1 sequences from 133 patients were obtained. Twenty sequence clusters were found. Thirteen clusters were of serial samples from the same patient, confirming their high similarity. Seven clusters between different patients were also found. In NS5b, seven clusters from patient serial samples and eight clusters from separate patients were identified. On average, NS5b clusters had closer patristic distances than Core-HVR1 clusters. One Core-HVR1 cluster was confirmed as a cluster in NS5b.

**CONCLUSION:** Sequencing HCV in different genomic regions affects cluster analysis. Identified transmission clusters may be further investigated by linking sequences to epidemiological data. Molecular epidemiology of HCV provides a powerful tool to identify transmission clusters in populations requiring prevention interventions.

### SP38

#### UTILIZATION OF *BORDETELLA PERTUSSIS* AND *B PARAPERTUSSIS* PCR TESTING IN A REGIONAL CHILDREN'S HOSPITAL

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**OBJECTIVES:** We examined the ordering trends and results of *Bordetella pertussis* (Bp) and *B parapertussis* (Bpp) PCR testing at the Children's Hospital of Eastern Ontario (CHEO) to determine if Bp and Bpp test requesting increases during viral respiratory season, and to determine the frequency of coinfections with Bp/Bpp and ≥1 respiratory virus.

**METHODS:** Data were collected from February 2012 to March 2013 from a CHEO laboratory database. Test ordering date, patient age, hospital

location, Bp/Bpp result, and viral studies result were collected on all patients. Testing for influenza A/B, RSV, HMPV, parainfluenzavirus, and adenovirus was done by DFA, culture, PCR or a combination thereof.

**RESULTS:** During the study period, there were 419 requests for *Bordetella* PCR. 318 (75%) of these were from the emergency department (ED) at CHEO. Of the 419 *Bordetella* requests, viral studies were also ordered in 226 (53%). Bp/Bpp results were available for 375 of all samples. Thirty-one of 375 (8%) were (+) for Bp (n=28) or Bpp (n=3). Viruses were detected in 98 of 226 (43%) of samples. Of the 31 samples that tested (+) for Bp/Bpp, 11 had requests for viral studies as well; only three of these were virus (+) (one each with RSV, Rhinovirus, and HMPV). Forty-three percent of testing for Bp/Bpp was in children <1 year of age. The average age for testing was 2.7 years and the average age of (+) Bp/Bpp patients was 5.7 years. There was a peak in Bp/Bpp ordering from October to December 2012; however, no seasonality to Bp/Bpp positivity was observed.

**CONCLUSIONS:** The majority of *Bordetella* test requests originated from the ED. While there was more ordering of Bp/Bpp in respiratory virus season, almost half of these samples were respiratory virus (+) only. There was no seasonal trend to *Bordetella* infection, and viral coinfection was rare in patients with Bp or Bpp.

### SP39

#### EVALUATION OF ANTIMICROBIAL PRESCRIBING IN HOSPITALIZED SOLID ORGAN TRANSPLANT RECIPIENTS THROUGH SERIAL REAL-TIME POINT PREVALENCE AUDITS

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**OBJECTIVES:** Solid organ transplant (SOT) recipients are high users of antimicrobials due to increased risks and incidence of infections. They are also disproportionately affected by multi-drug resistant organisms, prompting calls for antimicrobial stewardship (AS) in this population. This study aims to evaluate antimicrobial prescribing practices for concordance with AS principles, and the impact of infectious diseases (ID) specialists on antimicrobial prescribing.

**METHOD:** Four real-time audits were conducted on SOT in-patients in an academic hospital where all active antimicrobials, excluding routine prophylaxes, were independently assessed by two investigators. Each regimen was evaluated for timeliness of initiation, optimal empiric spectrum, dosing, route, de-escalation and duration using electronic and paper patient records. Each was categorized as 'concordant', 'discordant' or 'not assessable'. Proportion of AS-concordant regimens in ID versus 'no ID' consultation was compared using the  $\chi^2$  test.

**RESULTS:** One hundred and eighty audits were performed in 143 patients (28 patients [19.6%] had >1 audit) over four randomly selected days, at three- to four-week intervals. Of 180 audits, 110 (61.1%) involved at least one antimicrobial. Antimicrobials were most commonly prescribed in lung and liver transplant recipients, with 38.2% patients from each group prescribed at least one regimen, followed by kidney (11.8%), kidney-pancreas (8.2%) and heart (3.6%). Overall, 66.4% (77) audited regimens were AS-concordant; 30% (33) were AS-discordant and 3.6% (4) were not assessable. Inter-rater agreement (kappa) was 0.875. AS-concordant prescribing in patients with ID and without ID consultation was 77.2% and 54.7%, respectively (P=0.04). In those consulted by ID, SOT team's non-adherence to ID recommendation contributed to 67% of AS-discordant prescribing.

**CONCLUSION:** Serial point prevalence audit is a useful tool to evaluate antimicrobial prescribing patterns. ID consultation is a valuable point-of-care resource and ID specialists are key partners to a successful AS program.

## SP40

**SICKNESS PRESENTEEISM AMONG GENERAL PAEDIATRICS RESIDENT PHYSICIANS IN CANADA****K Mitchell, J Vayalumkal**

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**BACKGROUND:** Sickness presenteeism (SP) is the act of coming to work while sick. Sick health care workers are unique in that they pose a risk to the patients for whom they care. A small number of studies have looked at non-healthcare workers and factors affecting their decision whether to come to work sick; however, subjective factors affecting resident physicians have not been studied and no data exists in any capacity on SP among physicians in Canada.

**METHODS:** Ethics approval was obtained through the University of Calgary Conjoint Health Research Ethics Board. An online questionnaire was created and distributed to all general pediatric residency programs in Canada. SP was defined as coming to work while sick with symptoms including cough, fever, rhinorrhea, sinus pressure/congestion, sore throat, vomiting, and/or diarrhea. Data were compiled and evaluated using SPSS statistical software V.20 and Microsoft Excel.

**RESULTS:** Response rate of completed questionnaires was 50.3%. 96.9% of pediatrics residents identified as being sick during the study period stated that they had come to work despite being ill. 86.3% of residents who came to work sick reported to don extra personal protective equipment (PPE) prior to patient contact. Reasons for coming to work while sick most frequently selected as 'very important' were being on a high-service rotation, not wanting to cause extra work for or disappoint one's co-residents.

**CONCLUSION:** The vast majority of pediatrics residents come to work while sick, although many report using extra PPE prior to patient contact. The most important factors increasing a resident's likelihood to come to work sick related to perceived high work load.

## SP41

**WITHDRAWN**

## SP42

**ELEVATED S100A12 GENE EXPRESSION AT THE TIME OF INITIAL PRESENTATION IS ASSOCIATED WITH INCREASED SEVERITY OF ILLNESS DURING SEPSIS****N Lyle<sup>1</sup>, C Fjell<sup>1</sup>, D Hancock<sup>2</sup>, J Boyd<sup>1</sup>, R Hancock<sup>1</sup>**<sup>1</sup>University of British Columbia, Vancouver, BC; <sup>2</sup>Centenary Institute of Cancer Medicine and Cell Biology, New South Wales, Australia

**OBJECTIVES:** S100A12 is a pro-inflammatory protein found primarily in granulocytes. It is frequently cited as a marker of various non-infectious inflammatory diseases, and is classified as a damage-associated molecular pattern molecule. Its value as a diagnostic and prognostic marker in an unselected population of adult patients with suspected sepsis is unknown.

**METHODS:** In this prospectively conducted observational study, S100A12 gene expression levels were determined by RNA sequencing of the blood of adult patients with suspected sepsis (n=72) at the time of initial presentation to the emergency room at Saint Paul's Hospital, Vancouver, BC. Twelve patients undergoing elective surgery were enrolled as negative controls. Clinical data including the clinical adjudication of sepsis status among the sepsis patients was determined by retrospective chart review by an infectious diseases trainee who was blinded to the RNA sequencing results.

**RESULTS:** S100A12 levels were correlated with increasing signs of systemic inflammation in sepsis patients. S100A12 levels are elevated among patients with who were ultimately admitted to the intensive care unit with probable sepsis when compared with negative controls. Among patients who ultimately were not admitted to the intensive care unit, S100A12 levels were lower among patients who were classified as having localized infection or who were not likely infected. S100A12 levels were not associated with mortality; however, higher levels of S100A12 expression were strongly associated with subsequent development of organ failure (P<0.001).

**CONCLUSIONS:** S100A12 gene expression determination may be a useful diagnostic and prognostic tool in the management of patients with suspected sepsis.

## SP43

**ORGANISM IDENTIFICATION ON EARLY SUBCULTURE COLONIES BY VITEKMS MATRIX-ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT (VITEKMS) AND ITS IMPACT ON ANTIBIOTIC STEWARDSHIP****Y Yu<sup>1</sup>, W McCaffrey<sup>2</sup>, M McConnell<sup>2</sup>, N Irfan<sup>3</sup>, D Yamamura<sup>1,2</sup>**<sup>1</sup>McMaster University; <sup>2</sup>Hamilton Regional Laboratory Medicine Program; <sup>3</sup>Hamilton Health Sciences, Hamilton, ON

**OBJECTIVES:** Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF) provides rapid and reliable identification of clinically important pathogens. As part of our validation of the VitekMS™ (BioMerieux), the potential impact of MALDI-TOF organism identification on antibiotic stewardship was evaluated.

**METHODS:** The accuracy of the VitekMS to identify positive blood cultures from early subculture growth was evaluated. Eight-six positive blood cultures from April to June 2013 were subcultured and isolate growth as early as 2 h incubation was identified by the VitekMS. Results were compared to routine identification methods. A retrospective chart review was performed to estimate the potential impact on antibiotic therapy and stewardship.

**RESULTS:** The VitekMS correctly identified 78 of 78 isolates after an average 5.7 h incubation (range 2 h to 24 h, median 4.5 h). Time to identification by the VitekMS was an average 22.8 h earlier (range 2 h to 70 h, median 23 h) compared to routine methods. The isolates identified were: 51 (65.4%) GPC (*S aureus*, n=9; coagulase negative staphylococci n=23; *Enterococci* spp. n=11; various streptococci n=9; *Micrococcus* spp. n=1); 21 (28.2%) GNR, including four ESBL producing bacteria; three (3.8%) GPR; 3 (3.8%) yeast. Charts of patient from 66 of 78 isolates were reviewed. The average age was 64 years (ranging 10 months to 91 years). More rapid organism identification by the VitekMS would allow earlier modification of empiric treatment to narrow spectrum in 15 of 66 (22.7%), discontinuation of antibiotics in seven of 66 (10.6%) and improved spectrum for two of 66 (3%). In 42 of 66 (63.6%) earlier identification would not impact therapy.

**CONCLUSIONS:** Earlier accurate identification by the VitekMS could have a significant impact on antibiotic stewardship by discontinuing unnecessary antibiotic therapy and by narrowing antibiotic spectrum. However, it does not have a major impact on antibiotic resistant organisms such as MRSA and ESBL. Further research on methods to optimize antibiotic stewardship in conjunction with rapid identification by VitekMS is required.

## SP44

**'GRAINS' OF WRATH (CLINICAL VIGNETTE POSTER)****R Somayaji, L Berthiaume, K Fonseca, J Conly**

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**CASE PRESENTATION:** A 26-year-old man presented with three days of influenza-like symptoms, diarrhea and one day of acute dyspnea. He had no cough, neck stiffness, dysuria, joint pain, rash, recent illnesses or travel. He worked as an assistant grain elevator manager and had recently cleaned up "heated grain". He had rapid cardiorespiratory deterioration requiring intubation and vasopressors. He was started on veno-venous extracorporeal life support (VV-ECLS) for oxygenation failure. His hemoglobin was 78 g/L, WBC 38.4×10<sup>9</sup>/L with left shift, and platelets were 55×10<sup>9</sup>/L. He had mild transaminitis (ALT 108 U/L; AST of 196 U/L), evidence of hemolysis, and acute kidney injury with a creatinine of 234 μmol/L. Chest CT revealed peribronchial interstitial infiltrates with bilateral small pleural effusions. Blood, sputum, pleural fluid and urine cultures were obtained. Serum hantavirus antibody testing (performed at the National Microbiology Laboratory) was IgM+ and serum PCR testing was positive for the Sin Nombre virus. He required ECLS for four days following which he was successfully extubated. He required a pigtail catheter for a right pneumothorax and anticoagulation for line-associated bilateral upper extremity thromboses. His kidney and liver function improved allowing for discharge 10 days post-presentation.

**DISCUSSION:** Diagnosis of hantavirus pulmonary syndrome (HPS) can be made clinically in the presence of fever, hypoxemia and bilateral interstitial edema in conjunction with lab testing. In endemic areas, the

presence of thrombocytopenia, left shifted granulocytes without toxic changes, atypical lymphocytes and hemo-concentration have up to 98% diagnostic sensitivity in patients with typical clinical findings. Our case was unusual insofar that he had HPS with associated severe renal dysfunction. Hantavirus associated infections may be life threatening and must be considered in the differential of a rapidly progressive pneumonia in a healthy individual.

#### SP45

#### USING PHOTOGRAPHY-BASED SOCIAL MEDIA IN INFECTIOUS DISEASES EDUCATION: #A PICTURE IS WORTH A 1000 MICROBES #INSTAGRAM POSITIVE IN MEDICAL EDUCATION

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**OBJECTIVE:** Social media platforms may be used as learning tools, offering students the flexibility of accessing information anytime, anyplace, and in any space. We examined Instagram®, a photography-based online social networking platform, as a tool to reinforce learning related to a seven-week infection/immunity/inflammation course in medical school.

**METHODS:** A private Instagram® account (iimemoryhangers) was loaded with Memory Hangers (visual mnemonics with accompanying explanations, representing specific concepts discussed in each lecture). After reviewing ground rules, students could access the Instagram® Memory Hangers; comment on them or like them. The tool was evaluated with usage analysis and student surveys.

**RESULTS:** A total of 58 of 199 students (29%) followed iimemoryhangers. Over seven weeks, 35 Memory Hangers were uploaded with 85 likes posted by 26 different students. Of the 199 students, 125 responded to the survey (63%) and 43 of 125 (34%) used the Instagram® Memory Hangers. Of those 43 students, the majority looked at them at least weekly (n=21), at home (n=31), during the evening (n=27), but students also reported use at school, on public transit, and at all hours of the day and night. Thirty-one students felt the most useful aspects of the Instagram® Memory Hangers to be both the visual component and the explanation. Thirty-five students identified the importance of easy accessibility. The Instagram® Memory Hangers were useful for studying course content according to 36 of 43 students (84%).

**CONCLUSION:** The Instagram® Memory Hangers were a useful study aid, allowing students to easily access and review essential infectious diseases concepts using visual mnemonics and brief explanations.



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